

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
NATIONAL INSTITUTES OF HEALTH  
NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

MINUTES OF THE NATIONAL ADVISORY  
ENVIRONMENTAL HEALTH SCIENCES COUNCIL

September 1-2, 2010

The National Advisory Environmental Health Sciences Council convened its one hundred thirty-first regular meeting on September 1, 2010 in the Rall Building, Rodbell Auditorium, National Institute of Environmental Health Sciences, Research Triangle Park, NC. Dr. Linda Birnbaum presided as Chair.

The meeting was open to the public on September 1, 2010 from 8:30 a.m. to 5:30 p.m. and on September 2, 2010 from 8:30 a.m. to 10:00 a.m. In accordance with the provisions set forth in Section 552b(c)(4) and 552b(c)(6), Title 5, U.S. Code and Section 10(d) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2), the meeting was closed to the public on September 2, 2010 from 10:00 a.m. to 1:00 p.m. for consideration of grant applications. Notice of the meeting was published in the *Federal Register*.

**Council Members Present**

Stephen Baylin, MD  
Chris Bradfield, PhD  
Julia Brody, PhD  
Hillary Carpenter, PhD  
Thomas Gasiewicz, PhD  
Andrea Hricko, MPH  
Mary M. Lee, MD  
George Liekauf, PhD  
R. Stephen Lloyd, PhD  
Kenneth Ramos, PhD  
Palmer Taylor, PhD  
Nsedu Obot Witherspoon, MPH

## **NIEHS Staff**

Joel Abramowitz, PhD  
Bruce Androphy  
Kathy Ahlmark  
Janice Allen, PhD  
Beth Anderson  
Eddy Ball  
David Balshaw, PhD  
Martha Barnes  
Linda Bass, PhD  
Sharon Beard  
Linda Birnbaum, PhD, DABT, ATS  
Wanda Boggs  
John Bucher, PhD  
Matthew S. Burr  
Lisa Helbling Chadwick, PhD  
Pamela B. Clark  
Gwen Collman, PhD  
Caroline Dilworth, PhD  
Christina Drew, PhD  
Dorothy Duke  
Sally Eckert-Tilotta, PhD  
Lisa Edwards  
Benny Encarnacion  
Christine Flowers  
Mary Gant  
Barbara Gittleman  
Kimberly Gray, PhD  
Rachel Gross  
Tom Hawkins  
Heather Henry, PhD  
Jill Hesse, PhD  
Stephanie Holmgren  
Michael Humble, PhD  
Laurie Johnson  
Paul Jung, MD MPH  
Annette Kirshner, PhD  
Steven Kleeberger, PhD  
Cindy Lawler, PhD  
Chris Long  
Robin Mackar  
Carolyn Mason  
J. Patrick Mastin, PhD  
Elizabeth Maull, PhD  
Kimberly McAllister, PhD  
Rose Anne McGee

Liz McNair  
Aubrey Miller, MD MPH  
Sri Nadadur, PhD  
Sheila Newton, PhD  
Michelle Owens  
Jerry Phelps  
John Pritchard, PhD  
Leslie Reinlib, PhD  
Jim Remington, RN  
Margarita Roque  
Elizabeth Ruben  
John E. Schelp  
Thad Schug, PhD  
Daniel Shaughnessy, PhD  
Carol Shreffler, PhD  
William Stokes, PhD  
William A. Suk, PhD, MPH  
Claudia Thompson, PhD  
Chris Weis, PhD  
Mary Wolfe, PhD  
Leroy Worth, PhD  
Humphrey Yao, PhD  
Darryl Zeldin, MD

**Members of the Public Present**

Laura Bono, SafeMinds  
David Brown, SRA  
Kevin Coray, Coray Gurnitz Consulting  
Ernie Hood (Scribe)  
Sven Jordt, PhD, Yale University  
Jim Shannon, SRI International

**I. Call To Order and Opening Remarks**

Dr. Linda Birnbaum, Director of NIEHS and NTP, welcomed attendees and called the meeting to order. She noted that Council members Dr. Grace LeMasters, Dr. Sem Phan, and Dr. Jerald Schnoor, and incoming Council member Dr. Tom McKone were unable to attend this meeting. She welcomed new Council member Dr. Thomas Gasiewicz. She then asked all present in the room to introduce themselves, which they did.

**II. Review of Confidentiality and Conflict of Interest**

Dr. Collman then reviewed the Conflict of Interest and Confidentiality procedures, which had been provided earlier to Council members in written form, and went over various other administrative matters.

### **III. Consideration of May 2010 Meeting Minutes**

Approval of the May 2010 minutes was moved and seconded, and Council voted unanimously to approve the minutes. Dr. Collman also noted the dates of the upcoming Council meetings for members to put on their calendars.

### **IV. Report of the Director, NIEHS**

Dr. Birnbaum began her presentation by welcoming new staff in the Office of the Director. Dr. Ericka Reid is now on board as Director of Outreach and Education. Dr. Christopher Weis has been hired as toxicology liaison for the Bethesda NIEHS office. Chris Long is now serving as Acting Associate Director for Management/Executive Officer, as a nationwide search to fill that opening continues.

Dr. Birnbaum updated Council on FY 2010 and FY 2011 appropriations. Although the President's request and the House and Senate marks are essentially the same in terms of the 2011 NIH appropriation (\$32.007 billion), there is no language in the House mark directing specific expenditures. The Senate mark encourages many of the activities currently being conducted. In terms of the NIEHS 2011 appropriations, she pointed out that the Senate mark is \$1 million less than the President's request. Also, given that the President has directed agencies to prepare for 5% cutbacks, a 2-3% increase for NIEHS would actually be quite positive. Superfund shows a 3% increase in appropriation, with the President's and House's marks in agreement. The Senate has already marked up the annual \$10 million appropriated to supplement the NIEHS worker training program. She elaborated on some of the language contained in the Senate Appropriations report, requesting studies on effects of endocrine disruptors on women's health, exposures that may initiate or promote autoimmune diseases, risk associated with exposures to cosmetics and personal care products, exposures associated with increased time to pregnancy, intramural and extramural research related to the NRC report, "Toxicity Testing in the 21<sup>st</sup> Century," the Genes and Environment Initiative, and an update on the Sister Study.

Dr. Birnbaum updated Council on recent highlights from the Institute. First, she summarized recent work related to the Gulf oil spill. So far, approximately 100,000 workers have been trained by BP or its contractors using the materials prepared and provided by the NIEHS Worker Education Training Program. More than 8,000 copies of the NIEHS-produced guide, *Safety and Health Awareness for Oil Spill Cleanup Workers*, have been distributed to frontline responders, instructors and safety officials.

Several NIEHS/NTP officials participated in the oil spill workshop convened by the Institute of Medicine June 22-23 in New Orleans, including personnel from the Office of the Director, the Worker Education Training Program, Superfund, NTP and the Epidemiology Branch. The workshop sought to identify populations most at risk, determine current knowledge about oil exposure health effects, and discuss means of monitoring health effects.

Working with the Worker Education Training Program, NIEHS grantees at Dillard University were instrumental in arranging a visit to New Orleans on July 10 by HHS Secretary Sebelius, who participated in a community roundtable event to discuss the impacts of the oil spill. Among the roundtable attendees were New Orleans Mayor Mitch Landrieu and Representative Joseph Cao (R-LA), along with worker training graduates, members of the Vietnamese fishing community, and other community members.

Beginning with the announcement on June 15 by NIH Director Francis Collins that he would invest \$10 million to support oil spill health effects research, NIEHS has geared up to launch a major study of oil spill workers and volunteers. The NIEHS Epidemiology Branch is designing the Gulf Long-Term Follow-up Study (GuLF), which should begin in October. The Institute of Medicine will be holding another follow-up meeting on September 22, and NIEHS has begun a series of webinars designed to solicit stakeholder input on the design of the study.

Dr. Birnbaum briefly updated Council on developments related to the recent District Court injunction placed on federally-funded human embryonic stem cell research, noting that an appeal was to be heard on September 7, asking that the injunction be stayed. She mentioned that the ideal solution would be a legislative remedy, but that no action was expected until after the upcoming elections in November.

Dr. Birnbaum updated Council on some of the notable recent scientific publications by NIEHS staff or grantees. First, she highlighted a paper in *Nature* by Marcheva *et al* from Northwestern University, which showed that disruption of the pancreatic circadian clock modulating metabolism can lead to hypoinsulinaemia and diabetes. The second paper she described, which emerged from the intramural Laboratory of Respiratory Biology and was published in *Cell Metabolism*, sheds new light on the link between cholesterol trafficking and inflammatory response.

A recent article in *Environmental Health Perspectives* from researchers at the Children's Environmental Health Center at the University of California at Berkeley discusses an association between serum levels of the flame retardant PBDE and reduced fertility in women and girls in California's San Joaquin Valley. The children have been seen to

have higher concentrations of the flame retardant than the adults, and the women with high levels take longer to conceive than other women.

She described a recent publication in *Toxicology and Applied Pharmacology* that emerged from collaboration between the NIEHS Intramural Program and the NTP. The paper outlines a high-throughput method for assessing chemical toxicity using a *C. elegans* reproduction assay.

Another recent paper in *Environmental Health Perspectives* from McConnell and colleagues at the University of Southern California reported a clear association between childhood asthma incidence and traffic-related air pollution. Zanobetti *et al*, in the *American Journal of Respiratory and Critical Care Medicine*, described a newly-discovered link between particulate matter air pollution and sleep-disordered breathing such as sleep apnea.

Another paper emerging from the intramural program in collaboration with investigators at Johns Hopkins University, published in the *Journal of Allergy and Clinical Immunology*, showed that ozone activates pulmonary dendritic cells and promotes allergic sensitization, a mechanism associated with the asthmatic response to air pollution.

Dr. Birnbaum described another collaborative paper published in the *American Journal of Respiratory and Critical Care Medicine* that elucidates the role of PPAR gamma expression in protecting lungs against inflammation and oxidative stress.

Finally, she reported on NIEHS-funded work by Huh *et al* from Harvard, published recently in *Science*, which describes their development of a “lung-on-a-chip”—a model that recreates organ-level lung functions on a chip, opening many new possibilities for *in vitro* testing of toxicants and drugs.

Dr. Birnbaum continued by reporting on recent institute highlights. She mentioned that recent recipients of NIH Director’s Awards were Management Analyst Kent Stone, Administrative Officer Connie Riley, Dr. Karen Adelman of the Laboratory of Molecular Carcinogenesis, and Senior Advisor Dr. Chris Portier. Dr. Portier, she noted, has been named Director of the CDC’s National Center for Environmental Health/Agency for Toxic Substances and Disease Registry. She also told Council that 21 young NIEHS scientists had received NIH Fellows Awards for Research Excellence in July, out of 260 awards announced by NIH.

In terms of continuing efforts to fill out the permanent leadership team at the institute, searches are still underway for Deputy Director, Scientific Director, Director of the Division of Extramural Research and Training (DERT), and Executive Officer. Selections have been made for Deputy Director and DERT Director, and are currently in

process, with the hope being that they will be in place by the fall. The search committee has interviewed a candidate for Scientific Director, who will be interviewed in more depth soon. In the meantime, Dr. David Miller will assume the role of Acting Scientific Director on September 20, taking over for Dr. John Pritchard. Dr. Birnbaum took the opportunity to recognize Dr. Pritchard's service as Acting Scientific Director, his having come out of retirement to do so.

In other institute highlights, Dr. Birnbaum reported that the NIEHS with the National Cancer Institute has formed the 19-member Interagency Breast Cancer and Environment Research Coordinating Committee (IBCERCC) to review all breast cancer research efforts conducted or supported by federal agencies. The committee will develop recommendations for the Secretary of HHS, the NIH, and other federal agencies, and will do comprehensive planning.

Dr. Birnbaum described a recent Community Forum, held in April in West Harlem, NY, at which the newly-emerging issue of bedbug infestations was discussed. The next Community Forum will be October 20, 2010 in Louisville, KY, on Social Determinants of Health.

She reported a new funding opportunity announced by NIEHS and several other NIH ICs for research on exposures and diseases related to climate change. The first application receipt date for that FOA is September 28.

The Superfund Research Program has developed a draft Strategic Plan, which was published in the *Federal Register* July 1. With public input being analyzed, the plan is expected to be finalized within the next couple of months.

The Scientific Advisory Committee on Alternative Toxicological Methods met June 17-18, and discussed a wide range of topics related to validation issues and international acceptances of alternative methods.

Dr. Birnbaum described the workshop to be held on September 8 at NIEHS, *Autism and the Environment: New Ideas for Advancing the Science*.

She noted that DERT had received its 20,000<sup>th</sup> grant application July 22. It had taken the institute's first 30 years to get 10,000 applications, but just another 10 years to reach 30,000, reflecting the dramatic growth of the field.

Recent testimony by NIEHS officials included appearances by NIEHS Senior Medical Advisor Dr. Aubrey Miller at two separate hearings on the Gulf oil spill and the HHS response: June 15 before the Senate HELP Committee, and June 16 before the House Energy and Commerce Subcommittee on Health. Dr. Birnbaum testified on August 3 before the Senate Environment and Public Works Subcommittee on Children's Health

regarding the state of research on potential environmental factors in autism and related neurodevelopmental disorders. She will testify again before Senate committees later in September, once on endocrine disruptors, and once on Agent Orange and ischemic heart disease.

Dr. Birnbaum concluded her presentation by relating several major decisions recently made by Dr. Collins. Dr. Lawrence Tabak was named Principle Deputy Director of NIH. Dr. Sally Rockey was named NIH Deputy Director for Extramural Research. Dr. James Anderson was named Director of the NIH Division of Program Coordination, Planning and Strategic Initiatives. She also noted that the sixth annual NIH Director's Pioneer Award Symposium is scheduled to be held September 30-October 1 at Bethesda.

## **V. Legislative Report**

Next, Dr. Sheila Newton, Director of the Office of Policy, Planning and Evaluation (OPPE), updated Council on legislative activities at NIEHS.

Program Analysts for Legislative Affairs in the NIEHS Bethesda office Mary Gant and Leanna Kelly provide the NIEHS legislative liaison. They track information on legislation introduced in Congress, provide information to Congressional staff about NIEHS programs and research results, and keep abreast of issues important to Members of Congress. The OPPE works with the legislative liaison on testimony. The office also works with the Financial Management Branch on appropriations materials and documents, assembles briefing materials as needed, and coordinates information with program staff in DERT, DIR, NTP, and other entities within NIEHS, including individual scientists.

Dr. Newton said that preparation of Congressional testimony is a major function of the OPPE. Since her last appearance before Council in February, NIEHS had been asked to testify at five different hearings—the four previously described by Dr. Birnbaum, along with a hearing on *The Environment and Human Health: The Role of HHS*, a House Committee on Energy and Commerce, Subcommittee on Health proceeding in April. She noted that the written testimony prepared for each hearing is posted on the NIEHS website's "For Congress" page.

Another part of OPPE's and the Bethesda staff's jobs is to serve as Congressional Contacts. Two issue-related contacts took place recently. In July, there was a phone briefing for an Appropriations Committee staffer and a subsequent meeting with Congressional staff related to the styrene evaluation being conducted for the 12<sup>th</sup> Report on Carcinogens. In May, there was a briefing on dioxin exposure and ischemic heart disease for staff of the Senate Committee on Veterans' Affairs, which led to an



invitation for Dr. Birnbaum to testify at the September 23 hearing of the Senate Committee on Veterans' Affairs.

The legislative liaisons also track recently-introduced environmental health legislation, of which there has been a great deal this year. Dr. Newton described four current, pending bills that call for consultation with NIEHS, including:

- S2858: Brittany Wilkinson Mitochondrial Disease Research and Treatment Enhancement Act
- HR5320: Assistance, Quality, and Affordability Act of 2010
- HR5786: Safe Cosmetics Act of 2010
- HR5820: Toxic Chemicals Safety Act of 2010

Recent legislation of interest to NIEHS includes one bill that would require direct action by NIEHS—S3224: Radiation Exposure Compensation Act Amendments of 2010. The bill, which has been referred to the Senate Judiciary Committee, would require NIEHS to establish a program to study impacts of uranium mining and milling among non-occupationally exposed individuals. Another current bill of interest is HR6017: Gulf Coast Health Monitoring and Research Program Act of 2010. That bill, which would affect planned NIEHS research, would require HHS to establish short- and long-term comprehensive health screening, monitoring, and research programs for oil spill workers and vulnerable residents, and research on food safety potentially affected by the oil spill.

Other open legislation of interest includes:

- HR4160: Environmental Hormone Disruption Act of 2009
- HR4161: Women's Environmental Health & Disease Prevention Act of 2009
- HR4190, S2838: Endocrine Disruption Prevention Act of 2009
- HR4456, S753: BPA-Free Kids Act of 2009
- HR652: Skilled Trades Second Responders Act of 2009
- HR2084: Prevention, Awareness, and Research of Autoimmune Diseases Act of 2009
- HR3891: Safe and Healthy Housing Act of 2009

These bills remain in committee and are not expected to pass in the near future.

Dr. Newton concluded her remarks by relating excellent sources of information regarding NIEHS legislative activities, including the NIEHS public website, which incorporates a "For Congress" page, which links to a great deal of relevant information and databases.

Dr. Hricko asked Dr. Newton to elaborate further on the issues involved in the styrene evaluation being conducted by NTP. Dr. Bucher replied that there is evidence from animal studies, limited evidence from human studies, and mechanistic evidence of the potential carcinogenicity of styrene. He said that with many applications for styrene, including small, local industries, there is much Congressional interest. Dr. Birnbaum added that styrene is one of the chemicals proposed to be included in the 12<sup>th</sup> Report on Carcinogens as being “reasonably anticipated to be a human carcinogen,” and that any one of the animal, human, or mechanistic bodies of evidence would be sufficient to list the chemical. She noted that there is a great deal of economic concern about the labeling of styrene as “reasonably anticipated,” and that there has been considerable correspondence, including from Congress, on the issue.

Dr. Brody asked about Senator Lautenberg’s Safe Chemicals Act, which would overhaul the Toxic Substances Control Act of 1976. Ms. Gant replied that although it had not been included in Dr. Newton’s presentation since it does not contain language that directly impacts NIEHS, it would have a huge impact, and OPPE is following it closely. She also mentioned that the styrene industry had hired a very active lobbyist who had visited every Member with styrene-related industry in his or her district, accounting for the intense interest from Congress.

Dr. Carpenter asked what the lobbyists might be expecting NTP to do, given the scientific evidence in three categories, which should make the call “almost automatic.” Dr. Birnbaum replied that the concern is mainly about economic impact. She said the styrene industry group is funding research on the health effects of styrene, and thus is asking for a delay in the listing, which has been in process since 2004, pending the availability of data from the newer studies. She said that a recently-published meta-analysis concluded that there was limited evidence of human carcinogenicity, in agreement with NTP’s position.

Dr. Lloyd asked about the relationship, if any, between S2858, the Mitochondrial Disease Research and Treatment Enhancement Act, and the concept clearance regarding mitochondrial disease biomarkers to be presented to Council later in the day. Dr. Collman responded that NIEHS would be well positioned if the bill passes and an NIH Office of Mitochondrial Disease is formed, as it calls for. Dr. Birnbaum reiterated that the bill in this case is authorizing language only. Dr. Newton pointed out that developments in the scientific community were driving these efforts. Ms. Gant added that NIH traditionally opposes single-disease bills, and that if this bill ever comes out of committee, NIH would undoubtedly oppose it.

Dr. Liekauf sounded a note of caution, pointing out that NIEHS is in “a favorable time” with Congress at present, but that could change, and there could be vulnerability to even more severe budget cuts. He asked what the metric for success might be. Dr.

Birnbaum replied that NIEHS has hardly been unsuccessful, but that much is unclear in terms of the budget. She said that although there is no idea what the final numbers will be, it is clear they will not be large, with the President asking for every federal agency being asked to propose 5% cuts in their budgets. If NIEHS gets a 2.5-3% increase, that would be doing much better than many other groups. In the current economic situation, she noted, it would be unrealistic to expect large increases in any NIH efforts. Ms. Gant noted that each 302B allocation, the mechanism by which Congressional subcommittees are funded for all of their projects, was less than the President's request. So comparatively, she said, NIEHS came out well in its allocations from the three relevant subcommittees. Dr. Birnbaum added that NIEHS has been required to prioritize programs in light of 7% cuts, looking at FY 2012. So if the situation becomes drastic, it is likely some programs would not go forward by this time next year.

## **VI. Budget Process Briefing**

Laurie Johnson, Chief of the Financial Management Branch (FMB), briefed Council on the budget process.

The budget process, she said, goes through three phases: formulation, presentation, and execution. So at any given time, the FMB is going through three different phases related to three different fiscal years. Currently, FMB is in the execution phase for 2010, the presentation phase for 2011, and the formulation phase for 2012. It takes about a year-and-a-half from beginning to work on a budget to arriving at an appropriation.

The formulation phase begins in the springtime, with the NIH Director identifying philosophies and policies. The NIH ICs prepare a "commitment base," which identifies items that must be paid before any discretion can be exercised. In May, the NIH Director holds a Budget Retreat with the IC Directors, at which trans-NIH initiatives are identified. In May or June, NIH develops a preliminary budget, which is sent to HHS. With feedback from HHS, by August NIH submits a budget to the Office of Management and Budget (OMB). OMB evaluates that budget, and around Thanksgiving, they pass the budget back, and the appeals process starts, which can take a considerable amount of time. In December, the institutes prepare the President's budget and prepare Congressional Justifications. Then, the presentation phase begins.

In the presentation or Congressional phase, in February the Congressional Justification is presented to Congress. By April, Congress is supposed to have passed a Budget Resolution, which provides a blueprint for spending based upon revenues. During the spring, Congressional hearings are held before the House and Senate Appropriations subcommittees. In the summer, those committees mark up the President's Budget. Then, the budget goes to the full appropriations committees. Once it has passed them,

in July or August (theoretically) it goes to conference to resolve differences. Sometime in the September to December range, the bill passes and goes to the President for signature. By October 1, there should be an appropriation, but often that is not in place, and the fiscal year starts based on a Continuing Resolution, which is generally at the previous year's level. This is expected this year, for the first three-four months of the fiscal year.

On October 1, the start of the fiscal year, the execution phase begins. First, funding authority levels are established. Newly this year, those levels will be determined before the books actually open for business in mid-October. Sometime in the fall, NIH develops Operating Policies based on its impressions of Congressional parameters. Throughout the year, funds are obligated and spent for the various NIEHS programs. In early summer, there is an opportunity to reallocate funds if necessary. The fiscal year ends on September 30, when the books close for that year, followed by a short period of reconciliation. Then, official data, including financial reports and disease data, are collected.

Ms. Johnson then showed a slide depicting the FY 2010 and 2011 appropriations for NIEHS, NIH, Superfund, and the NIEHS DOE Training program, which Dr. Birnbaum had previously shown to Council. She reiterated that there remain some holes in the chart, as there is still some information lacking about FY2011 appropriations.

She then explained where NIEHS gets its money. Most of it comes from the Labor-HHS appropriation (\$663 million in FY 2009). Other sources are the Interior-Environment appropriation and the Department of Energy, both of which fund Superfund. In FY 2009, NIEHS and Superfund had an additional \$187 million in ARRA stimulus funds. NIH also provides funds, including monies from the Common Fund, other ARRA funding, GEI transfer funds, and other smaller stipends. NIEHS also receives funds from some other federal agencies, gifts, and small royalties.

Ms. Johnson showed a slide depicting the history of NIEHS appropriations, which depicted the leveling of funding in recent years, the ARRA stimulus, and post-ARRA drop-off in FY 2010 and 2011. There is concern about a funding "cliff," and hope for a "soft landing" so that the removal of ARRA funding will not be too disruptive to the grantee community.

Of the \$746 million appropriation in FY 2009 (excluding ARRA and GEI transfer money), the largest share, \$251 million, was spent on research project grants (RPG). \$39 million went to centers, \$11 million to other grants, and \$19 million to training. R&D contracts represented \$152 million, with \$176 million going to intramural research. Research management and support represented a \$20 million expenditure, and finally, Superfund received \$78 million.

In comparison with other institutes, it is evident that the mix of NIEHS expenditures is significantly different from other ICs and the NIH composite. That is largely due to considerable expenditure in R&D contracts compared with other ICs, mainly because of the NTP. Removing NTP from the picture, NIEHS expenditures on R&D budgets are roughly in line with several of the other ICs. NIEHS also spends more proportionately on intramural research, including NTP personnel, than f the other ICs.

Analysis of FY 2009 RPG distribution (excluding Superfund) shows that 67% of the expenditure went to R01 grants, with the other funding mechanisms sharing smaller portions of the remaining funds. In terms of the share of R01s compared to the other funding mechanisms, over the years from 2005-2009, that level remained relatively consistent, ranging between 61% and 70%.

Dr. Taylor asked Ms. Johnson the rationale for including NTP but excluding Superfund in the FY 2009 RPG distribution breakdown. She answered that, when the institute is being compared to other ICs, NTP has traditionally been part of the overall appropriation, and has typically been included in the calculations, but where Superfund funding is quite different, emanating from another committee, it is usually not included in the comparative tables.

Dr. Ramos followed up on Dr. Taylor's question, stating that perhaps there would be an advantage in not including NTP in the calculations, and that it would be useful to present the comparative figures that do not include NTP. Ms. Johnson replied that when Congressional justification takes place, the figures are inclusive, but that for display purposes to groups like Council it would be a good idea to prepare depictions without NTP. Dr. Ramos said that when presenting to Congress it would be equally important to make the distinction and show the breakdown that does not include NTP, to depict expenditures realistically. Dr. Birnbaum mentioned that this was "a ticklish issue," because the NTP appropriation has never been a line item. She agreed that it was important to pull out the NTP appropriation in discussions with Congress, to allow NIEHS to look more like the other ICs in terms of the overall breakdown. Dr. Newton explained that due to a recent change in reporting requirements, there is an opportunity to break out the NTP expenditures when showing NIEHS programs, allowing a more realistic depiction of the impact of NTP work on the overall NIEHS budget.

Dr. Liekauf asked about the fate of a previous idea to reduce center grants compared to R01s. He said the reduced center grants funding was supposed to have gone to R01s, but the R01s did not grow during the period shown by Ms. Johnson's chart. He asked where that money went. Dr. Birnbaum replied that the decision to reduce the number of centers was made years ago, and that she has not supported that move. Thus, the number of centers has now been stabilized, and they may in fact grow. She said there is a great deal of productivity coming from the centers, and that the evaluation later in

the day would show that. Dr. Liekauf stated that his main concern was the proportion of R01s in the portfolio, and that he has consistently advocated the elevation of investigator-initiated research, particularly R01s, to a 50% or greater share of the portfolio. Dr. Birnbaum said she would take Dr. Liekauf's assertion under advisement, and recommended that he consider the NIEHS breakdown without NTP or Superfund being included. She emphasized that the R&D contracts portion, which is largely NTP, is competitive, and that soon there will be competitive cooperative agreements commenced.

Dr. Taylor pointed out that inclusion of the NTP in the breakdowns may give an inaccurate impression from outside, and that the institute's external image could improve by removing NTP from the breakdowns. Dr. Birnbaum reiterated that NTP is not a line item in the budget, but that it would certainly be helpful with certain audiences to be able to show the percentage of budget, minus NTP, that actually is expended extramurally. Dr. Ramos agreed that NTP should be taken out of such pie chart breakdowns, in order to preserve a portrait of unique NIEHS elements that may otherwise be lost in the consideration.

Dr. Gasiewicz asked about the level percentage of R01s over time in NIEHS, and how that compares to the percentage trends at other ICs. Dr. Collman said she did not have that information at hand, but that NIEHS has gone to great efforts to pay out for scientifically meritorious R01s. She said that to get more R01s, it's not just a bean-counting exercise. Dr. Gasiewicz said it would be interesting to compare the percentage over the past several years with other institutes.

Dr. Lloyd asked why P30 grants were excluded from the RPG distribution pie chart that had shown. Dr. Collman replied that the center grants are not considered research grants, but infrastructure, and as such are in a different budget category. Dr. Liekauf pointed out that if the P30 grants had been included, the percentage devoted to R01s would be reduced even more. Ms. Johnson said there were several elements that could have been included, but they chose to focus on the RPGs. Dr. Baylin commented that the NIEHS paylines relative to quality are commendable and should not be given short shrift in these discussions.

## **VII. NTP Update: Connecting the Dots – Diseases, Genes, HTS Targets**

Dr. Bucher opened his presentation with a review of recent NTP meetings. The Board of Scientific Counselors (BSC) met May 10, 2010 to peer review a draft NTP brief on isoflavones in soy infant formula, to review a research concept for isoflavones in soy infant formula, and to review an approach for the Center for the Evaluation of Risks to Human Reproduction (CERHR) to evaluate low level lead. June 21-22, 2010, the BSC

met to consider listing profile reviews for the 12<sup>th</sup> Report on Carcinogens, a CERHR literature-analysis concept regarding chemotherapy during pregnancy, and a research-testing concept for hydroxyurea.

In upcoming NTP meetings, the BSC will meet November 30-December 1, 2010, to review Biomolecular Screening Branch programs and Tox21. There is a workshop on the influence of environmental agents on diabetes and obesity scheduled for January 11-13, 2011. A series of Technical Reports reviews are scheduled for January 25-26 and April 12-13, 2011.

Dr. Bucher mentioned that as of July, the FDA has signed on as a participating agency in Tox21. Dr. Bucher summarized past, present, and future Tox21 activities.

Up to now, in Phase I as he characterized it, the NIH Chemical Genomics Center (NCGC) has optimized assays and screened approximately 2800 NTP/EPA compounds for activity in more than 70 high-throughput screens, and has compared the sensitivity of 76 HapMap cell lines to 240 toxic compounds for genome-wide association analysis. EPA ToxCast has screened 320 compounds (mostly pesticides) for activity in more than 500 assays (including zebrafish embryos and *C. elegans*). There has been a major effort to develop statistical and informatic tools for analyzing and presenting the resulting data, and there has begun to be some success in identifying “fingerprints” potentially indicative of *in vivo* toxic effects.

Current NIEHS/NTP Tox21 activities include:

- Establishing a library of approximately 10,000 compounds with known structures for screening at the NCGC
- Evaluating the relationships between compounds, genes, pathways, and diseases, including planned NTP workshops
- Identifying the most robust and informative assays for screening compounds at the NCGC
- Evaluating the *in vivo* relevance of prediction models developed from Tox21 data
- Targeted testing through purchase of DrugMatrix database
- Identifying models for incorporating hepatic metabolism into *in vitro* screens

Beginning this fall, EPA’s ToxCast Phase II will test approximately 700 compounds in approximately 500 assays. Also, FDA will provide human drug safety assessment data.

Dr. Bucher reported that NTP has gone forward with a number of SBIR/STTR contracts, related to several aspects of the high-throughput screening activities taking place in Tox21:

- Development of Mid to High-Throughput Toxicological Tests Using Model Organisms
- Integrated Prediction Systems to Support Environmental Toxicological Assessments
- Incorporation of Metabolism into Quantitative High Throughput Screening Assays
- Development of Quantitative High Throughput Screens for the Detection of Chemicals that Modulate Gap Junction Intercellular Communication
- Monitoring *In Vivo* Gene Expression Changes after Exposure to Toxicants in *Caenorhabditis Elegans*

There are approved concepts for the 2011 SBIR/STTR competitive contracts:

- High Throughput Screening for Reactive Oxygen Species Mediating Toxicity
- *In Vitro* 3D Tissue Models for Toxicity Testing
- Application of 'Omics Technologies to Rodent Formalin-Fixed, Paraffin Embedded Tissue Samples

Dr. Bucher reported that NTP and NIEHS are purchasing the DrugMatrix® Database, a commercially available reference set of gene expression profiles for more than 630 drugs and chemicals with known toxicological profiles. The database includes 300 million gene expression measurements, *in vivo* histopathology data, clinical chemistry data, 124,000 frozen tissues, and the chemicals used to generate the database. Also included are drug signatures that have been developed relating gene expression with known biology, physiology or toxicology. As a service to the scientific community, the database will be made publicly available through the Chemical Effects in Biological Systems (CEBS) database.

Dr. Bucher elaborated on the elements of assay selection in functional genomics, which include pathways and networks, genes, and pharmacological signatures from databases such as DrugMatrix. These contribute to the selection of high-throughput screening assays.

He briefly listed recent publications from the NTP in-house screening facility called the WormTox, or *C. elegans* program, and from the High-Throughput Screening Branch.

Dr. Bucher explained in a bit more detail the NTP processes at work to link the output of high-throughput screens (HTS) with genes and with diseases. To make these linkages, particularly between genes and diseases, researchers employ literature mining, functional genomics, and genetics/genomics techniques. Literature mining resources include databases such as Copub, GeneCards and Entrez gene, Phenopedia (with its HuGE Navigator), and Ingenuity and GeneGo. Large amounts of functional genomics data can be accessed at NextBio, the Unigene Body Atlas, and GeneGo and Ingenuity.



NextBio is also an excellent source of classical genetics information. These databases can be mined to determine linkages between specific genes and diseases. Dr. Bucher showed an example of a compilation of information from the databases on genes related to obesity for which there is HTS data, and for which there is no HTS data, allowing a bigger picture to emerge and identifying potential therapeutic targets. These data mining techniques will be useful, for example, in preparing background information on chemicals and disease outcomes for the NTP Workshop on diabetes and obesity scheduled for January, 2011. The linkages information from the literature will be provided to a work group on Targets and Mechanisms at the workshop. It will be a starting point for those experts from several backgrounds, who will work to fill knowledge gaps in efforts to use HTS data to elucidate links between genes and diseases.

Dr. Baylin asked Dr. Bucher how deep NTP would go in terms of the experts in diabetes to be included in the workshop, particularly in the many aspects of the disease's biology, such as developmental events that influence muscle and fat development. Dr. Bucher replied that the hope is to include experts at that level and from that area, but that it remains to be seen how successful the effort will be, as the workgroups for the workshop are still in the process of being compiled.

Dr. Taylor asked about the process of making the DrugMatrix database available to NIEHS researchers. Dr. Bucher explained that it would in fact be made available to the world, not just NIEHS personnel. Dr. Taylor asked how that could be, in that the company is selling a commercial product. Dr. Bucher explained that the original owners had been acquired by another company, which was not interested in pursuing that particular database any longer. Thus, had NTP not purchased the database (at a "fire sale" price), it would have disappeared.

### **VIII. Scientific Presentation: "TRP Channels in Chemical Sensing and Environmental Disease"**

Dr. Sven-Eric Jordt of the Department of Pharmacology at Yale University Medical School, who is an NIEHS Outstanding New Environmental Scientist (ONES) awardee, presented an overview of NIEHS-funded research to Council.

In our environment, he said, we constantly encounter compounds that are irritating and toxic. For example, there is acrolein, the major irritant in cigarette smoke, which as a byproduct of hydrocarbon combustion is also found in automobile exhaust, diesel exhaust, and smoke from fires. It induces nasal constriction and respiratory depression in mice, causes lung edema, and at lower levels has been implicated in airway hypersensitivity, asthma, and COPD. Chlorine is another commonly encountered

irritant in the environment, whether in routine exposures such as household products or recreation, or in larger concentrations in accidental releases or warfare.

Exposures to such chemical irritants activate reflexive protective responses, which may be coughing, sneezing, tear production, or other responses. These responses are initiated by contact of the irritants with sensory nerve endings in the airways. These nerve endings from the nose, mouth, cornea and teeth terminate in the trigeminal ganglion, and send pain signals to the brain, activating reflexive responses. The lower airways are also densely innervated; those somatosensory neurons also sense noxious chemical and physical stimuli and communicate irritation to sites in the brain stem.

Sensory nerves have been characterized in three different types, depending on their sensitivity, degree of myelination, and diameter. Dr. Jordt concentrates on so-called C fibers, which are characterized by their sensitivity to capsaicin, the pungent ingredient in chili peppers. They are unmyelinated, have a comparatively small diameter, and sense chemical, mechanical, and thermal inputs.

The respiratory response can be modeled in the mouse and measured through a technique called plethysmography. The animal's respiratory flow is seen as a waveform, so response to an irritant can be visualized. When a mouse encounters an irritant, it lowers its respiratory rate via an extension of the End Expiratory Pause (EEP), a defined parameter between expiration and inspiration. This is thought to be a protective response. Humans do not do this, but have other protective, reflexive responses, such as cough.

Dr. Jordt displayed data from a study that showed that reflexive depression in respiration following chlorine exposure was significantly lower in mice pre-treated with capsaicin, suggesting that capsaicin-sensitive sensory neurons are responsible for the response to irritants. This effect can be recapitulated with several other irritants, including acrolein, formaldehyde, reactive oxygen species, and even some chemical warfare agents and scent compounds in perfumes.

The capsaicin receptor that mediates the respiratory irritation response to capsaicin and the other irritants is known as TRPV1. It is a Transient Receptor Potential ion channel activated by capsaicin. The channel conducts sodium and calcium. The TRP family of ion channels was initially identified in *Drosophila*. The classical TRP gene encodes for anion channels involved in the photoreceptor current, so *Drosophila* with a mutated form of the gene are blind, lacking the photoreceptor current.

In mammals, there are approximately 30 known TRP ion channels, including groups such as TRPV, TRPC, and TRPM. Dr. Jordt's lab has concentrated on research involving TRP receptors known to be involved in chemosensation and the pain pathway. Those ion channels are also important in sensing temperature, and are activated at

approximately the temperature at which mammals, including humans, sense thermally-induced pain. Dr. Jordt showed experimental data depicting results of tail immersion experiments with TRPV1 knockout mice. The animals' tails were immersed in a solution which was gradually heated, measuring the temperature at which the animal removed its tail, indicating thermally-induced pain. Compared with the wild-type controls, the knockout mice were for the most part not affected by the rising temperatures, indicating that knock-out of the ion channel made the mice insensitive to hot temperatures.

Dr. Jordt further explained that the TRP ion channels are connected in complex fashion to other signaling pathways, particularly inflammatory pathways. Thus, they become sensitized to exogenous stimuli, as the sensory neurons are chronically activated or sensitized by endogenous chemical stimuli. TRPV1 has in fact been found in sensory neurons innervating the airways.

Dr. Jordt added that after several years of research in his laboratory, he and his colleagues had hypothesized that another TRP ion channel, TRPA1, is actually the major target of respiratory irritants. They discovered, for example, that TRPA1 is the major receptor for mustard oil, the pungent ingredient found in mustard, horseradish, and wasabi, for example. Mustard oil, along with capsaicin, has been used for many decades in pain research to study the chemosensory properties of C fibers.

TRPA1 knockout mice showed no response to exposure to chlorine, nor to mustard oil, but did respond to capsaicin, because the TRPA1 channel was still expressed. These experiments indicated that for chlorine, TRPA1 is essential for the excitation of nerves. Activation of human TRPA1 by chlorine was found to be quite similar to the response seen in mice. Experiments also showed that the channel was activated by exposure to hydrogen peroxide. Acrolein was also found to be an activator of TRPA1.

TRPA1 was found to be a reactive irritant receptor for aldehydes and oxidants. For example, TRPA1 knockout mice did not respond to acrolein exposure. Similarly, the knockout mice showed very little response to chlorine exposure, where the wild type mice experienced profound respiratory depression. This supports the idea that TRPA1 is also a sensor for chlorine *in vivo*, and mediates the majority of the respiratory irritation response to oxidants. This was further confirmed by exposing TRPA1 knockout mice to acetic acid, engendering a normal response similar to the wild type mice.

It is believed that TRPA1 is a receptor that is activated through covalent modification of the protein, unlike the classic lock-and-key activation pattern found with pharmaceuticals. This helps explain why TRPA1 is responsive to so many different activating agents.

Dr. Jordt and colleagues have also studied TRPA1 in conjunction with a program (CounterACT) designed to identify chemical threat counter-measures. In that context,

they chose to explore exposures to isocyanates, such as methylisocyanate (MIC), the notorious agent released in the 1984 Bhopal industrial accident. Single channel recordings following MIC exposure showed that it activated TRPA1 very strongly. They also studied tear gas agents, which are very potent electrophiles. Knockout mouse experiments showed that they were insensitive to the acute effects of the agents, which had been seen to be the most potent activators of the TRPA1 receptor yet identified. This showed that TRPA1 is the essential receptor through which these compounds mediate their effects.

As they are involved in acute inflammatory pain, the TRP channels are of interest as therapeutic targets, and indeed the pharmaceutical industry is developing TRPA1 antagonists as novel analgesics, which are much needed in the armamentarium. One such compound is HC-030031. Administration of the drug to the mouse model significantly reduced pain response to the tear gas compounds and isocyanate, further confirming that TRPA1 is the important receptor mediating pain response to those highly irritating compounds.

Dr. Jordt proceeded to present some new data, showing that sensory nerves not only respond to irritants, but can respond to exposure to other compounds with counter-irritation, or a protective response, mediated by cold-sensing neurons. For example, menthol produces a cooling sensation, along with other natural products such as eucalyptol and camphor. Menthol is widely used to counteract pain and irritation, not only in remedies, but in mentholated cigarettes as well, which are marketed as having cooling, counter-irritant properties. Research findings shedding more light on response to menthol could have policy implications.

TRPM8 has been identified as the cold-sensitive TRP ion channel activated by menthol. TRPM8 knockout mice showed a significant deficit in menthol sensitivity. Dr. Jordt showed a movie confirming this concept behaviorally, as wild type mice preferred a warm plate to a colder one, while knockouts showed no preference, showing that they either could not discriminate between the temperatures, or did not sense the colder temperature as noxious or uncomfortable.

This led to a collaboration exploring the effects of TRPM8 on the respiratory irritation response. The researchers co-exposed animals to acrolein, the major irritant in cigarette smoke, and menthol to see if the addition of the menthol would depress the respiratory irritation response, which it in fact did. The irritation response was clearly blunted by the addition of the menthol, although menthol itself has a slightly irritating effect. Thus, it would make sense that mentholated cigarettes would be perceived as less irritating, which is a matter of concern as they are often marketed to beginning smokers.

In other work, Dr. Jordt is exploring the role of sensory neurons in inflammation and injury, beyond the initial reactive response to irritation. Accumulating evidence over the last several years indicates that sensory nerves are involved in shaping inflammatory response. The TRP channels can be activated by injury, responding to pathways involved in inflammatory response. Chronic TRP channel activation may trigger release of pro-inflammatory neuropeptides such as substance P or CGRP, two known modulators of the inflammatory response in asthma. This is known as neurogenic inflammation. Further, oxidative stress has been shown to produce endogenous TRPA1 agonists—compounds which structurally resemble acrolein. This represents another pathway by which immune factors can activate sensory neurons and inflame tissue, inducing either chronic pain or reflex responses. For example, chlorine is produced by neutrophils, in sufficient quantity to activate TRPA1.

With that understanding, experiments were conducted to see if modulating the TRP channel feedback response to neurogenic inflammation might interrupt the inflammatory response in asthma, potentially identifying a novel target for therapeutic intervention. This was in fact found to be the case, using mice sensitized by ovalbumin (OVA) injection, a common asthma model, which are later (days 17-20) challenged with intranasal installation of ovalbumin. In the TRPA1 knockout mice, there was very little inflammation, and the airways looked fairly normal, with small amounts of inflammatory mediators. The wild type mice, however, had a robust inflammatory response resembling asthma, as expected. Also, airway mucin transcript levels and interleukin-5 protein levels were reduced in the knockout mice. Several other cytokines and chemokines were also reduced in the knockout animals. These results all pointed to a profound reduction in the inflammatory response in the knockouts. Another experiment showed that the wild type asthmatic mice had a much stronger response to a cholinergic stimulus than the knockout asthmatic mice, suggesting that the lack of the TRPA1 channel reduced the airway hyperreactivity response to the cholinergic challenge. This effect on hyperreactivity in the inflammatory condition adds to the activities apparently modulated by TRPA1, which could have important implications in the treatment of asthma.

Expression studies confirmed that the channel is very specific to DRG sensory nerves, and is not expressed in lung tissue or white blood cells.

In mice exposed to a tear gas agent, there was much less release of neuropeptides in the lung in the knockout mice. So in an acute response the pro-inflammatory neuropeptides apparently require TRPA1 to be released into the lung. The OVA-exposed mice were found to have normal immune responses, and were not immune compromised. Experiments also showed that the TRPA1 antagonist HC-030031 blocked asthmatic inflammation and hyperreactivity in the OVA-challenged mice.

One of the major goals of the CounterACT program is to characterize airway injury upon high-level exposures. Dr. Jordt said it is very clear that the TRP ion channels are involved in the acute pain response to highly irritating chemical agents. Having seen in the asthma model that they are involved in maintaining inflammation, the question becomes what their role in injury might be. Mice were exposed to large doses of chlorine, and 8 hours later were treated with the TRPA1 antagonist HC-030031. At 24 hours post-exposure, lung injury and mechanical parameters were measured. There was initially a strong inflammatory response, which was reduced quite significantly by administration of the antagonist. Blood biomarkers of chlorine exposure indicating an ongoing inflammatory response were also reduced.

Ultimately, said Dr. Jordt, the message is that these TRP channels are involved not only in acute detection and response, but are also important modulators of the inflammatory and injury responses.

Dr. Baylin asked the role of the TRP channels in the respiratory epithelium, particularly at sites of self-renewal. Dr. Jordt replied that there are other TRP channels that are actually in the epithelium itself. He said injuries can cause changes in innervation, which can lead to chronic cough conditions similar to neuropathy. He added that these are very fine structures, often feet in length in their connection to the ganglia, and there is still little known about their cell biology.

Dr. Birnbaum suggested that in the context of CounterACT Dr. Jordt consider using higher doses of chlorine and holding the animals longer after treatment to see whether there was long-term damage following immediate amelioration of the effect. He replied that such experiments are difficult to get approved in the academic setting, as they are often lethal. Dr. Birnbaum said it would also be interesting to look at significantly lower levels to assess the long-term effects of very mild irritation. Dr. Jordt said that although he had not shown the data, his group has done some low-level exposure experiments. Dr. Birnbaum elaborated that the reason it would be interesting is because in an emergency response, there is often the question of when it is safe for responders to enter the site of a spill or release, or how long it is safe for them to be there. Dr. Jordt said that it has been seen that low-level chlorine exposure can induce an inflammatory response, as is the case with many irritants, and that the health status of the individual would be important in exposure decisions.

Dr. Kleeberger asked whether Dr. Jordt had looked at the question of cardiovascular effects from exposures to irritants. He replied that his lab had recently started doing so, particularly assessing serum levels of markers of inflammation, which could impact vascular function.

## **IX. Report of the Acting Director, Division of Extramural Research and Training**

Dr. Gwen Collman reported to Council on recent DERT activities, including updates on FY2010 ARRA funding, the Research on Research Integrity Program, and the Interagency Breast Cancer and Environment Research Coordinating Committee (IBCERCC).

Of \$168 million received by NIEHS under ARRA (along with \$19.4 million for Superfund), \$63 million remained for FY 2010. \$48 million was obligated for non-competing commitments made in 2009. \$10.2 million has been allocated to 14 new competing commitments, \$4.4 million for 103 administrative supplements, \$15 million for (institute-wide) R&D contracts, and \$6.7 million additional funding from the NIH Office of the Director. FY2010 NIEHS ARRA funding supported:

- Administrative supplements
- R56 grants
- AREA grants (R15)
- BRDG-SPAN Pilot Program (RC3)
- Building Sustainable Community-Linked Infrastructure (RC4)
- OppNet
- Director's Opportunity Grants (RC4)
- Breast Cancer Research and Development Contract

Administrative supplements have been allocated to assist consortia developed with ARRA funds in FY2009 to investigate the health effects of bisphenol A, to develop a consortium to standardize methods for assessing the environmental health and safety of engineered nanomaterials. FY2010 ARRA funds would also be used to for unforeseen opportunities and expenses.

ARRA funds supported renewal of a very successful program, Supplements for Summer Students and Science Educators, which puts students into scientific laboratories across the US. In 2010, 155 positions were awarded, including 27 high school students, 100 college students, 11 teachers, and 17 teachers taking part in a summer workshop. This was in addition to 100 total positions for the summer of 2010 occurring in two-year awards from ARRA 2009.

Dr. Collman described several ARRA success stories through the course of her presentation, providing specific examples of individual cases where ARRA funding had made a difference. First, she cited the Post-Baccalaureate Diversity Supplement, which supported opportunities for an economically disadvantaged student at the University of La Verne in California.

Dr. Collman provided more details on some of the ARRA 2010 RFAs.

The AREA (R15) grants, the Academic Research and Enhancement Awards, create research opportunities for scientists at institutions otherwise unlikely to participate extensively in NIH program. There has been \$1.3 million in NIH OD funding for three applications, and \$1.2 million in NIEHS funding for three applications.

The BRDG-SPN Pilot Program (RC3), Biomedical Research, Development, and Growth to Spur the Acceleration of New Technologies, is designed to address the funding gap between promising research and development and transitioning to market. Ten applications were assigned to NIEHS, three were scored, one application received \$2.7 million.

The RC4 program, Building Sustainable Community-Linked Infrastructure, is designed to support the development and expansion of infrastructures needed to facilitate collaboration between academic health centers and community-based organizations for health science research. NIEHS was assigned 32 primary and secondary applications, two primary and two secondary were scored, and one application received approximately \$1 million.

OppNet, the Basic Behavioral and Social Science Opportunity Network (which was presented in detail to Council in the May 2010 meeting), is a trans-NIH initiative to build collective knowledge on behavior and social systems. One application is pending for just over \$100,000 in NIH OD funding for a K18 award.

NIEHS expects to make two grants under the NIH Director's Opportunity for Research in Five Thematic Areas (RC4), totaling approximately \$3.6 million.

The Breast Cancer Research and Development Contract is designed to facilitate the translation of key findings emerging from the Breast Cancer and the Environment Research Centers to messages appropriate for a lay audience, including target audiences within the advocate community, to enhance dissemination efforts. One contract will be awarded this year, initially through ARRA funding, with option years to be funded by NIEHS as new findings emerge from the network.

Dr. Collman related more ARRA success stories. The first reported funding for an effort to assess the effects of asbestos exposure among young people in Libby, Montana. Next, she mentioned Council Member Dr. Grace LeMasters' ARRA-funded project to explore the link between tobacco smoke exposure and pediatric asthma. Finally, she discussed a Worker Training Program initiative taking place at the University of Medicine and Dentistry of New Jersey that is providing hazardous waste clean-up and green jobs training.

Dr. Collman then provided more details on the Research on Research Integrity Program, which is a ten-year partnership between NIH and the Office of Research



Integrity (ORI). The purpose of the program is to foster empirical research on societal, organizational, group, and individual factors that affect, both positively and negatively, integrity in research. NIEHS has become an administrative steward of the program, which has made more than 50 awards funding \$17 million in research in its ten years of activity. One hundred publications in prestigious peer-reviewed journals have resulted, and twelve NIH ICs have now participated. For 2011, Martha Barnes in the NIEHS Program Analysis Branch will be the coordinator of the program. In the 2011 FOA, the topics for the solicitation will be bias and public trust. Still being considered for the 2011 FOA are community engagement or CBPR, and cultural diversity. Dr. Collman listed the HHS, NIH, and NIEHS staff members involved in the program, including NIEHS Bioethicist Dr. David Resnick.

Dr. Collman concluded her presentation with more information about the IBCERCC, the committee that has been in development for the past two years and has now come to fruition. The committee has been chartered to investigate the environmental and genetic causes of breast cancer and determine the most effective allocation of breast cancer research funds. It consists of six federal scientists and one representative from the NCI Board of Scientific Advisors, six non-federal scientists, and six non-federal representatives of advocacy groups. NIEHS and NCI are partnering to coordinate the committee, which will hold its inaugural meeting September 30-October 1, 2010. The committee will:

- Share and coordinate information on existing research activities, and make recommendations regarding how to improve existing breast cancer research programs
- Develop a comprehensive strategy and advise the NIH and other Federal agencies in the solicitation of proposals for collaborative, multidisciplinary research
- Develop a summary of advances in breast cancer research supported or conducted by Federal agencies relevant to the diagnosis, prevention, and treatment of cancer, and
- Make recommendations to the Secretary of the DHHS within two years.

Dr. Collman shared the list of committee members, and then opened the floor for questions from Council.

Dr. Gasiewicz noted that there were many new DERT programs, and that the metrics of success for some, such as outreach programs, are not always readily apparent. He asked what the metrics might be, and if there would be follow-up programs for evaluation. Dr. Collman said that a strong evaluation component has been built into the Partners for Environmental Public Health program, and that new metrics will also be developed to better measure outcomes in public health. A set of four new metrics has

been developed; two are ready and the other two are still in development. When they are completed, they will be presented in detail to Council.

## **X. P30 Core Centers Assessment Results**

Dr. Christie Drew, Chief of the DERT Program Analysis Branch, reported to Council on the results of the study conducted to assess the P30 Environmental Health Sciences Core Centers. The charge for the assessment was to:

- Assess the Core Centers in keeping with the five-year Funding Opportunity Announcement (FOA) cycle
- Focus the assessment on programmatic and structural changes made for the Centers funded FY2007-FY2011
- Determine whether changes are needed for the next FOA
- Catalogue questions for future assessment

The study assessed six major components of the Core Centers, several of which were new or changed since the last assessment in 2004: Facility Cores (FCs), Director's Fund, Pilot Projects, Personnel/Career Development, Scoring/Review, and Community Outreach and Education Cores (COECs).

Before proceeding with the results, Dr. Drew acknowledged the hard work put in by the assessment team, with herself as chairperson, nine other DERT team members, and Drs. Lloyd and Taylor as Council liaisons. The process began in February, 2010 with presentation of the plan to Council, and as of this meeting, the report has been completed and presented to Council. October 2010 will see resulting changes to the FOA.

There were two major data sources for the study. The primary data sources were questionnaires developed for Core Center Directors and COEC Directors, respectively, both of which incorporated both specific and open-ended questions. Secondary data sources included applications, progress reports, and publication data from the Centers.

Dr. Drew pointed out that the Centers program is announced every year, so there is a new "cohort" annually, a total of five cohorts in every five year FOA cycle. Primary data for this study came from the cohorts that began in 2007 and 2008, or Cohorts 1 and 2.

Regarding the Facility Cores, the major assessment questions were:

- What changes resulted from the new Integrated Health Sciences Facility Core?

- Are the Centers more translational?
- How do the FCs leverage funds?
- What are the overall benefits and challenges of the new (2007) structure?

With the new structure implemented in 2007, the FCs were found to be more translational than under the previous requirements, including expansion of clinical and epidemiological studies, greater IRB expertise, improved biospecimen storage and processing, enhanced biomarker development, and improved data management and analysis. Increased flexibility, resulting in additional grants and new faculty and collaborations, emerged as a strongly-recognized benefit.

Although there was an existing NIEHS definition of translational research, in order to facilitate its analysis of the translational nature of the Cohort 1 Centers, DERT used a framework for analysis which staff had previously developed and that is based on the concept of five “buckets”: mechanistic understanding (MU), phenotypic validation (PV), clinical assessment (CA), application and intervention (AI), and emerging technology (ET). Cohort 1 FCs were analyzed according to each of these well-defined buckets, allowing the generation of a score with the ability to see changes over time, particularly before and after changes to the FOA. This exercise led to the committee’s first major recommendation, to clarify the definition of “translation,” particularly including references to public health applications and preventive strategies.

The assessment also looked at a component of the Centers added in the 2007 restructuring, the Director’s Fund (DF), examining what activities are conducted with DF expenditures, and how much money is allocated and carried over. The fund gives directors considerable discretion to respond quickly to emerging situations, for example, time-sensitive issue such as the Gulf oil spill and the Beijing Olympics. There was strong appreciation among respondents for the ability to provide small funds for new projects, new equipment, or other short-term exigencies. Over the three-year period from 2008-2010, there were 33 different projects funded with a total of \$760,000, including research projects, equipment, staff, career development projects, and others.

Pilot Projects have always been a core function of the Centers program, but for the cohorts that began in 2007, the ceiling was raised. The assessment found that Pilot Projects support the mission of NIEHS, address a wide range of topics and approaches, contribute strongly to the translation and career development aims of the program, and are resulting in subsequent funding from multiple sources, especially but not exclusively NIEHS. A full return on investment (ROI) analysis was not feasible with the data collected. This analysis led to the committee’s second recommendation, to change the parameters in Tables E1 and E2 in the FOA to facilitate ROI assessment.

The assessment included evaluation of career development activities, seeking to determine what career development activities had been undertaken, what results they have shown, and what new recruits, disciplines, and investigators have been added to the Centers. Meaningful training experiences for junior faculty and students that have been offered in the Centers have included salary and grant support, workshops, mentoring, and training. These activities have resulted in grant applications and awards, new collaborations, along with promotions and new positions.

The committee also examined scientific review criteria, looking at whether changes to the scoring process encouraged use of the full scoring range, and how the strategic vision compared to the overall score. Although the assessment did not provide compelling evidence for making changes in the scoring system at this point, Dr. Drew pointed out that the analysis was not tailored specifically to look at the scoring; a broad assessment of the scoring/review process was outside the scope of this endeavor. To do so, input would be needed from both funded and unfunded applicants, as well as from reviewers, neither of which were possible in the limited time frame for this assessment.

Finally, the team looked at the COECs, to determine who the target audiences of the COECs are, the impact of requiring a Community Advisory Board (CAB), and how those boards impact the research enterprise. Dr. Drew reported that the assessment discovered much evidence of meaningful dialogue and genuine partnerships associated with the COECs. There was considerable variety in the target audiences. The assessment found no major objections among the respondents to the formal CAB requirement, and that a range of creative strategies have been employed to establish CABs. For example, one COEC uses three individuals heavily in planning, developing, and implementing activities, where another group takes a town hall approach with their CAB, holding quarterly meetings with 100 or more invitees from the community. There is substantial evidence that the CABs do exert influence on community involvement and research questions.

In conclusion, Dr. Drew presented a list of options for future questions or analyses that the team had compiled during the course of the assessment.

- How has the use of “cutting edge” technologies in the FCs changed over time?
- Contextualize findings in relation to other large programs
- Fully evaluate ROI for the Pilot Projects
- Conduct future bibliometric analyses of scientific impacts
- Expand data collection for assessment of review/scoring changes
- Assess strengths and weaknesses of CAB recruitment procedures

Council liaison Dr. Lloyd complimented Dr. Drew and her team “in the strongest possible terms” for the job they had done on the assessment. He said he felt that the Centers had been quite thoughtful in their responses, and that they had been very positive in their response to the changes. He felt that there was no need for any significant changes to how the P30s are being set up. He praised the team’s flexibility during the process, saying they were genuinely open-minded and engaged. He said that it appeared that with the changes represented by the Integrated Health Sciences Facility Core addition, several of the directors may have been reluctant at first, but over time saw the added value for their Centers, allowing them to address questions they might not otherwise have had the opportunity to explore. It may have been a difficult transition for some of the groups, he said, but in retrospect they have seen the value. He felt that there had also been a very positive response from the COEC directors. There had been excellent feedback from the communities, and although as Dr. Drew pointed out there is significant diversity in how the CABs are set up, the overall assessment is that they are generating good will in the communities. Overall, he said, it was an impressive review with uniformly good news.

Dr. Taylor, the other Council liaison, echoed Dr. Lloyd’s conclusions. He felt the review process had been well-coordinated. He said the improvements in the program were, in his opinion, directly related to the flexibility added to the program, which had not been present previously. He expressed some concern that by broadening the diversity of the program, some of the mechanistic elements may have been diminished. He felt that the Directors obviously appreciated that flexibility, particularly in terms of the financing. He felt that it would be advisable to define *clinical* in terms of its role in translation, in that clinical research allows translational science to happen, as opposed to it being clinical science per se.

Dr. Liekauf said that environmental health is inherently translational, as it is addressing human health questions. He said translation should be viewed as a two-way street, rather than strictly from the basic scientists to the patient. He advocated that the phrase “and back again” be added to the proposed definition of translation. He was concerned that the Centers assessment had only involved successful applicants, and that there may be some unsuccessful applicant whose point of view would not be as positive. He said that given current financial constraints, requirements for clinical involvement are unrealistic, and that the emphasis should be on translation, in that proposed research should have implications for human health.

Dr. Baylin agreed with Dr. Liekauf’s comments on translational research including patient-to-bench feedback. Dr. Birnbaum also agreed, but felt that the formula should not be “bench-to-bedside and back,” but instead, “bench-to-public health and back.” That concept, she said, accentuates the need for community engagement as well. Dr. Carpenter observed that that would also bring prevention in, which is missing from the

clinical element. Dr. Brody felt that to bring public health into the translation formula, better literacy among the public on environmental health issues would be needed. Dr. Drew agreed that the role of public health in the mix depicted in her “five buckets” graphic needed to be explored further. Ms. Hricko asked whether the added language in the assessment’s first recommendation regarding the definition of translation had in fact been adopted and would be part of the application for Centers going forward. Dr. Collman said the institute was leaning toward that, but first wanted to hear Council’s opinions. Dr. Liekauf again pointed out that the phrase “and back again” was missing from the proposed definition. Dr. Collman agreed that it should be included, and pledged to incorporate that language into the definition, and in the RFA as well.

Dr. Liekauf mentioned that he had twice attempted to start Centers, only to discover that the budget being offered was inadequate to meet the requirements, including a clinical element. He felt that in a competitive situation, everyone should be given an equal chance. Dr. Collman noted that often university support is there to help get a Center started, and it is important that that type of support be shown in terms of sharing the potential risk associated with the project, even if the institutional support is eventually weaned back. She said that perhaps the issue Dr. Liekauf raised should be considered in future assessments of the program.

Dr. Gasiewicz asked how the new criteria had affected the review process. Dr. Drew said there had been two levels of structural change, and that due to the complexity of addressing that issue in the short time frame allotted for the assessment, consideration had been postponed. Dr. Birnbaum added that it would be important to be aware that there will be more evaluations.

Dr. Lloyd agreed that it might be useful to get feedback on the assessment from unsuccessful applicants in order to gain a different perspective, but that within the assessment process itself, that would have been outside the purview of what was taking place.

Dr. Ramos agreed about the importance of capturing the unique perspective offered by unsuccessful applicants, but pointed out that that the charge given to the assessment team was not to evaluate the progress of the Centers program, because that had been done previously. The charge, as he understood it, was to look at the program as it exists—thus, it would be most appropriate to speak with those who had been funded. Continuing, he mentioned that he found the comments on scoring to be confusing. He was concerned about changes in review guidelines causing chaos among reviewers. Dr. Lloyd replied that the data set on the new scoring system was so limited, it did create a degree of chaos, and that it would take years for the noise and error to diminish as people got used to the new system. Dr. Drew observed that the main body of the data in the report was from the first three cohorts, which had used the old system, so

there was some comparability. With the prospect of another, larger review in four or five years, one of the big questions to be addressed would be the scoring system, so she appreciated Council members' comments on that topic.

## **XI. Superfund Research Program Strategic Plan**

Dr. William Suk, Director of the Superfund Research Program (SRP), briefed Council on the recently-completed SRP Strategic Plan.

He said the SRP Strategic Plan, which will guide the program over the next five years, has three overarching objectives:

- To address issues of high relevance
- To maximize the impact of the program's investments, and
- To foster innovation

SRP was established in 1968 under the Superfund Amendments Reauthorization Act (SARA). The program is administered by NIEHS, and supports interdisciplinary research, as well as facilitating training, community outreach, partnering and technology transfer. Under SARA, SRP is mandated to support the development of:

- Advanced techniques for the detection, assessment, and evaluation of the human health effects of hazardous substances
- Methods to assess the risks to human health presented by hazardous substances
- Methods and technologies to detect hazardous substances in the environment
- Basic biological, chemical, and physical methods to reduce the amount and toxicity of hazardous substances (i.e., bioremediation)

A strategic plan was desired for SRP in order to provide a framework to guide the program over the next five years, including the ability to:

- Assess the scope of the science
- Establish approaches for attaining:
  - scientific balance and growth
  - enhanced research translation
  - community engagement, and
  - training
- Develop a framework for decision-making
- Clearly communicate objectives and goals

Although SRP has continuously reviewed the progress, direction and plans of the program, this strategic plan evolved in response to a recommendation in the External Advisory Panel's 2009 report. Also, the annual requirement of issuing an RFA demands continuous assessment, making a longer-term plan important for guidelines for decision-making.

The strategic plan process began with a staff retreat, where a timeline was developed, stakeholder groups were identified, questions were developed to facilitate discussions with stakeholders, and tools and venues for collecting input were identified. Throughout the process, plans and concepts were continuously vetted with NIEHS leadership, including Council. Ultimately, a web page was developed to receive input from stakeholders.

Stakeholder input included eight meetings, six of which were face-to-face, from November 2009 through January 2010. Stakeholders who have participated in the process included the lay public, environmental health researchers, sister Superfund agencies (EPA and ATSDR), other governmental officials (federal, state, and local), non-governmental organizations, the private sector, and policy makers such as Congressional staff.

The next step in the process was a facilitated retreat to process all of the input that had been gathered and form plans. A series of staff meetings followed, and ultimately, on July 1, the draft document was put into the Federal Register. Everyone who had participated in the process up to that point, including Council, received the draft document. Comments were incorporated and the strategic plan distributed to Council at this meeting was formulated.

Responses from all of the audiences mentioned indicated that the SRP should continue to:

- Support multi- and interdisciplinary research
- Support basic, mechanistic studies
- Emphasize the areas of health sciences
- Support community engagement as a component of the multi-project grants
- Encourage the translation of research emanating from the SRP
- Utilize the necessary resources and mechanisms to accomplish those tasks

The plan delineates Guiding Principles necessary for successful implementation of the goals and objectives. The SRP must be *accountable* to stakeholders and taxpayers, must be *coordinated* with other research and training programs, and must be *transparent*, emphasizing open communication.



Dr. Suk next went over the three objectives in more detail. The first, to address issues of high relevance, includes intent to:

- Encourage problem-based, solution-oriented research
- Promote interaction between SRP and its stakeholders, and
- Prioritize critical research areas, particularly ensuring that all mandate areas (health effects, risk, detection and remediation) are addressed

The second objective, to maximize the impact of program investments, involves the need to:

- Encourage investigator-initiated research translation
- Enhance coordination and collaboration between grantees
- Enhance the impact of training activities, and
- Disseminate Program successes and research findings

The third objective, to foster innovation, requires SRP to:

- Promote transdisciplinary science, and
- Encourage new technologies and challenge existing paradigms

In terms of the implications of the strategic plan, Dr. Suk reported that:

- Research will remain multidisciplinary
- There will be a focus on solution-oriented research
- Community engagement will be required in multi-project grants
- There will be increased emphasis on research translation at the project level, and
- Training experiences for the next generation of scientists will be enhanced

Dr. Suk said that fundamental science to address program mandates remains important. He concluded his presentation by adding that the objectives of the strategic plan have been designed to respond to the stated needs of the SRP's stakeholders.

Ms. Hricko asked about the SRP's mandate for development of basic biological, chemical and physical methods to reduce the amount and toxicity of hazardous substances, in the context of whether SRP was funding research into green chemistry as a way to reduce the amount and toxicity of hazardous substances. Dr. Suk confirmed that SRP is supporting that type of research, and that in fact the SRP annual meeting this year will have an entire session devoted to green chemistry. He said SRP has struggled a bit with the issue, since when it was established it was designed to address the end of the "pipeline," after a substance was already in the environment. He added that SRP has been working to move its activities higher up that pipeline, reducing

exposures by preventing them from occurring in the first place. Dr. Birnbaum added that other parts of NIEHS and NTP are involved in significant green chemistry efforts.

## **XII. Concept Discussion #1: Identification of Biomarkers for Early Detection of Mitochondrial Dysfunction**

Dr. Dan Shaughnessy presented the concept clearance regarding mitochondrial dysfunction biomarkers to Council.

He likened mitochondrial dysfunction to a canary in a coal mine—a harbinger of unseen threat. As the central source of energy in the cell, as well as calcium and apoptosis signaling, damage to mitochondria can be an early indication of damage to a cell before the emergence of a tissue phenotype.

Both environmental factors (e.g., smoking, nitric oxide, fungal toxins, pesticides, industrial chemicals, anti-retroviral and chemotherapeutic compounds, etc.) and genetic factors (e.g., nuclear DNA [nDNA] or mitochondrial DNA [mtDNA] variants) can cause mitochondrial dysfunction. Dysfunction can manifest in various forms, such as inhibiting oxidative phosphorylation or the complexes that make up the electron transport chain, inducing mtDNA mutations, causing redox imbalance, and increasing apoptosis.

Prolonged toxicity to mitochondria can lead to generalized clinical symptoms such as myalgia, fatigue, headache, fever, shortness of breath. In more severe cases, symptoms may include peripheral neuropathies or memory problems, and very severe cases may present with lactic acidosis. It has become clear that mitochondrial dysfunction can lead to rare diseases causing deafness, blindness, and movement disorders, and more common chronic diseases such as Parkinson's disease, diabetes, obesity, cancer, cardiovascular disease, asthma, and possibly autism.

It is known that there are more than 60 compounds, both natural and synthetic, that can inhibit complexes in the electron transport chain. They include the pesticide rotenone and a number of antibiotics. An excess of reactive oxygen species (ROS) that overwhelms the normal cellular anti-oxidant defense can also lead to mtDNA damage. This incursion can lead to a looping, cumulative effect that eventually causes apoptosis.

Several lines of evidence support the concept of a connection between environment and disease through mitochondrial dysfunction. One of the better-studied examples is the development of Parkinson's-like symptoms by animals exposed to rotenone. Apparently rotenone selective targets dopaminergic neurons. It has also been shown that genes associated with Parkinson's are involved in mitochondrial function and

respond to oxidative stress. Some studies, particularly with Parkinson's patients, have found reduced Complex 1 activity in the brain, platelets, and muscle.

As reported in a recent article in *Environmental Health Perspectives* (Schmidt, 2010), the study of mitochondrial dysfunction is an emerging field in environmental toxicology. Most of the data to date has come from drug studies. MtDNA is more susceptible than nDNA to damage from environmental toxicants due to the high background of ROS, and less efficient repair in mtDNA. Investigators interviewed in the EHP article called for a broader definition of mitochondrial toxicity, and better measures of mitochondrial function in whole organisms.

Dr. Shaughnessy reported that although there are currently several measures of mitochondrial dysfunction, including both clinical and imaging methods, they are not readily reproducible and lack the sensitivity and specificity needed for early markers of environmental exposures. Early, noninvasive biomarkers of mitochondrial dysfunction related to exposures are needed.

There are challenges to the development of such biomarkers, including heteroplasmy, the inherent complexity of mitochondrial biology, differences in response in target tissues versus surrogate tissues such as buccal cells or blood, and the ability to detect the effects of environmental exposures in the larger context of genetics, diet, exercise, temperature changes, etc.

In June 2009, NIEHS supported a workshop at the United Mitochondrial Disease Foundation annual meeting, which gathered many of the experts in mitochondrial research to discuss the issues related to mitochondrial research. The group's recommendations were to develop animal and other experimental models to:

- Identify environmental stressors that inhibit normal mitochondrial function
- Improve our understanding of the tissue-specific effects of mitochondrial toxicants
- Develop standards for analysis of mitochondrial endpoints
- Develop approaches and candidate markers to serve as the basis for developing early biomarkers of mitochondrial dysfunction in human population studies linking exposure to disease

The concept for the proposed RFA is to develop animal or other experimental models to address some of the fundamental, practical questions about how environment affects mitochondrial function, and then to use the resulting signatures to develop biomarkers of human diseases. Possible approaches include: why some tissues are specifically affected by toxicants, the thresholds for phenotypic changes associated with mitochondrial defects, measurement in surrogate tissues rather than target tissues, and

the development of genetic and transmitochondrial models to simulated different backgrounds. Possible endpoints include determination of proteomic or phosphoproteomic signatures, the use of metabolomics to develop biomarkers, the examination of changes in redox status, imaging techniques, and examination of novel mechanisms such as fission, fusion, and autophagy for signals that the mitochondria is undergoing stress.

These activities are anticipated to develop better candidate markers of mitochondrial dysfunction for use in human studies, and better understanding of how environmental toxicants induce mitochondrial toxicity.

The RFA would involve \$2.5 million in funding for 6-8 R01 grants. There is interest from NIDDK and NIA to partner on the program.

Dr. Bradfield, the first reviewer of the concept, suggested that projects under the concept might include high-throughput screens, and non-hypothesis-driven approaches. He was concerned that many grant applications might be rejected if they were not hypothesis-driven. Dr. Shaughnessy replied that he felt there was room for some of the more basic, mechanistic work, but that relevance to mammalian systems should be shown. Dr. Bradfield was concerned that the applications might be difficult to review objectively. Dr. Shaughnessy said there were many people working in this area on a basic level who might see the RFA as an opportunity to expand their investigations into an exposure model.

Dr. Lloyd, the second reviewer, said he was “extremely supportive” of the concept, and that it is in an area that has been overlooked in the past. He said that only in the last year or two has work begun to be published regarding mitochondrial proteomic profiling, with up to 700 proteins documented thus far. He observed that work at NIEHS and elsewhere has started to examine the susceptibility of mtDNA to environmental toxicants. He said that the studies looking for biomarkers to this point have been looking at severe disease endpoints, which are present after a great deal of mitochondrial damage has already occurred. With a lack of basic, fundamental data in this area, Dr. Lloyd concurred with Dr. Bradfield’s assertion that there should be caution in the review process in terms of overly skewing toward hypothesis-driven initiatives.

Dr. Liekauf wanted to ensure that the focus of the project was not exclusively on mtDNA. Dr. Shaughnessy replied that there was certainly much more going on in nuclear mutations as well. Dr. Liekauf agreed that there should be language in the RFA specifically encouraging discovery science and innovation. Dr. Taylor agreed as well.

Dr. Birnbaum called for and received a motion and second to approve the concept. The vote was taken, and the concept was unanimously approved.

### **XIII. Concept Discussion #2: Statistical, Bioinformatics, and Analytical Methods for Detection of Gene x Environment Interactions in Complex Diseases**

According to presenter Dr. Kim McAllister, the background for the need for improved methods for detection of gene-environment (G x E) interactions is the large numbers of genes and environmental factors that come together in complex pathways to cause complex diseases. Methods to incorporate multiple genes and multiple environmental factors have lagged behind, creating a bottleneck that has slowed the progress of knowledge in the area.

Although genome-wide association studies (GWAS) have become quite popular in recent years, the G x E hits detected have limited predictive power and are difficult to replicate. Also, it has been difficult to detect main genetic effects when strong G x G or G x E interactions are present. There has been much attention paid to this so-called “missing heritability” of common diseases in GWAS results. Lack of properly accounting for environmental factors may explain some of that discrepancy. Ultimately, it has become more apparent that there is a need to enhance detection of G x E interactions in common, complex diseases.

There are some examples in the literature of G x E interactions that show that a particular genetic variant combined with a particular environmental exposure can greatly increase risk for a particular disease. This combination can point to the subpopulations most susceptible to particular diseases. Dr. McAllister shared several pertinent examples from the literature, including examples of very strong environmental effect, of a genetic variant detected in GWAS only with the presence of an environmental effect, and an example of an EWAS—an environment-wide association study focusing on Type II diabetes. She also provided several examples of preventions/interventions in which the deleterious effects of genetic variation were blunted by modification of the environmental side of the G x E interaction, showing that there are feasible interventions or preventions that may have public health or clinical utility. These examples illustrated the idea that since very little can be done to modify the gene side of the equation, it is important to look at potential modalities to modify the environment or behavior to treat or prevent disease.

The objectives of the G x E Interaction Methods Initiative will be:

- To develop and test designs and analytical strategies for identifying G x E interactions in GWAS and other gene studies in complex diseases

- To develop and validate algorithms and new computational or bioinformatics approaches to identify individuals at high risk for developing disease based on both exposure patterns and genetic risk profiles

The reason for a separate initiative now is that there is no standing study section with expertise in biostatistics, bioinformatics, genetics, environmental health, epidemiology, computation, and computer programming—the requisite mix of skills to thoroughly address the important questions in the area. The initiative is very timely, in that:

- The post-GWAS era has begun, and it would be valuable to develop methods to re-analyze those expensive studies to glean G x E interactions
- It fits well with the next phase of the Genes and Environment Initiative
- It fits well with the current emphasis on prevention, in that environmental methods can potentially reduce disease burden
- Consortia and meta-analyses need G x E methods in that larger framework of data

Dr. McAllister related some of the lessons learned from previous G x E initiatives and workshops, which influenced the design and objectives of this initiative. She mentioned that the development of statistical methods and bioinformatic software came up as a barrier repeatedly in previous efforts.

She discussed some of the challenges that could be addressed by this initiative:

- New tools and computational approaches to further leverage GWAS human population studies for G x E interaction studies
- Statistical/analytical methods that incorporate continuous and/or long-term exposure measurements in G x E interaction
- Computational/bioinformatics methods for data mining/machine learning for incorporating prior knowledge into G x E studies
- Analytical or bioinformatics tools for data integration and harmonization of environmental data to allow G x E investigations

Grants issued would require the inclusion of at least one environmental exposure, the use of real human datasets to apply the proposed approach, that the use of an animal model be justified (with an inability to use a human dataset), and that software and methods developed must be user-friendly and made publicly available. Other NIH institutes that may participate include NCI, NIDA, NHGRI, NIMH, and NHLBI. Possible funding mechanisms could be 3-year R01s or R21/R33s.

Council reviewer Dr. Bradfield said that he was positive about the concept. He said he thinks of it as involving analytics and statistical tools, not animal model development. However, he thought animal models should perhaps not be so easily dismissed or

discouraged in the initiative, and that work in *Drosophila* or *C. elegans* may contribute in this area.

Reviewer Dr. Finnell disagreed with Dr. McAllister's assertion that GWAS had been conducted for virtually all common disorders, citing the need for GWAS in birth defects and other rare diseases with small available samples as an example. He expressed strong support for the concept, stating that the community very much needs the types of tools being developed. He agreed with the emphasis on the use of human datasets. Dr. McAllister agreed with Dr. Finnell's comment about rare diseases.

Dr. Liekauf suggested that given the short time frame, model datasets could be presented in contest format, encouraging development through a competitive model. Dr. McAllister was reluctant to endorse that idea. Dr. Liekauf elaborated that given a sufficiently robust model dataset, statisticians could be encouraged to apply their own methods, without the need to collect data on their own, which would be difficult in the program's short time period.

Dr. Taylor suggested that given the requirement for human datasets with exposures at different developmental time points, the only possible dataset might be twins datasets, with a large enough sample that could allow comparisons between twins exposed or not exposed to particular environmental factors at certain points in life. Dr. Lee suggested that NIEHS also consider involving NIDDK and NICHD as initiative partners.

Dr. Birnbaum called for and received a motion and second to approve the concept. The vote was taken, and the concept was unanimously approved.

#### **XIV. Concept Discussion #3: The Environmental Health Science Centralized Knowledge Base**

Dr. Elizabeth Maull presented a concept jointly developed by DERT and NTP, for the formation of an Environmental Health Science Centralized Knowledge Base.

A knowledge base, she reported, is a special kind of database for knowledge management, providing the means for the computerized collection, organization, and retrieval of knowledge. It is an archival and computational system that uses data, information and knowledge captured from experts to carry out tasks that create new information and new understanding.

The Environmental Health Sciences (EHS) KnowledgeBase (KB) would be an online, web-based, publicly available resource, featuring comprehensive, well-annotated data and analysis tools to inform the design and interpretation of environmental health studies. Information to be compiled would include data on chemicals, genes, pathways,

and environmental exposures, interactions and diseases. The EHS KB would be intended to promote understanding of the underlying mechanisms of environmental diseases. It would also be:

- A centralized, web-based tool for entry, query, display and communication of existing environmental health science information
- An interface to existing and emerging relational information describing interactions between exposures, genetic variation, and disease, with uni- or bi-directional linkages to existing and emerging relational information
- A tool for hypothesis generation and testing

Dr. Maull said the group believes that the Research and Development Contract Mechanism would be most appropriate for this effort, providing the appropriate oversight. DERT has a history of using R&D Contracts to provide resources for the EHS community, and NTP has access to R&D funds to support the effort. The contract would be divided into 3 major and 2 minor tasks. The major tasks are:

1. Identification, prioritization, and curation of toxic chemicals of environmental concern, environmentally responsive genes, and environmentally relevant diseases
2. Identification and incorporation of links to publicly available and complementary information resources
3. Application and/or development of appropriate statistical, analytical, and visualization tools, for analysis, interpretation and prediction capability

Under Task 1, the goal is curation of peer-reviewed EHS literature, looking for relevant information related to chemical-exposure-gene-disease interactions. For example, there would be curated chemical-gene-disease interactions, lists of genes and gene signatures from curated literature, and exposure information for prioritized chemicals. It would require the use of approved vocabularies for consistency throughout the KB, and consistency across curators. Flexibility would also be built into the KB, to allow rapid response to emerging environmental health issues.

Task 2 would provide centralized access to interdisciplinary information, such as primary data sources, metadata efforts, and data analysis tools. Potentially complementary data sources would also be included, such as ArrayTrack, PharmGKB, PubChem, and several more. Also, there would be an opportunity to link directly to other databases for complementary analyses.

Under Task 3, the powerful data analysis tools needed would be either purchased or developed if they are not readily available. This would include tools to:



- Compare associated datasets (e.g., pathways) for at least three chemicals, genes/proteins and/or diseases
- Generate lists of comparable chemicals and genes
- Generate network models (direct and inferred relationships)
- Identify GO annotations and associated pathways statistically enriched for genes, chemicals, or diseases of interest
- Provide user-configurable data reports
- Import data from complementary databases with the objective of manipulating data and conducting multiple entry analyses

The two minor tasks delineated in the initiative address requirements related to maintenance and expansion of the EHS KB infrastructure, emphasizing stability and flexibility, and outreach, marketing and evaluation activities.

The initiative is envisioned to be a collaboration between DERT and NTP, with each being an equal partner in terms of funding. The initial award is anticipated to occur in FY2012.

First reviewer Dr. Bradfield said he liked the concept, but that it was such a good concept it had been tried a few times previously, with varying degrees of success. He was concerned about what would actually populate the database as to its potential for acceptance, and with the composition of a potential advisory board. Overall, he said of the concept, “It raises as much excitement as it does concern.”

Second reviewer Dr. Ramos said that it’s “a great, great concept,” but that NIEHS had been down this path twice before and both efforts had failed. He recommended that the concept team go back and look at the criticisms of the prior iterations, and ensure that when this initiative is released, the same mistakes are not repeated. He said he was specifically referring to the CEBS (Chemical Effects in Biological Systems) database. He agreed with Dr. Bradfield’s assertion that the population of the database would be critical, in that in prior efforts, ability to interface data sets had proved to be a huge challenge. He stated that it was important for there to be considerable detail in the solicitation, so that the goals and objectives would be clear to all concerned, including applicants and advisors alike.

Dr. Liekauf asked Dr. Maull what was meant by “gene” in the proposal. She replied that this would be a knowledge base for curated literature, thus, information regarding linkages between expression levels of genes and diseases would be included, for example. She said that primary data sources would not be included. Dr. Liekauf replied that unless the knowledge base was built from the start to integrate from gene to protein to disease, it would be a stand-alone unit that would see very little use, “just like the last two iterations.” Dr. Maull explained that where they were referring to genes, they

actually meant something much broader than simply genes, for example, gene signatures, which would allow linkages with microarray data. She reiterated, however, that this is a knowledge base, not a data base as such with primary data included. Dr. Liekauf said he felt that this proposal was at the design stage rather than the implementation stage, and that the RFA should be soliciting knowledge base design assistance first. Dr. David Balshaw, one of the DERT officials participating in the project, replied to Dr. Liekauf question about genes by explaining that all of the aspects he had alluded to would be included, in as integrated a fashion as possible given currently available tools.

Dr. Gasiewicz agreed that the goal was laudable but complex. He recommended that future expenditures for maintenance, which could be considerable, be taken into account.

Dr. Birnbaum then asked for a motion for the concept to go forward. No motion was made.

Dr. Ramos reiterated his earlier comments that the concept is valuable, but that going forward with a contract without the goals being clearly defined would not be desirable. He asked Dr. Bucher for his thoughts on what NTP would like to get out of the KB, given NTP's role as a partner in the effort. Dr. Bucher said the KB would basically support many of the needs he had expressed in his update concerning having the databases available to make associations across larger and larger amounts of information. He said he shared many of the concerns that had been expressed about launching databases, but pointed out that CEBS had been resurrected as a much more valuable, improved tool. Dr. Kleeberger elaborated, stating that CEBS had improved considerably over the last two to three years. He and Dr. Bucher recommended that Council members examine CEBS if they had not done so recently, in that it is now populated with studies from a wide variety of sources and has become "a very, very useful tool."

Dr. Birnbaum pointed out that an advantage of using the contract mechanism is that with contracts, NIEHS can direct what is to be done. Also, contracts can be written in parts, or phases, and that decisions can be made to go forward upon assessing what has been done up to that point. Thus, a project such as the proposed KB can be done in so-called work order or task order fashion, so that leaders can be sure it is going in the right direction and providing the desired results.

Dr. Maull mentioned that she had discussed the concept with Dr. Ray Tice of the Biomolecular Screening Branch within NTP, and that he had said the availability of the exposure data would be of value in helping to prioritize which chemicals they would put through the High Throughput Screening program.

Dr. Collman commented regarding the proposed mechanism, saying that it is difficult to put together a project like this under a traditional study section or cooperative agreement. She said that based upon looking at existing databases and how well they have served the NIEHS/NTP priorities, it was felt that the contract mechanism would be best to help produce the next generation KB. She acknowledged that much work remained on the concept, and asked Council to consider that approval of the concept would allow that development to move forward, further fleshing out the statement of work that would ultimately go out on bid.

Dr. Lee said she thought the concept would need innovation, flexibility, and creativity because of how rapidly the information and technology are changing and growing, presenting a danger that the KB could be outdated from its inception.

Dr. Brody asked what EPA and NLM had been doing in this area, and how this KB might relate to those efforts. Dr. Maull replied that this KB of curated data is quite unique. She mentioned that some of the successes of the Comparative Toxicogenomics Database (CTD) from the Mount Desert Island Biological Laboratory in Maine had inspired some of the ideas incorporated in this KB.

Dr. Baylin pointed out that it appeared elements of the proposal were still being honed, and wondered if that work would essentially halt if there was no vote to go forward from Council at this meeting. Dr. Collman replied that the concept could be brought back before Council after more work, but that the solicitation process cannot start without Council approval of the concept. In response to a question from Dr. Ramos, she added that the contract process takes approximately one year from Council approval.

Dr. Liekauf said he did not understand why NIEHS has to use extramural funds for this effort, when it could be initially populated by NTP, proven in concept, and then brought into the extramural enterprise. He said he is not convinced that CEBS is the answer, or that the current strategy is the answer, and that a broader strategy may be necessary. He inquired about cost of the database and future maintenance. He reiterated his assertion that this should be considered to be at the design stage. Dr. Maull replied that the initial plan was to expend \$2 million per year of the five-year contract, \$1 million each from DERT and NTP annually.

Dr. Birnbaum said she was hearing a fair amount of concern from Council, and asked for a motion to give a sense of guidance. Council and Dr. Collman discussed several possible options for how to move forward with the concept, perhaps re-shaping it for reconsideration by Council at its next or a future meeting. Dr. Ramos approved of the idea of staff having an opportunity to think more deeply about some of the issues that had been raised, with the intention of rewriting the concept and re-presenting it to Council.

Dr. Liekauf said that if the concept was to be re-worked, he would be interested in more detail as to what NIEHS expected to gain from the KB, as opposed to NTP. Dr. Collman replied that DERT sees the KB as an important resource for the extramural community and the scientific community as a whole, while NTP would benefit by having the KB tailored to the other systems and information resources it uses on a regular basis. Dr. Ramos pointed out that this would also allow researchers access to the NTP database, which has been desired for years. Dr. Liekauf said he felt that NTP had more need and more resources at this time, and that they should develop it themselves for a couple of years and establish its value, and then he would be more likely to approve the idea for NIEHS expenditure. He expressed concern that the money expended would be coming out of extramural grants. Ms. Hricko disagreed with Dr. Liekauf's assertion that the KB should start at NTP, in that the viewpoint could end up being too narrow. She felt also that it would be more appropriate for outside contractor personnel to be working on the KB, rather than pulling NTP scientists out of their laboratories to do so.

Dr. Birnbaum said a vote was not necessary at this time, as Council had provided clear guidance that it was not yet comfortable to go ahead with the concept. She said internal discussion would follow, and there would then be a decision on how to proceed, and whether the idea would be brought back before Council at its next meeting in February 2011.

Dr. Taylor recommended consideration of another funding mechanism, since the KB would likely need to be supported in perpetuity, rather than being over in five years, as the contract would be. Dr. Birnbaum said that all of the many ideas expressed by Council would be considered.

Dr. Lee concluded the discussion by pointing out that the concept was clear, but that she was uncomfortable with the lack of delineation from past attempts, and that she would ask that any re-working of the concept include more information on those past attempts for the benefit of those Council members who may be unfamiliar with that history.

## **XV. Concept Discussion: Environmental Influences on Transcriptional Regulation**

Dr. Lisa Chadwick presented to Council in lieu of Dr. Fred Tyson, who was unable to attend.

Dr. Chadwick reminded Council that although all of the cells in our bodies share the same DNA sequence, there are hundreds of different cell types. Each cell type reads a slightly different subset of instructions, resulting in differing gene expression profiles.

Disruption of the instructions can lead to disease, and exposure to environmental toxicants has been shown to alter gene expression profiles. One way that occurs is through epigenetic mechanisms. In this context, she defined epigenetics as *changes to the DNA or the way it is packaged that affect gene expression*. In other words, epigenetics helps tell genes what to do. Among the major epigenetic “marks” are histone modification and DNA methylation. It has been shown that exposures can be associated with epigenetic changes, including exposures to toxicants such as arsenic, pesticides, fungicides, lead, alcohol, and many more.

Dr. Chadwick described NIEHS’s substantial investment in epigenetics, including the NIEHS Environmental Epigenetics Program, the Fetal Basis of Adult Disease program, being co-lead institute on the NIH Roadmap Epigenomics Program, and increasing numbers of extramural grants in the investigator-initiated portfolio.

Most of the funding to date has been focused on DNA methylation. Epigenetic marks are far more complex than DNA methylation alone, however. There are proteins that modify histones, other proteins that bind to those modifications and exert an effect on chromatin, various non-coding RNAs, and several other elements in the process. Many of those elements are potential targets for impact of environmental exposures. Also, if many of the elements exist in multi-protein complexes, the picture becomes even more complicated. Toxicants could also affect where in the genome these regulatory complexes go—another mechanism by which they might impact gene expression.

Although there is much research on epigenetics being conducted or supported at NIEHS currently, as mentioned previously, gaps in the portfolio have been identified. Again, there is considerable concentration on DNA methylation, looking mainly for associations with particular toxicants, without elucidating mechanisms involved. In other words, it may be known at this point that a certain exposure leads to changes in the expression of certain genes, leading to certain disease outcomes, but the process by which that takes place remains murky.

The proposed PAR would broaden this area of investigation to discover how exposures affect the function of:

- Various non-coding RNAs
- Co-activator or co-repressor complexes
- Chromatin remodeling complexes
- Functional genomic elements (retrotransposons/mobile elements, DNase I hypersensitivity sites, repetitive elements, imprinting regulatory domains, centromeres, telomeres or pericentric/subtelomeric regions, boundaries or insulators)
- Inter- or intra-chromosomal interactions

- Histones or nucleosomes

A range of systems would be accepted for these investigations, including *in vitro* studies, cell cultures, primary human or mouse tissues, or studies in model systems. The PAR will be open over three years.

Dr. Baylin began the discussion by confirming that this would be individual investigator-initiated grants, with a special study section, through a Program Announcement.

Dr. Liekauf asked why the program was restricted to only transcription, since the effects of exposures are much bigger than that alone. Dr. Chadwick agreed, but explained that this program was designed to build from the current portfolio, although there would certainly be room to expand it.

Dr. Taylor asked if there was a nomenclature issue, between the ideas of *epigenetic phenomena* and *transcriptional regulation*. Dr. Chadwick replied that those are all parts of the same larger process, and are intimately interconnected.

Dr. Ramos felt that Dr. Taylor was making an important point, and suggested that instead of calling the program “transcriptional regulation,” it might be more appropriate to refer to it as “genetic regulation” or “gene regulation.” Dr. Baylin said that in the field, the distinction is between calling things epigenetic when they’re actually signal transduction...transient phenomena which are not heritable. When they are permanent and heritable, you have epigenetics. Dr. Ramos said that supports his point that this RFA is not for epigenetics, but is focusing on the ways by which genetic expression could be controlled, either from DNA or epigenetically.

Dr. Birnbaum interjected that there did not seem to be agreement on definitions, and that while Dr. Chadwick and her group have been very specific about their areas of interest, that does not preclude other R01s coming in outside of this particular PAR.

Dr. Liekauf inquired how much this program would cost. Dr. Collman replied that for Program Announcements, money is not set aside in advance. As the initiative goes through review, NIEHS will look at the scores, and based on budgets and other priorities will pick the best science. Dr. Taylor asked if there would be a special study section. Dr. Collman replied that that question would be discussed with CSR. Dr. Baylin mentioned that it would be good for Council to hear a report on the previous work in this area. Dr. Birnbaum said it was a good suggestion and that it would be put on the calendar for the next Council meeting in February.

Dr. Birnbaum then asked for and received a motion and second. Council voted unanimously to approve the concept. Dr. Birnbaum adjourned the first day of the Council meeting

## **XVI. DIR Scientific Presentation: The Expanding Universe of p53 Targets**

Day two of the Council meeting began with a scientific presentation by Dr. Michael Resnick of the Chromosome Stability Group in the Laboratory of Molecular Genetics at NIEHS, briefing Council on his group's work in budding yeast and human cells investigating new and expanded consideration of the p53 tumor suppressor.

Dr. Resnick reported that his lab is concerned with issues related to genomic stability, including the consequences of internal or external perturbations, particularly the signaling process that takes place upon insult of the genome. Those processes largely determine whether the genome will effectively deal with the damage, or not. They are particularly interested in lesions called double-strand breaks—how they are induced, how they are processed, and the genetic consequences.

His lab also investigates the p53 tumor suppressor and the other genes it controls, seeking to understand the p53 master regulatory network, particularly the human network and its mutations. Those p53 mutations can lead to cancer and other diseases, so elucidating their mechanisms can lead to understanding of the associated health implications.

P53 is a master regulatory protein and transcription factor, and associates with many other cellular features to affect a variety of processes. Dr. Resnick said his talk would focus on the transcription factor feature, and the network related to the transcription factor.

He said that p53 “is involved in everything,” including meiosis, angiogenesis, embryo implantation, and much more, including innate immunity, which his group particularly studies. Various stressors (e.g., DNA breaks, UV radiation, oncogenes and others) can change the relationship of p53 with *mdm2*, a negative regulator of p53. In response to DNA damage, *mdm2* stabilizes p53. That stabilization allows p53 to act as a transcription factor, driving transcription of a wide variety of genes.

Fifty percent of all cancers have a mutation in the p53 tumor suppressor, and more than 90% of all cancers have altered expression of p53. It forms as a tetramer, binding to four different target sequences. It is a sequence-specific transcription factor. P53 consensus sites have been elucidated over the past two decades, which allows some idea of what to look for when seeking to identify genes in the network. However, nearly all response elements (RE) depart from the consensus, leading to the question as to the consequences of changing any one of the consensus bases, in terms of functionality. According to the available data, there is no binding to half sites in this scenario.

There are approximate 160 (and growing) validated sites in the genome where p53 has been shown to bind and lead to transactivation or repression of a particular target. In the p53 master regulatory network, the two essential elements are the p53 factor itself, and the target RE of the target gene, which p53 seeks. Thus, one of the questions being pursued by Dr. Resnick and his team is, “What constitutes a functional p53 RE?” That is, what is needed in terms of sequence inside the cell for p53 to see something and generate transactivation?

There is interest, he added, in human variations in functional p53 REs, as that can indicate variations in individual responses to stress. Also, there is interest in the effect of p53 mutations on transactivation of RE—on, off, and partial function. Interactions with other transcription factors is another area of investigation, as well as responsiveness to DNA lesions and other chromosomal stresses, human variations in p53 responsiveness, and the evolution of the network and what genes are brought into it.

In yeast, it has been possible to introduce human p53 (which is not native to yeast), and control its levels to help determine what constitutes a functional p53 RE. By turning p53 on to different levels, information can be gleaned about its ability to bind to different REs upstream of the reporter. Thus, functionality can be addressed *in vivo*, eventually working in human cells in similar fashion as well. With the ability to vary p53 levels 200-fold, its ability to transactivate sequences can be explored, as can the responsiveness of particular REs. This work has been shown that even small differences in sequence can dramatically affect levels of transactivation.

Dr. Resnick likened this to striking a chord on a piano, where you might have the same keys struck, but have different sounds emerge depending on how hard each key is hit...the keys being the REs, and the hand being the p53 master regulatory network. The next question becomes how variation in REs might affect the “chords.” SNPs in REs do have an effect, potentially resulting in completely different “chords,” or functional responses. Many different RE SNPs have been identified, significant among them in *toll-like receptor 8 (TLR8)*, which plays a fundamental role in innate immunity.

Knockout model without p53 have been produced, suppressing p53 activity altogether. But perhaps of more interest have been p53 mutants with altered transactivation, which have shown that by changing the spectrum of genes that are turned on, and the levels at which they are turned on, and that changes the biology. This led to another concept for p53, that it is a master gene of biological diversity.

Dr. Resnick and his team asked whether there were non-canonical sites in the genome that departed from the consensus, and also wondered about half-sites—whether they could bind p53 as a dimer and still lead to transactivation. After much exploration, they



determined that in fact just half-sites could support p53 function transactivation. Various half-sites were found that could support transcription almost as well as a modest full site. This discovery expands the p53 universe by showing that binding can occur with 10-base sequences, rather than requiring 20 bases. It has also been shown that this half-site binding is strongly sequence-dependent.

Dr. Resnick described work associated with p53 and the *FLT* gene (a.k.a. *VEGFR1*), which is involved in angiogenesis and had originally opened the door to the half-site discoveries. His lab found that there was a merging between the p53 network and the estrogen receptor, and that the two different transcription factors could act cooperatively to drive up expression. If there is an estrogen RE near a half-site, in combination with p53 and estrogen receptor, transcription can be driven up considerably. They asked whether this was a generalized phenomenon or unique to the *FLT* gene. Experiments with induced DNA damage at various half-sites showed that the synergistic effect was present elsewhere as well. Thus, “the damage responsiveness of the p53 network is influenced by estrogen receptor”—two very different networks coming together to produce a dramatic change in the responsiveness of several genes.

Dr. Resnick proceeded to present some information from preliminary, unpublished work emerging from his laboratory. With the newer knowledge about the involvement of non-canonical sites, the question remains about the true breadth of the p53 network and the consequences of DNA damage. A ChIP-Seq genome-wide analysis of human p53 binding sites was conducted, without using doxorubicin to induce high levels of p53. About half of the approximately 2900 identified sites were canonical—binding where there should be binding. About 25% were half-sites. The other 25% did not appear to have a p53 motif. After treating with doxorubicin, almost all of the binding was to non-canonical, or half-sites. This work is currently being conducted in lymphocytes.

In the final section of his talk, Dr. Resnick discussed the exploration of other functional full and half-sites in the human genome, or, as he put it, “p53 meets the innate immune pathway.”

There are ten toll-like receptor genes in humans, which are part of the innate immune set of genes, including *TLR8* mentioned above, and *TLR2*, the sequence of which appears to make it a p53 half-site. It also has estrogen REs nearby, so it resembles the *FLT* motif. It has been seen that both *TLR2* and *TLR10* are both responsive to p53, at levels comparable to a typical modestly-responding gene. Subsequently, other responsive half-sites and full sites were found in other toll-like receptor promoter regions.

The innate immune response provides the first line of defense against infections and triggers protective inflammatory responses. As part of the system, toll-like receptor

proteins can recognize particular patterns associated with pathogens, and they lead to the induction of signaling factors that trigger inflammation. Invasion by a pathogen activates the cellular TLR network, producing an inflammatory response that generates, among other things, reactive oxygen species (ROS). Looking at the p53 network, one of the factors that induces p53 is ROS—an intriguing convergence. Thus, the questions are:

- Does p53 activation alter TLR expression and then lead to further activation of the pathway?
- Do DNA-damaging agents induce TLR gene expression?
- Is p53 involved in the transcriptional regulation of TLRs?
- If so, what are the biological consequences?
- What are the effects on innate immune inflammatory response?
- Are there tissue-specific responses?

Although all of the questions have not yet been answered, some important information has emerged. The group has found, for example, that several of the TLRs are inducible by damaged p53 in cancer cell lines.

They extended their studies to primary cells in the innate immune pathway. They have collaborated with the NIEHS Clinical Research Unit to look at isolated human primary lymphocytes, to determine whether there is DNA damage responsiveness in TLR pathway. It has been found that p53 induced by nutlin treatment can induce expression of 7 or 8 of the members of the 10-member TLR gene family. DNA damage can induce all of the TLR genes. There is apparently considerable variation between people in these responses. The key point is that toll-receptor genes were found to be under the influence of p53. Also, TLR allelic variations have a profound influence on responsiveness to p53. Dr. Resnick said these findings have many implications, including for individual susceptibility and perhaps in personalized medicine.

He showed recent work positing the existence of a TLR-ROS-p53 feedback loop, by which it may be possible to modulate the entire system.

He closed with a brief description of work investigating “DNA niches,” areas where groups of genes have been captured into the p53 network. An evolutionary mechanism is apparently at work, as these genes do not appear in rodents. For example, the p53 control of TLR is present in primates but not in rodents.

Dr. Birnbaum asked Dr. Resnick how broadly his group has looked at mice. He described several experiments that led to the conclusion about the lack of the DNA repair niche in mice, and mentioned that there would be ongoing investigation of the evolution of pathways, with some of that work taking place in mice.

Dr. Birnbaum asked about the group's work looking at other response pathways in relation to p53. He said that ChIP-Seq allowed a more top-down genomic approach to those questions, which will be pursued.

Dr. Lee asked about the variability in response in the different toll receptors, wondering how much it might be affected by endogenous toll activation. For example, she asked, would there be p53 response in someone in the early, asymptomatic stage of an infection? Dr. Resnick speculated that there would, although the human samples had been taken from healthy volunteers. He added that he was amazed by the variability seen in the responses among individuals, implying a great deal of variability in the innate immune response.

Dr. Kleeberger asked if there were rules for other transcription factor family half-sites similar to those established for p53 half-sites. Dr. Resnick said he believed it was an approach that could be applied to other transcription factors.

From the audience, Dr. Jack Keene asked whether Dr. Resnick had considered looking at p53 in primate cells. Dr. Resnick replied that the evolutionary history of p53 is of great interest, particularly how it happens that entire clusters of genes are captured into the genome, seemingly suddenly.

## **XVII. Report of the DIR Board of Scientific Counselors**

The meeting's final open session speaker was Dr. Jack Keene of Duke University, chair of the NIEHS Board of Scientific Counselors (BSC), who briefed Council on the BSC's activities in relation to DIR scientists.

Dr. Birnbaum introduced Dr. Keene, and suggested to Council that they consider establishing Council liaisons to the NIEHS BSC, the NTP BSC, and to the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM).

Dr. Liekauf asked whether being a liaison would mean attending all of the group's meetings. Dr. Birnbaum said she would think it would certainly be an invitation to do so. She elaborated that the liaison would be a non-voting member, so the level of effort would be less than a voting member's, being more of an observer to report activities back to Council. Dr. Liekauf suggested having one of the BSCs' members on Council instead. Dr. Birnbaum answered that Council is the group designated to provide advice to the entire institute, the others are for segments only. She said an answer was not needed in this meeting, and suggested Council members think over the idea.

Dr. Collman added that another job is open, in the NIH Council of Councils, which includes a representative of every institute's council. It is the council to the Common

Fund, working on trans-NIH initiatives. Dr. Graziano has been the NIEHS representative, but pointed out recently that although he is no longer on the NIEHS Council, he has two years remaining on his term on the Council of Councils, suggesting that the terms should be aligned, serving four years on both committees concurrently. She asked for a volunteer from among those with three or more years remaining in their term on Council, and mentioned that if no one stepped forward, she would be contacting members individually. Dr. Birnbaum stressed the importance of having a strong NIEHS representative on the Council of Councils.

Dr. Keene reported that the BSC reports to the Scientific Director and to the Director, and to Council as well. He said he has been serving since 2008, and felt that the process has been going smoothly, with a new review template that has been working well. The board meets 2-3 times per year at NIEHS. He reported that there is a good mix of expertise on the board, with ad hoc members attending as needed.

He described the board's review process, commencing on Sunday nights with off-site discussions attended by the Director, then moving to the Institute for 1-1/2 to 2 days of review sessions, some being open and some closed sessions. The individual scientists present their data on their progress and sketch out their future plans. There are also poster sessions with trainees.

He reported that the success rate for continued funding far exceeds that of extramural investigators, which is important for the continuity of an institute such as NIEHS.

He went over the relatively new NIEHS BSC Review template, which had been put together by Dr. Schrader. It has been set up so that reviewers can know what is expected of them, and per NIH guidelines. Beyond the prescribed sections, there is a place for additional comments reviewers may wish to include. The BSC reviews tenured and untenured scientists. Training is a criterion, particularly with younger scientists, as are productivity and mentoring. There is a numerical scoring system, but the verbal descriptors are preferred. Criteria for tenure track are also included, if applicable.

The unique aspects of the intramural review process include an emphasis on quality over quantity of scientific progress, and encouragement of collaborations. Problems with the process include limited resources and budgetary issues, an aging senior staff, and the need for a plan of succession for each unit as leaders retire, as it is not always possible to bring in an external senior leader.

Dr. Keene identified key matters that the BSC wishes to advance, including:

- Emphasizing the mission of NIEHS

- The need to recruit members of underrepresented populations to the BSC itself and to NIEHS
- The need to foster interdisciplinary research efforts and excellence in mentoring
- The need to reward the highest-quality research

He reported that personnel from three laboratories and branches had been reviewed during his tenure, since June 2009, with the next review, of the Laboratory of Reproductive and Developmental Toxicology, scheduled for October 2010.

He said that most of the investigators in BSC reviews have received a rating of *excellent* or better. He said that within the past three years, one scientist was removed from tenure track, and three groups had been closed. One other group is slated to close, and a second is being substantially reduced in size. BSC assisted in those decisions, which were close to its recommendations.

In conclusion, Dr. Keene said the BSC is proud of its role in contributing to the critical decisions ensuring continued success at NIEHS.

Dr. Birnbaum said that she felt that the relationship between BSC and DIR had been going very well, and asked a round of applause for Dr. Schrader, who was a leader in refurbishing the review process.

Dr. Taylor asked whether funds and space were the major barriers in turnover decisions. Dr. Pritchard replied that funds do tend to be an issue, for example in recruitment of lab chiefs. Recruitment of tenure-track scientists has gone well in the past few years, he said, due to spots opening up. In terms of space, there are several suites of lab space appropriate for tenure-track scientists currently available. Dr. Taylor asked whether renovation of space was the main issue, or if it is more of a personnel issue. Dr. Pritchard replied that it is more of a personnel issue. Dr. Birnbaum added that the intramural program is large compared to the other NIH institutes, so that in order to bring in new people, there is a need for some of the more senior people to retire. She said that some retirements of senior investigators are anticipated within the next year, which should allow for recruitment of some new tenure-track scientists, although they may not be in the same research area.

Dr. Liekauf asked if there is a strategic plan in place for the intramural program. Dr. Birnbaum replied that the process will begin this fall for development of a new strategic plan for the Institute. In concert with that process, she said, a strategic plan would be developed for the intramural program. However, it makes the most sense for that process to await the arrival of a new Scientific Director.

Dr. Pritchard noted that he would be retiring shortly, with Dr. David Miller taking over as Acting Scientific Director, and it would be his highest priority to establish the initial steps

to determine DIR's priorities, so that when the overall strategic plan process starts, the Division will be in a position to contribute to it in a meaningful way.

### **XVIII. Consideration of Grant Applications**

This portion of the meeting was closed to the public in accordance with the provisions set forth in Section 552b(c)(4) and 552b(c)(6), Title 5, U.S. Code and Section 10(d) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2).

### **XIX. Adjournment**

Following the closed portion of the meeting, Dr. Birnbaum thanked Council for its efforts and officially adjourned the meeting.

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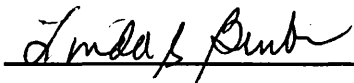
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Following the closed portion of the meeting, Dr. Birnbaum thanked Council for its efforts and officially adjourned the meeting.


The meeting was adjourned at 11:15 a.m. on September 2, 2010.

**CERTIFICATION:**

**I hereby certify that, to the best of my knowledge, the foregoing minutes and attachments are accurate and complete.**

  
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**Linda S. Birnbaum, PhD, DABT, ATS  
Chairperson  
National Advisory Environmental  
Health Sciences Council**

  
\_\_\_\_\_

**Gwen W. Collman, PhD  
Executive Secretary  
National Advisory Environmental  
Health Sciences Council**

**Attachment:  
Council Roster**