National Center for Toxicological Research

Science Advisory Board Meeting

June 11/12, 2001

Dr. Casciano convened the meeting at 1:00 p.m. on June 11. He announced Dr. Daniel Acosta as the new Chair of the National Center for Toxicological Research (NCTR) Science Advisory Board (SAB). Dr. Acosta announced the appointment of three new SAB members. The new members are: Dr. Jerry Kaplan, University of Utah School of Medicine; and Dr. Kenneth Tindall, North Carolina Biotechnology Center; the third newly appointed member, Dr. Elizabeth Barbehenn, from Public Citizen, was unable to attend today's meeting. He also announced that Dr. Marcy Rosenkrantz agreed to serve for an additional two years, and introduced Dr. Leonard Schechtman the new Executive Secretary to the SAB, and Dr. James MacGregor, the new Deputy Director for Washington Operations.

Board members present were Drs. Daniel Acosta, Catherine Donnelly, Nancy Gillett, Marcy Rosenkrantz, Jerry Kaplan, and Kenneth Tindall. SAB liaisons present include: Drs. Marilyn Lightfoote (by phone), CDRH; Patricia Hansen, CFSAN; Linda Youngman, CVM; and Richard Kennedy, UAMS. Other attendees included Dr. Bernard Schwetz, Acting Principal Deputy Commissioner, and NCTR staff.

Dr. Casciano, welcomed and introduced Dr. Schwetz who thanked the Board members, the liaisons from the other centers, ORA, and UAMS. He commented that this SAB has had more of an impact on what happens at the NCTR than any other Boards of Scientific Counselors or SABs in other Centers. He said that through these efforts, the SAB has helped to resolve problem areas and promote better communication and collaboration. Dr. Schwetz pointed out that peer review of Agency operating components was becoming more formalized. He also said that such peer reviews were conducted at NCTR when he was Director, and have since been initiated at other FDA centers. The Center for Devices and Radiological Health (CDRH) is undergoing review at this time; the Center for Veterinary Medicine (CVM), the Center for Drug Evaluation and Research (CDER), and the Office of Regulatory Affairs (ORA) are preparing for reviews; and the Center for Food Safety and Applied Nutrition (CFSAN) will undergo a review in the near future. He explained this was not a peer review of individuals, but of the entire science program within a given center. Centers that do not have a formal review program in place, as do NCTR and the Center for Biologics Evaluation and Research (CBER), will be reviewed “from the top down to the protocol level” for the first time.

Next, Dr. Acosta requested approval of the June, 2000 minutes. Dr. Hansen (CFSAN) said that she would like to expand the discussion on dietary supplement work and priority setting. She agreed to provide the Executive Secretary with written comments, which he will review, and insert if appropriate. A motion was made and seconded to accept the minutes as written, with this one possible modification.

Organization and Background

Dr. Casciano provided information on the organization of FDA and NCTR. He noted that FDA has five product centers and one research center (NCTR). Each center has a different mandate and mission and it is incumbent on NCTR staff to understand what those are so they can interface effectively with the other centers. He stated the mission of each of the centers and ORA and provided examples of NCTR interaction. He also reviewed:

- Resource information over various fiscal years.
- The full time employee (FTE) allocation over the last eight years.
- NCTR's attempts to maintain a level of productivity through a postdoctoral program and by using on-site contractors.
- Additional resources received from the National Institute of Environmental Health Sciences (NIEHS). through an interagency agreement (IAG) with the National Toxicology Program (NTP).
- How these additional dollars allow NCTR to maintain a critical mass of scientists.

Dr. Casciano said FDA requested additional dollars in FY 2002 and that the appropriated dollars included a percentage of what was asked for and the first cost of living increase in ten years. He added that an effort is being made by FDA to enhance its involvement and visibility in the area of proteomics. FDA has requested additional $2.5 M dollars in the proteomics/genomics area and additional dollars for antimicrobial dietary supplements. Food safety has a high visibility in the new administration, and there is a potential for obtaining additional funding. Areas that impact directly on NCTR’s mandate to respond to FDA needs are providing highly credible scientific data to the NTP Bioassay Program, thereby resulting in greater confidence in risk assessments. NCTR is also a major player in the food safety initiative. Dr. Casciano indicated that the Division of Chemistry has a system that allows detection of seafood decomposition, and it is attempting to adapt this system to detect decomposition in poultry. In the proteomics field, the analytical technology is transferable to bioterrorism as well as the food safety initiative.

NCTR is also doing work in the area of DNA adducts. The Division of Microbiology is working with an artificial GI tract that can be used to help evaluate the effects of dietary supplements and perhaps genetically modified food. The Center has done work in nutrition and on caloric restriction, efforts that have generated a number of new hypotheses. The Center has also developed and modified transgenic animals for use in detecting mutagenic and carcinogenic agents.

Addressing primarily the new SAB members, Dr. Casciano reviewed some of the Board's functions, which include:

- Advise the NCTR Director on science, budget and other issues.
- Review and approve site visit reports. Dr. Casciano said that SAB members might be asked to chair or be a member on a site visit team (SVT) if the program to be reviewed is in their specific area(s) of expertise. Ad hoc experts are also included as SVT members. Reviews are done on a cyclical basis, which averages about once every three years.
- Help in the decision making process on various undertakings within the Center. For example, a discussion on the possibility of moving a new subcommittee under the umbrella of the SAB was on the agenda, and Dr. MacGregor would present the rationale for the move. **(TAB 1**)

Dr. Casciano summarized the various technical approaches being pursued to assess toxicity and extrapolate results to the human. These range from cellular and biochemical mechanistic systems, various "-omics" technologies (e.g., genomics, proteomics), various other biomarkers, and transgenic models. The overriding interest is to develop better predictive models for the human. NCTR's efforts in these areas will result in a decrease in the use of animals as the dependence on in vitro systems relevant to the in vivo situation increases. There will be a tremendous amount of data generated from these various –omics technologies and computational science will need to be applied to aid in the understanding of data being developed. NCTR is building its infrastructure to accommodate development of proteomic-applicable analytical equipment, microarray chip technology, and the different toxicogenomic technologies.

Dr. Casciano then addressed recruiting and retention issues at NCTR, acknowledging that such efforts are difficult and that probably 20 to 25% of the staff will turn over in the next five years. He added that NCTR's location is not ideal for recruiting the staff that we're interested in, and we have to compete in a tight job market. He said that the individuals we're interested in generally have a professional spouse who is also seeking employment. We're looking for creative solutions and Dr. Schwetz mentioned some of them today. We're utilizing creative recruitment, recruiting individuals for a new group -- the cellular and molecular toxicology group, and we are in the process of doing some heavy leveraging.

Dr. Casciano then discussed NCTR's interactions with NIEHS, which has a toxicogenomics group and with which we are actively communicating to convey our interest in participating in this area. It is necessary for FDA to be a player in this area, since industry is already using these technologies, and will be developing safety data generated from these technologies in the near future. FDA needs to be ready to respond and if we're not, we will take the most conservative approach and become bottlenecks to the process and not be catalysts. He said NCTR needed to develop highly creative interactions with our colleagues in academia and industry. NCTR has the opportunity of purchasing academicians through the Intergovernmental Personnel Act, which allows us to hire academicians for a year or two. Under this program, they can spend their time here or work at their home institutions on problems that are of interest to the NCTR and/or the FDA. Any advice that the Board can provide regarding fostering positive relationships in these areas would be very appreciated.

The questions from the Board members focused on NCTR financial aspects. Dr. Casciano responded that FDA's ownership of the NCTR facility allows NCTR to better manage its funds. He said leveraging dollars from NIEHS and other FDA centers with which NCTR maintains strong ties (e.g., CFSAN, CVM), is another funding approach that will be pursued. Projected 2003 funding is expected to be adequate to sustain current activities and, in addition, a 4% increase has been requested.

Nonclinical Studies Subcommittee

Dr. MacGregor was next on the agenda; he proposed that the Nonclinical Study Subcommittee (NCSS) of the Advisory Committee for Pharmaceutical Science (ACPS) be moved under the SAB. He provided the Board with an overview on the NCSS and addressed the concept and related activities of this Subcommittee. Members of the Board asked for additional information and for time to study the concept. Dr. Tindall agreed to research the proposal and report back to the Board at it's next meeting. **(TAB 2)**

Endocrine Disrupter and Knowledge Base Program

The assembled group then reviewed NCTR's response to the SAB Site Visit Report on the Endocrine Disrupter and Knowledge Base Program's (EDKB). Dr. Sheehan provided a handout that included: (a) the program's fiscal year 2000 research

accomplishments (for comparison with the 2001 plan), (b) a list of 19 publications, and (c) the response to the SAB report. Dr. Sheehan then provided an update on the EDKB program. **(TAB 3)**

Dr. Rosenkrantz noted the absence of a response to the site visit team's (SVT) suggestion on mainstreaming the knowledge-based work into the rest of the activities of the Center. Dr. Sheehan said he thought that the Board's visit to ROW should have given her an idea of its work. Dr. Rosenkrantz said there was concern about how further studies would be funded through the Center and competed against other projects. She asked how that had gone and suggested that Dr. Casciano or someone else could answer, but she wanted to have this issue discussed. Dr. Sheehan said, he'd never seen the total expenditures from NCTR, but the $3,300,000 plus the $850,000 for the external validation had defrayed a substantial portion of the expenses associated with developing the models. He indicated that outside funding sources were a primary means of program support. Dr. Casciano said the androgen receptor studies and the EPA work were funded by EPA and NCTR's budgetary input is relatively small. Also, they are providing input into the genomics and proteomics activities in combination with investigators from chemistry, biology and biometry, and are directing the NCTR knowledge database into development for the DNA microarray technology. He said the SVT critique was taken very positively and the Center had responded to it.

Dr. Rosenkrantz asked for comments and there were none. She did not ask for a vote because people have not had a chance to read the Center response, and suggested that the vote be delayed to allow for the possibility of additional questions.

Division of Microbiology

Dr. Khan responded to the SVT Report in Dr. Cerniglia's absence and provided an update on the Division of Microbiology. The report provided general comments and recommendations:

- Strategic planning - The SVT asked the Division to develop more collaborative research projects with other FDA centers and ORA. Dr. Khan said the Division of Microbiology has about 20 different research projects (CVM-10, CFSAN-3, ORA-6 and CDRH-1).
- Food microbiologist - The SVT recommended that the Division hire a food microbiologist. Dr. Khan said that the position was advertised in February and an offer was made, but the applicant declined.
- Philosophical paradigm – The SVT recommended they carry out most of the studies based on the mechanistic approach. Dr. Khan reported that the Division followed the recommendation to establish the necessary collaborations.
- Work related to antibody resistance -- Dr. Khan explained that, based upon mechanistic studies, they can determine whether or not the risk of transferring antibody resistance is justified. This approach cannot be used for regulatory work, but can determine if antibody resistance can be transferred to other organisms. Division researchers have shown that poultry isolates can transfer resistance to human isolates and they now understand the role that CVM can play in controlling the use of antibiotics in poultry.
- Leadership role – The SVT asked what would happen if Dr. Cerniglia left and whether there is anyone being groomed for a leadership role? Dr. Khan said senior staff are given the opportunity to attend training and to build leadership skills. Also, the Division directorship was rotated among the senior staff whenever Dr. Cerniglia was on travel.
- Building – Dr. Khan added that the Division appreciates the recommendation that it needed space and reported the Division will probably move into the chemistry area after modifications are completed.
- Public relations and communications -- The SVT recommended that the Division develop a website. Dr. Khan reported that the site has been completed and that they have posted the skills, research interests, goals and accomplishments of the Division's principal investigators (PIs).

Next Dr. Khan addressed comments on the following research focus areas:

- Food borne pathogens, food safety, and methods development – The Centers focused in this research area are CVM and CFSAN. NCTR has ten projects with CVM, of which, seven or eight are on food safety. The SVT recommended that the Division try to quickly publish this research. Dr. Khan said that they want to publish quickly, but they want to avoid compromising the quality of the research. Before publishing they send the data to everyone involved in the collaborative process for review and comment.
- Standards and controls - Dr. Khan reported that whenever and wherever necessary the Division always tries to include standardized methods before publication.
- Determination of the role of intestinal microflora in activation or detoxification of xenobiotics – The SVT recommended that the Division strengthen this area. Dr. Khan responded that they have developed chemostat models that mimic the human large intestine to study the effect of low-level antibiotics used in veterinary medicine (e.g. in food-producing animals) on human microflora.
- Environmental biotechnology -- The Division is continuing its work in this area. Several researchers are studying the role of fungi and bacteria and how these microorganisms biodegrade pollutants in the environment.

?? Use of microorganisms as models to predict how drugs are metabolized - The Division is using a number of microorganisms to study the metabolic pathways. It has also begun collaborating with USDA and is trying to isolate bacteria from fish ponds. Division researchers are studying how antibiotics used in the fish industry are degraded and whether or not antibiotic resistant bacteria accumulate in the environment.
?? Microbiology surveillance and diagnostic support of research - The Division continues to offer surveillance and diagnostic services to NCTR and the other FDA centers.

Dr. Acosta asked Dr. Donnelly (SVT Chair) and Dr. Kennedy (SVT member) if they had any additional comments. Neither wished to comment at this time. Dr. Casciano said NCTR seriously considered the SVT's comments about public relations and communication. He reported that NCTR completed development of an "e-journal" that is being made available on its public website to enhance communications with other FDA centers, other regulatory bodies, and the international community. Staffers from FDA and other regulatory bodies will serve as its editorial board and can contribute to the journal as well. He also said that this journal is a regulatory research interest journal and could become a vehicle for the regulatory scientist to disseminate thoughts or ideas that they would like to develop and have others understand. The Division of Microbiology has contributed the first paper to the journal's inaugural issue. Dr. Donnelly asked the status of the food microbiologist position, because of all the recommendations in the report, she said this was the most critical and one the SVT strongly recommended. She added that this entailed not just hiring a food microbiologist, but a senior researcher who could direct research with applied outcomes. The incumbent in the position should be able to take advantage of all of the resources that appear to be coming to the Agency to deal with food safety. Dr. Casciano responded that the Division conducted a search and identified someone, but this candidate did not accept the position. He added that the Division will renew its search for a strong candidate to fill the position. In response to the SVT's comment on leadership, he said Dr. Cerniglia was making an effort to expose his senior scientists to administrative roles, as well as to representing him in critical meetings. Dr. Acosta asked Dr. Donnelly if she wanted to make a recommendation on acceptance. She moved to accept the report as submitted, the motion was seconded, and the report was accepted.

Division of Microbiology

Dr. Khan provided the following update:

?? The Division of Microbiology continues to serve a multipurpose function with specialized expertise to perform fundamental and applied research.
?? The Division responds to microbial surveillance and diagnostic needs for research projects within NCTR and FDA.
?? The surveillance and diagnostic program has three groups that offer services in the area of bacteriology, parasitology, mycology, virology, serology, and media preparation.
?? The virology and serology group offers its services for surveillance and support for research scientists. The surveillance branch maintains a breeding colony, quarantine facility, primate colony, NTP animal studies, non-NTP animal studies, environmental health, program assurance, and it oversees the animal husbandry and diet preparation contractors.
?? The Division provides research support to the other NCTR divisions such as Biochemical Toxicology, Chemistry, Genetic & Reproductive Toxicology, Molecular Epidemiology, and Neurotoxicology.
?? Division staff performs research in the areas of environmental biotechnology, food safety, microbial models in toxicology, and intestinal microflora.

There are many technical abilities and skills available from the Division. Its current work includes:

?? Collaborative projects with CVM, ORA, CFSAN, and CDRH. Included among the collaborative research efforts in the food safety area are the *in vitro* model and molecular analysis of competitive exclusion products, applicable to pathogens in the poultry industry.
?? Development of molecular screening methods for the determination of vancomycin resistance in selective competitive exclusion products, and characterizing gene markers in competitive exclusion products. Whether those markers are transferable and whether competitive exclusion is safe for use in the poultry industry. NCTR has ongoing studies on fluoroquinolone resistance in *camphylobacer sp.* and *Salmonella spp.* isolated from poultry.
?? The Division is using molecular techniques to screen animal feeds for proteins derived from mammalian tissue to detect possible bovine spongiform encephalopathy (BSE).
?? Developed and validated an assay for detecting BSE, which won the 2000 FDA Collaborative Science Award.
?? Developed molecular methods to identify and quantitate human food borne pathogens in the animal production environment and developed methods to detect the presence of these pathogens in a variety of food sources such as vegetables, poultry, fish, and salads.
?? Initiated, in collaboration with USDA, a project on biodegradation of veterinary drug residues. This project was undertaken in response to a suggestion from the SVT.

?? Developed a guidance document entitled "Assessing the Effects of Antimicrobial Residues in Food on the Human Intestinal Microflora."
?? Developed alternative microbial methods for studying the metabolism of drugs.
?? Developed a bioluminescent assay to determine the tuberculocidal activity of disinfectants.
?? Developed multiplex PCR methods for the determination of vancoymycin resistant genes.
?? Characterized the microbial cytochrome P-450s and glutathione transferase, the aerolysin toxins genes, and erythromycin resistance genes (erm) in *Staphylococcus* sp. isolated from chickens; azoreductase and nitroreductase enzymes in bacteria isolated from human intestinal tract; and fluoroquinolone resistant *Campylobacter* spp. from poultry samples.
?? Developed a method to isolate and characterize bacteria that are cutting undesirable antibiotic resistant markers.
?? Completed and validated a trial method for the detection of BSE.
?? Discovered an agent in oyster homogenates that is lethal to *V. cholerae* and *V. Vulnificus.*
?? Discovered new strains of bacteria and fungi that degrade environmental pollutants and explained novel biodegradation pathways for xenobiotics.

Plans for fiscal year 2003 include:
?? Initiation of a new chemostat experiment for testing veterinary fluoroquinolones.
?? Isolation and molecular characterization of fluoroquinolone resistant *Salmonella* spp. and *E.. coli* from chicken and turkey litter.
?? A study for the plasmid profile, genetic fingerprinting, and strain typing of vancomycin resistant organisms isolated from competitive exclusion products.
?? Characterize fluoroquinolone resistant camphylobacters at the molecular level using PCR-RFLP and pulse field gel electrophoresis and correlate the data that have been obtained from poultry litter with the environmental data available for human subjects.
?? Collaborate with USDA on biodegradation rates and metabolic fate of antibiotics used in aquaculture.
?? Working on diadzein and genistein to try to detect the specific bacteria (from the human intestinal tract) that convert these phytoestrogens to estrogenic and non-estrogenic end products.
?? Evaluate the effect of fluoroquinolones on anaerobic bacteria from the human intestinal tract. Including development of resistance, mechanism of resistance development, impact on metabolic activities, and the dissemination of resistance to bacterial pathogens.
?? Identify the metabolites produced from norloxacin and sarafloxacin by fungi grown on poultry litter.
?? Isolation of new strains of fungi from litter in poultry houses and screen for biotransformation of fluoroquinolones.
?? Complete *in vitro* assay of competitive exclusion products.
?? Maintain a world-class research program to solve current issues facing FDA, so the Agency can make sound science-based regulatory decisions on microbiology. **(TAB 4)**

Dr. Khan opened the floor for questions. Dr. Tindall asked if any of the researchers were thinking of pursuing patents. Dr. Khan said there were none, but that when they felt they had something they would definitely go in that direction. Dr. Tindall also asked about the practice for technology transfer. Dr. Casciano said the Center had a Technology Transfer Office that integrates with a similar group at the Agency level, and investigators are encouraged to use that office when they felt they had a useful discovery.

Dr. Donnelly observed that it would seem logical to extend the current work on S*almonella* DT 104 to *Salmonella newport*, because *S. newport* has now acquired that same gene cassette. Dr. Khan said that they isolated several strains of *Salmonella* from poultry litter and that he wasn't sure, but thought S. *newport* was being studied.

Dr. Acosta asked about external grants or other resources to do all of these projects. Dr. Khan said that most of this was money from FDA funds. Dr. Casciano added that there were directed funds from Congress and the Food Safety Initiative, which comes through CFSAN and CVM. He also said that when the dollars collide, we collaborate and leverage within the Agency. Dr. Acosta asked the percentage; Dr. Casciano said probably ten percent for this Division. Dr. Kaplan asked if NCTR received grants. Dr. Casciano said that because we are part of the Public Health Service (PHS) and that due to NIH policy, we cannot directly apply for grants, but we can serve as co-investigators and co-PIs. In addition, we can get some funds through either equipment or post docs. He also said that if we have an expertise that is unavailable in academia, or if there is a need for a fast track, there are mechanisms for obtaining dollars from other PHS agencies through an IAG.

Division of Neurotoxicology

Dr. Slikker, provided an update on the Division of Neurotoxicology. He explained that the focus of this Division is on the development and validation of quantitative biomarkers and to use these biomarkers to explain toxic mechanisms and increase the certainty of underlying assumptions used in risk assessments for neurotoxicants. Neurotoxicity can be defined as any adverse effect on the structure or function of the central and/or peripheral nervous systems. Within FDA, this includes biological, chemical, or physical agents. Adverse effects include any unwanted effects that diminish the ability of an organism to survive, reproduce or adapt to its environment. These effects can be permanent or reversible. He also provided the Board with copies of his slide presentation. **(TAB 5)**

Dr. Slikker identified various approaches used by the Division, which include:

- Multidisciplinary approach, because many of the relevant effects can be measured by neurochemical, neurophysiological, neuropathological, or behavioral techniques.
- Discipline-continuum approach, trying to link together the various kinds of approaches. Everything from psychological testing that can be done in human and animal models, through behavioral assessments, physiological assessments, and various kinds of motor function tests. This kind of linkage can be done using genomics, proteomics, also computational modeling. Neurobiological approaches including microdialysis allows the collection of information from the nervous system in a waking animal. Protein biochemistry is very critical with proteomics and cell culture approaches.

- Dr. Slikker discussed the disciplines represented by researchers within the division. These include a neurochemical specialist, a neurophysiologist, a developmental neurobehavioral toxicologist, a pharmacokinetic specialist, and an experimental neurohistologist. Several Division members have been trained and have experience in the areas of genomics and proteomics.

Dr. Slikker said the Division could provide the expertise needed to solve integrated problems within the area of neurotoxicology.

Next, Dr. Slikker provided specific examples for behavioral assessment tools. He said they had testing laboratories at the Arkansas Children's Hospital satellite lab and at the University of Arkansas at Little Rock. The tests are quantitative in nature. Since it is impossible to test every chemical in children, they have a primate center at NCTR that is capable of providing animals for general toxicology and pharmacokinetics studies. They also have a breeding colony, which can be used to study different stages of development during pregnancy. Dr. Slikker also said that they have the ability to behaviorally evaluate up to 90 primates a day. He said extramural funding had been good, consisting of over 0.5 million dollars per year over the last 13 years, and productivity has been excellent.

Dr. Slikker discussed ongoing studies within the Division, and provided examples of study drugs, which range from THC to chlorpromazine (major tranquilizer), Diazepam (minor tranquilizer), morphine and atropine, and marijuana. The Division is also collaborating with CFSAN and CDER. One protocol focuses on the use of clontech arrays to examine for gene expression alterations. The hypothesis is that substituted amphetamines may produce long-term ultrastructural and neurochemical changes and that this could be monitored by looking at specific DNA expression.

Dr. Slikker said that Division staff publishes in prominent peer-reviewed scientific journals and has published about 40 articles, reviews and book chapters per year, over the last six years. They also provide leadership on various kinds of committees, such as those that oversee CFSAN's Redbook II, the Reproductive and Developmental Toxicity group, EPA, and the WHO working group on pharmaceutical agents. In the last year, they have been on four committees dealing with risk assessment in children. The Division also serves on a CDER committee that evaluates various pharmaceuticals including anticonvulsant agents.

Dr. Slikker added that the Division believes in maintaining a diverse portfolio of leveraging opportunities, which include the following CRADAs:

- A primate study conducted at the NCTR, which is funded by NIDA to the University of Arkansas at Little Rock. One of our previous postdoctoral fellows is PI on this award and through a CRADA mechanism, reimburses NCTR at $250,000 a year, for five years.
- The NTP/IAG on Endocrine Disrupters, undertaken with EPA, that is valued at approximately $200,000 a year. This collaboration addresses quantitative risk assessment procedures and investigates some of the quantitative methods of comparing structure and activity.
- A CRADA with AstraZeneca, where the Division interacts with industry on broad questions of how anticonvulsants affect learning and memory. These CRADAs help supplement current NCTR/FDA funding.

Next, Dr. Slikker discussed projects and protocols that had either been approved or just begun in the past year.

- Use of multiple cDNA arrays to look at temporary changes after exposure to amphetamines. Collaborators include NIOSH, other divisions within NCTR, and Wake Forrest University.
- A long-term look at anticonvulsant treatment with AstraZeneca.
- Another protocol under review is on ephedrine-type compounds. This protocol is currently at the second stage of review. Collaborators include Syed Ali and Bill Girley from UAMS, who is an expert on dietary supplements.
- Another, in conjunction with Wright Patterson Air Force Base, looks at ephedrine-containing dietary supplements. These concept papers have been approved.
- Another, in collaboration with colleagues from Duke University, considers the developmental effects of nicotine in a rodent model.
- Another approved concept paper is on mitochondria energy metabolism to assess the claim that dietary supplements modulate metabolism

Future directions were also addressed.

- Thimerosal is an issue within CBER and CDER. CBER is pushing this through as an NTP compound. This is important because it is used as a preservative in many vaccines that children receive on a routine basis. NCTR and CDER think that it is very important to be able to evaluate thimerosal exposure in developing monkeys.
- Infant formula is being discussed with CFSAN.
- There is ongoing dialogue with CDER to look at the pediatric use of the dissociate anesthetic, Ketamine, and how to evaluate its safety.
- Imaging development with the other centers.
- Transgenics - the Division has shown the importance of using primates in certain studies.
- Genomics and proteomics are important areas to amplify for the future.

In closing, Dr. Slikker said that FDA working groups enhance communication and provide an opportunity to keep up with FDA needs, and help to anticipate future needs. One such group is the FDA Intercenter Neurotoxicity Working Group. The working group assists FDA in addressing neurotoxicity regulatory concerns. He also said there was an FDA intranet site that allows FDA researchers to communicate and schedule meetings. This approach of interacting with other agency researchers interested in safety assessment, especially neurotoxicity safety assessment, lets all the centers communicate about issues and try to come up with solutions.

Dr. Acosta asked for questions/comments. Dr. Rosenkrantz asked about the work of the computational science group. Dr. Slikker said that NCTR has been able to obtain funding from EPA, Research Triangle Park, to collaborate with them to develop a biologically based model. They hired a post doc and bought equipment to do the modeling. Dr. Slikker said that a manuscript was submitted and they are hoping for a good return from this effort. Dr. Rosenkrantz asked if ROW was involved and Dr. Slikker responded it was. Dr. Rosenkrantz asked if that included imaging as well. Dr. Slikker said imaging was more complicated and is being done in conjunction with JIFSAN (a consortium of CFSAN and the University of Maryland) and CDER (who has relationships at Duke University and various other universities), so the imaging is not at NCTR. Dr. Slikker added that NCTR does have imaging capability at UAMS and that they are trying to build on those relationships so that NCTR can have access to equipment. However, right now they are going through JIFSAN and the CDER relationship with Duke and other locations. Dr. Casciano said the proteomic and genomic efforts are interfacing with the bioinformatics group and that they are developing software to image and identify intensity for protein spots, plus lend creditability to the development of data sets. Dr. Casciano also said that this is a team effort and these developmental projects are applicable across various platforms.

National Toxicology Program (NTP)

Dr. Allaben provided an update on the NTP. He provided background information on the FDA/NIEHS Inter- Agency Agreement (IAG). He explained that originally this was a five-year concept, but it has been so effective that it's now an open-ended agreement.

In 1996, the NCTR entered into an agreement with NTP to do endocrine disruptor studies. We looked at five putative endocrine disrupters. These are complex, time consuming, multigenerational studies, and NCTR was the only facility that could perform these types of studies. In 1998 resources for developing a phototoxicity research and testing laboratory were added. This is a state-of-the-art facility that does not exist anywhere else in government. We are hoping to get appropriate resources from NIEHS for renovation and expansion of the phototoxicology research laboratory and for additional animal rooms to support these studies. Negotiations are ongoing.

Dr. Allaben continued by adding that in 2000, additional high priority FDA compounds, including some of the dietary supplements, were brought in under the IAG, and most recently we are entering in to a complex series of studies, the AIDS therapeutic mixtures study. Some of the compounds tested under the IAG are: chloral hydrate, fumonisin B1, malachite green, urethane/ethanol, riddelline, glycolic and salicylic acids. Dr. Allaben added that the endocrine disruptor chemicals, the phototoxicology nominations, dietary supplements, AIDS therapeutic studies, thimerosal and other important chemicals are coming in under the umbrella of the IAG. With regard to endocrine disrupters, we are looking at methoxychlor, genistein, nonylphenol, vinclozolin, and ethinyl estradiol. Aloe vera is the first dietary supplement under study. Dr. Allaben also explained that some of the nominations not included in the IAG include radio frequency, DNA based safety assessment of selected vaccines, therapeutics, and two antibiotics that the Agency needed genotoxic information on. He added that the NTP provided an opportunity to obtain information on P53 studies with Senna, a replacement for Phenylthalene and TG-AC studies with pilocarpine. These are all nominations that will be coming to NCTR for testing under the IAG umbrella.

Dr. Allaben said that the science we look at comes through our Toxicology Study Selection and Review Committee (TSSRC), a scientific oversight body that comments on the utility and the quality of the science not only from a pure scientific perspective but also from an Agency perspective. He added that scientists from all of the FDA product centers are at the table during the design phase. We go through a series of protocol reviews involving NCTR, the product center, and NIEHS. Dr. Allaben explained that the responsibility of the oversight group is to review research concepts and plans and the protocols are discussed and commented on. The committee recommends any protocol modifications with regard to dose level selection, design changes, and it meets twice a year to review new science projects and to monitor the ongoing. Dr. Allaben described the benefits of the TSSRC, which include enhancement of the regulatory decision process, support for quantitative risk assessments, encouragement for new/innovative research approaches, and speeding the research and testing process. He said that the TSSRC also provides data to the regulators who are at the table during the design, and during the monitoring of those studies, and they are the first to see the results of these efforts. He added that the process also utilizes NCTR scientific and contract staff, provides FTEs and post doc support, facilities renovation, equipment purchase, and provides resources for travel to scientific meetings. Dr. Allaben summarized his presentation by saying that this IAG provides a benefit package that is good for NTP and NCTR and is an example of leveraging to the best possible extent. **(TAB 6)**

Phototoxicity Research and Testing Laboratory

Dr. Paul Howard provided an update on the Phototoxicity Research and Testing Laboratory. He said there were four centers for research excellence within the NTP, which are:

- The NTP Center for Evaluation of Risk to Human Reproduction, which is composed of a panel of experts to develop expert opinions on the risk of certain chemicals to human reproduction.
- The Interagency Center for the Evaluation of Alternative Toxicological Methods, which works in collaboration with ICCVAM (the Interagency Coordinating Committee on the Validation of Alternative Methods) to evaluate the validation status of alternative methodologies.
- The National Center for Toxicogenomics (this is really not under the NTP; it is under the NIH).
- The Center for Phototoxicology, which conducts toxicological studies that expose animals to light emitting sources.

Examples of studies:

- Palmitate O, a sunscreen agent, is the dimethyl amino ester of PABA, and has been under the NTP study for about 2 1/2 years.
- Aloe vera, which was nominated for an NTP study by the National Cancer Institute. Aloe vera is absolutely everywhere. The question is what are the phototoxicological properties of this compound.
- Retinyl Palmitate - What is the effect on the skin of very high concentrations of retinol? Photocarcinogenic studies have not been conducted with retinol or retinyl palmitate and it needs to be needs to be looked at.

Dr. Howard added that the Aloe vera and retinyl palmitate studies would start this fiscal year.

Dr. Howard also provided an update on current studies, which include:

- Looking at a transgenic mouse with TP-rats, in collaboration with Lynda Chin at Harvard University.
- A CRADA with Argus Research Laboratories to understand dose response and to develop a historical database on photocarcinogenesis.
- Palmitate O, which is one of the most widely used sunscreen agents.
- Compounds related structurally to methoxy-psoralen. What phototoxicological properties do they have?

?? FD&C dyes 27 and 28, which are in lipstick and red based facial applications. The NTP asked if these compounds get out of red-based lipstick and red-based facial applications and into the skin? If they do, we will be studying these compounds. But only if pharmacologically valid.

Dr. Howard also reported that the group is working with NIEHS to look at potential biomarkers of PUVA-induced skin cancer. Very few things we know induce skin cancer, one is psoralen plus UVA, which induces basal squamous cell carcinomas and melanoma. He added that we are looking at developing biomarkers for skin changes. We are trying to meet the basic science research and testing needs of the NTP/NIEHS, and FDA. (**TAB 7**)

Dr. Kaplan, asked if they ever see cancer in the absence of inflammation from sunlight, and did they identify any intermediate steps on the way to the cancer outcome? Dr. Howard responded "yes and no". The TSSRC takes an extremely thorough look at these efforts, and one of the questions that came out of the review was where was this facility going. The TSSRC observed that this is satisfactory applied toxicology but questioned what other things are being done? One issue that came up was the need to understand the role of inflammation of the immune system in skin cancer development. Dr. Howard explained that immunosuppression leads to enhanced skin cancer incidence and that we need to address what is happening in our model and how we can adjust our model to best mimic the human. He posed the question of what would happen if we "immuno-enhanced" an animal with the same light regimen? Will or will not the skin cancers, etc. be induced? Dr. Kaplan said another question is, whether mouse models like the Tg AC mouse, which is expected to exhibit accelerated reactions to phototoxic carcinogenesis, could be employed. Dr. Casciano said NCTR was just starting the phototoxicity facility and such efforts were helping to justify its existence. He also said that the phototoxicity group is focusing on applied technology using systems that were acceptable for 20 or 30 years. Dr. Casciano explained that as we learn more about these systems, we have more questions. He added that Dr. Howard is expanding the areas of study by looking at other possible sources to answer the questions being asked.

The meeting adjourned for the day.

The meeting reconvened on June 12, 2001.

The first order of business was to revisit the EDKB discussion presented by Dr. Sheehan on the previous day. Dr. Rosenkrantz, in response to Dr. Sheehan's statement that the reason this information was not on the Internet was due to firewall issues, recommended that the SAB go on record saying this problem needs to be fixed. Dr. Rosenkrantz said that scientific work has to be communicated and the only way to do that on a broad scale is through the Internet. However, she said that she understood this was a result of FDA regulations. Dr. Sheehan agreed, saying that he is frustrated with the firewall issue. Dr. Casciano said recent leadership meetings have discussed transparency and that use of the Internet was one more way to enhance that transparency.

Commenting on the Microarray data analysis discussion, Dr. Tindall said that through NCTR's strong relationship with NIEHS, the Center should explore interactions with the new National Center for Toxicogenomics. Dr. Kaplan said he didn't understand why this group had to develop their own data analysis tool when there were so many available and recommended that they evaluate the available database software. Dr. Rosenkrantz said this would be an opportunity for the computational science group. Dr. Tindall said all of the programs were likely to become more and more involved in various aspects of genomics and proteomics and should evaluate the available software, servers, and storage. He also said that no software solution will be perfect, but it is important to have the infrastructure in place. Dr. Rosenkrantz's motion to accept the response to the EDKB Site Visit report was approved.

Division of Biochemical Toxicology

Dr. Beland provided an update on the Division of Biochemical Toxicology. He explained that this Division is interested in carcinogenicity and focuses on assessing carcinogenic risk and on introducing new techniques to assess carcinogenic risk. The Division has approximately 50 staffers, which include permanent researchers and investigators, several visiting scientists, and a varying number of postdoctoral students. The distribution of funds drives much of the Division's scientific research. Approximately 30% of the division's discretionary budget comes from FDA and is used for supplies, travel, and equipment. The other 60-70% comes primarily from NIEHS through NTP. The majority of the research conducted by the Division is funded externally. Dr. Beland added that the Division is meeting the Agency's needs, but obtains its funding from outside sources.

The Biochemical Toxicology Division focuses on four research areas: efforts in support of NTP nominated chemical compounds, the neonatal mouse bioassay, dietary folate/methyl deficiency, and analytical methods development. Within the Division, funding provided by the NTP IAG supports research efforts for fumonisin B1, chloral hydrate, urethane and ethanol, malachite green,

endocrine disruptors, phototoxicity studies, dietary supplements, and anti-retroviral agents. Dr. Beland discussed the following compounds and focus areas:

- Fumonisin B1, which was the first compound investigated under the IAG, and was nominated by CFSAN.
- Chloral hydrate, which is used as a pediatric sedative and was nominated by CDER.
- Urethane, which is in a number of food products. CFSAN needs better dose response data to set regulatory limits. The study is complete and the final report is being written.
- Malachite green is used without approval in the catfish industry as an antifungal agent. CVM nominated the compound to determine if enforcement action is necessary. The protocol information will be available for CVM within the next two years.
- Five compounds were selected for endocrine disrupter studies, these include genistein, methoxychlor, nonylphenol, ethanol estradiol, and the androgenic vinclozolin. Mechanistic studies are underway looking at alteration of hormone levels and the induction of cytochrome P450.
- Studies are being done on phototoxicology with alpha and beta hydroxy acids, aloe vera, and retinyl palmate.
- Dietary supplements under study include genistein, riddelliine, and aloe vera.
- Riddelline is a compound that is found in some herbal teas. The NTP asked if we could gather information to determine its genotoxic potential. It was found that ten DNA adducts were formed and two have been identified.
- Aloe vera is used in skin preparations and is also taken internally. The plant product includes several fractions, which include aloe vera gel, latex, and a whole leaf preparation. We know the fractions contain anthroquinones, which could be genotoxic.
- In collaboration with NCI, we developed an immunoassay for AZT and demonstrated the incorporation of AZT into DNA. The concern is that these agents are given to pregnant women to prevent development of HIV in the unborn child. Studies will be done to determine carcinogenicity, genotoxicity, and metabolism.
- The neonatal mouse bioassay responds to very genotoxic compounds, but the assay is also sensitive to other compounds. The following chemicals are undergoing tests, benzodiazepines, antihistamines, lipid peroxidation products, estrogens/antiestrogens, proton pump inhibitors, mycotoxins, known human carcinogens, and antiretroviral agents.
- Dietary folate and methyl deficiency studies to look at nucleotide pool imbalance and methylation deregulation during hepatocarcinogenesis, folate-dependent homocysteine metabolism, and methylene tetrahydrofolate reductase polymorphisms and Down syndrome. These studies have allowed for additional funding through the CDC and the Arkansas Children's Hospital.
- Two major laboratories working on analytical methods development, including immunochemical methods. The development of antibodies against fumonisin has allowed us to develop a purification system of ceramide synthase and to develop antibodies against specific DNA adducts. More recently the Division began studying catechol estrogens, with funding from the Office of Women's Health.
- The mass spectrometry group has done work with genistein and daidzein as part of the endocrine disruptors. We have developed mass spectrometry methods for oxidative DNA damage from DNA adducts from tamoxifen and we are expanding these studies to include DNA adducts that come from components found in hormone replacement therapy. (**TAB 8**)

Dr. Acosta asked if there were a decrease in external funding, how would the Division be effected? Dr. Beland replied he would go out and solicit funds from other sources. Dr. Acosta asked if the investigators were looking for sources of external research funds? Dr. Beland said this has always been encouraged. Approximately seven years ago a SVT suggested that the Division concentrate more on FDA funding, but a year or two later the focus shifted once again to external funding. Dr. Casciano said with the NIH budget on its way to doubling and the majority of that going towards extramural funding, NCTR was in a fairly stable situation. He explained that there are very few places in the world that can do what this group is capable of doing and our uniqueness allows us to be industrious participants. Dr. Casciano also said that the excellent mechanistic work that is being done is applicable to future FDA questions. He added that good science would continue to be supported. Dr. Beland said that the money they received from the Office of Women's Health served as seed money for research that has, in-turn, brought in additional funding from CDC.

Division of Molecular Epidemiology

Next, Dr. Poirier provided an update on the Division of Molecular Epidemiology. The major research areas of the Division include the identification of genetic polymorphisms that influence carcinogen metabolism, DNA repair, chemoprevention, and individual cancer susceptibility. The Division consists of approximately 25 people at NCTR with a number of collaborators at the University of Arkansas for Medical Sciences.

Studies and interests include:

- Genetic polymorphisms, DNA adduct detection in humans and molecular epidemiology studies.
- Developing a DNA microchip for large-scale population based studies.

?? Correlate cytochrome P450s with the onset of puberty in young girls.
?? A case-control study on colorectal cancer with respect to the H-glutathione transferase A-1 polymorphism.
?? A retrospective case study on survival of breast cancer after chemotherapy with respect to GS phenotype, and the recurrence of colorectal polyps with GST genetic polymorphism, MTHFR polymorphism, and glutathione peroxidase polymorphism.
?? A study examining the amount of GSTA-1 protein in the livers of humans compared to the amount of GSTA-2 protein in the same livers.
?? A study comparing the overall survival of women with each of the previous phenotypes with phenotypes of GSTA1.
?? Examination of hepatic DNA methyl transferase activity in smokers.
?? Determination of individual methylation profiles, gene expression, and enzyme activity of CYP1A2 in the human liver.
?? Elevation of DNA methyl transferase in the livers of smokers compared to nonsmokers.
?? Hypermethylation in the promoter region of the CYP1A2 gene and that it is associated with decreased expression.
?? Studies on *in vitro* toxicity of pancreatic cells in culture.
?? Research on biomarkers of pancreatic cancer.
?? Establishing biomarkers of cancer in high-risk groups such as smokers versus nonsmokers.
?? Develop *in vitro* predictive bioassays for chemopreventive agents.
?? Look at the toxicity of agents such as nicotine, soy, and tea components on pancreatic cells in culture.
?? Determine the mechanistic actions of such agents. A recent result from the study of genistein on the expression of K-ras on pancreatic cells in culture.

Two studies are being done in conjunction with an IAG with the National Cancer Institute:
?? Examining DNA methylation and cancer risk in humans and experimental animals as well as abnormal methyl metabolism in non-neoplastic diseases.
?? Examine the effects of dietary homocysteine on disease.

Future directions:
?? Breast cancer response to chemotherapy.
?? Increase the study populations that will be examined, looking at pharmacokinetics variations and altered enzyme kinetics.
?? Examine additional polymorphism and GSTs in the protein and the gene, and of tissue specific GST expression as potential factors in individual variation in the disease and chemotherapeutic response.
?? A large-scale study done in collaboration with the University of Arizona Cancer Center examining GST.
?? The validation of DNA snip microassay chip for examination of large-scale population-based studies.
?? An investigation of genetic and epi-genetic alterations of specific cells using laser-capture micro-dissection.
?? Determine hypermethylation of GST1 promoter as an early marker of prostate cancer in men. May include additional genes in studies of hypermethylation.
?? Undertake mechanistic studies on the biological and pharmacological actions of chemopreventative agents.
?? Conduct sight specific methylation studies over the promoter region of the IGF gene and lymphocytes from a case control study of colon adenomas.
?? Look at the global hypomethylation studies on H- and K-ras methylation patterns in human lymphocytes from a case control study.
?? Continue collaborating with NCI in case-controlled colon adenoma study. Extend collaboration on DNA gene methylation in rats undergoing hepatocarcinogenesis by methyl deprivation. Complete collaborative clinical studies on all abnormal methyl metabolism associated with non-neoplastic disease such as diabetes and arteriosclerosis.
?? Organize a workshop on diet, DNA methylation processes, and health. NCI has expressed interest in sponsoring a joint workshop on the different diseases and the overlapping mechanisms that seem to be impacted upon or involved with methyl deficiency. (**TAB 9**)

Division of Genetic and Reproductive Toxicology (DGRT)

Dr. Martha Moore provided an update on the activities of the DGRT. The Division has 31.8 FTEs (full time equivalent). The Division is composed of 14 principal research scientists, 3 staff fellows, 12.8 support scientists, and 2 administrative support personnel. They have four research focus areas, two disciplinary areas, (e.g., genetic toxicology and reproductive/developmental toxicology) and programs which cross-cut the diet and nutrition program (e.g., caloric restriction and dietary restriction program). The Division is expanding to move into the area of general nutrition and dietary supplements. Division researchers are working in genetic, developmental and reproductive toxicology, and are expanding to include genomics and proteomics. Dr. Moore said the Division's biggest challenge is how to use these tools to answer questions.

Dr. Moore explained that the Genetic Toxicology group is a Center for Excellence in the area of *in vivo* mutagenesis, and its current activities include:

- Development of the rat *hprt* gene mutation assay.
- Trying to understand mutations associated with the *in vitro* mouse lymphoma assay.
- Mitochondrial mutations.
- Developing the TK heterozygous mouse model.
- Quantitative mutagenesis, molecular mutagenesis, and most recently the Division used molecular techniques to quantitate genomic rearrangements.
- Development of new techniques for genotypic selection.
- Work with human lymphoblastoid cell lines and trying to understand mutation at the TK locus and also understanding how the p53 phenotype impacts mutation.
- A study on genistein in the p53 mouse.
- Development of the FIX *in vivo* gene mutation assay.
- Work with keratinocytes to develop an *hprt* gene mutation assay.

Reproductive/Developmental Toxicology is a smaller focus group with four members. They are working on:

- The EDKB models.
- Cell cycle kinetics and apoptosis.
- Understanding how folate impacts the development of the neural tube and the normal neural tube closure process.

The Diet and Nutrition group is a cross-cutting team involved in:

- Caloric restriction and dietary control studies.
- Studies with genistein, including one employing p53 mice.
- Studying folate as a dietary issue.
- Developing a study to understand the impact of severe malnutrition on the induction of somatic cell mutations.

The DGRT is moving into the genomics and proteomics area and has done work in the areas of:

- 2-D gels.
- Working with both micro- and filter arrays to further our understanding how filter arrays work and the problems in using filter array technology.
- Computerizing the data generated and normalizing the data.
- An international collaboration to try to understand the microarray technology.

The framework for DGRT is basic applied research, which includes hazard characterization and dose response assessment, to improve regulatory decision-making (risk assessment). In the process the Division:

- Generates chemical-specific information.
- Does research in support of the various centers and the Office of Women's Health.
- Participates in open dialogues to better understand the needs of the other centers.

In the area of hazard characterization, the DGRT is:

- Developing new methods (the TK+/- mouse model, phiX-174 gene mutation, keratinocyte *hprt*, MutEx/ACB-PCR genotypic selection, EDKB, fluorescent markers).
- Characterizing and understanding these new methods.
- Interpreting data (*in vivo lac-i* and *hprt*, *in vivo* mouse TK assay, human lymphoblastoid TK assay, filter arrays, mouse lymphoma TK assay) and using information for regulatory decision making.
- Studying the modes of action for toxicants in hazard characterization and selection of dose response models (DNA sequence analysis, chromosomal mutations, genomic rearrangements, gene expression and protein production, folate and neural tube development, impact of dietary restriction on somatic mutation and physiological parameters).
- Rodent/human extrapolation, response of hepatocytes to toxicants, response to dietary restriction and nutritional changes.
- Developing the necessary guidelines to provide information to the centers on how assays are done and which should be required (mouse lymphoma TK assay, *in vivo* gene mutation assays).

Dose response assessment is composed of several areas:

?? Relevant doses (dose selection, biomarkers, genomics/proteomics), susceptibility/variability (fetus/newborn/young child), repair deficiency.
?? PMS2-mismatch mice, diet (antioxidants, dietary restriction, phytoestrogens), the issue of rodent/human extrapolation (liver toxicity, biomarkers, diet and nutrition, genomics/ proteomics), cancer/non-cancer risk assessment.
?? The development of quantitative models (mechanistic commonality, genomics/proteomics).

DGRT is also involved in studies for chemical specific information on genistein, coumestrol, leucomalachite green, AZT (and other combination drugs for the treatment of AIDS), and UV light/phototoxicity studies. (**TAB 10**)

In closing, Dr. Moore said cross-agency collaboration was an important tool to complete what needs to be accomplished. The Division currently has active cross-divisional collaboration activities with the divisions of Biochemical Toxicology, Biometry and Risk Assessment, Neurotoxicology, and EDKB. In addition DGRT maintains cross-agency collaborations with the Office of Women's Health, CDER, CFSAN, and is talking with CBER. She added that DGRT has cross-departmental collaborations with NIEHS and NTP. Dr. Acosta asked if there was a central organizing group for the genomics/proteomics effort and was told that DGRT is starting one. Dr. Lightfoot asked if there was an Agency-wide genomics/proteomics working group and were they working and participating with them? Dr. Casciano responded by saying that they are working with the Office of Science to coordinate the efforts of the different centers and to generate common goals with minimal repetition.

Division of Chemistry

Dr. Robert Turesky provided an update on the Division of Chemistry. The Division has three units: 1) it supports a strong commitment to the NTP; 2) the Mass Spectrometry Branch, which conducts fundamental research and supports chemical mass spectrometry service work; and 3) the Analytical/Biomarker Branch. He continued by adding that the Division supports additional research efforts in analytical chemistry, toxicology, NMR spectrometry, spectroscopy, computational chemistry, and biomarker work. The Division's mission is to utilize chemical research techniques, including analytical chemistry, mass spectrometry (MS) and NMR spectrometry, spectroscopic and computational methods to implement intra-divisional, intra-center, and FDA-relevant research initiatives in toxicology, risk assessment, and regulatory compliance.

Historically the Division has been involved with providing essential support in analytical chemistry and spectrometry to the NTP as well as for other NCTR divisions. Key research projects of the Division of Chemistry include:
?? Spectrometric Data Activity Relationship (SDAR) Models for compounds binding to receptors of toxic responses (predictive toxicology).
?? NMR spectroscopy of drug purity with public health implications.
?? Rapid identification of intact whole bacteria based upon spectral patterns using MALDI-TOF MS (matrix assisted laser deionization – time of flight mass spectrometry).
?? Fresh Tag Sensor? technology for product safety, quality, and rapid screening of explosives.
?? Rapid screening and identification of complex mixtures by pyrolysis-mass spectrometry with pattern recognition methods.
?? Chemical characterization of critical chemicals, components, constituents, and biologics for selected botanical products.
?? Impact of dietary supplements on woman's health issues.
?? Comparison of principal components analysis (PCA) and artificial neural networks (ANN) for the prediction of qualitative and quantitative biological end points from spectrometric data.
?? Risk assessments of dietary contaminants.

The Division of Chemistry has a very strong support relationship for the NTP research programs. In addition, the Division has active collaborations with CVM in studying various drug residues including Amoxicillin, erythromycin, Lincomycin, and sulfa drugs, which require development of determinative methods that meet testing requirements for reliability.

Dr. Turesky said the Division is a strong proponent of using mass spectrometry for characterization and identification of various bacterial species and problems associated with microbial contamination. Working in support of the Food Safety Initiative, the Division uses mass spectrometry to differentiate strains of food borne pathogens; this is now a standard procedure used by FDA and others in the United States and around the world. Different milestones in this area include:
?? MALDI can differentiate bacteria by genus, species, and strain.
?? Specific Biomarkers for virulence can be detected by MALDI.
?? Biomarker proteins can sometimes be detected in contaminated media without pre-MS culture steps.

Dr. Turesky added that the Division would continue to adapt and use MS methods in other emerging areas such as proteomics and bioterrorism. Division researchers can detect proteins and identify biomarkers in intact bacterial cells and may detect protein/biomarkers in malignant cells or even *in vivo*. In the future they hope to:

?? Determine correlation of toxicity and strain types with MALDI spectra.
?? Develop more powerful MS methods (MALDI/FTMS).
?? Make more accurate assignment of biomarker (protein) identity.

Benefits to FDA, as described by Dr. Turesky, include:

?? The ability to differentiate strains from more Vibrio species.
?? Detection of biomarkers associated with antibiotic resistance.
?? Application of MS to FDA programs in bioterrorism, proteomics.
?? Characterization of various cell types, possibly malignant cells.

Current goals and objectives using MAB-TOF MS include:

?? Rapid chemotaxonomic strain-specific bacterial identification.
?? Development of bacterial databases and search strategies.
?? Applications to food/seafood borne bacteria, especially *Vibrio spp.* (CFSAN & ORA).
?? Development of patents for new methods.
?? Identification of bacteria without a prior cell-culture step.

Key Findings to date include:

?? Demonstrated that a multiplicity of laboratory variables distort mass spectral fingerprints.
?? Patented a simple algorithm to correct for method-related spectral changes. (The correction is more practical than using identical conditions.) US Pat. App. No. 60/239,549 filed 10/10/2000

Future Experimental and Computational Directions

?? ?-test Py-MAB-TOF- MS (from Dephy, Montreal) at NCTR.
?? Assemble and validate a 200-sample spectral database using bacteria from CFSAN and ORA reference collections.
?? License the patent on using a spectral correction method to mitigate laboratory-based variations.
?? Develop algorithms to transform spectra from environmental samples to equivalent laboratory spectra.

Dr. Turesky added that Dr. Ang, in collaboration with CFSAN has been establishing methods to isolate various bioactive components in medicinal or herbal medications. There has been a great deal of discussion and controversy because some of these chemicals contain components that may alter the pharmacological activities of a number of different drugs and medications. Dr. Ang has been asked to use her expertise in the chemistry of these compounds to work with Dr. Leakey who is looking at the activity of herbal medications on key enzymes, up regulation, down regulation, and metabolic activities. Dr. Ang has been able to isolate hyperforin, which is found in St. John's Wort (SJW). We are now able to conduct *in vitro* bioassays to better understand the biochemistry of these molecules. This work will be extended to looking at the metabolism of various components of SJW.

Objectives:

?? To develop human cell-based assays to determine whether a test substance affects key enzymes involved in the metabolism of pharmaceuticals.
?? To use these assay systems to investigate potential drug-herbal interactions between prescribed pharmaceuticals and dietary supplements.

Research Progress:

?? Extraction and LC methods developed for four SJW components in tea, fortified drinks, puffs and snack bars.
?? Methods developed for five phenolic compounds in echinacea capsules and tablets.

Preliminary Findings:

?? Developed methods for isolating hyperforin, the major active ingredient of SJW.
?? Developed or procured battery of cell lines expressing major isoforms of human drug metabolizing enzymes; used in inhibition assays.
?? Established that constituents of Echinacea inhibit enzymes conjugating estrogens.

Future Work:

- Develop human hepatocyte-based assay systems for measuring drug metabolizing enzyme induction.
- Isolate and identify the inhibitory constituents of Echinacea and SJW.
- Investigate the metabolism of active ingredients of SJW.
- Apply inhibition and induction assays to other herbal products.
- Establish microarray and proteomic technology for elucidation of mechanisms.

Dr. Turesky reported that Dr. Beger, in collaboration with Drs. Lay, Miller, and Wilkes, has been working on the relationship between structure-activity relationships (SAR) and spectrometric data-activity relationships (SDAR). There are some unique things that ^{13}C NMR can provide that other spectrometric methods may not, such as providing information on electron density molecules, configuration and confirmations. An example of some of the success of SDAR and quantitative spectrometric data-activity relationship (QSDAR) models include: (a) SDAR model of 108 compounds binding to the estrogen receptor using NMR and MS data, and (b) QSDAR model of 26 poly- chlorinated dibenzofurans binding to the aryl receptor using predicted NMR data.

SDAR Publications and Patents:

- ^{13}C NMR and EI Mass Spectrometric Data to Produce a Predictive Model of Estrogen Receptor Binding Toxicology and Applied Pharmacology. 169: 17-25, 2000.
- Producing ^{13}C NMR, Infrared Absorption and EI Mass Spectrometric Data Monodechlorination Models of Chlorobenzenes, Chlorophenols, and Chloroanilines J. Chem. Inf. Comput. Sci. 40:1449-1455, 2000.
- Developing ^{13}C NMR Quantitative Spectrometric Data-activity Relationship (QSDAR) Models to the Corticosteroid Binding Globulin. J. Comput.-Aided Molec. Design.
- Models of Polychlorinated Dibenzodioxins, Dibenzofurans, and Biphenyls Binding Affinity to the Aryl Hydrocarbon Receptor Developed Using ^{13}C NMR Data. J. Chem. Inf. Comput. Sci.
- Patent Pending for "Methods for Predicting the Biological, Chemical, and Physical Properties of Molecules from Their Spectral Properties."

Dr. Turesky then discussed the following future directions of SDAR:

- Protocol E0706801: "Continuing to develop SDAR models for the Ames test, neuraltoxicity (Neurotox), and other toxic endpoints."
- Protocol E0706811: "Developing new strategies for spectrometric models of toxicity" (ROW).
- Protocol E0708301: "Computational predictive system for rodent organ-specific carcinogenicity" (in collaboration with Biometry, CDER, ROW).
- Producing hybrid spectrometric models that incorporate three-dimensional structural information into the SDAR model.

Dr. Turesky then said that Dr. Buzatu, in collaboration with Dr. Lay, is taking complex chemical spectral data from ^{13}C NMR and setting up artificial neural networks and looking at the different predictive biological endpoints. One experiment using the QSDAR-ANN model reflected excellent results for 28 poly-chlorinated biphenyl, dioxin, and furan toxic equivalence factors (TEFs) using predicted ^{13}C NMR spectra.

Future Directions:

- Currently developing a quantum mechanical parameter based neural network model for the prediction of TEFs for dioxins and dioxin-like compounds.
- Developing an Internet parallel distributed neural network to handle large data sets as well as increasing the efficiency of the neural network.

Dr. Evans has been establishing methods to determine whether NMR can be used as a rapid screening tool for measuring adulteration and contamination of drugs using genistein as a model. The associated protocol "A New Approach to the NMR Spectroscopy of Drug Purity and the Public Health Implications" has the following objectives:

- Determine properties and develop procedures for use of NMR spectrometer under high dynamic range conditions.
- Develop concepts and methodology for applying spectroscopy to very-low-level impurities in drugs using results on genistein.

Mass Spectrometry Applications in FDA Research Initiatives were outlined to include:

?? Allergenicity
?? Bacteria (taxonomy/speciation)
?? Bioterrorism
?? Drug purity (chemicals and recombinant proteins)
?? Ion mobility - MS (protein confirmation determination)
?? Microbial biotransformation of drugs and antibiotics
?? Proteomics
?? Quality assurance and compliance programs
?? Rapid through-put analysis
?? Redox status (vitamins, lipids, proteins, DNA)
?? Risk Assessment (biomarkers, DNA- and protein adducts, DNA damage, metabolites)

Dr. Turesky explained that to have a successful infrastructure for proteomics the Division recently received funding to obtain access to additional MS instrumentation. In addition to the appropriate equipment and tools for proteomics, they need to be able to recruit and hire people with the appropriate skills. Dr. Turesky added that Dr. Alex Strasbourg is developing mass spectrometry methods using model compounds, which will have the potential to detect BSE.

He then outlined the following NMR spectroscopy applications in FDA research initiatives:
?? Computational chemistry
?? Metabonomics
?? Proteomics - Drug Interaction
?? Drug Purity

Dr. Turesky added that Dr. Beger, in collaboration with Dr. James, is looking at the metabolism modulation of tetrahydrofolate in Down Syndrome, which can only be done using NMR.

Dr. Turesky then listed the following research areas supported in FY-2001:
?? Ethinyl estradiol on bone growth in rats
?? Erythromycin from farmed animals
?? Malachite green/leuco malachite green in mice
?? Retinyl palmitate: isolation and detection
?? DNA adducts of Tamoxifen
?? Dietary supplements and herbals: identification of bioactive ingredients
?? Endocrine disrupters: genistein and daidzein
?? Phytoestrogen conversion to estrogenic compounds: genistein & daidzein
?? Fluoroquinolone biotransformation by fungi
?? Microbial degradation of drugs and feed additives in aquaculture
?? Antihistamine drugs in neonatal mouse cells (**TAB 11**)

Dr. Donnelly noted there was overlap with some of the initiatives in the Division of Microbiology, and asked if there were attempts to do formal collaboration and coordinate efforts. Dr. Turesky responded that there has been strong collaboration with Microbiology on microbial antibiotic resistance, which requires analytical chemistry and mass spectrometry. He said that the characterization of various proteins was initiated by this Division. Dr. Lay said this was larger than just an NCTR project, as the *Vibrio spp.* came from Dolphin Island, and they transported the technology to CFSAN. He added that they are now sequencing the proteins to differentiate the *Vibrios*. There are several microbiologists from NCTR and other FDA facilities involved in this as well as chemists. Dr. Casciano said this is a natural fit since the two groups have been collaborating for years. The Divisions of Microbiology and Chemistry have a support and research function and they leverage with each other to answer some of the questions. Dr. Donnelly suggested that there was additional work that could be done on identification of some of the poultry strains by applying mass spectrometry.

Dr. Rosenkrantz asked if there were other mechanisms for commercializing the FreshTag™? Dr. Miller said FreshTag™ is under license to Cox Technologies in Charlotte, North Carolina. He said they worked with Cox from the initial prototype. In terms of marketability, Tenneco is looking at building FreshTag™ into a packaging material. For example, with a zip lock bag, if you put a fish product or another food that produces ammonia, the bag will give a clear signal as soon as it decomposes. The process is autolytic and possibly enzymatically generated, which in turn forms aldehydes from the lipids. As it turns out, the

major compounds of decomposition, such as ammonia, acids in the case of carbohydrates, sulfurs, and aldehydes can be detected. Tenneco has expressed interest in detecting all of these end products in one step.

Division of Biometry and Risk Assessment

Dr. Ralph Kodell provided an update on the Division of Biometry and Risk Assessment. The Division has two Ph.D. researchers, three post doc mathematical statisticians, two research biologists, two computer specialists, a program support specialist, and a staff fellow.

He described the following research highlights:

- Fumonisin B1 risk modeling.
- An NTP IAG study in rats and mice.
- Risk assessment for the mechanistic data and traditional bioassay data.

Dr. Kodell also discussed the Divisions research projects, which include:

- *Cryptosporidium parvum*.
- Cumulative risk assessment.
- Computational toxicology.
- Looking at the 1,298 chemicals in the Carcinogenic Potency Database (CPDB).
- Photocarcinogenicity theory and methods.
- Analysis of cDNA microarray data. (**TAB 12**)

Dr. Gillett was interested in risk modeling of fumonisin and asked if they were planning to do this with any of the other NTP products; and if so, would the Division be involved in the protocol design to help ensure collection of the appropriate data at the earlier sacrifice time? Dr. Beland said they have wanted to be involved in the design of the NTP protocols, although they have not yet done so and do not have immediate plans to do so at this time. Dr. Casciano said that protocol development is reviewed closely and statisticians are involved. He also said that Dr. Kodell's group is thinking about applying the expertise they have in chemical dose response, database mining, statistical analysis, and statistical development to applications in new fields because we at NCTR consider this to be the future of toxicology. Dr. Gillett said the purpose of these large, expensive, bioassays is to assess risk to humans and it is important to get involved early in the study design. Dr. Beland said they would involve Dr. Kodell's group. He added that at this time, they have only completed fumonisin and chloral hydrate. An upcoming study of urethane and alcohol and how these two compounds interact is expected to be noteworthy.

Open Discussion

A general discussion session followed the presentations made by each of the Divisions.

Dr. Gillett asked if there was a provisional committee for genomics and proteomics at NCTR? Dr. Casciano said that the initial efforts had focused on identifying individuals who have an interest in using microarray technology and in building and evaluating the databases. He added that there are committees concentrating on computational science and another focusing on cellular and molecular methodologies. Dialog is ongoing with various centers regarding NCTR's technical expertise and how best to use these techniques to supply data for regulatory decision-making.

Dr. Lightfoot had a question regarding antiretroviral agents and a study in pregnant women. She said one of the few success stories for antiretroviral agents is that of pregnant women treated with AZT. What was the motivation for the research? Is NCTR looking to see if AZT is more mutagenic than it is therapeutic in the babies of women with AIDS? Dr. Beland said women and their babies are currently being treated with multi-drug therapy. The question is whether this is safer than treatment with AZT by itself, or if such treatment is going to cause future complications in children who are not HIV positive. He added that NCTR is not advocating discontinuing the treatment, but rather is questioning whether there are certain other treatments that may be safer. Dr. Lightfoot asked what motivated the study? Dr. Beland said the motivation was the demonstration that AZT is carcinogenic to neonatal mice when exposed transplacentally. He added that there have also been some mitochondrial toxicities and death reported in children who have been treated with AZT and 3TC in Europe.

Dr. Hansen said CFSAN was interested in pursuing more of the structure-activity approaches and approaches to constructing tier testing schemes. She said that these approaches could provide guidance for petitioners and for CFSAN staff. Dr. Hansen also said that the utility of the work being done in Biometry was excellent.

Dr. Youngman said her first visit to NCTR was a real learning experience and that she previously was unaware of the scope of the work being done here. She said she saw a huge potential for future collaboration with the Office of Research in Laurel. She said that she spoke to some of the Division Directors after the presentations and hoped they would consider pursuing new collaborations.

Dr. Donnelly said the last two days have been incredibly valuable. There is great work being done at NCTR, and how the Center communicates that work to its constituents is vital. She also said that while listening to some of the presentations, she got the impression that many of the initiatives and projects were PI directed, and was not sure they fit within the overall goals of NCTR. She added that a simple annual plan is needed to establish strategic goals. She observed that it seems that it would be very easy for NCTR to draft an annual plan with strategic goals and have each project fit within a strategic framework. An outside reviewer may get the sense that the projects are driven by PI interest or extramural funding. Dr. Donnelly added that it would then be easy to see that the project fits not only within the strategic goals of NCTR, but within the goals of CFSAN, CVM, or one of the other centers. This structure would build a constituency base across the centers. She closed by saying that her frustration is that there is some fundamentally excellent work going on, but perhaps NCTR staffers are the only ones that know about it.

Dr. Casciano responded that the Center has been trying to do that over the last nine years. The work going on relative to the NTP is a direct response to FDA needs, and each of the five product centers would like to have a specifically directed strategic goal. Each product center has different mandates and methodologies for doing something as simple in concept as risk assessment. Dr. Casciano continued by adding that NCTR establishes strategic goals from a concept statement or prepared paper. This concept statement can be one page or five pages, whatever it takes to communicate what the scientist is interested in doing. He said that if he understands and agrees with the concept, he sends it to the five product centers for comment. Dr. Casciano added that NCTR's scientists are very creative and in the last eight to ten years have developed better communications with the product centers and now have a greater sense of what their needs are. The rationale is to get early input from the regulatory centers. If there are three or four objectives that meet the needs or requirements of the centers, then the PI develops the protocol. We write strategic goals broad enough to cover the mandates of all of the centers. Dr. Donnelly responded that she thought that might be a nice framework for publicity. Dr. Casciano noted that NCTR could further improve its efforts at publicity and at conveying its strengths.

Dr. Rosenkrantz asked if there was a plan for the site visit teams for the coming year? Dr. Casciano said there would be a site visit in early fall or winter for the Chemistry Division.

Dr. Acosta said the Board appreciated the fact that almost all of the Division heads were present. He added that it is evident you are very proud of all of the hard work of your staff and that you have very high standards and operate in a very professional manner.

The meeting adjourned.

/Signed/ 08/09/02

Leonard M. Schechtman, Ph.D., Executive Secretary to the SAB Date

GLOSSARY of Commonly Used Abbreviations

3TC - lamivudine, an approved antiretroviral treatment
ACPS - Advisory Committee for Pharmacological Science
AIDS - acquired immune deficiency syndrome
ANN - artificial neural networks
AZT - zidovudine, an approved antiretroviral treatment
BSE - Bovine Spongiform Encephalopathy
C13 - Carbon 13
CBER - Center for Biologics Evaluation and Research
CDC - Centers for Disease Control and Prevention
CDER - Center for Drug Evaluation and Research
CDRH - Center for Devices and Radiological Health
CFSAN - Center for Food Safety and Applied Nutrition
CRADA- cooperative research and development agreement
CVM - Center for Veterinary Medicine
DGRT - Division of Genetic and Reproductive Toxicology
EDKB - Endocrine Disrupter and Knowledge Base
ERG - erythromycin resistance genes
EPA - Environmental Protection Agency
FTE - full time equivalent
GS
GST
GSTA
IAG - Interagency agreement
ICCVAM - Interagency Coordinating Committee on the Validation of Alternative Methods
LC - liquid chromatography
MAB -
MALDI-TOF MS - matrix assisted laser d-ionization – time of flight mass spectrometry
MTHFR
MS - mass spectrometry
NCI - the National Cancer Institute
NCTR - National Center for Toxicological Research
NCSS - Non-clinical Studies Subcommittee
NIEHS - National Institute of Environmental Health Sciences
NIH - National Institutes of Health
NIOSH - National Institute of Occupational Safety and Heath
NITA -
NMR - Nuclear magnetic resonance
NTP - National Toxicological Program
ORA - Office of Regulatory Affairs
PCA - Principal components analysis
PCR-RFLP - polymerase chain reaction---
PHS - Public Health Service
PI - Principal Investigator
ROW - company under contract to NCTR to conduct research
SAB - Science Advisory Board
SDAR - spectrometric data activity relationship
SAR - structure activity relationship
SJW - St. John's Wort
SVT - site visit team
QSDAR - quantitative spectrometric data-activity relationship
TEF - toxic equivalence factors
THC - tetrahydrocannabinol, a metabolite of marijuana
TSSRC -- Toxicology Study Selection and Review Committee
UAMS - University of Arkansas Medical School
WHO - World Health Organization