FDA National Center for Toxicological Research
SCIENCE ADVISORY BOARD MEETING
June 5/6, 2000

June 5, 2000

The meeting was called to order by the Chair of the Science Advisory Board (SAB), Dr. Marion Anders, University of Rochester. He welcomed new Board members Dr. Daniel Acosta, Dean of the College of Pharmacy at Cincinnati; Dr. Nancy Gillett from Sierra Biomedical; and Dr. Cecil Pickett, Executive Vice President for Discovery Research from Schering-Plough Research Institute. Other Board members present were Dr. Robert Anderson, West Virginia University-College of Agriculture and Forestry; Dr. Catherine Donnelly, University of Vermont; Dr. Steven Hecht, University of Minnesota; Dr. Marcy Rosenkrantz, Cornell University and the Air Force Research Laboratory and Information Directory; Dr. Charles Wilkins, University of Arkansas. Also present were liaisons to the SAB including Dr. Norris Alderson, CVM; Dr. Patricia Hansen, CFSAN; Dr. Leonard Schechtman, CDER; Dr. Marilyn Lightfoote, CDRH; Dr. Meredith Grahn, ORA; Dr. Richard Kennedy, UAMS, and staff from the various divisions/offices at NCTR.

Dr. Anders requested approval of the April 26/27, 1999 Science Advisory Board Meeting minutes; with the exception of one minor change the minutes were approved as written.

Dr. Casciano provided an update on the Center, which included a brief history of NCTR and how the Center fits within FDA's regulatory environment. He noted that NCTR was the primary research center within the FDA, and that part of the Center's responsibility was to try to understand the regulatory questions so that the hypothesis-driven research and fundamental research that provided at NCTR fits into the mission of the FDA.

Dr. Casciano presented an overview on each on the Centers and the Office of Regulatory Affairs (ORA) in order to provide the Board with an idea of their mission and various issues:

CDER assures that safe, effective drugs are available to people. Their issues include the Prescription Drug User Fee Act (PDUFA), adverse events reporting, gene therapy, monitoring drug information and advertising, drug quality, drug safety. NCTR, in addition to CDER's own research components, is interested in evaluating drugs for their safety and quality.

CFSAN is responsible for promoting and protecting the public health and economic interests by insuring the nation's food supply is safe, sanitary, wholesome, and honestly labeled. The program also insures that cosmetic products are safe and properly labeled. Also of interest is the interaction between drugs and dietary supplements and the resulting potential toxicity. NCTR has had a strong interaction with CFSAN regarding seafood decomposition and is now developing a collaborative effort with CFSAN evaluating drugs and cosmetic chemicals that may be photo-activated and potentially toxic.

CVM is responsible for insuring that animal drugs and medicated feeds are safe and effective for their intended use and that foods from treated animals are safe for human consumption. Their issues include food safety, antibiotic resistance, and aquaculture. The Food Safety Initiative is a joint effort between CFSAN, CVM and NCTR, in which the latter provided fundamental and applied research capabilities.

CDRH's mission is to protect the public health by providing reasonable assurances of the safety and effectiveness of medical devices and by eliminating unnecessary human exposures to radiation emitted from electronic products (medical, industrial, and consumer). Their present issues are reuse of single-use medical devices, medical device surveillance, FDAMA, response to re-engineering, and device GMP inspections. NCTR has participated with CDRH in such areas as tissue-based products, scanning devices (x-rays), ultrasound, and electromagnetic radiation.

CBER's mission is to protect and enhance the public health through regulation of biological and related products including blood, vaccines, therapeutics or related drugs, as well as certain medical devices according to statutory

authorities. The regulation of these products is founded on science and law to ensure their purity, potency, safety, efficacy, and availability. NCTR is developing an interaction with CBER in the areas of anti-terrorism, bioterrorism, research, compliance activities, action plans (blood, tissue, devices), and xenotransplantation.

ORA has one of their laboratories co-located at NCTR, i.e. The Arkansas Regional Laboratory. ORA is the lead office for all field activities of the FDA. NCTR interacts with ORA in such areas as international harmonization, seafood decomposition, and bacterial contamination.

NCTR's mission is to conduct peer-reviewed scientific research that supports and anticipates the FDA's current and future regulatory needs. This involves fundamental and applied research specifically designed to define biological mechanisms of actions underlying the toxicities of products regulated by the FDA. The research is aimed at understanding critical biological events, the expression of toxicity, and at developing methods to improve assessment of human exposure, susceptibility and risks. NCTR has three strategic research goals: (1) evaluation of method-, agent-, or concept-driven research, (2) knowledge bases, and (3) predictive systems. NCTR is divided by administrative functions and research functions. The SAB evaluates the science that is both proposed and ongoing in the research part of the organization. As part of this meeting's agenda the SAB will hear responses to the site visit reports of the Division of Biochemical Toxicology, the Division of Reproductive and Developmental Toxicology, the Division of Microbiology, and the Division of Molecular Epidemiology.

Next, Dr. Casciano described the functions of various areas within the Center:

Division of Microbiology serves a multi-purpose function with specialized expertise to perform fundamental and applied research in microbiology in areas of FDA's responsibility in toxicology. The Division also responds to microbial surveillance and diagnostic needs for research projects within the NCTR and FDA, microbial surveillance and diagnostic support of research, foodborne pathogen research, food safety, and methods development, human intestinal microflora research, environmental biotechnology, and microbial synthesis of compounds of pharmaceutical and toxicological interest.

Division of Molecular Epidemiology is interested in identification of genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy, conduct of epidemiological studies for post-market surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices. Other efforts include programs in human exposure biomonitoring, DNA adduct detection, extrapolation of the results of animal bioassays and of mechanistic studies to humans, and the development and validation of DNA microarray technology for human diagnostics.

Biochemical Toxicology Division provides major support to the NCTR and FDA through an FDA/NIEHS Interagency Agreement, whereby centers within the FDA nominate chemicals for evaluation in the cancer bioassay. Division staff function as the chemical managers as well as the principal investigators on hypothesis-driven research that is associated with each of these bioassays. They just completed an effort in evaluating fumonisin for the CFSAN. Dr. Beland recently went through an evaluation of the work he completed on chloral hydrate, which is a chemical nominated by CDER. The division is also actively investigating the interaction between urethane and ethanol for CFSAN. This group is also evaluating malachite green, which is an antibiotic used in the aquaculture industry in response to a need by CVM. A large effort evaluating endocrine active substances involves the Biochemical Toxicology Division as well as other NCTR divisions. A large study, which is part of an ILSI consortium effort, is evaluating the newborn mouse assay, as an alternative bioassay to the cancer bioassay. Of particular interest is (a) the characterizing of this system by evaluating genotoxic and nongenotoxic chemicals of interest to the CDER and (b) the ability of the system to detect genotoxins that act primarily through a clastogenic mechanism.

Phototoxicity Center NCTR has developed the first state-of-the-art FDA Phototoxicity Center and the second one of its kind in the world. They are assembling a program that will (a) address the mechanistic evaluation of chemicals that may be toxic, assess photo-reactive mechanisms and (c) respond to needs of FDA—(primarily CFSAN and

CDER). Staff are internationally recognized for expertise in studying DNA adducts (tamoxifen, riddelliine, nucleoside analogs [^{32}P-postlabeling, immunoaffinity, mass spectroscopy]), as well as other biochemical and molecular technologies to evaluate metabolism and gene expression.

Genetic and Reproductive Toxicology Division has two laboratories, a genetic toxicology lab and a reproductive toxicology lab. They develop and validate sensitive and predictive *in vitro* systems to identify, quantify and understand modes of action of potential human toxicants, especially carcinogens and mutagens, and develop and validate sensitive and predictive *in vivo* systems to identify, quantify and understand modes of action of potential human toxicants, especially carcinogens and mutagens. Their focus is to conduct fundamental research designed to define pathways from initial DNA damage to mutation, and to introduce new genetic techniques to assess carcinogenic risk. Their *in vivo* activities include (1) measurements to compare mutation frequency and mutational spectra of *Hprt* and *lacI* in splenic lymphocytes of nontransgenic and transgenic rats exposed to DMBA, thiotepa, and N-OH-2AAF, (2) evaluate mutation frequency and mutational spectra of *lacI* in the mammary gland of transgenic rats exposed to DMBA, (3) characterizing and determining the utility of the MX174 transgenic mouse system in collaboration with NIEHS colleagues, (4) construct a mouse heterozygous at the *Tk* locus using homologous recombination and characterizing its utility as an *in vivo* genotoxicity assay system. Their *in vitro* activities are using flow microfluorometric techniques to quantify viable, necrotic and apoptotic cells using transgenic human lymphoblastoid cells. They have a testing component that has evaluated the genetic toxicity of several drugs of interest to the FDA, and they are defining the *p53* status of several human lymphoblastoid cell lines. The Reproductive Toxicology laboratory of that division is primarily utilizing *in vitro* embryonic cultures to ask specific mechanistic questions which will help in assessing potential teratological activity of a variety of drugs that humans are exposed to. NCTR's computational toxicology efforts are largely driven by the division's evaluation of the Endocrine Disruptor Knowledge Base using estrogen-receptor binding.

Division of Neurotoxicology is interested in (a) *in vitro* cellular toxicity studies of ddI, ddC, 3TC and dT, (b) estimating quantitative neurotoxicity risks from domoic acid exposures, (c) implementation of molecular biological techniques for assessing changes in neurogrowth/neurotrophic factors after exposure to neurotoxicants, and (d) NIEHS/FDA interaction in evaluating the effects of estrogen disruptors on sexually dimorphic behaviors. There is an effort to study the effects of ibogaine on neurotransmitter systems, generation of free radicals and nitric oxide synthase activity and to examine any correlations with neurohistological evaluations in mouse and rat brain. This group is primarily responsible for NCTR's Primate Center as well. They have developed operant behavior testing (cocaine, Ritalin) methodology which is applicable to humans. They are involved in neurotoxicological and behavioral assessment of the HIV supressors ddC and thalidomide in rhesus monkeys, development of a non-human primate model for studying the consequences of long-term anticonvulsive medication on complex brain functions, and pharmacokinetic and metabolism evaluation.

Division of Biometry and Risk Assessment is presently interested in bioassay of shortened duration: developing statistical implications, statistical tests involving multiple tumor tests, sites and statistical analyses of tumor multiplicity data, tests of equivalence for dichotomous endpoints, and, recently, have become involved in the statistical tests to assess microarray data.

Division of Chemistry conducts analytical support and research. The Analytical Support Group is primarily for the support of the NTP Animal Bioassay and they collaborate with colleagues from other disciplines within the Center providing analytical support for NCTR experiments (antibiotics in feed and aquaculture; dietary supplements; amphetamine, dexfenfluramine, domoic acid). Some of their research involves mass spec/pattern recognition to identify components in complex mixtures, development of devices for determination of food/seafood quality (Fresh Tag®, etc.), development of MS methods for rapid identification of bacteria, detection of trace impurities in drugs by NMR techniques. Dr. Dwight Miller and his group were instrumental in the development of the Fresh Tag® and Dr. Jack Lay's group developed the component for the identification of bacteria.

Division of Veterinary Services provides oversight and veterinary management to all of the lab animals and housing facilities at the NCTR. This group provides professional and technical support to all of the research divisions. They are the project officers for the pathology service contract, the animal care and diet prep contract, and the feeding and bedding contract.

- The Pathology Services recruit certified pathologists and technicians who conduct routine pathology and a variety of special procedures that respond to requests from the principal investigators that have a need for these specific procedures.
- The Animal Care/Diet Prep program is accredited by AALAC. The program employs certified veterinarians and technicians and maintains specific-pathogen-free barrier conditions. They maintain 86 animal rooms and maintain a quarantine facility for rodents, rabbits, and non-human primates. The laboratory animal feed and bedding is purchased via the contract through the Division of Veterinary Services.

Dr. Casciano discussed NCTR's funding for the last five years, which includes (a) congressional appropriations, (b) external funding (which is a breakdown of IAG [Interagency Agreement] funds, which are primarily those from interagency agreements with NIEHS and is a leveraging effort by NCTR and NIEHS), and (c) a small amount of income that comes through cooperative research and development agreements (CRADAs). He noted that all FDA Centers have had a major decrease in funding from Congress. In order to maintain some level funding over a period of time, he said NCTR had increased its creativity by marketing its experience and unique facility to leverage dollars for the FDA through the NIEHS inter-agency agreement. Breaking this down, he said 74% of our funding comes from FDA and about 26% comes from external sources.

He said that the status of the various NCTR components were as follows:

- Molecular Epidemiology group remained relatively level throughout the five-year period
- Nutritional Toxicology was re-organized in 1997: the group was dissolved and the people placed in various divisions
- Chemistry realized a decrease in the support
- Microbiology has held its own
- Biometry Division realized a decline in Biometry and Risk Assessment because the project on Caloric Restriction was halted after FY'97;
- Reproductive Toxicology and Genetic Toxicology groups remained relatively stable
- Biochemical Toxicology Division has experienced growth, primarily via the IAG with the NIEHS
- Neurotoxicology program has also seen some growth.

Dr. Casciano said that one question that is continuously asked is “What does NCTR do for FDA?" He said NCTR has almost 30% or their resources directed toward NTP bioassays which are a direct response to FDA needs. There's an active program that responds to directed messages received from the FDA regarding the Food Safety Initiative. Dr. Dwight Miller's invention of Fresh Tag® is a real boon for the FDA and the NCTR. The Center has responded to an FDA mandate involving bioterrorism. NCTR is regularly called upon by colleagues in the various centers to provide information and data. Other efforts perhaps not so evident, but that result in efforts that eventually end up on the FDA table, is work on the development of methods to identify DNA adducts in model systems, to understand chemical reactivity and potential toxicity as well as application of that technology to the human. Although not a direct mandate from the FDA, the Agency utilizes this technique in their safety assessments. Work with the artificial GI tract was developed because of Dr. Cerniglia's interest and foresight, and CVM is very much interested in the effects of antibiotics on this GI tract. The estrogen and computational toxicology and the estrogen database have allowed NCTR to develop a computational toxicology presence that can be translated to any chemical evaluation that will be useful to the FDA. CFSAN is very interested in the work we've done with the Project on Caloric Restriction and understanding the effects of nutrition on the longevity of test animals that are used in cancer bioassay. Regarding NCTR’s transgenic animal work, the gene chosen was primarily as a result of the need for an *in vivo* system capable of providing information regarding the value of the data that was being developed in *in vitro* systems. DNA chip technology is beginning to develop at NCTR and is already of some usefulness; this is being developed in

collaboration with CDER for identifying individuals in the clinic who may be susceptible to different chemicals. The Biometry Group has been developing risk assessment procedures for the toxicological community for the last 20+ years. The technologies that have been developed are being used and evaluated by FDA. The Operant Behavior system that was developed in the Neurotoxicology group will have usefulness in understanding the effect of various neural depressants on children.

Dr. Casciano commented on the role of the SAB. He stated that one function of the SAB is to advise the Director on science and budget issues. Another important function is that members serve not only as full SAB members for review of site visit reports, but that they also serve as chairs and committee members of program division site visit teams. Dr. Casciano said this helps the Center in the development of the scientific infrastructure. He thanked the Board for their efforts, saying they lend credibility to what the Center does.

Division of Biochemical Toxicology, Dr. Beland

Next, Dr. Beland responded to the Division of Biochemical Toxicology, August, 1998, Site Visit Report. He assured the Board that the Division was living up to its commitments, as stated by Dr. Casciano in his preceding remarks regarding fumonisin and chloral hydrate. Dr. Anders, addressing the new Board members, stated this was one of the things he found rewarding, he said “many of us serve on advisory boards and most of us don't get listened to very often, but at NCTR very serious consideration is given to the recommendations made”.

Questions followed Dr. Belands presentation.

Dr. Hecht said he thought the question about riddelliine was the extent of human exposure, and asked if there was something new on that. Dr. Beland responded that after the site visit, representatives from the NTP and CFSAN visited, to discuss this issue, and that there was sufficient concern within CFSAN to go ahead with it.

Dr. Anders asked if this is a model for the pyrrozilidine alkaloids, and how prototypical was it. Dr. Beland responded it goes to the retronecine-type base; two of the DNA adducts have been characterized from this Preliminary data indicate that the side chain on all of these other compounds is lost and the same adducts from other types of compounds can be found.

Dr. Wilkins asked if they had any specific dietary supplements in mind, and what kind of approach they planned to take Dr. Beland respond that they were presented with a list, echinacea, aloe vera, ginseng, and a fourth that he couldn't remember. He said echinacea could be a carbon source for a rat and couldn't possibly kill it, so there was nothing to study; ginseng has been used by the Chinese for eons; aloe vera, he said was interesting, and could be a tumor promoter. Therefore, among these, the compound of choice would be aloe vera. Noting that there is both topical and oral exposure, the studies could also involve the phototoxicity facility. Dr. Wilkins asked if the first step to study would be the biological activity, or, is it to find out what these things are? Dr. Beland responded, the active component of aloe vera had been defined, and said it's sort of what they faced with fumonisin. Do you look at the mycotoxin or the products of mycotoxin? Do you pick a marker compound in it? It seems to be easier to regulate upon what you consider to be the active component, and said he'd push to look at that one component, then they could look at a mixture, too. First, they’d have to know from the product center what they were going to do with the information, saying this helps design the study.

Dr. Rosenkrantz said in previous discussions, when the possibility of doing dietary supplement work was talked about, she thought it was about the effects of dietary supplements on drug interactions with regulated drugs. This, she said makes more sense, since the FDA has regulatory authority over drugs but not over dietary supplements. Dr, Casciano responded, there was some concern, but the center that has aegis over these chemicals is the CFSAN. Dr. Patricia Hansen (CFSAN), said there are issues about chemical characterization, active ingredients, interactions with drugs, possible heightening of adverse reactions or interactions, as well as other endpoints of toxicity. Both the concerns and the relative priorities are being considered. Dr. Beland pointed out that a given bioassay costs literally millions of

dollars. He said there is an office within CFSAN that deals with dietary supplements. Dr. Beth Yetley, director of this office was at the Center to discuss this issue. The major endpoint is addressed in the Biochemical Toxicology Division is cancer. The question is, is this the major issue of concern with dietary supplements? If it's neurotoxicity, then the Biochemical Toxicology Division is the wrong group to look at this. This is why they need the product center to determine what they want to know, then it can be decided which is the appropriate group to conduct the studies. Regardless of which division does the studies, the question remains, how do they go about answering the questions? Dr. Leonard Schechtman (CDER), said that obviously dietary supplements is a new area that we entering into, inasmuch as we have been historically involved with other products and regulated drugs. He said CDER was also concerned, not only with the effects of the dietary supplements themselves, but also with the way these interact with pharmaceuticals. One of the questions is how, with the input of the different product centers, are we going to prioritize these dietary supplements in light of all of those that are marketed? Another issue is what are the questions that should be addressed and who should perform the studies necessary to answer those questions?

Dr. Pickett also had a question about the dietary supplements, particularly with regard to drug-drug interactions. Is no one internally looking at the ability of any of these supplements to inhibit cytochrome P450 3A4, 2D6 to begin to predict what types of drugs they may in fact interact with? Dr. Casciano responded that Dr. Leakey was working with different dietary supplements and looking at the effects of Phase II metabolism in *in vitro* systems. Dr. Picket asked if the rat bioassays were two-year, and if the toxicokinetic support of the assays were done internally by the group? Additionally, since human exposure is not known in some cases, how does one predict the doses to use in the carcinogenicity tests? Dr. Beland responded that with chloral hydrate they used typical pediatric doses anywhere between 15-50 mg per kg, and then they went up to what they considered to be a maximum tolerated dose.

Genetic Toxicology Program Update, Dr. Feuers

Next, Dr. Feuers presented an update on the Genetic Toxicology Program. Dr. Ritchie Feuers, said at the time of the site visit, he wasn't actively involved in any of the studies ongoing in the Genetic Toxicology laboratories. He remarked that the site visit report was extremely thorough and was favorable in many cases to the efforts that were put forth by the staff. In fact, the results of the site visit stimulated the group to continue its efforts and go forward. In instances of possible disagreement or concern, those particular recommendations and critiques were acted upon in a very positive manner by the division. Those criticisms or critiquings were used as guide posts to develop new strategies to pursue what they consider good and valuable research that would be useful for supporting FDA-related initiatives.

Dr. Feuers said he was going to go through the individual projects that were presented, tell a little bit about what happened at the site visit, and what the response of the individual investigators has been. There are very specific points that need to be addressed. Much of the effort of the division is being devoted to defining the mutagenic pathways resulting from initial DNA damage and mutation expression, thereby leading to an understanding of mechanisms. The goals associated with this kind of strategy are designed so as to yield a product that is useful to the FDA. Such goals include: (1) validation of sensitive and predictive *in vitro* as well as *in vivo* systems, some of which have gotten to the point where protocols can be written up and used directly; (2) to identify, quantify and hopefully understand the mode of action of potential toxicants, especially where they might relate to human toxicity, with specific emphasis on carcinogens and potential mutagens.

Genetic Toxicology Program Projects

During the site visit, a number of Genetic Toxicology Program projects were presented to the Board.

- Dr. Suzanne Morris

Dr. Suzanne Morris presented a discussion on apoptosis and mutation. She presented four topics:

1. Molecular Analysis of The TK^- Mutations

The primary goal of this effort is to determine the molecular nature of those mutations. Dr. Morris had been asked for a clarification of rationale, to provide some detail for the analysis, and to formulate specific experimental plans. She has assembled the details of analysis and they are in the process of being further developed before she initiates any of the particular parts of the study. It's important to note that she is currently screening the *Tk* mutants for loss of heterozygousity (LOH). Recently, she and her colleagues have determined that the mutagenicity of coumestrol appears to be due to this LOH phenomenon. Now, she has experiments underway to characterize those *Tk* mutants and analyze the *Hprt* mutants using the multiplex PCR approach.

2. *In Vivo* Mutagenesis Studies with Genistein in the $p53^{+/-}$ Mouse

One of the strengths of this study is that the analysis of thymic lymphomas and *Hprt* mutations in lymphocytes are performed in similar tissues, allowing for comparisons in cancer and mutation induction. Dr. Morris has submitted a protocol to the NTP and the dose range-finding study has now been approved for NTP funding. The chemical is ordered and purity and structural analysis are currently underway. The dose range-finding study is to proceed at the completion of that chemical analysis.

3. *In Vitro* Toxicology of Coumestrol

Based upon the presentation of this project, it was noted that the experiments would be straight-forward and were analogous to other previous work, and no particular problems were identified. The protocol has been submitted and is now approved. The coumestrol study has now been completed and shown the agent to be an effective *in vivo* mutagen in human lymphoblastoid cells. Dr. Morris recently prepared an addendum for the project for the molecular characterization of those particular mutants and this work will be ongoing.

4. Apoptosis as a Mechanism of Modulating Mutagenesis

A particular piece of evidence that was put forth is that amiloride blocks radiation-induced apoptosis. The question is does this also increase mutagenesis, and if that's the case, does p53 affect mutation yield by regulating apoptosis? The team asked that Dr. Morris consider and rule out other possible effects of ionizing radiation. At this point she submitted and has an approved protocol. This project will be revisited in the near future.

- Dr. Ben Aidoo

Dr. Aidoo has done considerable work concerning the lymphocyte *Hprt* assay, specifically in the rat model. This assay is now being done successfully in human lymphocytes. The work has expanded well beyond anything that was presented 1-1/2 years ago and includes a number of other studies and cross-species-types of comparisons, which will be extremely important for support of a number of FDA-related issues in the future. The idea is to develop a tool to detect, quantitate, and evaluate the spectrum of chemically induced genetic damage. The recommendations were quite positive for this proposal. The site visit team (SVT) put a very high priority on continuing this effort, and had recommended studying cross-species applications; this particular recommendation had been followed. The SVT asked if Dr. Aidoo and his staff could put some effort into attempting to make the assay more user friendly so that it could be applicable in a broader range of environments. He said this particular assay requires a fair component of hands-on skill, that not just anyone could go into the laboratory and make this assay work. He indicated that scoring clones was a problem; it was being done with a light microscope, but now a fluorescent spectrophotometer is in use to replace the scoring technique. This has made a big improvement on where they are with the assay and how efficiently they can apply it to particular problems. They have designed a protocol to use the rat *Hprt* assay from mouse spleen lymphocytes for species comparison. The original presentation described studies with rat lymphocytes; now the work is being done in lymphocytes from mice and humans. As requested, there has been an effort to expand and address the concerns of the FDA, primarily to make the assay a bit more hands-on and user friendly. Those are moving forward.

- Dr. Mugimane Manjanatha

Dr. Manjanatha discussed the Big Blue transgenic rat project. The idea was to evaluate the sensitivity of this Big Blue rat transgenic mutation assay for the purpose of detecting *in vivo* mutations, mutations at *Hprt*, and tumor-related genes. The SVT suggested that it might be useful to try the Rat 2 cell line in initial mutation studies, because of possible availability problems of the Big Blue Rat. A concern was raised about the high background mutation frequency with the particular assay they were using. Another point made was to measure of DNA damage and repair to the *lacI* gene using PCR-based techniques. In response to these suggestions, the group has incorporated those ideas into its approach and have made significant progress in those areas. With the assay now in use, they have begun to gain more extensive experience with it. They have also designed a protocol that included tissue-specific measurements of DNA damage and repair in the *lacI* gene.

- Dr. Carrie Valentine

The next project was presented by Dr. Carrie Valentine, the ΦX174 transgene mouse assay and its development. The goals of this assay are to develop an *in vivo* screening assay for all tissues. There are a number of important, potential positives that make this possible. Single burst has a potential for greater sensitivity and execution as a microplate assay and could improve its usefulness. The point was made, at that time, that it might be the only transgenic rodent mutagenesis assay with these particular potentials, and as such was viewed as an important idea to consider. The concern was that the AM3 reversion assay may not be broadly useful. There might be a limited DNA target size and sequence sensitive agents might even be missed. The SVT thought there might be little potential for using this type of approach for doing mechanistic related studies. In doing this kind of genetic toxicology it is important to be able to have mechanistic insight. The SVT also asked that the mutation versus cancer issue using either Big Blue or GPT Delta be addressed. With these types of comments, decisions had to be made as to how to proceed. As a first step, Dr. Valentine made the decision to continue the reversion assay. The alternative was to go with the forward mutation assay, which is termed single burst assay, to characterize different sets of mutants via sequencing. A considerable amount of progress has been made: she is now establishing sensitivity in the animals and there are 15 target sights that have been identified. ENU spectra differ from spontaneous mutation frequency. The input from the Board has thus helped move this project forward in a positive way. Microarray technology may now be used to sequence the mutants. Dr. Valentine is also now using the GPT Delta to address those mechanistic questions.

- Dr. Vasliy Dobrovolsky

The next project centered around the work that Dr. Dobrovolsky has done with development and creation of the thymidine kinase transgenic models. This is very important work for the Genetic Toxicology laboratories and for NCTR, in general. The idea is have a method for the evaluation of mice heterogeneous for the endogenous thymidine kinase gene that could serve as a model for *in vivo* mutation detection. The SVT thought this was an excellent complementary assay to the L5178Y mouse lymphoma cell assay. The SVT felt that the approach was well thought out and the protocols were well designed; furthermore, there was a very significant possibility of successfully completing this kind of project based on the early work. Another point that was made was that the system does allow genotyping of heterozygous mice and the *Tk* mutant cells. The *Tk* knockout mouse was identified as an important model to understand and it resembles human conditions; it was recommended that this project should be supported. It was pointed out that there was a potential need for further optimization of cell culture conditions, mutation selection, and related conditions. There was some concern for mutation response to some clastogens and the SVT suggested that there might be a need to use some other alternative clastogens to further develop the procedures. Dr. Dobrovolsky responded to these recommendations by implementing the SVT suggestions, and doing so has helped him to proceed in a very positive way. Bleomycin has now been used as an alternative clastogen.

- Dr. Barbara Parsons

Development of the genotypic selection methods was presented by Dr. Barbara Parsons. The idea is to develop DNA-based methods to detect rare mutations. The SVT commented that the approach was an innovative strategy for genotypic selection. This sort of positive input was useful in helping gain momentum to move this work forward.

The sensitivity of the method for a very small target size was questioned and was an issue that needed to be considered as Dr. Parsons moved forward. The SVT suggested that it might be of value for Dr. Parsons to consider communications with other groups in developing collaborations to leverage the effort to move forward at a more rapid rate. In response, a priority had been established in her laboratory to determine if the method is sensitive enough to detect spontaneous mutations in three strains of mice. In addition, interactions with other laboratories are in process or being pursued.

New Initiatives Presented to the SVT

- Creation of a new transgenic mouse model with florescent markers (Dr. Debrovolsky).

The idea is to construct a system that is capable of detecting cymatic mutations based on florescent detection of altered expression of a reporter gene. The target gene would be constitutively expressed repressor. The reporter gene would be under negative transcriptional control of the repressor. The mutation in the expressor gene unlocks expression of the reporter gene. Detection of enzymatically amplified florescence could be potentially a very powerful tool. The recommendations/questions that the SVT asked that this group at least consider, was what the tightness of repression of the *lacI, lacO* combination might be? What the toxicity of the repressor proteins *in vivo* might be? What is the degree of induction of expression of the reporter gene? There were unresolved technical issues that were identified using flow cytometry. In response, Dr. Dobrovolsky has considered other repressors and reporter combinations. He is also considering the issue of detection, and the possibility of detection of mutation using direct florescence instead of the enzyme amplified technique. He is also considering chemical selection of mutant cells and is initiating *in vitro* studies as well. The SVT-specified planning is underway in preparation for the initiation of the *in vivo* work. This is another example of where the investigators have followed up with the recommendations of the SVT and are using those inputs to establish their future directions.

- Dr. Angela Harris

Dr. Harris presented her study on "Gene Expression and Carcinogenesis." The idea is to develop techniques to detect alterations and expression of a large number of genes. The SVT noted that the use of arrays makes this a realistic strategy, provides good mechanistic information, and is relevant to humans using human primary hepatocytes. The SVT wanted to make sure that the staff was aware of the pitfalls involved with the development of this kind of new technology. This has been well noted. The idea is to progress systematically, to be careful about each step, and to coordinate with other groups. In an emerging technology this is an important consideration. In response, Dr. Harris concurred with the SVT recommendations. She is currently identifying genes responsive to both aflatoxin and acetaminophen and is establishing collaborations.

Dr. Anders called for questions. Dr. Pickett said the National Cancer Institute has a cancer anatomy project, where they are using microarray technology to look at initiation progression. Dr. Feuers commented that it's not just NCI, but there are other places where this kind of work is being done. He said they were attempting to make contact with some of those groups. Dr. Harris's comments were inaudible for documentation. Dr. Pickett raised the issue of validation, questioning if Tackman or a similar technique was being used. Again, Dr. Harris's response was inaudible for documentation. Dr. Donnelly had questions about the transgenic mouse models with florescent markers and the technical problems with flow cytometry. She asked if those issues were being overcome and whether one of the problems was simply a labeling issue. Dr. Dobrovolsky responded that they hadn't started the protocol yet, and the short answer is that these technical difficulties have not been resolved yet.

Molecular Epidemiology Program

Next, Dr. Anders asked Dr. Poirier to provide the update on the Molecular Epidemiology Program. Dr. Poirier noted he would be highlighting specific portions of the written response, particularly those involving the principal

investigators within the division. He said there were several presentations by people outside the division, and the projects of the other principal investigators would be alluded to during the course of his presentation.

- Metabolic Polymorphisms and Cancer

The recent accomplishments on this project include (a) the demonstration of the role of the slow N-acetyltransferase-1 in increasing both amine adducts to DNA and to cancer risk and smoking-related pancreatic cancer; and (b) the positive association between the combination of the presence of the fast trans-acetylase-2 and high meat consumption in the recurrence of colon cancer. The genotyping that was done in the present study made use of both classical and microarray technology. He said he would discuss the microarray technology later in the presentation. There was a 99% concordance between the results obtained by the two systems.

- Glutathione Transferases in Carcinogenesis

The principal investigator, Dr. Brian Coles, concurred with one of the critiques of the SVT on the problems associated with the use of human hepatocytes *in vitro*, and has thus focused his studies on the use of human liver. Recent results have compared the effects of glutathione transferase A-1 versus that of glutathione transferase A-2 taken from human liver. The results show a clear pattern that there is not a very good correlation, however, if the different polymorphs of glutathione transferase A-1 are taken into account. Three different linear correlations are seen depending upon whether the A1A homozygotes, the A1AB heterozygotes, or the A1B homozygotes are being compared with glutathione transferase A-2. Dr. Robert Delongchamp, their biostatistician, assisted with the interpretation of the results. The significance of these findings are that the polymorphism in hepatic glutathione transferase indicates that the linear correlations previously observed with lung and pancreas, for example, in the expression of the different glutathione transferase alleles in other organs is also seen in liver. The glutathione transferase expression is organ-specific, influenced by allele and subject to considerable inter-individual variation. The ratio of the two forms, GSTA1 to GSTA2, is also predictive of the polymorphism of GSTA1 in liver and, they hope, also in blood.

- COX-1 and COX-2

Work on the COX-1 and COX-2 project was performed by Frederick Wiese who has since obtained his Ph.D. and has gone on to work in industry. The work is being completed and, as recommended by the SVT, is in various stages of preparation for publication. One practical outcome of these studies is a grant proposal recently funded between Dr. Fred Kadlubar of NCTR and Dr. David Alberts of the University of Arizona to examine COX-2 and glutathione transferase in colon polyp recurrence using celecoxib and selenium as potential chemopreventive agents. An aim of the projects to which Dr. Kadlubar will contribute is to determine whether the polymorphism of MTHFR (methylenetetrahydrofolate reductase) modifies the effects of selenium and celecoxib, when administered separately or in combination, on the recurrence of colon tumors or on COX gene expression. Another aim of the project is to look at glutathione transferase genotypes and phenotypes to see if they also modulate the effects of selenium and celecoxib on the recurrence of colon tumors. The working hypotheses being examined are that S-adenosyl-methionine blocks COX-2 activation by reacting with nitric oxide and that the stimulation of cell proliferation in fatty acid hydroperoxide metabolized by the eicosanoids pathway will be modulated by glutathione transferase. There are a number of different forms of glutathione transferase that will be looked at.

- Gene Activation and Chemoprevention

The principal investigator for this project is Dr. Beverly Lyn-Cook. Since the time of the site visit she has published two papers describing the results presented to the SVT. These publications have incorporated suggestions made by the team on the delineation of hypotheses and on additional controls. Also, a protocol on the mechanistic actions of chemopreventive agents in pancreatic cancer is being prepared. In line with the SVT recommendations, the protocol describes the rationale, the FDA relevance, and the hypotheses for the potential mechanisms being explored. Other

contributions from this project include (a) the observation of a significant rise in COX-2 in the pancreas of heavy smokers, and (b) the publication of a collaborative study with Drs. George Hammons, Fred Kadlubar, and Lionel Poirier, describing an elevation in the enzyme DNA-methyltransferase in the livers of smokers.

♦ Methylation and Chemoprevention

Dr. Lionel Poirier serves as principal investigator for this project. Recent developments are concerned with elevated DNA-methyltransferase and its association with carcinogenesis. The collaborative study demonstrating an elevation in the enzyme DNA-methyltransferase in the livers of smokers was referenced previously by Dr. Lyn-Cook. Another study showed a rise in the enzyme in the livers of rats fed a methyl-deficient diet. This finding extends other observations made with liver carcinogens in other similar diets. The enzyme, however, as pointed out at the time of the site visit, behaves abnormally during the course of carcinogenesis. These studies are also being extended by Dr. Vlasova. She has shown that the inhibition constants of this enzyme obtained from pre-neoplastic liver are different than those obtained from normal liver. In the study conducted in collaboration with a Japanese group, methyl-cobalamine, an abnormal substrate with the enzyme DNA-methyltransferase, was observed to inhibit gene transcription in a transformed cell line but not in the corresponding normal line. Finally, there's an inverse association between caloric intake and methyl-group availability. Again, in the study that was being conducted with Dr. Chou, feeding the methyl-deficient diet decreased S-adenosyl-methionine levels and DNA methylation in liver and also produced pre-neoplastic foci in the livers of the methyl-deficient animals. Dietary restriction in animals fed the same diet increased the availability of S-adenosyl-methionine, diminished the extent of DNA hypomethylation, and inhibited the formation of the pre-neoplastic foci. Related findings seem to occur in humans.

Dr. Poirier reported on a collaborative ongoing study with Dr. Rashmi Sinha of the National Cancer Institute. Earlier portions of this study were presented at the time of the SVT; these are more recent findings. In this study, the blood levels of S-adenosyl-methionine (SAM) and homocysteine (HCYS) were examined in normal men and women and were compared with several other parameters, including weight and dietary intakes. The HCYS levels of blood were positively associated with the body weights and with the body mass index of the individuals. In addition, the SAM levels were inversely associated with fat and with caloric intake. This is apparently the first metabolic linkage made between two separate risk factors for cancer in humans, energy intake and methyl deficiency. Dr. Poirier said that the accumulation of HCYS can cause a hypomethylating environment.

♦ Molecular Epidemiology

Dr. Christine Ambrosone reported on the work being conducted on the molecular epidemiology project and expressed her thanks to the SAB for their very positive critique of her work. As recommended by the SVT, there has been an expansion in the number of subjects in the breast cancer study. Also, there has been an extension of the studies to confirm the association between breast cancer risk and polymorphism of manganese superoxide dismutase in women with low anti-oxidant intake. Dr. Ambrosone has been genotyping for carcinogen metabolizing capacity and oxidative stress enzyme in the ovarian cancer study, and has undertaken two separate studies with Dr. Bart Barlogie of the Arkansas Cancer Research Center. One of these studies concerns the role of polymorphisms in chemotherapy of multiple myeloma and another concerns the role of polymorphisms on the susceptibility to multiple myeloma. The studies on the effects of glutathione peroxidase P-1 polymorphism on the efficacy of chemotherapy in the clinical progress of patients with breast cancer, have shown that the patients who are homozygous for the valine mutant (val/val), of GSTP-1 have significantly better survival than those who are homozygous for the iso-leucine (ile/ile) of the enzyme or the heterozygous form of val-ile polymorph. The valine mutant form of glutathione transferase P-1 is less active than are the other two forms and, hence, permits the chemotherapeutic alkylating agent cyclophosphamide, with which these women were treated, to persist in the body longer.

♦ DNA Adducts and Cancer Susceptibility

Next, the project on DNA adducts and cancer susceptibility. The work on DNA adducts and cancer susceptibility was begun by Dr. Patricia Thompson, and since her departure is being continued by Drs. Ambrosone, Kadlubar, and Doerge. The overall aspects of this study were highly regarded by the SVT. The SVT did express concerns about the quantitation and identification of the DNA adducts. These issues are currently being addressed by Dr. Doerge employing liquid chromatography and sequential mass spectrometry methods. A recent finding is a significant linear correlation between CYP 1A2 and interleukin-6, but not between estradiol and interleukin-6. The CYP 1A2 may be induced by the interleukin-6. These data were obtained from women who used oral contraceptives. The SVT was highly enthusiastic about the DNA microarray technology presentation, a collaborative project of Dr. Kadlubar's. The current list of genes whose allelic forms will be examined by this technology include enzymes involved with the metabolism of drugs and carcinogens, the metabolism of hormones, DNA repair, growth control and immuno-surveillance. Some of the examples have already been seen, the N-acetyl-transferases, the glutathione transferases, the cytochrome P450s, two enzymes responsible for the metabolism of methyl groups, catecholamine O-methyltransferase, methylenetetrahydrofolate reductase (MTHFR), as well as sulfatransferases and uridine-glucuronosyltransferases.

Dr. Anders asked if there were polymorphic variants of the DNA methyltransferase that have been identified. Dr. Poirier responded that to his knowledge there were not. Dr. Hecht, questioned if is it known that GSTP-1 catalyzes detoxification of cyclophosphamides. Audience response …not the cyclophosphamide itself… (rest is unintelligible). Another question (source unidentified and partially unintelligible) was whether, in the GST experiments, the A-4 gene and its protein were going to be looked at. (A discussion between staff member, Dr. Brian Coles, and the questioner ensued but the details were inaudible.) Some of the comments made by Dr. Coles that could be transcribed were as follows: "We have done some work… We have only seen GST A-4 expressed in any of the tissues which we have examined, which are …, lung, pancreas, liver. We've only seen it expressed at high levels in one or two pancreas tissues in which case GST A-1 was also highly expressed. We think that GST A-4 perhaps only expressed at high levels in tissues which perhaps are in oxidative stress. Indeed, I agree that it's important, but it seems to be a very small component in most of the tissues that we've examined. But we are studying it; we're certainly not neglecting it." There were no more questions.

June 6, 2000

Dr. Anders reconvened the second day of the meeting.

- Endocrine Disruptor Knowledge Base (EDKB) Project

The Endocrine Disruptor Knowledge Base (EDKB) project was reviewed previously in January 1997 and again in June 1999. Dr. Bernard Schwetz, in the 1999 review, charged the SVT to look at a range of issues: current status, quality of the science, and whether the computational approach was effective and could be transported to other systems, whether the computational approach was effective but not suited to the EDKB, whether the computational approach was flawed but the biological research was meritorious, and finally, whether sufficient progress had been made to answer these questions. The SVT developed a number of objectives to examine.

Addressing the issue of endocrine disruptor assay validation, the SVT was impressed with the work of the project team. The validation of the assay and its comparison with other assays were considered to have been well done. The computation models were interpretive and not extrapolative, and therefore other areas needed to be looked at to ensure things were being done correctly. They were cautioned to look at other tools that are in use in the pharmaceutical industry. There was discussion about the filters being used in assembling the database. For example, the Phase 1 filters looked at whether the molecules had an aromatic ring structure, and the way the structure is entered would have an effect on whether the Phase II filter was effective. The SVT recommended that the project information should be made available on the Web quickly, at least for the very early tests to see if the compound was active or inactive. This would be useful for those who just wanted to check a structure. Some

problems that were identified included (a) the way a structure is entered into the database, (b) the different formats that are used to enter a structure into the database, and (c) how a query is executed.

Extensibility/transportability of the knowledge base system was also considered with respect to whether the approach could be used to look at the activity of other types of molecules besides estrogens. The problem was in the different types of filters that would be necessary and the biology of the systems would be more complicated. Basically, however, it was felt that the idea is sound and that the system could be transportable.

Dr. Pickett asked whether there would be biological endpoints that are going to be monitored to predict estrogens or anti-estrogens because of the complexity of the biology *in vivo*. Dr. Anders said that the present focus is on *in vitro* methods in the estrogen receptor-binding assay, and that Dr. Pickett was correct in noting that both agonist and antagonists could bind. An in vivo endpoint is the uterine weight assay.

Dr. Sheehan said they'd acquired a substantial database from NIEHS on estrogenic activities of chemicals. Using that database together with some literature data, regression analyses of RVA values versus uterine weight were performed. There was a significant correlation between outliers measuring up to 2 orders of magnitude. The approach is usable for some limited purposes; e.g., if information on appropriate dose selection for use in a uterotrophic assay were desired, then the method could provide a range of values where activity would be expected. To improve that kind of model one should incorporate models for other determinants of potency that are extra-cellular and possibly even intra-cellular, that would include binding to the serum estrogen binding proteins. There are a number of ways to dissect the mechanistic sequence model and then put it back together in the same sequence.

Dr. Pickett said their experience has been that it is almost impossible, once you have a competitive assay to predict in animal models whether these compounds will in fact be agonist or antagonist depending upon the tissue in which there is activity. You can have a pure antagonist that has agonist activity on bone and lipid levels. Dr. Sheehan agreed. He said he wanted to make sure that it was understood that this is not a complete package, but is supposed to provide information that will lead one to conduct other sorts of experiments. The uterotrophic assay would just be one of those, and would have to be run a number of different ways to look for potential antagonists. Mr. Perkins said based on what they know, there is some understanding at the molecular level of that antagonist; if you have sufficient data on a specific tissue you could build a QSAR model.

Dr. Schwetz asked about transportability of the technology. With the experience we have now, with the computational capabilities, and the knowledge that we've gained, it would be good if there were criteria extracted from this experience to give guidance on whether or not to attempt other hormones or other receptor mediated activities. Dr. Sheehan pointed out that his model at best represents a single event, i.e., receptor binding. We had a range of discussions about this. To try to model for a phenomenon for which you do not have a discrete single event would be very difficult if not impossible. Dr. Schwetz said that would be one criterion and questioned whether there are others that could be added to that. Dr. Sheehan said he thought their experience had shown them that the literature from diverse laboratories using different types of assays could not be relied upon to provide data that is appropriate to develop models. Unless such data are available, one has to include the assays, assay validation, and collection of a large amount of data over a wide diversity in ADR range. The models, while they will be new, should be more quickly developed just because of the experience gained; a large number of models have been evaluated to see which ones would work the best together.

Mr. Perkins said since the inception of the program in late 1996, predicting early metabolism and toxicology has been of great interest. The pharmaceutical companies are very interested in this and apply these types of models early in their lead discover and development programs to eliminate "bad actors." Dr. Rosenkrantz said that the question of transportability still remains in systems where the biology is much more complex, when you don't have a single event where you get a "thumbs up" or "thumbs down." When you have several competing events you have a real problem.

Dr. Pickett said we now measure plasma levels and we understand similarities between the animal models that we use vs. human models. He pointed out that in reality, there is nothing that is predictive until you get into the pharmacokinetic properties based on his experience using human hepatocytes, human microsomes, or human P540s, 3A42D6, 2C9, 2C19. After all of the modeling one does, it is not absolutely predictive as to what will happen in people after oral administration of a drug. One still has to study people and find out; one still needs to do the experiment.

Dr. Anders said at the time of the site visit, little had been done to market the EKB. Two questions that arose were, can it be done and is there a need for it? The answer to the first question was a definite yes. The answer to the second question was we don't know. It appeared that the EPA would be a major user of this because of their mandate to look at tens of thousands of potentially endocrine disrupting chemicals. As Dr. Sheehan indicated, though, EPA is apparently not interested in this. There is an issue of the marketing of this and the issue of who are the potential users.

Dr. Sheehan offered clarification, saying that the EPA was interested in their models, but was not interested in the database. This became an interagency issue; EPA said they did not want the database they were working with to be on an FDA computer. A decision was made that they couldn't use the database, and for that reason they would house a database at EPA. Dr. Rosenkrantz asked whether they have their own copy? Mr. Perkins said they had no interest in the database that FDA built and that they built their own. Dr. Sheehan said, in any case, EPA was very interested in our models, and has entered into an agreement with FDA with a commitment for a substantial sum of money.

Dr. Schwetz asked what was used by EPA to populate their own database. Dr. Sheehan said they have the priority setting data that include more ecological measures than biological data, such as production volume, half-life in the environment and in animals, persistence and fate. Of the some 86,000 chemicals nominated to be studied, only a few thousand had any biological data associated with them at all, and that was mostly pesticides and herbicides which is required testing. There is no biological data for the majority of the chemicals to be used for priority setting for entry into the database, which is why they are so interested in NCTR's data. Mr. Perkins said that EPA also indicated it was not going to publicly disseminate its own database.

Dr. Anders introduced the next issue to be considered, which was the low-dose/threshold hypothesis, and pointed out that the pilot project team had challenged the assumption that a threshold exists for estrogenic compounds. It was the view of the SVT that the question bore no relationship to the EDKB project and was not relevant to the development of the knowledge base. As such, the SVT recommended work on the low threshold hypothesis not be continued under the aegis of the pilot project. The next issue addressed was resources and future plans. The SVT summary was positive, although marketing had not been done to the extent that the SVT would have liked.
Dr. Sheehan then presented a discussion of the NCTR toxicological knowledge base. That knowledge base, as defined by NCTR, is an *in computero* aggregated set of the most germane literature citations and biological activity datasets, together with computational models which correlate those activities with chemical structure, and, ultimately, models for risk assessment. It is also important that this is a "circular process," i.e. one in which models can be improved upon as one is doing other related studies, such as, validating the model. The first learned was that use of data in scientific papers is not an appropriate way to develop models, at least for the estrogen receptor. Instead, it was necessary to perform our own binding studies, validate the assay, and then conduct diversity analysis on the chemicals that were included among those that were assayed. A training set has been designed that meets the criteria for structural diversity in a wide range of receptor binding agents. The intent here is different than that in the pharmaceutical industry where high affinity ligands are sought. Rather, in this case of environmental chemicals, 8,000 of which are regulated by FDA, these generally have significantly lower binding affinities than do pharmaceutical products. The model has to be able to accommodate that kind of difference. From there one can develop the models and have a training set for drawing correlations between structure and activity. There are two ways to validate the model, i.e. cross validation and test set challenge. Once such a validation has been performed, one can define new data needs and research hypotheses when consistent errors in the prediction compared to the

measured values are encountered for different chemicals. It also means that data derived from an external test set that are also used for external validation can be incorporated into a training set and merged with the initial training set so as to increase even further the structural diversity of the chemicals included.

An integrated approach composed of four phases has been developed:
Phase I - Uses several simple rejection pre-filters to eliminate a significant number of chemicals which are most unlikely to bind the ER due to molecular weight or absence of a ring or other criteria.
Phase II - The chemicals not ruled out in Phase I are assigned as either active or inactive using three sets of computational approaches. Inactive chemicals will be eliminated in this phase. In this phase there are about a dozen different models involved, for a chemical to pass from Phase II to Phase III they have to only be hit by one of the twelve models. Much effort is being directed at protecting against false negatives by setting stringent criteria in this phase. Only active compounds will go on to Phase III for further evaluation.
Phase III - In Phase III, quantitative prediction is applied to these chemicals using multiple QSAR models.
Phase IV - In this phase, the information gained in Phase II and III will be combined, data that are quantitative or categorical can be used for priority setting for entry into the screening and testing. In addition, other information related to the chemicals should also be considered in this phase.

Three objectives were defined, i.e. (1) to develop a prototype knowledge base for estrogenic chemicals, and particularly for endocrine disruptors; (2) utilize this knowledge base for application to FDA estrogen issues; (3) help develop other knowledge bases to expand this approach to other types of FDA issues.

— Development of a Prototype Knowledge Base for Estrogenic Chemicals/Endocrine Disruptors

Towards this objective, the estrogen receptor models are developed and being used now. There was an external peer review sponsored by the Chemical Manufactures Association (CMA) and CMA hired Dr. John Patsenellenbogen, University of Illinois, to review the NCTR report. He is the world's expert on ligand binding to the estrogen receptor. His review was very flattering and bolstered CMA's confidence in the effort. Those factors necessary to complete the models include external validation, refinement of the Phase II models by looking at what is being included/excluded, and the "black box" issue. Dr. Tong, the leader of the computational chemistry group, has investigated the "black box" issue. She's said it can be done but will require a great deal of time and additional resources. It will also require that the computer software vendor allows us to work within their proprietary code in order to establish the necessary interface and have the "black box" work.

— Application of the Knowledge Base to FDA Estrogen Issues

Towards this objective, a joint venture with FDA and the NIEHS began with six natural products. It involved some 50 cosmetic ingredients (CFSAN) and the ~ 10,000 chemical databases from CDER. Recently we made contact with individuals in CDER concerned about the wide range of chemicals that are present in Premarin. Premarin is used for postmenopausal therapy and is named after pregnant mares' urine, which is collected, dried down, coated, and marketed. There are numerous other steroids and chemicals in this urine besides estrogens and it is not clear, from the work that has been done, how many of those estrogens have been identified through receptor binding. The plan is to make predictions on that large dataset on Premarin in order to determine which other chemical components might also be estrogenic. In addition, in collaboration with Dr. Fred Beland, efforts will be directed at developing potential carcinogenesis models for estrogen, which would be a foray into an entirely different kind of modeling.

— Application of the Knowledge Base to Related FDA Issues

An arrangement has also been entered into with the Food Contact Chemical Branch of CFSAN. Food contact substances are chemicals that come in contact with food by way of their packaging material, e.g. through plastic film, the lining in cans, etc. Having heard a presentation of the potential of the NCTR model, CFSAN wanted to acquire the technology and add it to its own list of models. CFSAN has instituted a pre-market notification process by which

a company can submit to the Food Contact Chemical Branch a statement that they plan to market a given chemical. The FDA then has the responsibility, within 90-120 days, to tell them they can't market it. This is a very short turn-around requirement for chemicals that have very little biological information available. Therefore, these models are viewed as very useful approaches in this endeavor. NCTR has agreed to provide training to, access to, and use of the models.

Among the new issues since the report was written is a possible use of the androgen receptor (AR) model for athletic "doping" chemicals, an interest expressed by a member of CVM who is also a member of the International Olympic Committee Doping Subcommittee. Such chemicals are used in racehorses and athletes and have become a major problem. With the model, one should be able to predict the RBA for the AR anabolic agents. In addition, common interests have been identified between CVM and NCTR through discussions with Dr. Steve Sundlof, Director, CVM that will require further exploration. Dr. Tong has comprised a list of seminars that we can give, either here at the Center or at any of the other agency Centers, that is an extension of some of our modeling plus some areas of information processing techniques.

— Development of Other Knowledge Bases

Work on the androgen receptor (AR) assays is about to start and will involve validation of the assay and the assay of a sufficient number of chemicals to meet the criteria for model development. That will run approximately six months, then an AR knowledge base will be developed using the ER as an example in terms of where the data goes and how it is stored, etc. The cost-effectiveness of this project should improve due to the infrastructure and experience gained from the ER model.

Marketing Strategy has been an issue that we have been addressing since we were first asked. Initially our marketing strategy was through individual contacts for predictions of a relatively small number of chemicals. In the beginning we only had the CoMFA model, and we didn't want to get into the business of providing service with the CoMFA model when we were trying to develop other models. Rather, we would do predictions for small numbers of chemicals. Later, this was supplemented with larger scale marketing to groups rather than individuals.

Recent Marketing Efforts include a one-day mini-symposium on Computer-Aided Toxicology at the FDA Science Forum. Although 30 people were expected, more than 80 attended. There were four talks given, two from CDER, one from CFSAN, and one from NCTR. Ms. Jeanne Anson chaired a committee that issued an FDA Vision Statement (handout) on computational computer-aided toxicology in the FDA. This vision statement is available in the response to the site visit team. It also included a software demonstration. An FDA Computer-Aided Toxicology Working Group Website (with discussion forum) has resulted so that people across the FDA who are interested in this topic may communicate on general and specific issues.

The ability of the FDA centers to lease "seats" for commercial software (MSI) was negotiated as part of NCTR acquiring more software and leasing such software rather than buying it. We have a Computational Toxicology Laboratory Website which describes projects, hardware, software, and seminars. The website requires some modification and will need to be approved by a website committee prior to access. As indicated, there is potential for an AR model for "doping" chemicals.

— New and Anticipated Funding.

About one month ago NCTR received $120,000 from the CMA to fund the androgen receptor CoMFA (QSAR) model. The EPA is in the process of writing an Interagency Agreement (IAG) to fund Phase I and Phase II AR models and for priority setting support. At this point we are in negotiation for funds. We have also worked with CMA for funding of the "black box." They are very interested in having a "black box" available for the same reasons that we were aware of and as pointed out in the site visit report. In addition to using this to prioritize chemicals for a one-time only event, it can also be used to monitor chemicals in development in the way drug companies do and to determine, in

this case, whether they are likely to have unintended hormonal activity. The external validation is important; you can validate the models internally but what people really understand are false positives and false negatives. We have to either do the validation ourselves with CMA funding, or it may be more preferable to have the CMA establish a contract to run the chemicals and then provide us with the data. NCTR, in turn, would provide the predictions.

Dr. Lightfoot (CDRH) said that Dr. Tom Umright of her Center's Health Sciences Branch was working on estrogen receptor assays and asked if NCTR was interacting with that individual. Dr. Sheehan said they were working with a scientist who was working on pharmacokinetic models on bisphenol A, but wasn't aware of Dr. Umright's work in this area and said that he would contact him.

Dr. Schwetz asked why reference was still being made to a "black box". He questioned the next product being a "black box" considering all the years of intensive mechanistic work. Dr. Sheehan said that there are several pitfalls in a "black box" and that it was with some reluctance that an evaluation of its feasibility was undertaken. Dr. Rosenkrantz asked if the "black box" was just for the likely/unlikely test, and whether it served no other purpose. Dr. Sheehan responded affirmatively, and that they were not considering, at this point, trying to develop a "black box" for the CoMFA (QSAR) model or other 3D QSAR models. Dr. Schwetz said he was troubled about the concept, in that, after decades of his deriding the "black box" approaches to decision making, all of a sudden we're introducing one. Dr. Sheehan said they were responding to the SAB and to the CMA in this regard. Dr. Schwetz said that his point was a semantic one, and that consideration should be given to calling it something else.

Dr. Anders called for a motion to accept or reject this report: moved by Dr. Rosenkrantz, seconded, and accepted.

Microbiology Review and Initial Program Response

Dr. Donnelly chaired the SVT for the Microbiology Program review, and SAB members Drs. Anderson and Kennedy joined her. She said they were very impressed with the work going on within the Division, and the comments offered in the report were mainly made to strengthen the already excellent work that exists.

In going through the report, it wasn't clear to the SVT that there was good strategic planning going on within the FDA. As you read the SVT comments it might seem that they are more directed to the Division of Microbiology, but she said those comments were made to research planning at the other centers. Frequently during the SVT, it became apparent that there could be better communication between CVM and CFSAN and some of the other centers regarding the work that is going on at NCTR. A second general recommendation was the need for a new philosophical paradigm. One consistent with the mission of NCTR refereed journal publications is clearly an outcome of the good science that is going on. The SVT felt there was an opportunity to also view outcome as the potential for the research being done at NCTR to have real world impact. The SVT believed that a lot of advances could emerge from the work being conducted in the Division of Microbiology in the area of methods development. Now, with the Arkansas Regional Laboratory (ARL) being co-housed on this facility, the SVT felt that this was an incredible opportunity to transfer developments from the research lab to the ARL through pilot testing and further validation. Second in the area of regulation, specifically risk assessment. The SVT also asked bout the applicability of the microbiological research being conducted at NCTR to the larger issue of risk assessment.

Regarding the issue of leadership, the SVT was very impressed with Dr. Cerniglia's work and productivity. The SVT was concerned that they should try to build additional leadership capacity within the division, that this would be very positive. Analogous to Dr. Campbell serving as Deputy Director of Surveillance, consideration is being given to a position such as the Deputy Director of Research in the Division of Microbiology in order to create some shared leadership. Under the aegis of building capacity, is the need for a food microbiologist, an applied scientist, to further advance the good basic microbiology being done in the group. It was felt that the addition of a food microbiologist, especially a senior scientist, would suddenly develop an extraordinary communication channel within the population of food microbiologist. Some creative solutions, in light of the zero-base budget scenario, might be to identify someone

already at CFSAN that might want to take a position at NCTR. This is viewed as the most significant and critical need that came out of the SVT review.

With regard to the area of public relations and communication, Dr. Donnelly said the SVT had talked about that issue within NCTR, but that the SVT wanted to echo the need for more attempts at communicating the very good work that goes on at NCTR to a broader base, so that others within the FDA understand the capabilities and types of work that go on at the Center. The SVT sees a need for better communication in the area of methods development with the Office of Regulatory Affairs (ORA) and the Arkansas Regional Laboratory (ARL).

A unique area of strength cited as existing within the Microbiology Division is the gastro-intestinal (GI) microbiology and anaerobic microbiology capability. It is a unique resource within the FDA and the SVT believes it is an area that could be further stimulated and grown. In the area of gastro-intestinal microbiology the SVT believes that there is potential for understanding the interaction between human enteric pathogens and normal GI microflora. Again, all of the research capacity is positioned to make significant progress in that area. Another area of interest within the FDA is the effect of human probiotics on GI microflora; again NCTR has the capacity to really understand a lot of the science behind that. Other areas to be studied include dietary supplements and GI microflora interactions, the effects of antimicrobials on human GI microflora, and competitive exclusion systems for food animals. The SVT feels all the research capacity that exists at NCTR is just right for exploitation of that capacity and advancement within the FDA, and the human intestinal microflora program should remain a top priority.

Dr. Miller from CFSAN would like to see expanded research to include problems associated with plant products, fruits, vegetables, nuts, and grains, which are becoming a big focus within the FDA in terms of regulatory concerns, such as antibiotics that are used on plant products and the impact on human health of the residues entering the food chain. This helped reinforce the point made by the SVT regarding the hiring a food microbiologist.

The bioremediation program is of a very high scientific quality. But the SVT believes that the goals of the FDA would be better served if this program were redirected to examining and carrying out risk assessment studies of the by-products of the conventional bioremediation processes. They saw a real need for that type of work.

Finally, Dr. Donnelly said the group seemed to be working in very crowded conditions. The SVT noted during the review that some of the difficulties may be a direct function of the limited space and the inability to restrict contamination between samples. The type of work going on in the Division of Microbiology is very equipment intensive and there is literally no bench space left because equipment is occupying all of that square footage. The SVT was hopeful that perhaps there would be shared space available in the ARL that could help decompress the conditions.

Dr. Anderson said the SAB members were impressed with the research going on in the Division of Microbiology, but they raised a few questions about the possibility of being able to transmit some of the critical, excellent findings, specifically the results of the rapid methods developed. This information is real time monitoring information and is very useful in monitoring e.g., imported fresh cut produce and other types of products. The SVT made a recommendation that this information be disseminated as best as NCTR can to USDA and others. He said the SAB supported the need for a food microbiologist, while questioning just what a food microbiologist was. Several definitions and variations of the theme are possible. Dr. Anderson recommended that serious consideration be given to someone who already has a career impact on food microbiology, someone not only with a Ph.D. but someone who has been out in the field for 8-10 years. That can help you tremendously because that person can come in and “hit the ground running” with a complete program that is already in tune with national needs. This is not to take anything away from CFSAN, which is thought to play a very critical role in providing user insights and providing what the customers on the outside need. Lastly, some of the fingerprinting work that has been done is very useful. Here again is an opportunity for information dissemination; the Communicable Disease Center for Prevention is very interested in retrospective-type of epidemiology. Dr. Hansen (CFSAN) said she was heartened to read the recommendation and said she thought it would foster the communication between the two Centers. Dr. Anders had

an observation about the leadership development. He said the SVT talked about it mostly in the context of succession; but he would argue that leadership is a career development issue and should not be targeted at succession but to develop a cadre of leaders.

Dr. Donnelly said she wanted to say that she did not comment much about the microbiological surveillance and diagnostic support of research only because the SVT thinks that program is wonderful and the review was very positive. All of the SVT members on the review were jealous that their own institutions don't have a similar working program.

Dr. Casciano commented that this was a creative team, and noted that Dr. Donnelly asked the liaisons to participate in writing the SVT report. He asked if there were any comments on the advisability of continuing that effort. Dr. Donnelly said, she thought that anything that improves communication between the different Centers within the FDA helps, and this was a great way to engage those communications on a review level. She said the effort was very positive and that CVM had some very good things to offer in terms of commentary on the research agenda. Furthermore, that this might have been the first and only time that CVM had the chance to evaluate a research program. She hoped what would happen now was that individual protocols might be shared with scientists within CVM, instead of in the context of a research program. She said that the approach was a very positive way to help communications within the agency as well as strengthen the quality of review. Dr. Cerniglia agreed with what Drs. Donnelley and Anderson said.

Dr. Cerniglia said he had some questions, and some suggested revisions. One issue was in regards to strategic planning. Dr. Donnelly had alluded to the fact that it didn't seem like the group had a strategic plan, but in fact had worked extremely hard. He said a few of the program weaknesses were somewhat contradictory to what was in the report and they were corrected. Another issue was about rushing to publication. Dr. Cerniglia said that in his entire scientific career he had never asked anyone to rush for a publication and requested that the comment be deleted. Because these reports go many places, he didn't think that comment was reflective of the program's excellence. Dr. Donnelly said she fully supported all of the changes, some of which were just semantics. Dr. Anderson said the points are well taken as far as semantics. Dr. Cerniglia also commented on the approaches being used as far as public relations and dissemination of information. One such approach is a program document that was assembled and distributed to the officials with FDA, USDA, EPA, and scientists across the country so not only scientists but also policy makers would be familiar with the program. They are also working with the Information and Planning Group and with Dr. Jackson on a web-page and with Mr. Brand of the Federal Consortium to get this information even more disseminated. A newsletter, *Newslink*, from the Federal Consortium, has published an article on "NCTR Detection Methods Help Ensure Meat and Poultry Safety" about the food safety initiative which covers the work going on at CFSAN and other things that have gone out nationwide as well. The comment about information dissemination has been taken very seriously and measures are being taken to correct that.

In terms of the leadership comment, Dr. Cerniglia agreed with what was said. One always wants to maintain the continuity of a program and strive to maintain an excellence of work. One way to do that is to have a plan to develop your own leaders. NCTR does have leadership training available, and efforts will be made to work harder to make sure individuals attend such training, and that within the program they are given more responsibilities. NCTR has a very rigorous peer review system, and often people want to stay at the bench and not get to involved in the management aspects of a job. When it comes down to it, management doesn't count very much in this very rigorous evaluation system that we have to get people promoted. As a mentor and supervisor, Dr. Cerniglia said that he was reluctant for people to do this kind of thing, for the simple reason that you want to make sure they can get their work done and you're not abusing them. He agreed that it works both ways, having a planned program to hire someone at a senior level position while developing people within the program.

Dr. Anders commented that if your promotion system negatively rewards you for leadership activities your promotion system needs to be examined. Dr. Cerniglia said it's different in terms of scientific leadership, which rewards you quite nicely if you're working hard in your respective area and you are a scientific leader in your specific discipline.

Rather, he said that he was thinking more in terms of administrative leadership. In terms of the organizational structure of the division, Dr. Campbell leads the surveillance diagnostic program and as far as the research program, Dr. Cerniglia functions as Division Director as well as being in charge of the research. It was recommended that it would be useful if there were an additional person in available to be in charge of research aspects.

Dr. Sheehan said he'd like to follow up on our peer review system for promotion, which was established over 20 years ago, in 1978, and has since been revised at least once to try to reflect the need to have FDA impact, as well as to conduct research. He said, he thought it still was not adequate from the perspective of giving proper credit to scientists that engage in those activities, as well as in management activities. This has been, in the past, an active discouragement to individuals when they are being assessed for their scientific expertise and the quality and number of their publications. Thus, there is a reluctance when they are asked to do something outside of that scope and are not given sufficient credit for it in the terms of the number of hours they have to spend doing this kind of work. Dr. Casciano said this was true, early on we were in the publish or perish mode. When Dr. Schwetz took over the directorship he tried to instill an atmosphere of greater scientific impact, both in one's scientific field and within the FDA; so now the teams being selected for review of an individual are reflecting those kinds of events. To respond to Dr. Cerniglia, he said there is no gratification for being an administrator at NCTR.

Dr. Cerniglia commented on the building of staff capacity that was mentioned by Dr. Donnelly. He said there were two issues; one was the position, which he agreed with. He met with the staff about the report, one-on-one and he also had a group meeting. He said they were involved with the write-up of the response, and there has been good interaction. They agree it would be good to have a food microbiologist, or a good microbial physiologist with a strong food background, or an applied microbiologist, as well as some support scientist. From a space standpoint, it was comforting to have had the space problem acknowledged by independent observers. Their observations were their own, and it is clear that we really do need more space. It is also clear that we do not have a plan to acquire more space. We have established a very good relationship with the ARL and it is very nice to have people from the field working here. In the future, it should be terrific for scientific interactions with respect to some of the recommendations made by the panel, e.g. being able to test out our methods and validate and standardize these. We have been working closely with these people, but at this time they don't have space for us; rather, we've been letting them use our space and facilities to help them operate. At the same time, there is some reluctance to dedicate any new space in the new building because they don't know how much they are going to need. Dr. Casciano asked Mr. Victor (Pete) Attwood to respond to this issue.

Mr. Attwood said they were in negotiations with ORA regarding space in the new building. The lab consolidation issue in total is over a period beginning last year and running through 2014. The Kansas City and Denver labs are not scheduled to close until 2010 and 2014, and then they would consolidate here. He said the building was built to absorb all of the five consolidated labs. NCTR's concern was more than just moving into new space; he said the microbiology and chemistry facilities desperately need some renovation from environmental standpoints and otherwise. NCTR saw this as an opportunity to move into new space, and NCTR would help support the new building while we renovated our old space, he said it would be less disruptive to move than to renovate around the workspace. He said he understands ORA's position of not wanting to give up anything at this point in time, although he did point out that it would be temporary while NCTR completed renovations. He said they're pushing hard to get both chemistry and microbiology into the building, not only for the space but also to facilitate the collaborative scientific efforts.

Dr. Anderson said he would like to expand on his description of the food and microbiology position. He said he didn't think a post-doc position would solve the SVT's concern. He doesn't think they should be a recent graduate. Dr. Donnelly agreed. She said the linkages that were needed to foster good collaboration were with food microbiologists who would be members of the International Association of Food Protection, the American Society for Microbiology, or the Institute of Food Technologists. There are individuals who consider themselves part of the food microbiology community. Selecting an individual who belongs to the food microbiology community to join this group could far

advance the communication and dialog with food applications of the work that is already ongoing; it would really catapult this effort forward.

Regarding the strategic planning, Dr. Cerniglia said he would like to see a coordination of microbiology research across the Agency. Each Center has their own priorities and issues and their own critical needs. He said for us to be effective in terms of collaboration and to be effective in strengthening FDA microbiology, it is important to develop more mechanisms to set FDA research priorities. From a global standpoint there are many microbiology issues, and with a staff as talented ours we can certainly keep busy and do pioneering work.

Dr. Schwetz suggested that while they consider filling a vacancy on a long-term basis for this kind of microbiologist, they consider a short-term solution by bringing in somebody from one of the other Centers on a detail assignment. That will give an immediate bridge for someone to come to NCTR and get involved in something that may be related to what they have been doing in their own Center. Such a person could be somebody from CFSAN, CVM, or ORA with the credentials that you want who would come in and specifically work with the group; at the same time, you will get a feel from what that person could bring to the group.

Dr. Schwetz stated that one of the important Agency issues that he is helping to steer is the issue of antibiotic resistance. Geomycin was identified as an antibiotic used on plants in response to a question on that point. Dr. Schwetz questioned whether that topic is included in our antibiotic resistance plan. Since we also have exposure from plants, it would be important to understand what impact that might have? It was pointed out that this is really an EPA issue. Dr. Schwetz said that certain of the agents FDA regulates (chemicals, metabolites of drugs, or other items) that are going to enter the environment are going to come back to the EPA for additional regulation. As an example, he cited the presence of hormones in drinking water that result from therapeutic uses of hormonal drugs or their excreted metabolites. This is something we are going to have to deal with more seriously as an Agency than we have in the last few years. It is now newspaper press as opposed to an isolated manuscript, and is an issue that we should not lose sight of.

Dr. Anders said the SAB could vote to accept, modify, or reject the report or can vote to accept the amended report. Dr. Donnelly said she'd like to share the amended report with members of the committee to gain their concurrence. The changes are not substantive but rather semantics and she said she was comfortable with the proposed changes. Dr. Anders said he would entertain a motion to accept the report as amended. A vote was taken and the report was accepted. Dr. Donnelly said she'd like to add a statement to the oral record of the meeting that pertains to the Division of Microbiology review. In the written report she said they had bulleted an issue they though was very important, and she wanted to reiterate it. She said a representative from the ORA was invited to participate in the site visit and they elected not to, and similarly they were invited to at least hear the report. Again, anything that can facilitate communications, especially now that ORA is here, is important and she viewed the absence of ORA as a real missed opportunity. Dr. Anderson agreed with Dr. Donnelly's statement, stating that if the individual in charge could not be here it would have been very nice if an appropriate representative could have attended.

Future Research Directions and Opportunities

Dr. Anders asked Dr. Casciano to proceed with the topic of Future Research Directions and Opportunities. Dr. Casciano said he wanted to remind everyone of the current budget situation, and said that he would be presenting some of the requests made for FY2001 and 2002. His hope was to see budget increases considered small relative to the FDA budget but significant relative to the NCTR budget. He also wants to provide some insight what happened to the FTE allocation. We have experienced a reduction in FTEs, not only here at NCTR but across the Agency. Commissioner Henney is highly supportive of science and is interested in increasing the capacity of science within the Agency. She has requested increased funding for the science activity of the Agency and we hope to see such increases from Congress. In addition to the dollars we receive from congressional appropriations, we supplement our budget with external funding. It is important for us to maintain our interaction with the NTP and attempt to increase that support. We believe, as others do, that the NIEHS will be funded to a relatively high level for the next ten years,

so we think that stable funding via the NIEHS would also occur through the interagency agreement we have with the NIEHS.

We are going to have to find some mechanism for evaluating dietary supplements. Besides the initial discussions regarding how this would occur, we have had discussions with the CFSAN and they are promoting the analytical evaluation of these components through an agreement with the University of Mississippi and they are very interested in doing the toxicology here at NCTR. We have had very little interaction with the CBER in the past 15-20 years but that is going to change. They have asked us to lend them some intellectual resources, as well as our unique utilities here, to help them evaluate the toxicity of a variety of vaccine adjuvants. Yesterday we heard from Dr. Beland regarding photo-reactive drugs and cosmetics. Dr. Allaben is the head of our FDA Toxicology Study Selection and Review Committee (TSSRC) that nominates chemicals for NTP type evaluations and he is working hard on providing new nominations.

During the FY2001 budgeting process, an extensive amount of effort was expended that unfortunately only resulted in a $10 million increase within the Agency for research. The NCTR was allocated $500 thousand and two FTEs, and those dollars will be directed toward development of methodology to evaluate the safety of products coming to market. The Food Safety Initiative is being supported to a high degree through presidential initiatives and we have a request in for $2 million, which will primarily be funneled through activities of the microbiology program. We have a request for $1 million and one FTE for the counter-bioterrorism effort. This is being directed toward the purchase of a new mass spectrometry apparatus to define and discriminate various peptides on surfaces and intra-cellularly in various pathogenic organisms that are thought to be used in the bioterrorism arena.

In the FY2002 budget, which we are still working on, we have asked for $7 million in additional funds and 14 FTEs in the pre-market review area. To improve product reviews we have asked for $617 thousand; to reduce entry of substandard foreign products, we have requested $834 thousand; for product safety, we asked for $1.1 million; for issues of sustained public confidence, we requested $1.9 million; for science and risk assessment issues, we asked for $1.4 million; for managing food safety, an increase of $3.7 million was requested; and for bioterrorism, an increase of $1.8 million was requested. The dollars that were identified for pre-market review will be allocated in support and leveraging dietary supplements; we will participate in collaboration with the NIEHS and also in development of other toxic endpoints to evaluate the toxicity of these compounds. We are actively searching for a computational individual (government employee) to help in extending the excellent activity that has been developed through the EKB. We are also asking for a relatively large increase in funds to amplify and initiate more activity in the DNA microarray arena. We want to expand the present knowledge base capacity to include data prediction of additional endpoints and to recruit additional computational toxicologists.

In the application of DNA microarray, we have a CRADA with Genometrix where we are developing a “risk chip”. We would like to develop a consortium within the FDA in terms of developing a chip that would have DNA of interest to the FDA that may not be of interest to other organizations that are getting into this area. Dr. Cerniglia has communicated with our colleagues on using the chip for pathogen detection. We have communicated with colleagues at CBER to look for growth promoters such as cytokines that are a function of inflammatory reactions. We are very interested in enhancing our gene expression capacity here. We have a small effort that is ongoing where we are evaluating the effects of drugs on gene profiling and we would like to expand this effort. This is occurring in the genetic and reproductive toxicology arena. We would like to encourage other disciplines at NCTR to utilize this technology to begin thinking about using these DNA and RNA based technologies to predict their toxicity.

In managing food safety we have begun an effort to develop microbial dose response models and risk assessment methods. This was a recommendation put forward by the site visit team and we have plans to follow it through in a greater way. Dr. Cerniglia’s will continue ongoing studies on the mechanism of fluoroquinolone resistance as well as in salmonella species and campylobacter, as well as the continued analysis of competitive exclusion products.

Under the strategy of sustained public confidence, it is becoming increasingly apparent to the FDA and NCTR of the requirement to being transparent to stakeholders, which include the public. There is a large effort that is starting to develop regarding enhancing information processing through the Internet. The FDA understands the requirement for this and we do as well. We would like to be able to utilize some of the new funds to enhance that tool so that information would become much more available to colleagues in other Centers as well as to stakeholders within the FDA.

In the bioterrorism arena, we continue to develop novel mass spectrometry techniques to detect and identify pathogens through analysis of specific peptides. We will continue to modify PCR-based techniques to identify pathogenic microbes and generate a consortium to consider DNA chip technology to genotype various pathogen isolates.

Dr. Rosenkrantz asked if the bioterrorism work NCTR is interested in would warrant some support from FEMA. Also, in the Air Force there are a series of battle labs and there is one on chemical and biological warfare. They might therefore be interested in helping to support that work.

Dr. Casciano noted that FDA had a stake in this and are going to have to participate because of potential entry through the food supply. Dr. Cerniglia said they were working with FEMA, pointing out that there is a regional representative in Conway, Arkansas. So in this particular region, if there is some type of a biological or chemical attack… we are working closely with FEMA, the FBI, and with the CDC as the lead organization involved. We have monthly regional meetings. Dr. Rosenkrantz opined that his might be an agency to might be able to support morally on a public relations issue and they do have money for technology transfer. They may see this as a technology transfer opportunity and support it in that way. Dr. Cerniglia said we're involved from the public relations standpoint in the sense of training individuals from the Arkansas Health Department. They do not have the skills or capabilities that we have at NCTR so we've been training them in the microbiology program. They do not have money at this time, and they get very little funds to build their program; hopefully in the future that will change. Dr. Anderson said, regarding competitive exclusion, the Center certainly has a role in that particular area of research and has the expertise available. He asked if NCTR had an opportunity to contact the people in Georgia (Dr. Stan Bailey, Dr. Nelson Cox, Dr. Norm Stern) who have been working with these organisms on this particular project for eight or ten years. He said there is a need for this because there are knowledge gaps. Dr. Casciano agreed with him in terms of the importance the issue. He said we were working closely with the manufacturers involved in this product and with CVM scientists. Dr. Alderson noted it was an important area for CVM and the issue is what are the standards these products have to meet to be approved? There is a very narrow line that the FDA walks in terms of doing things that contribute to approval of new products versus development of the standard. We cannot cross over that line of developing the data that the product needs for clearance, we have to be very careful in that arena. Dr. Anders said he didn't think his comment was intended to engender that particular response, we all agree that certain standards and regulations have to be abided by. Nevertheless, there has been an amount of knowledge that has already been generated, whether the Athens group agrees with your standard or not. He suggested to Dr. Casciano, when working with campylobacter in the future, that consideration should be given to arcobacter also.

Dr. Schwetz said he wanted to speak to the source and level of outside support through IGAs and CRADAs. In the past there was a single large source of money, about $3 million, coming that was used to support a little bit of research and a large amount of infrastructure. After Dr. Allaben got the interagency agreement working with NIEHS to support NTP, FDA-related work through the NTP has built up to $11-$12 million per year. We no longer receive support from the Institute on Aging, so we are still in a situation where a large percent of our overall budget comes from outside, but again from a single source. He agreed that NIEHS does well while NIH does well, and said when you're doing well is a good time to consider what you would we do if you did not have NIEHS providing us $10-$12 million per year, considering NCTR is a $40 million a year organization. He encouraged the SAB to consider sources of diversification. He said there were a number of options to look at, and at a time when you are not in trouble is a good time to look at them.

Dr. Casciano said these discussions had begun internally and that they would be brought before the SAB. In addition to looking for other funding sources and increasing diversity NCTR is looking at what we're spending our money on, as well as whether there are programs and projects that we could wind down if we wanted to amplify in another area.

Dr. Acosta noted that NCTR has individual groups, a budget allocated for salary and operating expenses, and, he assumed, at the beginning of the year it is determined how much each of the directors will get to fund their existing projects and newly proposed projects. There is also a source of money in the Director's budget. He said that he did not have a clear understanding of the budget and allocation process. Dr. Casciano said in the last 5-6 years there was a very open mechanism for budget allocation at the division levels. There is an annual meeting with the division directors and they present their accomplishments, and where they're going the next year, including FTE and post-doc requirements. During this process they are meet with NCTR planning and budget staff, identifying specific dollar requests for projects, equipment needs, and discretionary funds. The directors hear the needs of the other divisions, learn what the competition was, and understand the rationale regarding why they got funding different than that sought. He said it is a fair system and some of the discussion is driven by what is requested for the next year. He said he was supporting DNA and RNA based technology, and molecular toxicology, and they're smart enough to understand that so they are going to come back with protocols and ideas in those specific areas. During the process they are completing experiments and generating new concepts. Dr. Acosta said in looking at the budget for the past 5 years you get the same amount from FDA, $35 million per year, each year you are reallocating from one area to another to keep within the $35 million because you are not getting any increase. Dr. Casciano said we are actually losing money, because we are not receiving current services. In essence, Dr. Acosta said, you are taking money from each division; you make a decision about who gets money and who doesn't, so when the SVT makes a recommendation to hire another person or do another project, how do you handle that? Dr. Casciano said within this process a director comes and presents the SVT recommendations and defends the position, and depending on how well he does that we respond. There is an infrastructure in place where we can increase and decrease contract support activities. This is mainly a function of what will be a generator for the type of protocols we do. We do fewer animal-intense protocols and do more *in vitro* protocols, and there are other ways of maintaining productivity; these people are very creative.

Dr. Anderson said for the future, that he did not know who is doing any research on detection of these circulating autoimmune antibodies or cytokines. Literature reviews could be done to identify who in the gerontology arena might be doing work on this. Dr. Casciano said his view was that we're all developmental biologists and what we are doing is perturbing the developmental program at different times of the development. When we expose an animal, cell, or culture we are learning to understand the effects of that perturbation on that developmental program relative to a control. Every individual at NCTR can develop a paradigm for evaluating their particular question in any part of that developmental sequence; there is no inhibition. Dr. Anderson said he realized there was no inhibition but he didn't know if anyone had determined if there was a need for any additional research in this particular area, and if so, does NCTR have a role. Dr. Casciano said NCTR had a large role in the project on caloric restriction, which was a ten year project where we looked at the effect of nutrition on longevity. He didn't think anyone had looked at a microbial endpoint/biomarker within that context; rather, the approaches have mostly been biochemical or molecular ones.

Dr. Gillette commented on the need for diversity in funding to grow the dollars in order to stay viable. She said the biggest focus should be to increase the core FDA program funding. To the extent that the FDA can increase funding to support the FDA's interest in research at NCTR, it will remain a strong organization for the FDA. Dr. Casciano agreed, saying we do animal bioassays, if attached to doing that is mechanist work that is developed by the various PI, that means the NIEHS will support a post-doc coming in to do a particular piece of work, and we have a chance to do hypothesis driven research within a particular project and a chance to look at an individual who may be a future candidate for employment.

Open Public Session

Dr. Anders requested any members of the public who were present and waned to make comment to identify themselves. Hearing none, the Open Public Session was closed.

Dr. Rosenkrantz commented that one of the risks NCTR faces is that of seeming less relevant to its own organization if it is perceived that the Center is getting so much money from source X and questions arise as to "what have you done for me recently?" Alternatively, it could be perceived that NCTR has so much money from the outside that it doesn't need any money from within. There is a real danger in relying on too much outside funding. Dr. Casciano said NCTR is only 3% of the FDA budget and they don't think about that 3% as competition.

Dr. Schechtman said he'd heard comments at this SAB meeting regarding significant progress in the different scientific disciplines, that there are protocols already available and others ready to be generated for transportability of assay systems, enabling testing that could be used elsewhere. There is a genuine opportunity here to get the word out that NCTR is doing as much as it is doing and has lots to offer the Agency and other stakeholders. Marketing being very much a part of fund raising, NCTR would be wise to take advantage of the opportunity made available through ICCVAM, the Interagency Coordinating Committee for the Validation of Alternative Methods. This committee, which is comprised of fourteen federal regulatory and research organizations, was established under the auspices of the NIEHS to provide guidance on the validation and regulatory acceptance of alternative toxicological methods. ICCVAM provides a mechanism by which to get those protocols and test systems into that domain, such that once the methods have gone through the ICCVAM process and are determined to have been validated, you can get buy-in by fourteen different federal agencies. Once that happens that will, in effect, serve as an entrée that carries that protocol or assay system into the OECD process. An OECD-endorsed protocol provides international buy-in of twenty-nine different countries and nations. The result is an NCTR test or protocol that has both national and international exposure; talk about marketing. Here you have a mechanism already in place to assess the validation status of your method, endorse it, and recommend its use. Dr. Schechtman said he was the FDA point person for ICCVAM and he encouraged all of the program directors to start looking at this system as a channel through which to start funneling their assays, their test protocols, their laboratory techniques to gain broad regulatory acceptance and implementation of their methods. This would further serve to broadcast NCTR's capabilities and attract funding as well.

Dr. Kodell commented on the need to increase core funding. The FDA is a very lean agency with 0.1% or less of the Federal Budget so that from a regulatory perspective, the work that we do is amazing. NCTR is no different that the rest of the FDA, all of the Center's are hurting for funding and they have a regulatory mission. With the explosion of these new food issues and drug therapies he asked whether Dr. Schwetz could provide any guidance as to how NCTR could move the agency forward to get a constituency to support the Center. Dr. Schwetz said Dr. Henney had done more to interact with Congress than he'd seen in previous years. There is a much greater effort for the Agency to interact with the staffers and committees and other points of contact in addition to our appropriations committees. The FDAMA, which was mentioned yesterday, makes it clear that the Agency needs to be more interactive with our constituent groups. Outreach, leveraging, and a number of approaches have been translated out of FDAMA to increase our visibility among the people we serve. The Agency was starting from a very low level to have higher visibility among the public; even though we have done a lot, we have a long way to go. We are nowhere near the success of NIH; everyone else comes in and sells themselves to NIH. One of the things Linda Suydam has done is to orchestrate the area of response to stakeholders and to emphasize leveraging. The whole budget process has been designed to earmark parts of our future growth for these areas. My reason for raising the question of diversity was only from the standpoint that much of our spending in somebody else's hands and in one agency's hands. I'm comfortable with the NCTR having that level of outside support, although it makes me nervous that if something happened at NIEHS we would be vulnerable.

The meeting was adjourned at 11:30 AM.