NCTR SAB Subcommittee Review of the Division of Biochemical Toxicology
May 12-13, 2021

The National Center for Toxicological Research (NCTR) is a research facility of the Jefferson Laboratories of the Food and Drug Administration (FDA). The Jefferson Laboratories, which include both NCTR and the Arkansas Laboratory (ARL) of the Office of Regulatory Affairs (ORA), are located near Jefferson, a rural community in south-central Arkansas approximately 30 miles from Little Rock. The Division of Biochemical Toxicology (DBT) is one of six research divisions within NCTR.
The DBT conducts fundamental and applied research designed specifically to define the biological mechanisms of action underlying the toxicity of products regulated by, or of interest to, the FDA. This research centers on quantifying the toxicities and carcinogenic hazards associated with specific chemicals and introducing new assessment techniques to enable regulatory agencies to evaluate better the risks associated with exposure to chemicals. The hazard assessment research is firmly rooted in mechanistic and exposure assessment studies focused on the understanding of toxicological endpoints, an approach that allows greater confidence in subsequent risk assessments.

Mission: To conduct fundamental and applied research designed to define the biological mechanisms of action underlying the toxicity of FDA-regulated products.
Goals: To characterize the toxicities and carcinogenic hazards associated with chemicals, specifically those of interest to FDA.
Strategies: Bioassays, mechanistic studies, and computational modeling

As part of the annual Scientific Advisory Board (SAB) review of NCTR for 2021, the DBT received an in-depth review by the Subcommittee. The Subcommittee review occurred virtually on May 12-13, 2021. The Subcommittee comprised Patricia Ganey (chair, SAB member), John-Michael Sauer (co-chair, SAB member) and subject matter experts Drs. Bodour Salhia, Cecilia Tan, and Neera Tewari-Singh. Five focus areas were reviewed: Covid-19, Dermal, Toxicological Assessments, Epigenetics, and Computational Modeling. The Inhalation Toxicology efforts were not reviewed as these undergo a separate review within the Center for Tobacco Products. Key points that the Subcommittee focused on were evidence for integration of ongoing research with the overall mission of the FDA and NCTR and the quality of the science. Strengths and opportunities were identified.

Comments for the DBT
The DBT is a productive unit that engages in basic research in important areas and provides support to FDA Product Centers. Intramural and extramural funding for the Division has been fairly steady, except for a couple of temporary decreases. The quality of research conducted within the DBT is excellent. The DBT has averaged about 50 publications per year over the last 5 years, speaking to peer recognition of the quality of science being conducted. The productivity over the year prior to the Subcommittee Review was maintained despite the challenges associated with conducting science during the COVID pandemic. How the projects fit into the larger mission was not always clear. It was also thought that some opportunities are being missed. These concerns are addressed in comments within the specific research areas and in the recommendations.

Recommendations for the DBT

- The DBT is a valuable resource for the NCTR, providing expertise to conduct both basic and applied research to evaluate biochemical mechanisms of toxicity of products relevant to the FDA. This strength is recognized within as well as outside of NCTR, as evidenced by the

important collaborations established within NCTR and with other Centers (CDER, CBER, CDRH, CFSAN, CTP, CVM) and other federal institutes/agencies and universities. There is a need to establish priorities regarding research questions and strategies to address them. This requires leadership within the focus areas to bridge communications with other units and to assess the most pressing needs.

- The DBT has experienced recent retirement of 3 key scientists. One was Dr. Jeffrey Fisher, the head of the computational modeling group. Before retirement, he had assembled an outstanding group of young scientists with expertise in modeling. This effort would be best served by appointment of a senior scientist, especially one who has safety assessment experience or to lead the program to develop its core competency and establish an integrated computational modeling-data collection program with other laboratories within the NCTR. Another option would be to provide opportunity for existing personnel to train with advisors in the regulatory programs to gain experience in safety. The two other individuals are Dr. Daniel Doerge and Dr. Mary Boudreau. The decision to replace these individuals is currently tied to future needs for pharmacokinetic studies and NTP funding issues. As mentioned in the previous paragraph, some of the focus areas would benefit from more senior leadership, and it is recommended that the voids created by departure of Drs. Doerge and Boudreau be used as an opportunity to obtain that leadership.

- Recent changes in the Interagency Agreement with NIEHS/DNTP stand to jeopardize maintenance of key infrastructure (facilities and personnel) necessary to conduct studies that support FDA Product Centers. It is recommended that a strategy be devised to prevent a disruption in service or a need to rebuild these capabilities.

- It was mentioned both during the general SAB meeting and the Subcommittee Review that the DBT could take a leadership role in providing results from animal studies that could be used to evaluate the usefulness of alternative *in vitro* and *in silico* approaches that are being adopted. It is recommended that the DBT give this consideration.

- Dermal studies conducted by the DBT are essential to expand knowledge of the extent of dermal absorption, and 3D-bioprinted human skin is an exciting new approach to evaluate skin absorption for product development and risk assessment purposes. Efforts to optimize this approach should continue.

- Efforts to develop methods using metabolically competent liver cells are viewed as valuable.

- The epigenetics effort within the DBT would benefit from an expert in epigenetics and epigenetics data analysis. The goals of the individual projects, their novelty within the field and the link to the overall mission should be articulated.

- There is the potential for the time and effort of the computational modeling group to be taken predominantly by projects in support of other Centers as opposed to research instigated within the DBT or NCTR. The DBT should decide strategically if this is the primary function of the computational modeling program.

- The objectives of the studies being conducted by the computational modeling group should be aligned more closely with the scientific questions to be addressed.

Comments for individual topic areas

Research Focus area 1 – COVID-19

The DBT should be commended for its flexibility with respective to initiating studies on COVID-19 so quickly after the identification of the issue. Two projects were presented within Focus area 1: Surveillance of SARS-CoV-2 in wastewater as a complementary tool to estimate the viral spread in Arkansas (Dr. Silva), and Flow Cytometric Analysis of Anti-SARS-CoV-2 Antibodies in Human Plasma (Dr. Fang).

Integration into FDA Mission
This area of research is a response to the COVID-19 pandemic. Efforts to understand more about the virus and its transmission are within the scope of the FDA mission.

Quality of Research
The research quality in this focus area is reasonable.

Specific comments for each project

Project 1: Surveillance of SARS-CoV-2 in wastewater as a complementary tool to estimate the viral spread in Arkansas
The first project had been underway for a year at the time of the review, and a method has been developed to detect the virus shed in feces in wastewater. The aspects of the project that are still ongoing are monitoring the dynamics of concentration of the virus in the wastewater, the impact of the vaccine on viral concentration in the wastewater and relating known and new variants in the wastewater to those identified in the community. The significance is that this method could be used to identify new cases early in an outbreak, provide information to public health officials to help contain the spread of the virus, and evaluate the effect of the vaccine on viral shedding. These are admirable goals, but there is yet no assessment of sensitivity of the assay (how many people must have the virus and how many virus units must they shed for detection). Nonetheless, the titer seems to change with social behavior (holidays, children returning to school), suggesting some utility. This approach has been used for other viruses, and it was agreed that it could be a useful tool in the public health arsenal for SARS CoV-2 if appropriate epidemiological data could be obtained.
Project 2: Flow Cytometric Analysis of Anti-SARS-CoV-2 Antibodies in Human Plasma
The second project is an effort to develop a flow cytometric method to analyze the profile of anti-SARS CoV-2 antibodies and to determine if there is a difference in viral protein recognition among the antibodies. SARS CoV-2 proteins have been isolated and coupled to microspheres. Several technical issues were encountered including those related to selection of the microspheres and nonspecific binding of plasma to the microspheres. Conceptual problems exist as well. There was no articulation of the value in knowing the immunologic profile as opposed to the currently used antibody methods. There was no suggestion that a longer-term goal could be to use results from this method to understand pathogenesis or to direct therapy. Finally, the alignment of this project with the mission of the DBT was not clear.

Research Focus area 2 – Dermal Studies

Investigators in DBT conduct both in vitro and in vivo dermal toxicity studies. These studies include in vivo pharmacokinetic assessments of tattoo pigments and cannabidiol, an assessment of the carcinogenicity of triclosan, and in vitro percutaneous absorption experiments with 1,4-dioxane and avobenzone using human skin. The novel and exciting aspect under this research focus area is analyzing the use of 3D-bioprinted human skin equivalents for assessing dermal absorption. The presentations given during the review were on Tattoo pigments (Dr. Boudreau) and Percutaneous absorption (Dr. Camacho).

A major organizational change on dermal studies could be the retirement of a senior scientist, Dr. Boudreau, who has contributed substantially towards NTP-funded studies including those on retinyl palmitate, aloe vera, aloin, and silver nanoparticles. A decision will have to be made regarding her replacement, and recruitment of a senior scientist to replace her could be beneficial to this program.

Integration into FDA Mission
Dermal studies conducted by the DBT are essential to expand knowledge of the extent of dermal absorption, bioavailability, and pharmacokinetic parameters of ingredients within cosmetics and topically applied drugs, as well as potential toxicity. These are key considerations for safety assessment and therapeutic profiles. The research conducted by the DBT is helpful for the evaluation of FDA-regulated topical drugs and cosmetics, and to ensure that cosmetics are not adulterated with detectable presence of contaminants. Furthermore, research on injected pigments used in tattoo inks will assist FDA in addressing significant data gaps and developing an approach for future testing requirements. The presentations and review reflect that comprehensive evaluation of 3D-bioprinted skin equivalents in *in vitro* skin absorption studies is a highly useful tool for evaluating skin absorption for product development and risk assessment, better assisting the regulatory needs.

Quality of Research
With qualified and experienced investigators, the research quality is high and relevant to the mission of FDA.

Specific comments for each project

Project 1: Biodistribution and Transplacental Transfer of Tattoo Pigments in Normal and Plug-Positive Female SKH-1 mice
Data were presented regarding biodistribution and placental transfer of three commonly used azo tattoo pigments in mice. The tattoos were applied to pregnant and non-pregnant SKH-1 mice with 14C-labeled tattoo pigments. This study is significant because the highest rates of tattooing is reported in women of child-bearing age, and it is hypothesized that exposure of the unborn fetus to tattoo pigments may occur via placental transfer. There is a lack of epidemiological data supporting this hypothesis, which weakens the rationale for the studies. A concern was raised that the mouse might not be the best animal model to adequately replicate pigment biodistribution in humans. Pigs or minipigs should be a considered, although the availability of pregnant pigs could be a challenge. An additional valuable area of extension for this project would be to determine what factors, including combinations of pigments, influence the distribution of these chemicals.

Project 2: Percutaneous Absorption
The skin absorption studies are highly relevant since the safety and efficacy data can help FDA product centers in their regulatory decisions. Both the projects presented are in the early stages; however, the

potential of 3D-bioprinted human skin equivalents to provide reliable skin absorption data and serve as a tool to support the regulatory mission of FDA is novel.

a) *Pharmacokinetics of cannabidiol (CBD) and its major metabolites in Sprague-Dawley rats exposed dermally to CBD*

The aim of this study was to characterize the pharmacokinetics of CBD and its major metabolites in Sprague-Dawley rats upon dermal exposure to CBD formulated in oil and cream vehicles. The data from the pilot study to determine the dose of CBD show that the serum levels of the absorbed CBD are below or close to the limit of quantification in the high CBD dose (10%). There is a concern that formulating a cream vehicle with more than 10% CBD may be challenging. The optimization of the analytical method to improve the quantification of CBD and its major metabolites will be important before proceeding for further dermal studies.

Any information on inter-species PK differences (e.g., *in vitro* skin absorption data obtained using rat and human skin) should be discussed, so that study results obtained in rats can be extrapolated to humans.

b) *Performance of 3D-Bioprinted Human Skin Equivalents for In Vitro Dermal Absorption Studies*

This is an exciting new approach to evaluate skin absorption for product development and risk assessment purposes. For this project the aims and the experimental approach, as well as limited preliminary data were presented. The preliminary data suggesting that the barrier function of 3D-bioprinted human skin equivalents may be weaker than that of native human skin could be of concern, and further evaluation is needed. It will be of value to determine the maximum time for which the 3D-bioprinted skin can be successfully cultured. The stratum corneum of the representative 3D--bioprinted skin shown appeared to be much thicker than the human skin. Since the 3D-bioprinted human skin is intended as a surrogate for estimating *in vivo* absorption, it makes more sense to compare the 3D-bioprinted skin absorption with available *in vivo* absorption data. Concordance *in vitro* dermal absorption studies using 3D-bioprinted human skin equivalents and excised human skin are planned. Although the excised human skin is currently the "gold standard" for *in vitro* skin absorption studies, the concordance between the two *in vitro* systems does not necessarily suggest the 3D-bioprinted skin is a good surrogate for predicting *in vivo* skin absorption.

Research Focus area 3 – Toxicological Assessments

As the research of the DBT centers on quantifying the toxicities and carcinogenic hazards associated with specific chemicals and introducing new assessment techniques to enable regulatory agencies to evaluate better the risks associated with exposure to chemicals, the work within this focus area is key and impacts all the NCTR. This effort has produced highly impactful publications since 2014, attesting to the productivity. Long-term efforts to understand mechanisms of pyrrolizidine alkaloid-, ricin- and abrin-induced toxicity continue.

More recent efforts were presented at the Subcommittee meeting. These include efforts to elucidate mechanisms of male reproductive toxicity caused by cannabidiol (Dr. Chen) and metformin/glyburide (Dr. Delclos) as well as efforts to construct metabolically competent HepG2 cells and physiologically relevant 3-dimensional hepatocyte cultures for evaluation of drug-induced liver injury (Dr. Guo).

Integration into FDA Mission

These studies are highly integrated into the mission of the FDA. Results of the studies provide approaches that lend confidence in risk assessments to support the needs of FDA support centers. Recently initiated studies using both *in vitro* and *in vivo* investigations into the risk potential of

cannabidiol and protocols being developed to assess the toxicities of metformin/glyburide on the male reproductive system, are notable. Developing and using metabolically competent human liver cells could lead to a high-throughput screening platform for assessing the toxicities associated with specific drugs. Drug-induced liver injury is a specific concern for the FDA, and toxicogenomic approaches will provide sensitive and predictive new safety assessment techniques.

Quality of Research

The high quality of research in this focus area is evident from the quality of publications and their support to the FDA centers.

Specific comments for each project

Project 1: Cannabidiol (CBD) and male reproductive toxicity

CBD has recently received FDA approval for use as treatment of epilepsy in patients one year and older. Data in monkeys and rodents suggest that CBD is toxic to the male reproductive system. A question being addressed is whether CBD or one (or more) of its metabolites is responsible for its toxicity. This question is relevant for choosing an appropriate animal species for further studies, as there are species differences in CBD metabolism. The plan is to use primary human Leydig and Sertoli cells and compare to primary murine cells and cell lines. Human Leydig cells are not currently available, but it was not clear whether the group plans to develop them from iPSCs in collaboration with others in the Center, as has been done for other cell types.

Preliminary studies indicated some difference in sensitivity between species. Cytoskeleton reorganization occurs during treatment with CBD, but neither the rationale for this endpoint nor the consequence relative to the toxicity was presented. There were some concerns raised regarding use of available data on the toxicity and concentration of CBD in humans and on species difference in tissue distribution.

This project would greatly benefit from better *in vitro* to *in vivo* linkages. It appears that there are gaps in understanding differences in the metabolism of CDB across species and its effect on testicular toxicity. Perhaps the development of PB/PK models could help to define the relationship between testicular cell injury and exposure (parent and metabolites), as well as better define the reproductive safety of CBD in humans. With respect to effects of CBD on Leydig cell viability, a deeper understanding of the potential mechanisms of decreased cellular growth and division needs to be sought. Although traditional cytotoxicity endpoints were evaluated, it is unclear if the pharmacological effects of CBD (receptor-mediated effects) were evaluated.

Project 2: Metformin/glyburide and male reproductive toxicity

This is a collaborative project with investigators from CDER /OPQ/OBP/DBRR having expertise in reproductive biology. Metformin and glyburide are being used off-label for gestational diabetes mellitus. There is some limited evidence from rodent studies that metformin produces male reproductive toxicity. These drugs are known to cross the placenta, but there is little follow-up in exposed infants. The plan is to use an animal model of human gestational diabetes mellitus. The inclusion of a pilot study to evaluate the effectiveness of the dosing strategy is important. The pilot toxicity is well outlined with vehicle, metformin, glyburide groups but there were no results as of the time of the review. Furthermore, the design of the pilot study should ensure, as much as possible, the potential for translation to humans.

Project 3: Metabolically competent liver cells

Drug-induced liver injury is an insidious problem and challenge to pharmaceutical companies and FDA. Modalities with which to study mechanisms are lacking. A reproducible, high-throughput model would

be valuable. The aims of this project are to evaluate the stability of established metabolically competent HepG2 cells, construct new cell lines that co-express multiple CYPs, and establish a high-throughput screening platform. The project is ambitious and has potential for high impact. A key will be to engage the pharmaceutical industry to use these platforms early in development. The outline of study aims presented to assess drug-induced liver toxicity using this new approach was reasonable.
However, an important aspect to this research should be evaluating the comparability of HepG2 cell model to currently used *in vitro* systems (including those under development) for regulatory decision making. The application of the proposed system in the pharmaceutical industry will require easy access to the model, as well as acceptance of the system by the FDA

Research Focus area 4 – Epigenetics

A major theme within the Division of Biochemical Toxicology has been to investigate the importance of epigenetic alterations in various toxicities and diseases. Three presentations were given during the review. One major area of emphasis has been to elucidate the genomic and genetic determinants associated with nonalcoholic steatohepatitis (NASH) (Drs. Pogribny and Tryndyak). A second area of research has been to assess the epigenetic effects of nanoparticles (Dr. Hammons). A further area of focus has been to understand the role of epigenetic changes in triple negative breast cancer and lupus (Dr. Cook).

Integration into FDA Mission
There is no question that epigenetics plays a critical role in human health and disease, and the decision to include this field of study in the Division is essential to support NCTR's mission to develop translational research approaches that provide the FDA with science- and data-based methods to improve public health. Some concerns include the broad nature of the topics, which are loosely tied to the mission of the both the Division within NCTR and the FDA. The goals of the research need to be better defined as they pertain to the mission. For example, how the specific epigenetic findings support the mission of the FDA and translate to improve human health should be articulated as opposed to "we need better biomarkers for NASH or LUPUS".

Quality of Research
The overall quality of the epigenetics research within the division is fair and addresses broadly important questions. However, a major concern stems from the lack of strong epigenetics expertise in the division which can be noticed in the publication history. The lack of expertise was also evident from the data and study designs presented and from questioning during the presentations where there was insufficient information provided on the methodologies and analytical methods used to study DNA methylation or other epigenetic changes. The division would benefit from an expert in the field.
The program would also stand to benefit by having clearer and more focused research goals. In particular, the program to understand the role of epigenetic changes in triple negative breast cancer and lupus was too broad and lacked focus. Lastly, the research questions being proposed in epigenetics were by and large not novel and have been widely studied and reported previously in the literature.

Specific comments for each project

Project 1: Non-Alcoholic Steatohepatitis: Genomic and Genetic Determinants of the Susceptibility to Nonalcoholic Steatohepatitis in Mice* and *Development and Evaluation of a Novel In Vitro Noncoding RNA-Based Screening Model System for the Hazard Identification of FDA-Regulated Products

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the USA affecting of 18.8-40% of the general population. The number of NAFLD cases is projected to increase by 21% by 2030 in the USA. NASH, an advanced form of NAFLD, is the fastest growing cause of hepatocellular carcinoma (HCC) in the USA. Molecular mechanisms of NAFLD heterogeneity and progression of NAFLD to NASH are not completely understood. There are currently no FDA-approved pharmaceutical therapies or biomarkers for NASH. This makes NASH an important area of study for the FDA.

Current findings from a study performed to identify phenotypic and epigenetic changes in NAFL-resistant and -prone mice were presented. Several studies have already been published on NASH and DNA methylation. It will be important to highlight what is new about this study and how it will contribute to our broader understanding that would enable better therapies or biomarkers.

The use of Collaborative Cross (CC) mice, a high-diversity mouse population, to investigate strain- and sex-related differences in susceptibility to NAFLD and its progression to early NASH is a strength in the study but it was unclear how the investigators planned to differentiate between susceptibility of differentially methylated regions (DMRs) vs those induced by specific diets (high fat/high sucrose, HF/HS) used in the experiment. The data need to be interpreted to address the main point of the study which is understanding predisposition to NAFLD/NASH.

Pathway analysis is useful but a more in-depth analysis of DMRs is important to decipher the meaning and impact of DMRs after HF/HS diets in mice and also male/female differences.

The lack of human samples is viewed as a problem, especially given previously published data. Different data measurements (RNA, methylation, etc.) need to be integrated. It was unclear which genetic measurements as stated would be studied? Only epigenetic changes are measured. In addition to liver tissue analysis it could be useful to also measure other tissue types collected (e.g. muscle) from mice.

Project 2: Nanomaterials: Assessing Epigenetics Effects of Nanomaterials in Human Cells

Most studies examining the effects of titanium dioxide have been primarily conducted based on nanoparticle models such as P25. In contrast to food grade titanium dioxide, these nanoparticle models are strictly nanosized. Given that it is food-grade titanium dioxide that is most used in FDA regulated products, and its epigenetic effects have not been characterized, the proposed study builds on and extends on previous work to provide an assessment of epigenetic effects of exposure to this nanomaterial in cellular systems.

Overall, this study aligns well with the mission of the FDA and the Division. One concern is that it does not address the potential impact of epigenetic alterations in response to titanium oxide exposure. Are the changes deleterious or passive? It might be useful for this reason to also add gene expression changes.

The methods proposed for epigenetics are outdated and have several limitations. For example, it is recommended that a BeadArray or sequencing based approach be used for DNA methylation in lieu of a PCR array. Understanding the effects of titanium oxide on human tissues will be necessary and valuable.

Project 3a: The Role of Epigenetic Mechanisms in Re-Expression of ER, PR, and HER Receptors in Triple Negative Breast Cancer: Effects of Vorinostat and Indole-3-Carbinol

The purpose of this study is to enhance the understanding of epigenetic regulation of triple negative breast cancer (TNBC) and expand the paradigm of treatment of current FDA-approved targeted therapies for this subtype of breast cancer. Studying the potential for epigenetic therapies in the treatment of TNBC is valuable. One concern is that not enough due diligence was conducted to understand this space. Vorinostat and other histone deacetylase inhibitors have been widely studied in TNBC, and clinical trials have been conducted as well. Re-expression of ER and HER2 have been reported previously after vorinostat treatment in TNBC. These findings were not acknowledged in the study design presented. The study needs to define its novelty and improve scientific rigor given previous published data.
Only cell lines studies are outlined which will offer very little in terms of impact.

Project 3b: Epigenome-Wide Association Study of Peripheral Blood Mononuclear Cells in Systemic Lupus Erythematosus: Identifying DNA Methylation Signatures Associated with Interferon-Related Genes Based on Ethnicity and SLEDAI

Systemic lupus erythematosus (SLE or lupus) is an unmet medical need; despite the progress made with managing the disease symptoms, the etiology remains unknown. A potentially important study was described looking at a methylation signature in SLE between African Americans and European Americans. A better understanding of how and why the selected markers were chosen should be demonstrated. Simply filtering beta values in BeadStudio is inadequate and incorrect for selecting regions even with a Bonferroni-adjusted value. What methods will be used to conduct the proposed studies outlined in Aims 1 and 2.

Recommendations for the Epigenetics Research Focus area

1. **New technologies/approaches that should be considered**

   Consulting with or hiring an expert in epigenetics should guide the choice of methods in DNA methylation and histone analysis. This is especially important when trying to design and interpret results. Bisulfite-based methods are best in class with Illumina BeadArrays or sequencing being the most widely used. Choice of assay, analytical approach and data interpretation of epigenetic results would be greatly improved by aligning with domain experts.

2. **Areas less relevant to the FDA's public health mission**

   All research projects have the potential to significantly contribute to the FDA's public health mission if the deliverables of the research are clear and milestones are laid out.

3. **Recommendations**

   - An expert in epigenetics along with expertise in epigenetics data analysis is needed for this program. If hiring such a senior scientist is not a feasible option, providing opportunities for the investigators to train with senior advisors will be beneficial to the program.
   - Access to patient samples and animal models will improve translational impact of many of the proposed studies. Facilitating access to these resources via academic collaborations will be useful. Alternatively, commercial sources are available.
   - Since biomarkers are proposed for a few projects it would also be prudent to partner with experts who are capable of designing biomarker studies and potentially credentialing such biomarkers for clinical use.

Research Focus area 5 – Computational Modeling

One of the major focus areas within the Division is developing computational tools to integrate available data for safety assessment. Five presentations were given during the review, including a physiologically based pharmacokinetic (PBPK) model for nicotine (Dr. Mehta), a generic peri-natal PBPK model (Dr. Li), an artificial intelligence pregnant woman modeling suite (Dr. Fairman), collaborations related to modeling activities (Dr. Lumen), and an approach to select complex mixtures for toxicological evaluation (Dr. Gamboa da Costa).

A major organizational change to the computational modeling program is the retirement of a senior scientist, Dr. Jeffrey W. Fisher in 2020. The pharmacokinetic modeling program established by Dr. Fisher has grown, and the program has demonstrated its ability to establish effective collaborations with internal and external partners, as well as secure extramural funding. While the computational modelers are well-trained and skilled, the program would benefit from recruiting a senior scientist who has safety assessment experience or training with advisors in the regulatory programs to gain experience in safety assessment.

Integration into FDA Mission

The computational modeling capability in the Division is essential to support NCTR's mission to develop translational research approaches that provide FDA with science- and data-based methods to address regulatory questions. The computational modeling program is uniquely poised to integrate toxicological, mechanistic, and pharmacokinetic data generated within or outside of NCTR for safety assessment. At the same time, this program can also help testing 'what if' scenarios using modeling tools to guide better design of experiments. Since there are only four computational modelers in the group, the FDA will benefit more from this program if this modeling group can develop a core competence/product. From the review, it appears that PBPK modeling for pregnant women and during early life stage is a niche for this modeling group. If the computational modeling group can develop a modeling suite, with a modular design and capability to customize, for predicting pharmacokinetics of substances in pregnant women, fetus, and infants, this product/modeling competency will facilitate FDA approval/review of safety and efficacy of drugs/chemicals.

Quality of Research

The quality of the computational modeling work is high. The computational modeling program has several highly qualified computational modelers.

A major concern about some of the research efforts is not about the quality of the model, but about the purpose or design of the research. For some of the presentations, it is not clear how the computational models address the science questions being raised as motivation for the research. It is recommended that all research projects start with problem formulation to ensure that the scientific question(s) are clear and specific, and that the computational tool being developed can be used address these scientific questions.

Specific comments for each project

Project 1: Multi-pathway PBPK model for nicotine in humans

The value of a PBPK model in refining human health risk assessment lies in the ability to predict the delivered dose available for interaction in the tissues, given the scenario that is relevant to human exposures. In this project, a respiratory tract compartment is included in a PBPK model to simulate a route that is important and relevant to human exposures to nicotine. However, it is not clear how the

model outputs will be linked to toxic effects or addictive properties of nicotine. As stated in the background document, the motivation for developing a multi-pathway PBPK model for nicotine in humans is to support the FDA's plan to consider lowering nicotine levels in cigarettes to "minimally or non-addictive levels", thus decreasing the likelihood of future generations becoming addicted to cigarettes and allowing currently addicted smokers to quit. However, there is no discussion on how to use the PBPK model to predict an internal dose metric that is relevant to addiction endpoints. For example, if dose-response studies for evaluating nicotine addiction are available in animals, an internal dose associated with an no effect level in animals, which can be predicted using a PBPK model for that test species, can be used as the basis for estimating an equivalent no-effect level in humans. Also, in the background document, it states that "knowledge of the internal dose in tissues, such as liver, brain, and kidney can in turn provide additional, robust science to inform regulatory activities", but there is no information provided on which toxic effects are related to the internal dose in liver, brain, and kidney or how the predicted internal dose in tissues will be used to "inform regulatory activities".

Same comment applies to Specific Aims 3-5; the proposed research activities stop short of explaining how model predicted outputs will be applied to inform safety assessment of tobacco use. For example, will the predicted distributions of nicotine concentrations in tissues be used to estimate a minimally or non-additive level (Specific Aim 3)? Are there any data that identify common toxicologic effects from nicotine and other tobacco constituents, or is there any evidence that shows kinetic interactions (such as competitive metabolism) between nicotine and other tobacco constituents (Specific Aim 4)? Furthermore, how can predicted intake of nicotine based on metabolite biomarkers "extrapolate the dose-response relationship established in experimental animals to humans" with only the human model (Specific Aim 5)?

Project 2: First-generation in-house PBPK model-based tool

The proposed in-house perinatal life stage PBPK model, potentially with a graphical user interface, will address the current challenges related to model transparency and accessibility. This tool has the potential to be the core product of the computational modeling program in DBT to support various Centers in the FDA when internal dosimetry predictions are needed for pregnant and pediatric populations.

Project 3: Artificially intelligent virtual pregnant woman modeling suite

The description of the study design requires more detail. It is unclear whether artificial intelligence and machine learning (AI/ML) are proposed to inform the selection of influential model parameters, based on specific drug class, to be included in a PBPK model; or to predict some PK data (e.g., AUC, plasma conc over time?) or some PK changes (e.g., fold changes in AUC?) during pregnancy for either a specific drug or a specific drug class. It is also unclear if the purpose is to replace PBPK models with an AI/ML informed "virtual pregnant women platform" or to support the development of PBPK models. Finally, this pregnant woman modeling suite is proposed to solve the problem that developing a pregnancy PBPK model for one computer at a time is time- and labor-intensive. This objective can also be achieved with the proposed in house PBPK model-based tool. A joint effort between the two projects could result in a better product.

Project 3: Computational modeling collaborative projects

The five projects showcase the program's capability to offer a wide range of computational modeling support to various FDA Centers and initiatives.

Project 4: Selection of complex mixtures

It is unclear how this project is related to computational modeling. If computational modeling approaches are used to merge analytical data, the details of such approaches are not provided in the presentation.

Recommendations for the Computational Modeling Focus area

1. **Recommendations for the New technologies/approaches that should be considered**
   The computational modeling program has done a good job using technologies/approaches that are appropriate for the needs of different research projects. For example, software platforms, such as Multiple-Path Particle Dosimetry model, are used to predict the deposition of nicotine in respiratory tracts; an open-source programming language, R, is used to develop an in-house modeling platform that is transparent; AI/ML are considered to predict pharmacokinetic changes during pregnancy; and a proprietary PBPK modeling platform, Gastroplus, is used for *in vitro* to *in vivo* extrapolation. Given that some of the projects focus on developing generic modeling tools, more *in silico* tools (such as the Gastroplus' ADMET predictor) that can be used to generate physio-chemical properties and ADME parameter values for PBPK modeling can be explored, especially the open source options. Also, cheminformatic tools (such as structural similarity analysis and classification algorithms) that can be used to generate early ADME predictions can be incorporated into the various modeling suites that are being developed in the program.

2. **Areas less relevant to the FDA's public health mission**
   All research projects have the potential to significantly contribute to the FDA's public health mission, if the modeling work can clearly link to human relevant exposures, human relevant toxicity/drug efficacy, or both.

3. **Recommendations**
   - A senior scientist is needed to lead the program to develop its core competency and establish an integrated computational modeling-data collection program with other laboratories within the NCTR. If hiring such a senior scientist is not a feasible option, providing opportunities for the investigators to train with senior advisors who use computational modeling in drug safety/chemical risk assessment will be beneficial to the program.
   - Problem formulation is an important first step that is often ignored. Currently, several research projects are technically strong, but modeling efforts do not necessarily answer the science questions that need to be addressed. These modeling efforts will have a much higher impact if the investigators have a clear and specific scope/purpose and testable hypothesis prior to deciding which model design is the most appropriate for the intended purpose.
   - The investigators have demonstrated their ability to support a wide variety of computational modeling needs from various Centers within the FDA. One potential concern is that their success in these collaborations may lead to many smaller modeling projects that take up too much of the investigators' time. It depends on how DBT envisions the primary function of the computational modeling program. If the primary function is to offer modeling services to the FDA, then the investigators can focus on marketing their modeling capabilities. If the primary function is to develop modeling tools to fill an important gap (such as pregnancy PK modeling), then the investigators should be given adequate resources, including time, to contribute to the development of a core product.