NCTR SAB SUB-COMMITTEE VISIT
DECEMBER 4-5, 2019
REVIEW OF THE DIVISION OF NEUROTOXICOLOGY

Subcommittee Review of the Division of Neurotoxicology

Two members (Drs. Michael Aschner and Gregory Lanza) of the NCTR Scientific Science Advisory Board (SAB) participated in the review. Drs. Aschner and Lanza served as Subcommittee chair and co-chair, respectively. All other reviewers participated as subject matter experts in content areas of interest to the Division of Neurotoxicology. These were Drs. Pat McConville (InviCRO), Edward Levin (Duke University Medical Center) and Wei Zheng (Purdue University).

Comments Specific to the Neurotoxicology Division

Overall, the members of the NCTR SAB Subcommittee appreciate the leadership Dr. Sherry Ferguson brought to the Division of Neurotoxicology (DNT) the past 3 years. The DNT continues its tradition of excellence in research and discovery, the foundation laid by Dr. William Slikker and his successors. It is deemed that work carried out in the Division of Neurotoxicology is well aligned with the US FDA mission, with major translational value for regulatory decisions. It was also apparent that ongoing collaborations with other FDA Centers are informative to regulatory decision processes. The Subcommittee acknowledges that internal and external collaborations are invaluable for the NCTR, and should be pursued as much as possible when advancing future research programs.

Specific recommendations for DNT as a whole include the following:

- DNT continues to perform an outstanding service to NCTR, the FDA and the field of neurotoxicology in general with its research programs to not just test toxicant exposure effects on neurobehavior function, but to develop a better understanding of the key processes of neurobehavioral function that are vulnerable to disruption by toxicants. This strategy provides immediate answers concerning the individual drugs and chemicals under study, but more importantly builds a foundation of understanding to facilitate neurotoxicology investigation going forward.
- It is recommended that DNT continue to integrate neurotoxicological investigation across levels of analysis from in silico and in vitro models through, invertebrate, aquatic and rodent models through nonhuman primate (NHP) models to human studies. This is a current strength but could be enhanced by collaboration with researchers using invertebrate models such as C. elegans or drosophila either within or outside NCTR.
- It is important that DNT have the leeway to balance between providing service studies for specific toxicity questions for the FDA and more fundamental studies to advance the

field of Neurotoxicology and facilitate future investigations. This should provide an optimal approach to protect the public against neurotoxic risks.

- Increased drug applications for treatment of brain diseases such as Alzheimer's Disease (AD), Parkinson's Disease (PD), attention deficit hyperactivity disorder (ADHD), and traumatic brain injury (TBI) require FDA to develop the means or standards (to be included in drug application dossiers) to assess the brain availability of any given CNS drugs. The time is ripe for launching a highly interdisciplinary group within NCTR with a unique focus on the blood-brain barrier (BBB). The underlying rationales are multifold. First, the **vision** of this BBB group shall be a pre-eminent world-class research group embracing the conceptual research and practical evaluation of CNS drugs and its impact on human brain health. The **mission** is to discover, create, and implement knowledge in BBB biology, physiology, and methodology for accurate assessment of CNS drugs' efficacy and safety in order to better treat brain diseases and disorders, and in the meantime to protect against neurotoxicities. The task of this BBB group fits well with the FDA's central mission in drug review and post-market evaluation for better efficacy and better protection. Second, the DNT has the skilled investigators in the BBB research area including Syed Ali, John Bowyer (retiring), Sumit Sarkar and postdoc fellow Andrew Shen. Their personal interests in the BBB and therewith the drive is essential to the success of this interdisciplinary research entity. Third, there is an extensive technical knowhow in the DNT. The Division has already established several in vitro models to mimic the BBB and the equipment to assess permeability. Finally, DNT has the existing high-end facility readily applicable for BBB research. BBB permeability can be evaluated by DCE-MRI or DCE-CT; the BBB can be modeled by induced human stem cells; and the knowledge from vasculature research can also be applied to other cardiovascular drugs. The current research led by Dr. Ali has built the sound ground; but more is needed in human resources. The BBB group is not only built for screening and testing of CNS drugs by using in vivo, in vitro and in situ models, but also for understanding the mechanism by which a potential drug works in treatment of brain diseases. For example, a new anti-AD drug, abucanumab, has shown promise in clinical trials; yet how this large-molecule-weight antibody comes across the BBB and how the resulting conjugates of the drug with fragments of amyloid plaques are removed from the CSF are completely unknown. The in-house testing would add a great strength in FDA's review and approval of this potential impactful drug.
- Reviews of DNT research projects (i.e., protocols) seem to be conducted within the Center and approved by the senior leadership. It will be beneficial to make use of the Subcommittee members of the SAB as the external peer review to comment on the strengths and weaknesses of the submitted protocols.
- It is unclear what is the rationale to conduct the gender differences in nicotine by using MRS. This project, while relevant to FDA tobacco regulation, does not seem to have a significant impact on the product *per se* or its regulation.

Review of Specific Research Areas:

Research Area-1: Imaging in Neurotoxicology (Liachenko, Zhang)

The Bioimaging core consists two PIs providing their services to five areas of investigation, including Drug Safety and Biomarkers, Drug Addition, Drug Delivery, Translational Imaging, and Mechanisms of Toxicity. There are four major projects in this core's research portfolio, i.e., gender differences in neuronal reward circuit activation by nicotine and tobacco smoke using MRI led by Dr. Liachenko (E751001), Inveon microPET/CT and its application to in vivo monitoring of neuronal apoptosis led by Dr. Zhang (E758601), functional correlates of gadolinium deposition in the rat brain led by Liachenko (E762501), and neurotoxicity assessment of desflurane in neonatal rats led by Dr. Zhang (E768001). Dr. Liachenko focuses on MRI, while Dr. Zhang focuses on PET/CT and each of these two PIs appears to have a limited technical staff of 1-2 personnel to assist with scanning and related tasks.

1. Integration into FDA Mission

The Imaging in Neurotoxicology research area is primarily involved in discovery, development and application of imaging biomarkers that have the potential to identify and quantify neurotoxicity through non-invasive imaging. The potential impact of the imaging research and services within NCTR is significant and unambiguous. The overarching goal of this research is to provide significant improvement to what is already available (clinically) by increasing efficiency, sensitivity, accuracy or applicability using novel imaging biomarkers. The research fits directly within the FDA mission by aiming to improve public health and also by developing technologies that facilitate FDA approval/review of safety and efficacy of novel agents, especially in terms of potential neurotoxicity of novel agents and devices.

2. Quality of Research

The quality of the research is high and the imaging equipment impressive. Medical imaging modalities provide unique noninvasive approaches to understand the actions of drugs or toxicants on human tissues and organs, particularly the brain. DNT possesses the state-of-the-art imaging equipment and highly qualified technical personnel.

The MRI capability is particularly mature and diverse, and has leveraged the strengths of MRI across a number of impactful projects. The PI (Dr. Liachenko) is a recognized researcher with strong track record in MRI and has a strong understanding of the challenges of translating technologies to the clinic via FDA approval, and related pitfalls of related safety issues. Dr. Liachenko has pioneered the application of the MR brain T2 relaxation time as a marker of neurotoxicity and comprehensively shown its validity across a broad range of toxicants. He has also explored its ability to delineate the brain regional location of neurotoxicity, aiding the

knowledge of mechanism. Work of note includes T2 based imaging of neurotoxicity associated with:

- Pediatric anesthesia
- Gadolinium contrast agents
- Iron nanoparticles
- Nicotine/tobacco

Areas outside of the brain that have been examined include liver toxicity, cardiac toxicity and mitral valve imaging.

The MRI work has led to new developmental work associated with:

- MRI super-resolution
- other MR based biomarkers that may be used to measure neurotoxicity including metabolite concentrations quantified through MRS
- application of the T2 biomarker in AD and PD diseases

The PET/CT imaging program is newer and in a more developmental stage. Despite this, good progress is being made in PET research, driven predominantly by a fruitful relationship with Marc Berridge and/or 3D Imaging that provide radiochemistry for compounds being imaged at the DNT/NCTR. Focus so far has been predominantly on apoptosis imaging agents and related SUV based quantification of anesthesia associated apoptosis in rat and NHP brain. This includes 18F-FEPPA, 18FAV133, 18F-FESP, and 18F-FPCIT, an impressive array of novel PET compounds. PET based apoptosis imaging has also been applied to examine neurotoxic side effects of methylphenidate (MPH), and 18F-FDG has also been used in this regard. The quality of the imaging data is high and Dr. Zhang has done a great job of finding potential 18F labeled compounds that can be used to detect and image neurotoxicity, as well as seeing them into production by a third party for her facility.

Both the MRI and PET labs are predominantly focusing on broadly applicable, but relatively non-specific biomarkers. This has the advantage of greater potential for use and positive signals stemming from neurotoxicity. The disadvantages of this approach are that there are broad non-specific mechanisms that could affect or drive the signal modulation. For example, water content, density change, edema, necrosis, apoptosis, and blood flow changes could all affect measured T2 and could in certain cases diminish sensitivity through opposing effects. PET based measurement of apoptosis will depend on BBB penetration of the compounds, blood flow, necrosis and apoptosis and the approach has similar limitations to T2. These limitations will likely limit the ability to understand the mechanisms of apparent neurotoxicity detected through their application.

Despite its limitations, the T2 biomarker is approaching potential FDA qualification. This represents a tremendous achievement. In addition, several end points, such as apoptosis applications have become a standard in imaging, and they are a relatively easily applicable platform for PET-based assessment of neurotoxicity. Studies carried out by the several research groups in DNT could incorporate such validations to their current and future programs.

3. New technologies/approaches that should be considered

Expanded image analysis and access to analysis expertise and hardware/software. The imaging core has become relatively mature in terms of it establishing foundational biomarker platforms for routine application into almost any area of FDA need regarding neurotoxicity. The resource would now strongly benefit from enhancing its image analysis capability. As of now, analysis is handled by the PIs, which both limits new imaging applications they could be exploring and limits the return and power of the data, since there are sophisticated tools that are standard in image analysis that would improve the efficiency and quality of the end points from imaging studies.

The lab appears to be lacking industry standard analysis software and hardware for storage, image reconstruction, backup, archiving, visualization and quantification. Commercial products and/or external collaborations will need to be leveraged to realize the full potential of the imaging technologies. This includes examination of the potential of machine learning/AI. The volume of data from the MRI lab and, it seems soon, the PET lab may lend itself to AI approaches. The imaging core is generating valuable data which can be used in machine learning and in developing AI models for prediction. In particular the T2 biomarker research with an intended scalable approach is likely to generate large datasets, which are ideal for machine learning (ML) and AI modeling and prediction. In order to do this, it is recommended that the PIs look to collaborate with existing labs that have ML expertise.

PET/CT biodistribution imaging. This is a very large area of translational imaging and a large area of focus in many PET/CT imaging labs. It would not take a larger effort or expense to expand PET imaging applications in the DNT imaging core to include longer lived PET isotope based labeling, and in vivo tracking and biodistribution imaging. While this has been most prominently used for larger molecules that are not brain penetrant (e.g. antibodies), it has also been used extensively for neuro-targeted compounds that are brain penetrant or become so in a disease setting. It is also commonly used to examine non-specific brain accumulation (and related neurotoxicity) of compounds that are targeted elsewhere in the body. We encourage DNT to examine potential here under the FDA, NCTR and DNT missions, as this would be a potentially impactful way of leveraging the impressive PET, and external radiochemistry capabilities that are already in place.

AD/PD Research. To the extent that AD/PD disease based research is a priority for DNT, it is recommended that the PET/CT imaging lab examine the 3 FDA standard approaches for a-beta PET based assessment of disease progression. This is an area where limitations in these applications are limiting the FDA's ability to review and approve new AD therapies. New PET tracers have great potential, and the NCTR imaging lab may be able to play a critical role in examining some of those coming along, such as those targeted to tau protein (AD) and in the PD setting, alpha-synuclein. This would complement the work being done in the MRI lab and by other PIs such as Dr. Sarkar in DNT. Similar comments pertain to TBI, where DNT has an area of

focus. MRI and PET can facilitate AD, PD and TBI research being done in other labs at DNT, and this should also better leverage internal collaborations.

Blood-Brain Barrier research. The BBB is critical to neurotoxicity and related protection and drug delivery and has very direct and well-established applications in MRI and CT in particular. These applications will also directly aid the development of imaging biomarkers which are often BBB dependent (notably including the T2 and apoptosis applications which rely on delivery of toxicants and/or imaging agents). It is recommended that BBB imaging platforms and/or applications be considered and implemented. This could provide a valuable platform for the NCTR and the FDA.

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

Exploration of disease mechanisms are arguably less directly related to the FDA mission (e.g. the imaging work being done in AD and PD), though notably it is relevant to the general mission of the FDA to improve public health. Therefore, it is recommended that the NCTR, DNT and imaging core consider and re-examine priorities in this regard. To the extent this area of research remains a priority, the imaging core should focus on method development to support research by other groups (both at FDA and at NCTR and DNT), and less on self-driven mechanistic studies. Especially for projects exploring disease mechanism, imaging projects should almost always have a collaborator at DNT who is providing support for the disease biology and/or therapeutics being examined (and correlative data generation in vitro, and ex vivo), with the imaging lab focusing on the imaging technologies, platforms and applications.

5. Recommendations

- There are positive indications of collaborations between the imaging core and other DNT and NCTR labs (and also with the FDA centers). This is to be commended, and there have been significant improvements in recent years of this integration as outlined by the DNT Division Director, Sherry Ferguson. However, further enhancement and scaling of cross lab collaborations at DNT, secondarily NCTR and also with FDA centers should be a major priority.
- It is noted that CDER is highly active in support of MRI collaborations, and it was not clear which centers support PET imaging projects. It makes sense that CDER will be one of the more prominent sponsors for imaging research, but it is also encouraged that the imaging PIs proactively assess and discuss immediate and evolving needs of the other centers and potential collaborations to address these needs.
- The resource and personnel/FTEs in the imaging labs do not match the level and scale of hardware resource (4 impressive imaging systems) and is not sufficient to fully leverage the lab's potential. Currently there are two PIs and three supporting staff in the imaging core and minimal analysis and data handling resources. While there is one new hire on the way (MRI) it is recommended that personnel resource be examined. It may be possible to see successful transfers of staff from other labs or sharing of FTEs between

labs as a method of addressing this without as negatively impactful an expense for NCTR.

- It is expected that the level of data generation even with the existing staff will soon outstrip the ability to keep it well annotated, organized and archived. These issues should be included in immediate and future planning. Some or maybe even most of this issue could be addressed by collaborations with other labs and/or purchasing of relatively inexpensive platforms for data handling.

Research Area-2: Neurotoxicology Research on Alzheimer's Disease, Parkinson's Disease, and Traumatic Brain Injury (Sumit Sarkar, Syed Imam, Hector Rosas-Hernandez)

This research area is relatively new for DNT; but it has been focused and off to a commendable start, and the results have led to significant accomplishments in a relatively short time period. The core has four PIs (Drs. Syed Ali, Sumit Sarkar, Syed Imam, Hector Rosas-Hernandez), currently conducting research in areas of AD etiology (serum exosomes, transgenic mouse model of AD, and Aβ clearance and vascular dysfunction), neurotoxicology of PD (nicotine-Nano therapy, and lipid malfunction as PD biomarkers), and traumatic brain injury (TBI) (in vitro stretch model of TBI and BBB injury, and early TBI with later development of brain disorders). Researchers in this core have developed unique techniques suitable for the stated projects. Their past work has primarily focused on rotenone and MPTP, both of which are dopaminergic neurotoxins that may be used to model PD. More recent studies on PD have shifted to therapy and biomarkers, which meet well with FDA's mission. In addition, the core has invested aggressively on AD etiology, by working on transgenic rodents strains to study chronic neurodegeneration. Finally, the core has also incorporated new studies on TBI. Expertise in the various techniques are on site, and the topics studied are timely and meritorious.

1. Integration into FDA Mission

The research into processes of neurodegeneration and their treatment is key to the mission of the FDA. The investigations into the biological processes of PD, AD and TBI advance the FDA evaluation concerning drug safety and efficacy with more than just individual tests of each drug put forward, but rather better understanding of the neurobiology underlying the impairments and how they can be well treated with minimal side effects. In particular, the vascular dysfunction is known to underlie all three diseases that are the focus of this core. Thus, an in-depth research effort and therewith the investment in this area, i.e., neurovascular biology, physiopathology and toxicology along with in vivo and in vitro models and assisted with various imaging modalities, are well warranted.

2. Quality of Research (approaches/methodologies) – includes strengths and opportunities

The quality of the science in this core is quite high in several aspects. First, the core has made several novel discoveries, such as serum exosomes as a potential biomarker for AD, increased BBB permeability under the condition of cerebral amyloid angiopathy, and c-Abl activation in Parkin-mediated PD etiology. Second, the core has developed the critical in vivo and in vitro models for their next phase of research and discovery. These models include Tg-SWDI mice and F344-AD Tg rats, in vitro bioaxial stretch model of TBI, microelectrode array (MEA) for neurotoxicity screenings, and microphysiological system (MPS) for BBB modeling. Finally, the core has established a collaboration with the imaging expertise in Research Area-1 to study lipidome and peripheral biomarkers of PD by using 3D-MALDI IMS and SeleXion Lipidyzer. These studies have taken advantage of existing cores within the Division. The studies include an array of techniques and are deemed to be of great translational value.

3. New technologies/approaches that should be considered

The relationship between behavioral function, mechanistic imaging and molecular mechanisms could be more thoroughly investigated and cross-talk between the individual programs should augment the return on investments.

More specifically, research on transgenic mouse and rat models should not be limited solely on vascular pathology or microbiome, but include also neurobehavioral assessment of the changes in typical AD phenotypes. Using the MRI and/or CT to assess the disease onset or progression, and efficiency of any potential treatment in cerebral amyloidal angiopathy (CAA) should be implemented. In addition, the current research in neurovascular unit has employed the dynamic microfluidic model and human-induced pluripotent stem cells (hiPSC) to model the BBB. It is recommended that researchers in this research area adapt these advanced technologies in their proposed neurovascular projects.

4. Identify areas that are less relevant to the FDA's public health mission

The research concerning traumatic brain injury addresses an important research need. It is less clear how this fits the needs of the FDA. While it is recognized that TBI can lead to neurodegenerative conditions, such as chronic traumatic encephalopathy pursuant AD and tauopathy, the rationale for these studies should be better articulated.

While gender differences in AD is worth investigation, it is unclear how important the gender difference in TBI would contribute to drug development for treatment of TBI.

5. Recommendations

- The core is well suited for the mission of FDA. Collaborations with the imaging core, and others, with skillful experts and high-end research facilities should be broadened when opportunities are available.

- Combining the current on-going research projects (about 7-8 in the core) with more than a dozen projects listed as future studies, the core (with 4 PI's) appears to be stretched too thin with too many directions. The solution could be to either narrow down the focus to several high impact projects among three research groups (AD, PD and TBI), or increase the investment in FTEs and resources in the core. The Subcommittee recommends that DNT leadership develop a priority list that will guide investigators in this core for next 5-years of research enterprise, taking into account the emerging areas aligning with FDA's core missions in approval of drugs and medical devices.
- Apply the sophisticated mechanistic science to an array of current and proposed treatments for the syndromes under study including Alzheimer's disease, Parkinson's disease and traumatic brain injury.
- A recommended approach would be to avoid modeling complete disease syndromes, but rather to study neurobehavioral processes that are impacted by chemical exposure.
- It is recommended that this line of research continue its strong program, taking into account the opportunities addressed above .
- Increased drug applications for treatment of brain diseases such as AD, PD, and TBI require FDA to develop the means or standards (to be included in drug application dossiers) to assess the brain availability of any given CNS drugs.
- Considering vascular dysfunction as a drug treatment target, studies on vascular integrity in AD, PD and TBI are deemed appropriate and necessary. It is recommended that research area-2 include studies with transgenic mouse/rat model, pericytes in amyloid angiopathy, and Aβ clearance, among others. These efforts should continue. In addition, the research on exosomes should also include the analysis of bioactive molecules other than Aβ, such aSyn.
- Recent advancement in MRI and other medical imaging techniques has allowed for more accurate assessment of Taupathies; the effectiveness of abucanumab in treatment of AD in clinical trials also supports the Aβ-based AD theory. It is, therefore, important to study the neurofibril formation in the core's AD models.

Research Area-3: Developmental Neurotoxicology (Sherry Ferguson, John Talpos, Josna Kanungo, Cheng Wang, Shuliang Liu)

This core has five PIs (Dr. Sherry Ferguson, Josna Kanungo, Shuliang Liu, Cheng Wang, John Talpos), conducting research in areas of arsenic toxicity (E220601 and E220901), perinatal anesthetic toxicity (E728501, E728511/E728521, E760101, E763901, E767501, E767901, and E768601), neurological device toxicity in children (E734301), ADHD drug safety in children (E765501), and pain medication (C19056 and C19060). The core has developed in vivo and in vitro models for evaluation of developmental neurotoxicities associated with FDA-regulated drugs and products.

1. Integration into FDA Mission

The research in this area hits the core of the interests of the FDA, namely what are the risks of chemical exposure to neurobehavioral development?

The projects not only characterize the functional behavioral impacts of developmental neurotoxicity, but also contribute mechanistically.

The research concerning the developmental neurotoxicity of ketamine is very important to the FDA mission, particularly because ketamine-like drugs are not only used as anesthetics, but also treatments for depression and other psychiatric impairments.

2. Quality of Science (approaches/methodologies) – includes strengths and opportunities

The researchers in this do an excellent job covering a broad array of neurobehavioral tests and investigations of the mechanisms of effects.

Developmental neurotoxicology is a longstanding outstanding strength of the division.

<u>Additional Strengths noted by the committee include:</u>

- The seminal research by investigators in this core on the developmental neurotoxicity of anesthetics is laudable. It has led to the labeled warning on the use of general anesthetics and sedation drugs in young children and pregnant women in 2016. Studies performed at the NCTR were crucial in generating the data used to craft this warning, and research describing the consequences and molecular mechanisms of these exposures continues at the NCTR.
- The core investigators have access to a variety of animal models suitable for developmental neurotoxicity studies, from zebrafish, rodents, to NHPs. There are also existing stem cell lines from human and rodent sources available for developmental research.
- The current collaboration with Mayo Clinics in Mayo Anesthesia Safety in Kids (MASK) studies is commendable for its impact on clinical practice of general anesthetics.
- With the established in vivo models, the core has planned to apply new methodologies such as resident intruder, home cage locomotor activity, and medical imaging techniques to study neurobehavioral changes in children. These approaches are appropriate and likely to assist the evaluation of developmental neurotoxicity of drugs used in treatment of ADHD.
- Acetaminophen has been used in clinics for years and extensively investigated for its hepatotoxicity. Yet, its developmental neurotoxicity has only recently become noticed. Epidemiological meta-analyses show a probable relationship between prenatal uses of acetaminophen and declined IQ or increased risk of ADHD. For its worldwide over-the-

counter availability to the general population, investigation of its developmental neurotoxicity coming along with the potential label warning is well justified.

- Zebrafish model, recommended by the last review, has been well developed in the core and used to study nine different FDA-regulated drugs and products.
- The core has established and maintained several stem cell lines and used them to screen drug's interaction with cell differentiation and proliferation. The 3D culture model is currently under development.

3. Identify emerging technologies/approaches that should be considered

- It would be beneficial to include in the hierarchy of approaches using invertebrate models such as C. elegans or drosophila.
- Other non-rodent mammalian models of neurodevelopment such as mini-pigs could be used to complement the NHP research.
- A great advantage of NHP is their extended juvenile and adolescent life stages. This could be quite useful for the evaluation of psychiatric drugs beyond methylphenidate such as antidepressant and antipsychotic drugs that are being increasingly used in younger people.
- Study of persistent neurotoxicity of adolescent nicotine could be of great use to the FDA tobacco branch.

4. Identify areas that are less relevant to the FDA's public health mission

DNT has pioneered studies into the neurotoxicity of developmental exposure to anesthetics. This has provided important information particularly concerning risks of NMDA blockade on neurodevelopment. At first it might appear that further mechanistic studies into the causes of these impairments might be less relevant to the FDA mission than the identification of the impairment. However, we would argue that these mechanistic studies are quite in line with the FDA mission. Determining the mechanisms of neurotoxic injury provides the biologic understanding to develop better ways to provide therapeutic effects with lower neurotoxic risk. With each project it is recommended that there be a dual focus on tests of potential neurotoxicity as well as investigation of the mechanisms of neurotoxicity to facilitate future research with other drugs.

5. Recommendations

- The core is well suited for the mission of FDA for safe regulation of drugs, medical devices and other FDA-regulated products. Their in vivo and in vitro studies in multiple fronts are likely to generate a large quantity of useful data for regulatory guidelines. It, therefore, becomes absolutely necessary to establish a close collaboration with the Division of Bioinformatics & Biostatistics. This type of collaboration takes advantage of

the Center's unique strength capable of bringing together in one place the highly skillful experts and high-end research facility to tackle the challenging issues.

- Beyond anesthetics, acetaminophen and abused drugs, the core leadership is suggested to conduct the search in the existing drug inventory for those long-term used drugs or medical devices. The neurodevelopment toxicity should be conducted in these products as well.
- Early stage exposure can lead to late-life development of AD, PD or other neurodegenerative disorders. The current projects can include the end-point assessment of certain AD and PD markers such as proteins, enzymes and/or peptides known to participate in pathoetiology.
- The introduction presentation has indeed discussed the importance of developmental neurotoxicological research in the Division and the relationship between animal models and human brain development. However, the introduction did not address the research focus and the existing lines of investigation pertinent to FDA-regulated drugs and products, and no mentions on personnel arrangement, facility needs, and the current and perspective collaborations within and outside of the core.
- While the zebrafish model is useful to predict the development toxicity, there remains the gap from fish to human outcomes. Thus, it becomes necessary to incorporate the fish data into those generated from rodent and NHP studies, by deep learning/machine learning, to generate meaningful regulatory recommendations.
- The same is also true for their stem cell models. There is a need to conduct the in vivo rodent studies by pause-chase of specific markers to delineate the impact of drugs on neural cell proliferation, differentiation, migration, and maturation.
- The current acetaminophen study is conducted primarily in rats. Some neuronal structure changes can be monitored by MRI or MRS. This in-house collaboration has yet been established.
- Integration of the existing strength within the NCTR such as Bioinformatics and Biostatistics is deemed inadequate .
- It is recommended that this line of research continue its strong program to determine the vulnerability of the developing nervous system to neurotoxic insult. It would be important to follow up the study of the impacts of ADHD medication on neurodevelopment with study of the impacts of other psychiatric drugs inasmuch as they are increasingly being used in patients of younger ages. The study of CBD is very important given that this drug is being much more widely used despite very little information concerning its safety. Just because it is not intoxicating does not mean that it is not toxic.
- The broadest perspective of the Subcommittee's review of DNT reveals that it has consistently increased its collaborative research across the FDA Centers with the trend for interactions continually increasing. The Division has excellent scientists, equipment and staff producing quality research in peer-review journals, despite continuing

professional recruitment challenges. DNT research collaborations with the institutions external to the FDA were also numerous and intra-NCTR optimization and synergy of resources and expertise were very notable and documented by numerous internal collaborative projects and co-publications.

- Artificial Intelligence (AI) and Machine learning (ML) has become a current fascination and fear of society as its potential capabilities to impact our lives through new products expands. The most frequently cited examples reflected efforts to improve workflow efficiency and manage internal and external information to assist the review process. At the NCTR, AI expertise through Dr. Tong et al is rapidly evolving to enable AI-enhanced bioinformatics as documented by many high-impact peer reviewed journal papers. DNT should further develop relationships with the Division of Bioinformatics & Biostatistics and tap into its AI expertise to enable programs tailored to link large and diverse datasets. Investment in the AI capability at NCTR as a whole and DNT in collaboration with other already strong intergovernmental AI programs, as in the Department of Defense, would allow the DNT to harness this tool.