SOT2024 (March 10-14, 2024) Report

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The SOT 2024 was my first SOT event that I had attended. I was impressed by the large amount of support for the event and participation from around the world. The event had both public entities and private companies who were actively involved research but also creating new regulations and new scientific techniques to discover and utilize for the betterment of society. The event was around one week and held at Salt Lake City, Utah in the US. I was very impressed that SOT had large number of scientists from around the world at the event. The event consisted of chemists, biologists, clinicians, environmental scientists and others who all presented the latest topics in toxicology. This event gave me a very positive view for science moving forward even after concerns of the post COVID-19 era. People are more passionate to share their expertise and tackle issues not just in the USA but also the world about chemical use in daily life but also in the industrial setting. New discovery of toxic chemicals such as new emerging PFAS, pesticides and more offer new insight to what is necessary in the foreseeable future in order to create a healthier society. More events like SOT should take place around the world to ensure healthy promotion of science but also increase our understanding of the environment and technology we use moving forward for the greater good.

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I. POHaD (Paternal Origins of Health and Diseases)

Symposium Session: It Takes Two! Paternal Exposures and Their Impacts on Offspring Health

1. Paternal Environmental Exposures and Their Influence on Development via the Sperm Epigenome. by Dr. Kimmins from McGill University (Abstract # 1215)

The first topic was introduced by the presenter who specialized on the paternal germline. According to Kaati et al., too much food availability before puberty in grandfathers (8-12 y.o.) was associated with increased risks of diabetes and cardiovascular diseases in their grandchildren. This suggested that there was the possibility of environmental reprogramming on the sperm epigenome through food. The paternal germline is the genetic material passed from the father to the offspring. The functional genotype that was focused on were altered sperm, transmission at the fertilization stage and the escape of reprogramming cellular memory exposure in subsequent generations. The presenter looked at sperm epigenome and paternal histone modifications which would influence the histone environment. Histones are essential proteins which provide structural support for the chromosome.

An important histone mentioned was H3K4me3, which is sensitive to paternal folate deficiency and alters offspring development. It was observed that sperm genetic material changes are transmitted and persisting in the embryo along with an association with embryonic gene expression. The presenter has shown that 25% of promoters that have altered H3K4me3 in sperm and embryos have shown deregulated gene expression.

The presenter then changed the topic to DDT used for malaria vector control which was associated with male reproduction health. The presenter has also mentioned that the accumulation of DDT and DDE were detected even in the arctic due to bioaccumulation in the region. It was also revealed that DDT and

DDE are health risks that is associated with neurodevelopment and neurofunction. The presenter also mentioned that DDT exposure has an association with differentiation of H3K4me3. This is seen by environmental changes in sperm and a reduction of H3K4me3 occurs in developmental genes. The presenter concluded that we should focus on male health as an important subject to study for paternal health.

2. Aberrant Sperm DNA Methylation following Wildfire Smoke Exposure in Mice and Humans. by Dr. Montrose from Colorado State University (Abstract #1216)

The presenter has shown data that in recent years there has been an increase in wildfire activity around the world. According to the presenter, in 2023, the Canadian government has spent over one billion dollars on fighting wildfires which was the most ever recorded for the government. There is also an increase of Particulate Matter (PM) 2.5 due to wildfires across the US and Canada regions. The presenter has shown that there are 3 categories of PM which consist of size, chemical composition and dose. An example of occupational hazard was mentioned when firefighters working in the wildfire environment were associated with health risks of neurocognitive dysfunction and fertility issues.

Smoke primarily affects the lung and heart which causes cytokines and chemokines to be released into the body to fight the effects of smoke in the body. The presenter has shown the data that PM is linked to a deterioration of sperm quality. A study on wildfire firefighters in Portland, Oregon has found that lower sperm concentration was detected after wildfire exposure. It was also mentioned that female firefighters were about 2 times more likely to have a miscarriage than nurses.

In a mice study, they found that exposure to wildfire smoke led to sperm DNA methylation caused by the exposure led to epigenetic inheritance to be plausible, however more studies are needed to confirm. The presenter has concluded that more studies of wildfire smoke are necessary as it is a growing risk of concern.

II. Micro- Nanoplastics

Platform session: Micro- and Nanoplastics

1. Understanding the Health Risks of Micro- and Nanoplastics exposure in Humans by Dr. Hernandez from Autonomous University of Barcelona (Abstract #2077)

The presenter has explained that Micro-Nanoplastics (MNPs) have similarities to nanomaterials which have a high capacity with biomolecules and are used in the medical field. It was also emphasized by the presenter that support for

better policies of risk evaluation is needed to identify more MNPs and to address knowledge gaps.

The presenter showed a list of items which need to be addressed to improve the understanding of health risks which MNPs impose. Such as the difficulty of hazard assessment due to lack of regulations and specific rules, lack of existing human models and biomarkers which make it difficult for scientists to have a definitive conclusion on the impact of human health of MNPs. It was also stated by the presenter that there is a need to study measuring equal cell exposure of MNPs along with the study of kinetics. This is due to absence of understanding of the mechanisms of MNPs related to health risks in the human body. Also, the author has emphasized that there should be more studies of chronic exposure of MNPs to look for in health risk assessments. At last, the author introduced the large-scale EU project, “PlasticHeal” (https://www.plasticheal.eu/en) which is aimed at developing novel tools to study MNPs’ mode of action and impact of MNPs in human health. The presenter also mentioned that more studies in genotoxicity, methodologies and complex in-vitro studies should be pursued to continue the evaluation of health risk assessment of MNPs in human health.

2. Utilization of Non-targeted Chemical Analysis and In-vitro Studies to Assess the effects of Primary-sourced and Secondary-sourced Microplastics Dosed in Simulated Lung Leachates and Extracts by Rios-Colon from RTI International (Abstract #2079)

The presenter gave an explanation that primary microplastics were related to microbeads, while secondary microplastics are found from products. Primaary and secondary microplastics have aged and unaged microplastics particles. MNPs have the potential to be physically or chemically affected. It is also known that tire rubble is detected by FTIR (Fourier Transform Infrared Raman Microspectroscopy) for the aging process of MNPs. In the research presented, it was revealed that increase in tire rubble concentration was linked to a rise in Reactive Oxidative Stress (ROS) in lung cells. Aged tire rubble particles were associated with a rise in inflammatory biomarkers which increased the dose and exposure of MNPs. MNPs leachate studies have concluded that there is a difference between specific shredded polymers and manufactured microbeads in MNP studies. The presenter concluded that measuring both physical and chemical properties were needed to understand MNPs in more detail affecting the human body.

3. In-vitro Toxicity of Microplastic Fibers to Human Lung Epithelial Cells Cultured at an Air-liquid Interface by Dr. O’Conner from University of Florida (Abstract #2080)

The presenter has mentioned that in the lungs, the most common microplastics are Polypropylene (PP) and Polyethylene terephthalate (PET). In the lungs it is also found that the most common forms of the plastics are fibers and films. An emerging issue of Azobenzene Disperse Dyes (ADD) was mentioned, which is

being used in 70% of industrial dye, used to color synthetic fiber. The color violet is the most toxic color used which affects dendritic cells and causes IL-1β, IL-8 and IL-10 activation. In the study, it was also noted that the violet color was more associated with cancer compared to other dyes and none dyed plastic samples. This is due to the association with ferroptosis (programmed cell death by iron and accumulation of lipid peroxides) signaling pathway along with NRF2 mediated oxidative stress response. It was also found that undyed fibers were associated with inflammation, negative effects on wound healing process and ROS. Dyed fibers are known to be associated with DNA mutation effects and a risk of cancer. The presenter concluded that dyed samples of plastics might be partially responsible for plastic film and fiber risks in the human lungs.

4. Assessing Toxicity of Amorphous Micro- and Nano-plastics on Bronchial Epithelial Cells using Air-liquid Interface Models by Dr. Gosselink from the School of Nutrition and Translational Research in Metabolism (Abstract #2081)

The presenter introduced the topic that MNPs can be found in the human lung and be able to cross the bronchial epithelial barrier. The presented study mentions that PP exposure induced Interleukin-8 (IL-8) protein secretion in a submerged test of nanoparticles. Polyamide exposure was mentioned to negatively affect IL-8 levels and apoptosis when tested in nanoparticles. A droplet test was also conducted in the study of air exposure effects of nanoparticles. The results have shown that IL-8 levels in air exposure had no adverse effects on IL-8 levels, it only seen a slight negative minor impact on bronchial cells when affected in air exposure environment. The presenter concluded that the variety of MNPs in polymer type, size, exposure length tends to have different outcomes when it comes to the research about MNPs. It was also mentioned that more particle studies should be conducted when it comes to human health as more data is needed.

5. Cellular Responses to Nanoplastics: Implications for Exosome Release for Extracellular Nanoplastics Transport, and Cellular Toxicity by Dr. Lee from University of Iowa (Abstract #2082)

The presenter gave an introduction of the role of exosomes (nanosized organelles) which are released when exposed to nano-plastics (NP), this was shown in a dose-dependent model. It was shown that exosomes were able to remove NPs to the extracellular space to maintain cellular homeostasis. Due to this, a disruption of glucose homoeostasis and the increase of insulin was observed when tested in mice. An increase of weight and overall adiposity was seen including adipose immune cells. Gene expression changes were also seen where it saw a promotion of adipogenesis and showing signs of hyperglycemia. Gut microbiome changes were also observed and a decrease in beta diversity, which looks at the ratio between regional and local species diversity in the gut environment. As a result, leads to weight gain in the mice.

Signs of atherosclerosis were also seen when a decrease of propionic acid was observed. Propionic acid is responsible for the metabolic breakdown of fatty acids, this can affect high blood pressure leading to CVD symptoms. It was also mentioned that altered gene expression which have seen increased leukocyte activity is also linked to CVD symptoms. To try to mitigate the symptoms, the presenter tested delphinium which is a purple flower that has antioxidant and anti-inflammatory properties. The delphinium testing was able to see weight gain loss and saw deregulation cAMP and PPAR signaling in adiposity. To conclude, the author stressed that more studies are needed that look at exosomes and other nanosized organelles to further understand the impact of microparticles in the human body.

Hot Topic Session: Translational Insights into Reproductive Impacts of Micro- and Nanoplastics Contamination

6. Challenges and Solutions to Quantifying Micro- and Nanoplastics in Biological Specimens by Dr. Yan, Columbia University (no abstract number)

This session looks at how to quantify Micro-Nano plastics (MNPs) in the environment and the biological systems. To get the samples from the environment, the presenter used modern laboratory techniques such as gravity separation and removal of natural organics with water. The presenter also showed the use of hyperspectral dark-field microscopy, a novel technique which is cheaper and quicker for looking at nanoparticles from the size of 5nm. This is then used for Identifying the MNPs and further testing is done with a novel technique called Py-GC/MS (Pyrolysis- Gas Chromatography-Mass Spectrometry). This technique allows samples to remove macromolecules using acids and bases manipulation.

The presenter also looked into novel technique like Nile Red Staining, which is capable of looking at lipids in microorganisms and FTIR (Fourier transform infrared Raman microspectroscopy) which can look at MNPs but also can look at lipid collection from cells which can identify microorganisms' behavior within MNPs. This could give us great insight into how the body can potentially be affected by these MNP interactions with living organisms. The presenter concluded that with the combination of novel techniques mentioned should be standardized by the National Institute of Standards and Technology (NIST) due to the importance of having MNP research more accredited and be able to produce more data with confidence.

7. Comparative Quantitation of Testicular Microplastics Accumulation in Dogs and Humans and Associations with Sperm Count. by Dr. Yu from University of New Mexico (no abstract number)

This session started with the topic of reproduction system levels of MNPs stating that studies are still scarce and the association of sperm quality is relatively unknown. It was found that there was an increase in TDS (Testicular

Dysgenesis Syndrome) which was linked to testicular cancer and poor semen quality. In the study, the author was able to look at a correlation between an increase of plastics to a decrease in sperm count. The authors collected 47 male reproductive tissues from routine dog neuter surgeries. Human testis samples from 23 deceased individuals, aged 16-88, were also obtained. They quantified 12 types of plastic polymers by using Py-GC/MS from both canine and human samples. In dogs, it was revealed that different microplastic contamination profiles were seen in testicular tissues in individual canines. Mean total MNPs levels were 122.63µg/g in dogs and 328.44 µg/g in humans. They were able to observe that PVC, PET PC, but ABS (Acrylonitrile butadiene styrene) had the most significant impact on sperm count. It was also revealed that dogs share more MNP concentrations and compositions compared to humans. The presenter concluded that increased global awareness and development of mitigation to safeguard male fertility is crucial for reproductive health.

III. PFAS

Risk Communication of PFAS: Challenges and Opportunities: Informational Session

1. PFAS Risk Communication from an NGO Perspective by Ms. Benesh from Environmental Working Group

PFAS risk has been mentioned since the 1950's from industrial review of PFAS use. This was followed by the 1960's discovery of PFAS linked to animal toxicity. It was stated that regulators like FDA (Food and Drug Administration) knew of the existence of PFAS risk as early as 1966. The Department of Defense (DOD) has also shown that it knew the risk of PFAS but does not ban its use due to quoting "can't live without PFAS". This is due to number of products used in PFAS such as plastics, gaskets and fabrics which have water and oil repellent qualities. In conclusion, Ms. Benesh argues for PFAS bans on drinking water along with tap water to resolve the PFAS issues.

2. Global Collaboration on Managing PFAS Chemical by Ms Eeva Leinala from OECD (Organisation for Economic Cooperation and Development)

This session explained the implementation of the guidelines for terminology for PFAS. This was based on standardized tools needed to improve communication of molecular structural traits when it comes to PFAS. Another implementation measure suggested was the inclusion of synthesis reports should be noted on polymeric PFAS. Such as life cycle, hazard, occurrence, exposure and etc. Perfluoropolyesters and fluoropolymers were also mentioned for synthesis reports. Considering OECD policies, they would like to incorporate alternatives of PFAS for items like food packaging, cosmetics, coatings and varnishes. This session also mentioned risk management implementation such as registration

of chemicals, collaboration with the business sector, increased information sharing across government and private sector, proprietary and/or voluntary approaches alongside with regulatory approach. In conclusion, Ms. Leinala argues that PFAS regulation is the upmost importance to make sure that global collaboration for PFAS management is done for benefits for citizens and the scientific community.

3. Updates and Recent Activities Related to the US EPA National PFAS Testing Strategy by Mr. Dawson from EPA (Environmental Protection Agency)

This session mentioned that an important roadmap called the EPA's Commitment of Actions 2021-2024 was created by the EPA council for PFAS.

This initiative was created to deal with the PFAS exposure in the United States to Research, Restrict and Remediate PFAS.

One of the mentioned topics was raising the drinking water standards, this is due to the prevalence of PFAS in the water system. Another topic mentioned was the regulation of PFAS.

The initiatives also emphasized on the TSCA (Toxic Substance Control Act) which is a regulatory framework for data gathering of any PFAS manufactured since 2011 including pesticides. This data gathering is done by the national PFAS testing strategy which looks at mixture testing of PFAS and around 90 categories including centroids and other substances. Under TSCA testing orders, it also looks at chemical structure and toxicokinetics.

Also, emerging issues on pesticide packaging was mentioned during the presentation. It was discovered that active ingredients of pesticides and PFAS were prevalent in pesticide packaging before the emergence of the EPA council for PFAS. The creation of this council during the EPA Commitment of Actions 2021-2024 has successfully eliminated 12 PFAS from pesticide packaging. The council will continue to monitor the situation for pesticide packaging.

The final section of the presentation mentioned conflicting views with stakeholders about PFAS, this is due to the lack of scientific information and data gathering issue. To combat this, the EPA is planning to create more publications for supporting scientific evidence and to have webinars and workshops for further information on PFAS awareness and exposure. For the EPA, this is an important issue, and it wants to continue to promote the importance of its strategy in dealing with PFAS exposure in the US.

Mechanisms of Per- and Polyfluorinated Substances Action: PPARα and Beyond Symposium Session

4. Integrating Drug Development Tools to Predict Key Mechanisms of Per- and Polyfluoroalkyl Elimination and Clearance by Dr. Slitt, University of Rhode Island (Abstract #1039)

This session mentions that recent data shows that over 200 million people in the United States rely on tap water that is contaminated by PFAS. In the session it is mentioned that PFAS comes in all varieties of items such as textiles used in the Department of Defense, and water system used in the US. PFAS was also detected in the human liver. The presenter wanted to know which PFAS were currently present in the human liver.

The session went into detail of the biological process of how PFAS comes into our body. This includes how PFAS is well absorbed in the GI tract with little change of biotransformation, the presenter also mentioned that the liver has the highest amount of concentration of PFAS. This is likely due to the high binding affinity for serum albumin which is linked to the detection of liver disease. It was also mentioned that it also has tissue binding affinity and reabsorption by renal transporters which both are used in drug development tools. The session also mentions Organic Anion Transporter (OAT) and Organic Anion Transporting Polypeptide (OATP). OAT and OATP play a role in the kidneys which are involved with bile salts, thyroid hormone and steroid signaling.

With this information, a new form of classification system was created to bring more transparency of PFAS in the body. It is called the Extended Clearance Classification Systems (ECCS). This looked at three important points, Permeability (how much substances it allows to pass through with other coexisting substances), Ionization (how molecules charge is gained or lost depending on electrons) and molecular weight (the weight of the substance). The changes of these points mentioned depend on the metabolism, renal clearance (Kidney clearance rate) and hepatic uptake which predominantly is associated with transporters such as OAT and OATP which can be used to detect hepatotoxicity.

In the session, it was also mentioned that PFAS could interfere with OAT transporters, which could cause a disruption of normal transporters activity in the liver. This is due to PFAS being an OAT1PB1 inhibitors causing the disruption of transporters. It was also mentioned that ABCG2 mediated transporters, a known multidrug resistance contributor is linked as a substrate for PFAS. These transporters fit to all the categories of the ECCS. It was mentioned that the ECCS classification system could provide PFAS, PFOS retention with albumin playing a crucial role. This is due to the system's ability to identify transporters and mechanisms which provide useful data for scientists working on *in vivo* models.

5. Importance of Evaluating Mode of Action and Human Relevance in Assessment of Human Health Risks of PFAS: Case study with Short-Chain HFPO-DA by Dr. Heintz, ToxStrategies LLC (Abstract #1040)

It was revealed by the presenter that there was absence of evidence in the mode of action of specific endpoints (a direct marker for disease progression) in humans but recently the EPA has made a toxicity review of Hexafluoropropylene oxide [an organofluorine chemical] (HFPO-DA) as a class

of PFAS that affects the most sensitive endpoints in mice, which is the liver. This process is influenced by actions such as apoptosis and hepatotoxicity. It was also revealed that HFPO-DA did not bioaccumulate much in mammals. Despite this, it was shown to have negative effects on rats which saw tumor growth with chronic exposure.

The presenter wanted to know the significance of HFPO-DA on humans. The presenter has seen the effects of HFPO-DA on PPARα (Peroxisome proliferator activated receptor alpha) which looked at cell growth pathways, survival of cells, clonal expansion and liver tumors. The presenter found that PPARα lowered lipid metabolism regulation, increased in β-oxidation affecting mitochondrial fatty acids, tumor growth, decreased birth weight and higher mortality rate in birth were discovered in rodents exposed to HFPO-DA.

It was revealed by the presenter that PPARα in humans has no significant enrichment process like rodents do. It was also found that this could be due to an enhanced upstream regulator analysis which humans process compared to rodents. The presenter also found that humans carry a higher concentration of PPARα which has less effects for treatments on the human body. Despite the findings, the author concluded that there was a lack of human relevance based on current research. The presenter also highlighted the importance of mode of action and human relevancies when looking at PFAS toxicities in human health risk assessment studies.

6. Is PPARα the Molecular Initiating Event Driving Human-Relevant Toxic Effects of PFAS? By Dr. Schlezinger, Boston University (Abstract #1041)

PPARα (Peroxisome proliferator activated receptor alpha) is a prominent molecular initiating event that is associated with PFAS-induced toxicity. The presenter gave an explanation that PFAS exposure was linked to increased LDL cholesterol, CVD and birth weight. This is due to the liver being associated with lipid homeostasis with PPRE (Peroxisome proliferator response elements) playing a role for gene expression. PFAS can change transcription of lipid movement in genes thus creating lipidemia due to this change affecting lipid homeostasis.

A study in mice have indicated that PPARα in males seem to only be affected by lipid regulation disruption, while females are affected by PPARα mechanisms which did not regulate their lipid homeostasis. This led to the team finding out the mode of action for the duration of exposure to see any changes in the lipid accumulation in the liver. This is because the presenter discovered that lipid homeostasis in the liver depends on exposure time along with sexes and types of lipids involved in the process.

It was found during this process that PFOAs were not significant in the mice study unless they undergo treatment manipulation and genotyping for PPARα in the mice. They have also discovered that PPARα affected females more than males in serum cholesterol. The discovery revealed that there was a decrease in HDL levels in males while females have an increased amount of LDL while

not having significant HDL changes. This had led scientists to believe that the suppression of CYP7a1 (an enzyme for cholesterol) might play a role in affecting PFOA serum cholesterol in the liver. This led to the conclusion of the presentation that scientists can finally uncover the complexity of molecular interactions of PFAS in human relevant toxicity.

7. Mechanisms Linking Mixtures of Per- and Polyfluoroalkyl Substances to Increased Circulating Cholesterol and Cardiovascular Disease Risk by Dr. Petriello, Wayne State University (Abstract # 1042)

This session focused on the issues of PFAS affecting cardiovascular disease (CVD) and cholesterol. It is known that atherosclerosis development starts in early childhood due to apoptosis and reactive oxygen species (ROS). The biomarkers used for CVD are total cholesterol count, C-reactive protein and Trimethylamine N-oxide (TMAO, an organic compound found in gut microbial metabolism) and the microbiome. These were looked at for diet manipulation based on phenotype line of metabolic and high cholesterol studies. To grasp a better picture, they used a high cholesterol to low fat diet model to use for the PFAS investigation in mice. PFAS mixtures were measured in mass spectrometry.

A study from occupational exposure from environmental exposure of PFAS from West Virginia found that there was an increase of cholesterol in residents living in the exposed occupational area. When PFAS were investigated in mice, they found that they had an increase in overall cholesterol and bile acids. They have also found that TNF-α (Tumor Necrosis Factor alpha) is a cytokine responsible for immune cell response and IL-6 (Interleukin-6) found in female mice.

The study also found that PFOS showed the strongest sign of affecting cholesterol over other PFAS groups. In the study of mice, they also found that PFAS mixtures likely reduced endogens cholesterol formation as well as decrease bile acid excretion. It is also understood that PFAS mixtures increased the amount of bile acid transporters despite the decrease of excretion of bile acids. This led to the question that despite having some indication of PFAS mixtures affecting the bile, the presenter emphasized that PFAS studies on the intestines are still understudied and should be investigated.

Looking at the absorption and excretions of PFAS and cholesterol, it was revealed that the ABST inhibitor (used for non-alcoholic fatty liver disease treatment) is positively associated with decrease of PFAS toxicity related to cholesterol. It is also revealed that PFAS mixtures modulated genes related to cholesterol role of absorption and excretion. This also influences the microbiome which controls sterol homeostasis and impact secondary bile acids produced from bacteria. The presenter concludes that a multi-omics approach could allow scientists to identify mechanisms of cholesterol and bile acid circulation to find a link between PFAS and atherosclerosis risk.

8. Alternate Mechanisms of PFAS Toxicity by Dr. Apte, University of Kansas Medical Center (Abstract #1043)

HNF4α (Hepatocyte nuclear factor 4-alpha) is a master regulator of hepatic differentiation as well as responsible for metabolism regulation and proliferation of liver and intestinal epithelial cells. The presenter states that HNF4α showed degradation with interaction of PFAS and PFOAs. The degradation of HNF4α is shown to affect drug metabolism, coagulation factors, nutrient metabolism, bile acid synthesis as well as transport and no cell deaths.

The presenter then mentioned that stability in proteins of fatty acid binding was linked with HNF4α which resulted in steatosis (excess fat buildup of liver cells). The presenter also found that this type of activity can be seen when myristic acid and PFOA interact with HNF4α. This led to the discovery that DNA response elements like protein-binding sites are induced by PFAS exposure which affects the circadian rhythm of mice. Overall, the presenter wanted to show that new hypotheses needed to be considered when dealing with mechanisms of PFAS toxicity.

IV. Neurotoxicology

Symposium Session: The State of the Science Linking Environmental Chemicals to Age-Related Neurocognitive Disease

1. The Effects of Chronic Exposure to Ambient Traffic-Related Air Pollution on Alzheimer's Disease Phenotypes in Wildtype and Genetically Predisposed Male and Female Rats by Dr. Lein from University of California Davis (Abstract #1123)

The presenter introduced to the audience that in epidemiological studies, Alzheimer disease (AD) and TRAP (Traffic-Related Air Pollution) have a strong link to one another. The presenter explained that AD from genetic factors was less than 5% and underlying factor of the disease might come from environmental factors, lifestyle or other disease related factors. The presenter advocated the implementation of effective public health policies to reduce the prevalence of AD. Further details of TRAP mechanism, the identification of factors and chronic exposures of AD were presented. The presenter created an experiment with rats of exposure chambers in real time with control of filtered air.

The experiment revealed that TRAP increased inflammatory response and rat data on neuroinflammation was closely related to human AD pathology. The data has also shown that women tend to have AD more than men. It was also revealed that TRAP guidelines tested were within guidelines of WHO and the EPA limits for PM2.5. During the experiment, they found plaque formation on the hippocampus of rats exposed to TRAP compared to rats in an air filtered environment. It is also found that rats have increased pTau (biomarker protein in the CNS for AD) in male rats and found exacerbated neural cell loss in 15-

month-old rats which is approximately 45 years old in humans. The experiment has also found that Aβ42 (amyloid beta 42) a biomarker for AD has toxic effects on AD when the rats are in an exposed TRAP environment, Aβ42:40 ratio balance is essential for cortical reproduction. There is also evidence that TRAP promotes cognitive deficits in male rats when it comes to learning and memory. This might be due to refractive particles being present in the hippocampus of the brain. The experiment also has found fewer refractive particles in other regions of the brain in TRAP-exposed animals.

The presenter has concluded that the standards of PM2.5 and other TRAP-related measures are not sufficient for protection of the aging brain.

2. Uncovering the Complex Relationships between Air Pollution, Social Determinants of Health, and Aging by Dr. Ward-Caviness from US EPA (Abstract #1125)

The presenter showed air pollution and SDoH (Social determinants of Health) as a growing environmental issue for the EPA. The presenter showed data of adjusted hazard ratio, which looks at hazards related to the environment and social deprivations. An example given was the difference between air mixtures in high and low social classes. This led to a topic of having clean air solution to have more of an impact on low social deprivation communities than higher social classes. The presenter then showed data showing epigenetic aging based on DNA methylation analysis such as the biomarker Age Acceleration Difference (AAD) to compare between social classes.

The EPA also looked at neighborhood quality indicators such as street cleanliness, graffiti and greenspace. It was shown that neighborhood quality was significantly associated with epigenetic aging biomarkers like AAD. It was also shown that increase in greenspace like tree count has shown positive effects on slowing down AAD. The reason for the positive effect is still not known. A mice study conducted by EPA has shown that housing depletion has been associated with depleting body fat percentage based on housing condition. Low housing conditions have shown that there was an increase of high fat, higher LDL cholesterol and plasma glucose. While high housing conditions has shown lower fat percentage than the low housing counterpart. The study has also shown that short term exposure has shown that no significant epigenetic aging. Another concern is that EPA has found that air pollution and dementia has been linked with the social environment.

Symposium Session: Neuroinflammation as a Central Mediator of Neurotoxicity: Implications for Environmental Links to Chronic Neurodegenerative Diseases

3. NLRP3 Inflammasome Function in the Response to Parkinson’s-Associated Pesticides by Dr. Havrda from Dartmouth College (Abstract #1080)

NLRP3 is an important innate immune system protein that is responsible for responding to microbial infections and cell damage in the human body. When it is active in the body, it becomes an inflammasome which can have negative effects. The effects can include ROS (Reactive Oxidative Stress), cell death, mitochondrial dysfunction due to genetic mutation by environmental exposure, misfolded protein and lysosome dysfunction which can lead to negative outcomes. In animal studies, it is found that NLRP3 can have positive effects such as protection from neuron loss. This experiment was done by looking at specific NLRP3 mouse models. They found that exposure to rotenone (a fish piscicide) led to neurotoxicity in mice with signs of neurodegeneration and cognitive dysfunction. Measure of NLRP3 can be indicated by blood test looking at haptoglobin levels.

The importance of NLRP3 being responsible for Parkinson's disease being linked to pesticides is an issue that is present today. The mechanism of NLRP3 and other immune system responses should give scientists a better understanding of the mechanisms involved in Parkinson's-associate pesticides for the development of therapeutic methods and diagnostic testing.

4. Innate Immune Responses to Alpha-Synuclein Facilitate Its Aggregation in Parkinson's Disease by Dr. Panicker from Cleveland Clinic Lener Research Institute (Abstract #1082)

The presenter started with a basic information regarding Parkinson's disease (PD) which is associated with dopamine neuron death. PD is the fastest growing neurological disorder and treatment for PD consists of dopamine antagonist.

An important protein called α-syn (alpha synuclein) plays a crucial role for regulating neurotransmission and trafficking. It is thought that the pathogenesis of PD derives from α-syn dysfunction. The presenter tested an α-syn Pre-Formed Fibrils (PFFs) model which was injected in the mice's brain to induce α-syn aggression. This led to Lewy body holes which led to the death of dopamine neuron, causing PD. It is found that microglia (non-neuro cells in the CNS) mediated inflammatory response were seen in the development of PD. It was also found that inflammasomes from microglia are present in human cells and were associated with misfolded proteins which are supported with α-syn interactions. The interactions occurred by having the inflammasome like NLRP3 being active in the brain caused the secretion of IL-1β which is a known neurotoxic inducing interleukin.

The presenter concluded that the combination of NLRP3 and α-syn were interlinked together for the implication of PD development. More studies are needed to further understand the mechanisms behind this process to help reach the underlying cause of action and to help scientists with diagnostic and therapeutic solutions for PD.

5. Extracellular Vesicles as Biomarkers and Mechanistic Mediators of Aging and Age-Related Neurodegenerative Diseases by Dr. Lu from Harvard University (Abstract #1122)

The presenter has shown his research in Extracellular Vesicles (EV) being used as a biomarker and functional mediator for environmental and disease research, this is due to the large amounts of EV's circulating in bodily fluids. It was also explained that EV heterogeneity found difficulty of tissue of organs and subtypes such as biogenesis and composition. It was also revealed that EVs from a mouse brain revealed that by using proteomics, it was found that EVs contain plasma membranes for proteins. It was revealed by an EV subtype called ARMMs linked protein which were associated with increase expression of stem cell differentiation. It was also mentioned that EV neural progenitor cells in metals are linked with ARMMs. Cadmium and arsenic inhibition of ARMMs and leads to an increase in both cadmium and arsenic toxicity due to not being able to digest cadmium and arsenic properly. It was also discovered that the transfer of antioxidant proteins could have protective effect of ARMMs in cadmium neurotoxicity. The author concluded that novel EV pathways and signatures could explore a new way for potential biomarkers and functional systems which can lead to more discovery in neurodegenerative research based on the environment.

V. Emerging issues

Hot Topic Session: Where the Rubber Meets the Road: Impact of Tire Wear Particles on the Environment and Human Health

1. 6PPD-Quinone and Beyond: Tire Rubber Additives and Transformation Products as Emerging Contaminants by Dr. Tian from Northwestern University

The presenter started the presentation with the death of coho salmon in northwestern US. Storms that carry 6PPD chemicals are dipped in the river where coho salmons thrive and then experience Urban Runoff Mortality Syndromes (URMS). The increased death of the salmons is due to not only the specificity of 6PPD chemicals but also the rise of urbanization and traffic intensity, which 6PPD chemicals are associated due tire use. The study was able to create a non-targeted analysis which could detect contaminants without identifying every chemical. This was done by tracking chemical signatures from collected water containing 6PPD chemicals which track specific chemical signature for both treatment and contamination sources.

A method called effect directed analysis was created to link observed toxicity with non-target pollutants. They use tire wear leachate which looked at toxic chemicals for use of mass spectrometry for identifying and following HPLC which found the only chemical which linked to coho salmon death. It was discovered that an unknown chemical ($C_{18}H_{22}N_2O_2$) which has an appearance of a purified pink-magenta solid. The scientists were able to

rearrange the unknown chemical to a known chemical with similar characteristics, what they discovered was 6PPD. The scientists were also to rearrange the 6PPD further using Nuclear Magnetic Resonance technique which led to the creation of 6PPD-Quinone, which was shown to be more toxic and affecting larger variety of fish such as rainbow trout.

The mechanism of 6PPD has shown cerebrovascular effect linked to leak blood brain barrier, O- glucuronide metabolites. It was also observed that not high soluble along with being moderately hydrophobic and has similar sorption properties with other plastics. Since 6PPD is found in water, there is a high chance that it is susceptible to human toxicity. In zebrafish testing, it has been found to decrease head size and facial deformation. In conclusion, they have found that short term acute exposure of tire wear particles showed signs of hyperactivity. An increase in exposure, the scientists have found that there was increase of motor activity in zebrafish. The reason for the hyperactivity was due to the ion chamber modulation when interacting with tire wear particles. The presenter concluded that more studies on tire wear particles needed to be done to find other potential unknown chemicals which could cause biological harm to the environment and people.

2. Coordinating to Fill Critical Knowledge Gaps on the Tire Anti-Degradant Contaminant of Emerging Concern 6PPD-Quinone by Guiseppi-Elie from US EPA

The presenter made an introduction concerning of 6PPD-Quinone emergence in the US. It was discovered that accumulation of 6PPD comes from storms, which were collected for water treatment for finding 6PPD chemicals. The rise of chemicals in the US found that tire manufacturers are competing for making innovative chemicals for tire use. This has led to the US EPA creating a cross coordination of other agencies to investigate 6PPD chemicals. The presenter introduced an initiative called the Office of Research and Development (ORD) StRAP, a strategic research planning project for 2023-2026 with six national research programs into the investigation of environmental health with input from EPA partners. The presenter concluded that 6PPD along with other chemicals of unknown origins should always be monitored on a multi-organizational level to make sure that it can quickly detect and research potential health risks it poses to humans and the environment.

VI. Microbiology

SOT/EUROTOX Debate: Can the Microbiome Mediate the Toxicity of Environmental Chemicals? Presented by Dr. Tamala Tal from the Helmholtz Centre for Environmental Research, Germany and Dr. Karsten Beekmann from Wageningen University, the Netherlands

The SOT representative, Dr. Tamala Tal started the debate by introducing ingested xenobiotics like additives, pesticides, drugs make their first contact

with the gut microbiome which affects the biological half-life of xenobiotics. An argument made was that chemical exposure can alter the microbiome which can be influenced by over 124 chemicals. It was also found that chemical selected microbiomes have shown altered function in toxicokinetic properties. It was also mentioned that around 2/3 are bio transformed by microorganisms. This interaction in the microbiome affects the structural activity of the relationship between microorganisms in the microbiome. This leads to altered toxicodynamics, this results in microbe dependent drug interaction which can cause dose limiting in the GI (Gastrointestinal) tract toxicity. An example of this was the drug called Irinotecan which is a drug used to treat colon and rectum cancer. The drug is primarily in the gut lumen, which as a result has a drug limiting toxicity which might not be as effective at targeting the intended molecular drug target.

Dr. Karsten Beekmann, a representative for Eurotox, has argued that exposure of microorganisms is not predictive of composition due to differences between composition (biological material) and function not always aligning one another. The presenter explained that there are over trillions of bacteria which introduces of thousands of possibilities for interactions which we could not follow. It was also explained that there is a difference of epithelial gut microbiota for each individual person, thus not all microbiome focused targets can be reached to people. It was also mentioned that stressors and microbe space are also different between people. This causes a difference in environmental chemical differences among individuals. Dysbiosis association was also mentioned which is linked to disease in people. It was also argued that it would take many years to understand the microbiome due to the amount present in the human body which also includes other living organisms with the microbiome. The presenter concluded that risk, hazards, correlation vs causation and limited understanding persists in research thus argues that microbiome cannot exhibit toxicity of environmental chemicals.

Dr. Tal has argued that Microbial Associated Genomics can show examples of bad microbe environments such as salmonella having a negative correlation with the human microbiome. The presenter also agreed that the microbiome is too complicated to study with examples such as model systems. Human Associate Microbiomes are microbes that can be cultured for research which can be used for not only humans but also with other living organisms like bees and zebrafish. Dr. Beekmann had argued the models of study and individual differences, methods and challenges of limited understanding make it difficult to gain an understanding of environmental chemicals and microbiome interactions. Dr. Tal had argued that microbiome is not only present in the gut but also the skin and lungs which has a potential to explore the bigger potential of microbiome research. Also, it was concluded that differences in ecosystem change can have a potential to affect the microbiome environment in not only humans but other organisms in the ecosystem.

VII. Risk Assessment

SOT/EUROTOX Roundtable: Is Mixture Risk Assessment Now Established Regulatory Practice? Introduced by Dr. Rider from NIEHS

The session's aim was to assess human health risk when it comes to combined exposures of mixtures. International bodies including WHO, OECD, US EPA, EFSA and ECHA have made attempts to work together to implement a way to have evidence-based risk assessments to exposures to chemical mixtures which can come from the environment or food. The current situation of assessment is inconsistent and where it is mandated, it is often very restrictive due to requirements of scenarios and chemical groups. Recently, the European Commission's new initiative the "Chemicals Strategy for Sustainability Towards a Toxic-Free environment" has gained attraction of debate whether there is an unrecognized issue regarding health effects in human populations of exposure to mixtures. The session's mains focus is on regulatory toxicologist which present mixture risk assessment and address the problem whether established regulatory practice across regulatory domains.

1. The first presenter of the day was Dr. Herzler, who works for the German Federal Institute for Risk Assessment (BfR).

The aim of the topic was to give an overview of US and Europe but also introduce the German regulatory system done at the federal level. There is a difference in naming when it comes to mixture assessments. In the US, the preferred name of mixtures is combined mixtures which looks at a mixture that has two or more substances which each substance retains its own chemical identity. In Europe, the term "Intentional mixtures" is used to address chemical mixtures which look at "Well-Defined substances". The "Well-Defined substances" is the definition which can identify well known chemical compositions and properties. Another is the definition of Unknown or Variable Composition, Complex Reaction Products, or Biological Materials (UVCBs), this looks at unknown mixtures and tries to characterize and identify the mixture. To further analyze these compounds, a Mixture Assessment Factor (MAF) is used to test mixtures of industrial chemicals in the EU's Chemicals Strategy for Sustainability. The final definition is Environmental Risk Assessment which looks at pesticides and background concentrations of substances based on the life cycle of a given environment.

In German federal standards of assessment, they look at three important principles which rely on health risk exposure assessment. They look at the proportionality principle, which looks at the fairness and justice of restrictions imposed by a measure, acts and good that can be achieved for chemicals. The German government also focuses on common toxicological targets, which looks at chemical-based toxicity which have physicochemical characteristics of a mixtures affecting cellular or metabolic pathways. Off target assessment looks at targets on other biological species or the environment. The final principle is

the chemical viability for systemic uptake. This principle looks at a substance that is absorbed into a living system made of physiological activity. Even with these stated principles, it still has its limitations. One of the mentioned limitations where human biomonitoring, which is not stated in any of the principles, this is due to not having a clear specificity on humans' assessment of chemicals. The presenter concluded that this leads difficulty of exposure assessment in relation to human health due to the guidelines not being clear of having a definitive principle when looking at human health specifically.

2. Presenter Dr. Wilks from the University of Basel.

He presented the EFSA (European Food Safety Authority) which is an EU agency which focuses on scientific evidence on food systems across Europe. The main principles presented at EFSA were the Cumulative Assessment Groups (CAGs) which have four levels to identify compounds that exhibit toxicological properties in a system or organ. Level 1 of CAGs looks at the toxicological target of an organ or system which assess what mixtures or compounds are specifically targeted in the living organism. Followed by this Level 2 focuses on the phenomenological effect of the mixture which looks at whether the effects of the mixtures are toxic, neutral or beneficial to a living system. Level 3 focuses on the mode of action which primarily looks for a mixture which can have functional or anatomical changes in the living organism. The final level that is assessed is the mechanism of action, which looks at the specific biochemical interactions which a substance or mixture can produce an effect. This final level looks at molecular targets, enzymes and receptors that would interact with the given mixture or substance. EFSA has a separate cumulative exposure assessment which is done by primarily by a software called SAS which is a standardized statistical program. This leads to the final assessment of risk characterization which quantifies the exposures and toxicity of the given mixture. The presentation concludes that there is an action plan initiative between the EFSA and the EC (European Commission) for pesticide residues regulation and control by the year 2030.

3. Presenter Dr. Matheson by US Consumer Product Safety Commission

The aim of this presentation was to look at medical products which have links to substances or mixtures which are risk assessed at the commission. The Chronic Hazard Advisory Panel (CHAP) was established to look at risk associated with consumer products which focused on phthalates. The method of assessment is the analysis of phthalate exposure and risk this possesses for women of reproductive aging using NHANES and SFF for children which are biomonitoring data from the CDC of National Report on Human Exposure to Environmental Chemicals. The presenter concluded that more focus is needed in product safety of chemicals to find out more risk assessments when it pertains to human health exposure to mixtures.

It's Not Easy Being Green: Applying Alternatives Assessment to Create Safer Consumer Products

4. Development and Implementation of the National Academies Framework to Guide Selection of Chemical Alternatives by Dr. Dorman from North Carolina State University (Abstract #1157)

This session focused on chemical alternative assessments which were developed to identify chemicals that are most likely to harm human and ecological health, and to provide a framework which the industry can develop and adopt safer alternatives. The framework starts with the science policy field which discusses the matter for adaptation. This is then followed by looking for a chemical of particular concern and summarizing the unintended consequences. The current assessment of chemical alternative assessments does not consider cost, performance, human health or environmental health. The presenter suggested a framework which can be broken down into three categories which are: A (unacceptable alternative), B (preferred alternative) and C (acceptable alternative). This framework was assessed by National Academies of Sciences, Engineering, and Medicine (NASEM) which looked at the function and performance requirements for the framework initiative.

The presenter also stated that we must consider the life cycle thinking of possible impacts of a chemical at all stages. This includes production, use, performance, economic and disposal assessments. This can include tradeoff risks in chemicals such as product functionality, efficiency, process safety and resources which need to be considered for the framework initiative. Case studies include computational and mechanistic data from the chemical alternative assessment. The presenter concluded that the framework needs to be implemented more in the industry to not only help understanding of chemical products but also the health of humans and the environment must be considered, when using alternative chemicals in production.