



IIT UPDATE

INSTITUTE FOR INTEGRATIVE TOXICOLOGY



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Poole Hardy Selected for K99/R00 Award from the National Institute of Diabetes and Digestive and Kidney Diseases



EITS alumna and former IIT training grant postdoctoral fellow, **Dr. Lauren Poole Hardy**, was recently awarded a K99/R00 from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) for her project, “Novel mechanisms linking blood coagulation to liver fibrosis.” The K99/R00 program is designed to facilitate a timely transition from a mentored postdoctoral research position to a stable independent research position with independent NIH or other research support at an earlier stage than is currently the norm.

part because there are no therapies to delay progression or promote resolution of hepatic fibrosis, the end stage pathology of chronic liver disease. Activation of the blood clotting cascade is associated with chronic liver disease progression, but the specific mechanisms linking clotting factors to deposition of scar tissue (i.e., collagen) in the injured liver are poorly understood. The primary goal of Poole Hardy’s K99/R00 research proposal is to uncover precisely how blood clotting factors interact with collagen-producing cells in the injured liver so that new therapies can be designed to delay the progression of liver fibrosis in patients.

Poole Hardy is currently a postdoctoral fellow in the laboratory of Dr. James Luyendyk. “Receiving this award is absolutely a dream come true,” commented Poole Hardy. “Since my first undergraduate research experience, being an independent academic researcher has been my only career goal, and this award plays a significant role in facilitating this transition. I am so excited to spend the next five years uncovering new mechanisms driving chronic liver disease progression, as well as starting my own research program to train new scientists.”

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Blevins and Team Publish Exciting Research on Dioxin-like Compounds Effects on the Human B Cell and Immune Response



IIT-affiliated faculty member, **Dr. Lance Blevins**, along with colleagues, Dr. Norbert Kaminski, Dr. Jiajun Zhou, and Robert Crawford, are working on exciting research on dioxin-like com-

pounds effects on the human B cell and its natural immune response properties. Dr. Blevins' research since graduate school has typically focused on regulation of the adaptive immune response, particularly how dioxins modulate the immune response, especially in the human B cell. Dr. Blevins joined the Kaminski laboratory as a postdoctoral mentee in 2016 and then joined the IIT as an assistant professor earlier this year. Dr. Kaminski has been studying dioxins and their relation to B cells for several decades. Together, Drs. Kaminski and Blevins are now studying primary human B cells that they have isolated from the blood and recently published a ground-breaking paper in the journal *Frontiers in Immunology*, "Identification of a Sensitive Human Immunological Target of Aryl Hydrocarbon Receptor Activation: CD5+ Innate-Like B Cells."

"Of all the different cell types that make up the immune system, the B cell appears to be the most sensitive cell type within that system, especially with respect to affecting its function," commented Dr. Blevins. The B cell contributes to immunity in the body by producing antibodies.

Dioxin-like compounds can interfere with the production of these antibodies which has led to Dr. Blevins current research focus - how this specific subset of B cells (innate-like B cells) function when exposed to dioxins. Innate-like B cells are very similar to innate immune cells in that they are the body's frontline defense against pathogens and produce natural antibodies. Innate-like B cells also help to regulate the immune response in the body.

One of the more interesting findings from Dr. Blevins' research is that dioxin seems to be taking advantage of innate-like B cells regulatory capabilities in order to suppress the immune response. PD-1, a protein widely studied in the context of cancer, is a receptor that works like an on/off switch for immune cells. "We actually found in this study that dioxin's appeared to have the capability of causing these innate-like B cells to artificially upregulate this protein, PD-1, on their surface, making them more sensitive to being turned off, to flipping that light switch and affecting immunity," said Dr. Blevins.

"This is one of the first times this protein, PD-1, has actually been shown to be involved in a mechanism of immune toxicity where a chemical is causing a change in the immune system. Just like cancer cells can flip that switch with PD-1 to turn off the immune system and to evade it, now we are also showing that chemicals can make these innate-like B cells more sensitive to having that switch flipped against immunity as well," commented Dr. Kaminski.

Natural antibodies are very important early in life before the immune system is developed, and then as humans age, the

immune system begins to wane and people become more sensitive to pathogens. If people are then exposed to dioxin-like compounds, it may be of greatest consequence to the very young and the very old, whose immune systems are at their most fragile state. "This research has been very relevant to our Superfund Program efforts in understanding the effects of dioxins around the Tittabawassee and Saginaw River Valley area where very high concentrations of these contaminants have been found."

Dr. Blevins' research has looked into a subset of B cell that has been previously under explored. "Starting to unravel this mystery of how AhR, which is the pertinent protein in relation to dioxin contamination, may actually be mediating some of these effects in the B cell that Dr. Kaminski's lab has been studying for twenty plus years has been really gratifying and exciting," Dr. Blevins commented. He is looking to continue this vein of research by drilling down even further into the innate-like B cell subsets, to focus on an even narrower niche of cells that seem to contain most of the regulatory capabilities within the cell pool. Dr. Blevins will continue to delve deeper into how these dioxin like compounds are actually mediating the decrease in the antibody response within these specific cell populations. 🍀

Recent CRIS Blog Topics

The Center for Research on Ingredient Safety continues to use their expert knowledge to research, fact check, and supply the global community with the latest science-based information about the ingredients in food and cosmetics. Here are some of CRIS's most recent blog post topics:

- » Food, Medicine and Cosmetic Packaging - Overview

- » Packaging - Plastic Wrap
- » Packaging - Plant-Based Plastics
- » Packaging - Active Biofilms, Biofilms, and Films
- » Insect Repellent - An Overview
- » Insect Repellent - DEET
- » Fragrances - Overview
- » Hand Sanitizer - Overview
- » Trending - Chlorpyrifos
- » Trending - Aflatoxins

- » Regulation - Understanding Proposition 65
- » Cultivated Meat and Seafood - Background
- » Cultivated Meat and Seafood - Technology

Read more on all of these topics at: <https://www.canr.msu.edu/cris/news-views/>.

Three IIT-Affiliated Faculty Receive Promotions

The IIT would like to congratulate three affiliated faculty members who recently received faculty promotions. Dr. Karen Liby was promoted to Professor and Dr. Jamie Bernard and Dr. Gina Leininger were promoted to Associate Professor.



Dr. Karen Liby is a Professor in the Department of Pharmacology and Toxicology. The goals of Liby's research are to 1) learn how inflammation and the immune system contribute to

carcinogenesis and 2) develop effective drugs that intervene in this process for the prevention or treatment of cancer. Carcinogens found in cigarette smoke and related chemicals are used to initiate or accelerate cancer in some of the laboratory's in vivo models. Synthetic triterpenoids, rexinoids (ligands for the retinoid X receptor), HDAC (histone deacetylase) inhibitors, and bromodomain inhibitors are all potent anti-inflammatory agents. These novel drugs are effective in experimental models of estrogen-receptor negative (ER-) breast

cancer, lung cancer and pancreatic cancer. Identifying molecular biomarkers and investigating the importance of the immune system for the biological activity of these compounds are ongoing areas of study in the Liby laboratory.



Dr. Jamie Bernard is an Associate Professor in the Department of Pharmacology and Toxicology. The Bernard laboratory studies the mechanisms that underlie the onset of carcinogenesis, so they can find

new targets for prevention. Bernard has discovered that intra-abdominal (visceral) fat can promote carcinogenesis in in vitro models and animal models of high-fat diet-induced obesity by releasing fibroblast growth factor-2 (FGF2) and activating FGFR1. The Bernard laboratory aims to identify specific mechanisms of obesity-promoted cancer with a focus on visceral fat inflammation. They are now exploring the translational relevance of FGF2 as a biomarker of adiposity-associated cancer risk. Additionally, the laboratory has interests in screening

to identify new compounds that target their pathways for chemoprevention and identifying modifiable risk factors as biomarkers of adiposity-associated cancer that are amenable to prevention and early intervention measures.



Dr. Gina Leininger is an Associate Professor in the Department of Physiology and the Neuroscience Program. The Leininger laboratory studies how the brain controls behaviors (feeding, drinking,

moving) that alter body weight, with the long term goal of identifying strategies to effectively treat obesity and eating disorders. The Leininger lab has characterized specific populations of neurons and circuits that promote weight loss. Now, using a combination of genetic and pharmacological approaches, they are defining the precise circuits, cells and signals that modulate feeding, drinking and physical activity to understand and how they might be leveraged to modify body weight. 🍷

McCabe Named New Assistant Vice President for the Office of Regulatory Affairs at MSU



IIT-affiliated faculty member, **Laura McCabe**, was recently appointed the new assistant vice president for the Office of Regulatory Affairs (ORA) at MSU. Her appointment began on August 16, 2021.

McCabe has been serving as director for special projects in ORA for the past year, working closely with J.R. Haywood, who most previously held the position.

McCabe has been actively involved

with COVID-19 research response efforts, coordinating with all units under ORA to develop safety plans for research continuity and reactivation. In her new role, she will be responsible for all current units under Regulatory Affairs and, additionally, under Export Control and Trade Sanctions. She will report directly to Doug Gage, vice president for Research and Innovation, and serve on his leadership team.

An MSU Foundation Professor in the Department of Physiology, McCabe's research program has focused on preventing osteoporosis and identifying ways to regulate bone mass. She has published extensively on novel mechanisms and therapeutics that target the intestines and treat or prevent bone loss that occurs from

conditions such as menopause, intestinal dysbiosis, stress and diabetes.

"MSU is extremely fortunate to have Dr. McCabe at the helm of the Office of Regulatory Affairs," Gage said. "Regulatory compliance is critical for the research mission of the university, and it requires nimble responses to changes in requirements at the federal and state level for environmental health and safety, animal care, and human subject protection.

Read more of this story that originally appeared on the College of Natural Science website at: <https://natsci.msu.edu/news/laura-mccabe-tapped-to-lead-regulatory-affairs-at-msu/>. 🍷

EITS Student Morgen Clark Featured on MSUToday in Student Perspective

EITS graduate student, **Morgen Clark**, was recently featured on MSUToday for her student perspective on joining a research lab at the start of a pandemic. Clark is a third-year doctoral student in the lab of Dr. Gemma Reguera, studying microbiology and environmental toxicology in the Microbiology and Molecular Genetics Program and the EITS Doctoral Program.

Clark's student view:

When I scheduled a rotation in the Reguera Lab, I knew that it would come with a steep learning curve. While the lab works in my much-beloved field of microbiology, its focus on the genus *Geobacter* — a group of anaerobic, environmental bacteria — would be a departure from my previous pathogenic and mainly aerobic work. The learning curve only became steeper when the pandemic hit and shut down the university less than two weeks after my previous eight-week rotation ended and I had officially joined the Reguera Lab.

Although the shutdown stifled my ability to work in the lab, it presented an opportunity many new graduate students do not have: Time to dive into



the literature surrounding my new research topic without any distractions. And because of that, I was able to write a review with Dr. Reguera encompassing topics critical to my research. Concepts such as extracellular electron transfer (a mechanism used by *Geobacter* and certain other electroactive bacteria to complete their metabolism) soon became intimately familiar, and this foundation of knowledge has been extremely important as I design experiments and analyze data during my research.

Returning to the lab was a relief, but also quite daunting. My project

depended upon equipment not used by any current lab members and, even when I used techniques common in the lab, there often was no one else present as we observed proper COVID distancing rules. With that being said, careful planning was essential and accepting inevitable mistakes was equally important.

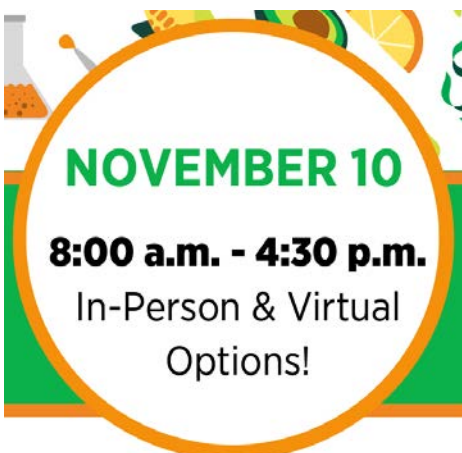
For instance, the little things like learning my way around the lab became a solo venture. It turns out there were a lot of drawers and cupboards to search each time I needed to locate a piece of equipment I had not used previously.

Eventually, though, I hit my stride and achieved progress with my research. A little over a year after joining the Reguera Lab, my first, first-author research article has been accepted for publication.

Despite the struggles I faced joining a lab in the midst of a pandemic, I learned many invaluable lessons from the circumstances and became a better scientist for it. I guess there really is a silver lining in every situation.

To read the original article on MSUToday, please visit: <https://msutoday.msu.edu/news/2021/student-view-joining-a-research-lab-at-the-onset-of-a-pandemic>. 📄

CRIS Science Day 2021 - Packaging Ingredients



The 2021 Center for Research on Ingredient Safety Science Day will focus on food and cosmetic packaging ingredients specifically looking at

nanomaterials, contaminants, and sustainability. In-person and virtual options are available to attend. Two sessions will be held:

Session One: Nanomaterials in Packaging

- » Sabina Halappanavar, Ph.D., *Nanomaterials safety assessment: challenges and opportunities*
- » Raymond Briñas, Ph.D., *Safety Evaluation of Food Contact Substances Containing Nanomaterials*
- » Timothy Duncan, Ph.D., *The migration of engineered nanomaterials out of nanotechnology-enabled polymers and into foods*
- » Maria Rubino, Ph.D., *Including functional nanoparticles for the implementation of active surfaces*

Session Two: Contaminants and Chemical Hazards in Packaging

- » Olivier Jolliet, Ph.D., *High Throughput Risk Screening of chemicals in packaging and food contact materials*
- » Karen Hagerman, MS, *Sustainable Packaging: Toward Safe and Circular Materials*
- » Devon Wm Hill, Esq., *A Focus on the Impurities (NIAS) Assessment in Food Contact Notifications*

Communication Presentation

- » Katya Hantel, MS, *How to Talk About Science so People Will Listen*

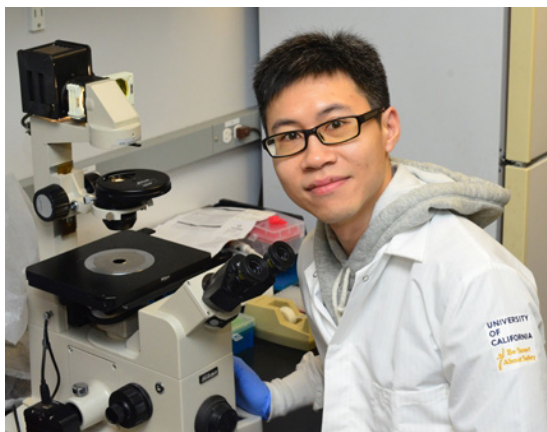
For more information and to register, please visit: <https://www.canr.msu.edu/events/science-day-2021-packaging-ingredients>. 📄

Kin Sing Lee and Graduate Student Morteza Sarparast Awarded Pearl J. Aldrich Endowments

IIT-affiliated faculty member, **Kin Sing Lee** (pictured), as well as his graduate student, **Morteza Sarparast**, were both recently selected for 2022 MSU Pearl J. Aldrich Endowment awards.

The Pearl J. Aldrich Endowment in Gerontology was created through an estate gift by Pearl Jackson Aldrich, Ph.D., who passed away in 1997. Dr. Aldrich was a long-standing faculty member who devoted nearly 36 years to MSU's Department of Family Ecology before retiring as a Professor and Associate Dean for research in 1978. These one-year awards are competitive and are intended to support Michigan State University graduate students and faculty who are committed to or strongly considering a career in a discipline that relates to aging. Awards may be used to support new research initiatives or ongoing projects that have a clearly identifiable focus that relates to gerontology or geriatric medicine.

Morteza Sarparast was selected for his proposal, "Recusing Effects of the



Soluble Epoxide Hydrolase Inhibitor AUDA on Neurodegeneration Induced by A β and/or Tau in *Caenorhabditis elegans*."

Lee will use his award to investigate how omega-3 and omega-6 fatty acids modulate age-associated neurodegeneration. Specifically, Lee will investigate how endogenous omega-6 dihomo-gamma-linoleic acid induces ferroptosis in neurons. Ferroptosis is a novel

non-apoptotic programmed cell death pathway that is iron dependent and is characterized as an increase of membrane lipid per oxidation. Due to the challenges of investigating ferroptosis and aging in mammalian models, in this proof of concept study, Lee and his laboratory will use *Caenorhabditis elegans* as their model organism to investigate the specific mechanism of aging and neurodegeneration induced by dihomo-gamma-linoleic acid. Because *Caenorhabditis elegans* is genetically homologous to humans, it has a short life span for aging studies and vast imaging and genetic tools available for mechanistic studies. Lee's results from this project will help identify novel targets and mechanisms to maintain neuronal health, particularly for the elderly population. In addition, the tools that they develop from this project will build a strong foundation for future mechanistic studies. 🍷

Hui Li Leads Team Awarded \$2 Million EPA Grant to Study Impact of Current Biosolid Treatment Methods



IIT-affiliated faculty member, **Hui Li**, is leading a team of researchers recently awarded a \$2 million EPA grant to study the impact of current biosolid treatment methods used by wastewater treat-

ment facilities on a variety of pollutants in soil, water and plants. Li will work alongside IIT-affiliated faculty members, **Wei Zhang**, an associate professor in the Department of Plant, Soil and Microbial Sciences, and **Courtney Carignan**, an assistant professor in the Department of Food Science and Human Nutrition. The team will also include James Ippolito from Colorado State University, Qingguo Huang from the University of Georgia,

and John Norton Jr. from the Great Lakes Water Authority.

With the new funding, the first step is for the team to survey current biosolid treatment processes for per- and poly-fluoroalkyl substances (PFAS) and pharmaceutical and personal care products (PPCP) concentration and leachability. Then, through field and laboratory experiments, Li and his team will look to fill knowledge gaps in the fate, transport, occurrence and plant uptake of PFAS and PPCPs. This data collection will help in the development of models that quantify exposure risk to humans, which will be shared with communities and agencies responsible for biosolid treatment.

For agricultural purposes, some growers use biosolids as a soil amendment to provide plant nutrients and improve soil health, but there is mounting concern that they inadvertently introduce harmful chemicals such as PFAS and PPCPs.

PFAS, in particular, has become a

growing public concern in recent years as a threat to human health, resulting in an increased emphasis on research from both funding agencies and scientists. To that end, MSU has created the Center for PFAS Research, which brings together scientists from across the university to quantify and communicate PFAS risks while working to mitigate its impact.

Developing quantitative models for human exposure is the ultimate goal of the new project, followed by communication of the information via MSU Extension and other outreach initiatives.

Read more of this story by Cameron Rudolph that originally appeared on the College of Agriculture and Natural Resources website at: <https://www.canr.msu.edu/news/msu-led-team-receives-epa-grant-to-explore-biosolid-treatments-and-the-effects-of-chemical-pollutants-such-as-pfas>. 🍷

EITS Student Jessica Moerland Awarded the Barnett Rosenberg Endowment Research Assistantship



EITS graduate student, **Jessica Moerland**, received the Barnett Rosenberg Endowment Research Assistantship for the academic year 2021 - 2022. Moerland is training with

IIT-affiliated faculty member Karen Liby in the Department of Pharmacology and Toxicology. Moerland will receive a stipend of \$34,000 for the upcoming academic year, which includes a standard graduate assistantship to cover health insurance and tuition waiver.

The recipients of this distinctive and prestigious award are generally advanced students with a distinguished record of accomplishment at MSU. Barnett

Rosenberg was a chemistry professor at MSU who recognized the anti-cancer potential of the platinum-based compounds cisplatin and carboplatin. These drugs produced over \$325 million in royalties for MSU, providing funding for Barnett Rosenberg Endowments.

Moerland's research focuses on the immunomodulatory effects of Nrf2 pathway activation in the lung tumor microenvironment (TME). Nrf2 is a cytoprotective pathway which protects healthy cells from malignant transformation, but when upregulated in cancer cells can promote drug resistance and tumor progression. Tumor cells in up to 30% of human non-small cell lung cancer (NSCLC) patients have an activating mutation in the Nrf2 pathway. However, pharmacological Nrf2 activation has anti-tumor effects in preclinical mouse models and whole-body Nrf2 knockout results in increased lung tumor burden and an unfavorable immune signature

in the lung. The role Nrf2 plays in activation or polarization of immune cells within the TME is underexplored, and how activation of this pathway affects tumor cell/microenvironment crosstalk is unknown. Nrf2 activators are in clinical trials for multiple diseases, so it is important to study how these drugs affect immune cell populations and the potential implications this has in lung carcinogenesis.

Moerland was honored to receive the Rosenberg award, since Dr. Rosenberg's work is very relevant to her current research and personal interests. "As the discoverer of cisplatin, a powerful and commonly used chemotherapeutic," commented Moerland, "his contributions to drug discovery and cancer pharmacology have motivated me to be more creative in my research, as well as to not be afraid to take on difficult projects."



EITS Graduate Kathryn Wierenga Selected for New Investigator Award at ISSFAL 2021 Virtual Congress



Recent EITS graduate, **Kathryn Wierenga**, received the New Investigator Award from the International Society for the Study of Fatty Acids and Lipids (ISSFAL) 2021 Virtual Congress. Wierenga was awarded for her presentation,

"Single cell RNA sequencing reveals DHA-induced macrophage polarization toward an anti-inflammatory pheno-

type." Wierenga trained with IIT-affiliated faculty member James Pestka who is appointed in the Departments of Food Science and Human Nutrition and Microbiology and Molecular Genetics.

ISSFAL is an International Scientific Society established in 1991, of more than 500 members from more than 40 countries. ISSFAL members are scientists, medical professionals, educators, administrators, communicators and others with an interest in the health effects of dietary fats, oils and lipids; members include researchers carrying out studies on the health effects of omega-3 and omega-6 fatty acids, conjugated linoleic acids (CLA), saturated and monounsaturated

fatty acids as well as other lipids. ISSFAL is the foremost international scientific society dealing exclusively with the health impact of dietary lipids.

Wierenga will be continuing her education as a postdoctoral student in the laboratory of Dr. Alain de Bruin at Utrecht University in the Netherlands. The de Bruin laboratory is interested in understanding the pathobiology of aging and cancer and how changes to the cell cycle influence these pathways. Initially, Wierenga will participate in a project using single cell technologies to investigate how individual cancer cells respond to replication stress induced by anti-cancer drugs. 🌟

IIT Welcomes New EITS Students

The IIT is pleased to welcome the following students who have joined the EITS program in the past several months:

- » **Brianna Finn**, training with Dr. Nobert Kaminski
- » **Ying Guo**, training with Dr. Courtney Carignan
- » **Romina Gonzalez-Pons**, training with Dr. Bryan Copple
- » **Jessica Moerland**, training with Dr. Karen Liby
- » **Warren Sink**, training with Dr. Timothy Zacharewski

Recent EITS Graduates



Christine Ponnampalam

Genetics

Mentor, Brian Gulbransen

Dr. Christine Ponnampalam received her Ph.D. after completing the dual major program in Genetics and Genome Sciences and Environmental Toxicology. Her dissertation was, “*Novel impacts of host-environment interactions on enteric glia through sequencing and in situ expression.*”

As a MD/Ph.D., Ponnampalam is now working in Grand Rapids, MI doing clinical rotations at Spectrum and Mercy hospitals. She will continue to finish up her Ph.D. publications and may potentially complete some clinical research if she comes across an interesting project.



Nikita Saha Turna

Food Science and Human Nutrition

Mentor, Felicia Wu

Dr. Nikita Saha Turna received her Ph.D. after completing the dual major program in Food Science and Human Nutrition and Environmental Toxicology. Her dissertation was, “*Co-exposure of aflatoxin and fumonisin in Nigerian maize and the non-carcinogenic risk of aflatoxin in southwest Nigerian children and adults.*”

in Vancouver, BC, Canada. Her responsibilities include identifying environmental hazards, exposure levels and environmental health issues in response to requests from regional health authorities, ministries, and healthcare officials. She is also responsible for providing expert advice on complex questions related to environmental health issues, food and water safety, exposure assessments and toxicology. Saha Turna’s position also requires her to lead multi-disciplinary project teams and monitoring project team activities in identifying problems related to public health and solving them.

Saha Turna is now a Toxicologist at the British Columbia Center for Disease Control (BCCDC)



Jenna Strickland

Pharmacology and Toxicology

Mentor, Bryan Copple

Dr. Jenna Strickland received her Ph.D. after completing the dual major program in Pharmacology and Toxicology and Environmental Toxicology. Her dissertation was, “*Cytokine Dysregulation Disrupts Macrophage-Mediated Liver Repair, Triggers Hepatic Encephalopathy, and Increases Mortality in Acet-*

aminophen-Induced Acute Liver Failure.”

Strickland is now a Toxicology Study Director at LabCorp in Madison, WI. In her new role, she helps to conduct complex studies to generate high quality scientific results on behalf of Covance clients and plays a key role in developing new strategies, techniques, and instrumentation for new ventures and critical issues in a variety of toxicology specialties.

EITS Student Azam Sher Leads Public Health Outreach in Pakistan



Kher-Kun, which means “well-wisher” in Arabic, is a nonprofit founded and headed by Dr. Azam Ali Sher, an EITS graduate student. Sher is mentored by IIT-affiliated faculty member, Dr. Linda Mansfield, University Distinguished Professor and Albert C. and Lois E. Dehn Endowed Chair for the MSU Department of Large Animal Clinical Sciences. Kher-Kun’s mission

aims to provide public health education to far-flung Pakistani villages with outreach efforts that include, among other avenues, webinars and virtual trainings.

Sher led a recent Kher-Kun initiative called “Student Leaders in Public Health in Pakistan,” which was enacted in collaboration with the U.S. Mission to Pakistan and the Pakistan-U.S. Alumni Network. The semester-long project was entirely coordinated and funded by Sher while he was working on his research and PhD.

The goal: recruit passionate students from diverse academic and regional backgrounds to train in public health topics with public health experts and Fulbright scholars overseas.

To read more about Sher, his work with Kher-Kun and his research interests, view the original article from MSU CVM at: <https://cvm.msu.edu/vetschool-tails/spartan-researcher-leads-public-health-outreach-in-pakistan>.



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