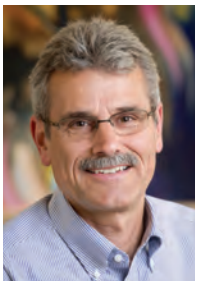
OSU Oregon State University

The Linus Pauling Institute

SPRING-SUMMER 2015 **RESEARCH NEWSLETTER**



From the Director

Balz Frei, Ph.D.

LPI Director and Endowed Chair
Distinguished Professor of Biochemistry and Biophysics
Joan H. Facey LPI Professor

The Linus Pauling Institute recently added four new investigators to its faculty roster, who joined the Institute as Adjunct members: Drs. Claudia Maier, Arup Indra, Robert Tanguay, and Siva Kolluri, all from Oregon State University. Their role as Adjunct Faculty is to support and help advance LPI's research program by collaborating with other LPI faculty on research projects, grant proposals, and shared methodologies. Dr. Maier is a Professor of Chemistry and an expert in mass spectrometry applied to the analysis of biomolecules, including proteins and metabolites (called proteomics and metabolomics, respectively), and the study of the interactions of these proteins and metabolites in human blood and cells (for an article on Metabolism, see our Spring/Summer 2013 Newsletter). Dr. Indra, an Associate Professor of Pharmaceutical Sciences, is studying melanoma, inflammatory skin diseases like atopic dermatitis, and natural compounds for effective therapeutic interventions. Dr. Kolluri, an Associate Professor in the Department of Environmental and Molecular Toxicology, is developing novel agents that selectively kill cancer cells, but not normal cells, and is devising optimal combinations of therapeutic agents for the prevention and treatment of different types of cancers. You can read more about Dr. Tanguay's exciting research in the world-class zebrafish facility that he built at OSU in this Newsletter (at right) and will hear more about the research in the other Adjunct Faculty's laboratories in future issues of the Newsletter.

Claudia Maier

Arup Indra

Siva Kolluri

Robert Tanguay

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Fish Toxicology

An Interview with
Robert Tanguay, Ph.D.
LPI Adjunct Faculty

Q. You earned your Ph.D. from the University of California-Riverside about 20 years ago. What was your thesis?

A. Post-transcriptional regulation of gene expression.

Q. What does that mean?

A. Prior to that era, few scientists thought that gene expression could be regulated outside the nucleus or after messenger RNAs were produced. Messenger RNA (mRNA) conveys information from DNA to the cellular machinery where proteins are made. My advisor Daniel Gallie and I proposed that there are elements within RNAs that regulate how much protein is produced from a given messenger RNA. More specifically, we proposed for the first time a circular translation model where the ends of mRNA were looped backed and intimately connected through proteins. Our work and a number of other papers demonstrated that our hypothesis was correct, and now it is well established that post-transcriptional mechanisms are very important in all species. Ultimately, my interest in gene expression really stemmed from the RNA universe I was studying as a graduate student.

Q. Where did you grow up?

A. I'm from the upper peninsula of Michigan. I lived there until my junior year in high school, and then we moved to California, where I finished high school, my undergraduate training in biology at California State University-San Bernardino, and graduate school in biochemistry at the University of California-Riverside. I did my post-doctoral training for 3½ years at the University of Wisconsin in Madison with Richard E. Peterson. That is where I developed the zebrafish model for use in toxicology in 1996. I got my first faculty appointment at the University of Colorado Health Sciences Center in the

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Continued from cover — From the Director


Joe Beckman

I am also very pleased to report that LPI's Joe Beckman has recently been awarded a $2.1 million grant from the Department of Defense, entitled "Development of Copper ATSM as a Therapeutic for SOD-Familial and Sporadic ALS" (amyotrophic lateral sclerosis, also known as Lou Gehrig's disease). This project is based on Dr. Beckman's recent breakthrough discovery that the copper complex, Cu-ATSM, is exceptionally effective in delaying the onset of ALS in a mouse model of the disease. The DOD grant will allow Dr. Beckman to design new chemical derivatives of Cu-ATSM targeted specifically to the mitochondria of motor neurons, where the copper is released and triggers the protective mechanisms against the neurons' death. This research will help move Cu-ATSM and these novel derivatives towards human clinical trials to test their safety and efficacy. Although we still have a long road ahead of us, if successful, this could become the first treatment ever for Lou Gehrig's disease.

Two other projects have kept us busy in the Linus Pauling Institute in recent months—the re-design of our website and the eighth installment of our Diet and Optimum Health conference. Re-designing the LPI website and its Micronutrient Information Center (MIC) would not have been possible without the painstaking work of my assistant, Barbara McVicar; LPI's MIC manager, Victoria Drake; and many others in the Institute. I invite you to visit and explore our new website (lpi.oregonstate.edu), including the MIC (lpi.oregonstate.edu/mic), and send us your feedback and suggestions for further improvements!

The 2015 Diet and Optimum Health conference, to be held September 9-12 on the OSU campus in Corvallis, Oregon, emphasizes dietary and lifestyle approaches, including exercise and dietary supplementation, to advance human healthspan and prevent age-related diseases. We are excited to have been able to get the commitments of many outstanding scientists from around the world to present their work at our conference on such important topics as "Vitamin E: A Critical Nutrient for Development and Health," "Extending Healthspan: Basic Concepts, Underlying Mechanisms, and Role of Diet and Lifestyle," and "Cancer Chemoprevention." The conference also features a session open to the public focusing on "LPI's Rx for Health: The Role of Diet, Micronutrients, and Dietary Supplements in Human Health and Disease." The complete program of the conference can be found on pages 12-13 of this Newsletter—which I now invite you to enjoy reading! LPI

Continued from cover — Fish Toxicology

School of Pharmacy in 1999. I was then recruited to direct the Sinnhuber Aquatic Laboratory and to join OSU's Department of Environmental and Molecular Toxicology as an Associate Professor in 2003.

Q. What led you to Oregon State University?

A. Whenever you move there is push and draw. The push was I had large plans to grow my program that could not be accommodated in a typical School of Pharmacy. The draw was that I saw tremendous potential everywhere on this campus, and I thought I could help lead advancements. I'm the type of person who likes to build and nucleate programs. I enjoy collaborating with others, and OSU has a tremendous collaborative spirit that can be addictive and rewarding.

Q. Some of your initial research was on plants. What did you work on?

A. In plants, I was trying to understand how an RNA sequence could possibly modulate how much protein is made. I'm a reductionist, really, and like to understand processes at the most fundamental level; I had little inherent interest in plants but was really interested in understanding evolutionarily conserved molecular machinery. Funding also influenced my decision to cross over into a vertebrate model.

Q. About 15 years ago you developed an interest in zebrafish and trout as useful models in toxicology. Why fish?

A. Good question. When I was offered an NIH fellowship to go to Wisconsin, I had a choice of 20 labs to join on the training grant. I interviewed Richard Peterson, who has a long track record in evaluating the toxicity of environmental chemicals in applied research using lake trout and rainbow trout. He determined how much of a chemical was killing these fish in the Great Lakes. This led to some dramatic changes in regulation and saved fish populations in the Great Lakes. But at that time the Peterson lab had limited experience with molecular explorations in those models. When I went there, I didn't want to work with trout because I became aware of a new model—zebrafish—in toxicology and human health. I didn't know anything about toxicology. I had never taken a toxicology course and still haven't, but I just saw there was a huge potential to solve problems in toxicology with this new model.

Q. Is the zebrafish a good model for human diseases like cancer and heart disease or is it limited to toxicology?

A. I think that the field has advanced faster outside of the toxicology realm. There are hundreds or thousands of investigators using the zebrafish model for various

aspects of human disease, including heart disease and cancer, for example, in the discovery of tumor suppressor genes, oncogenes, and therapeutics.

Q. What are the specific attributes of the zebrafish model that make it more compelling than a rodent model?

A. They are numerous, and we try to exploit all the advantages and avoid the disadvantages with a lot of discipline. For example, we now know that the zebrafish genome is remarkably well conserved, sharing about 70% with humans, and of the human genes known to be involved in disease, 84% are also found in zebrafish. If you look at individual biochemical pathways, they are all there. And then there are massive technical advantages. We can investigate almost every human disease in this model at a fraction of the cost and a fraction of the time, with very few limitations. Most of the limitations are truly just matter of perception or unintended biases by scientists unfamiliar with the strengths of the model.

Q. Is it more economical to use zebrafish rather than rodents?

A. Absolutely. You still have the same animal care and use considerations and the same compliance requirements, but the cost of housing is dramatically lower. We have several hundred thousand zebrafish that are supporting dozens of researchers on this campus.

Q. I imagine that the low cost and high numbers of fish increase the statistical power of these experiments.

A. It certainly does, but more importantly, it frees your imagination. If you talk to researchers who are primarily using rodents for their research, the number one consideration that stops them from doing an exciting experiment is cost. I do not like barriers to my imagination.

Q. What kinds of toxins have you studied using zebrafish?

A. We have tested thousands of compounds, including pharmaceuticals, dietary constituents, pesticides, industrial chemicals, herbicides, chemical libraries, environmental contaminants, and real-world mixtures.

Q. Are the concentrations of chemicals to which you expose the zebrafish embryos relevant to those that humans might be exposed to?

A. All of our studies are completed at relevant ranges, but because of the low cost, we don't limit it just to that. We evaluate broad ranges; we may do a typical study with five orders of magnitude evaluations in large numbers of fish, giving massive statistical power. It allows comparative analysis of different chemicals based on their structure.

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Zebrafish

I just saw there was a huge potential to solve problems in toxicology with this new model.

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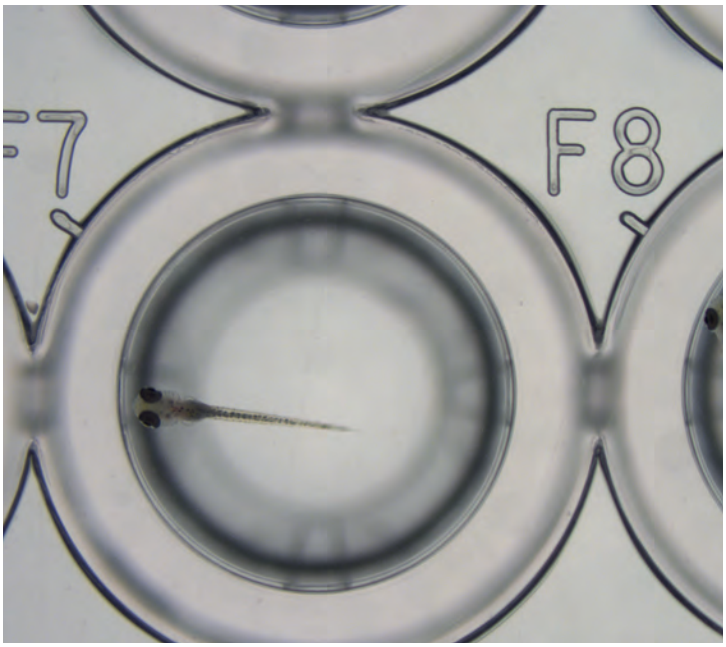


A five-day-old zebrafish in a microwell plate

Q. Could some of your results be applied to amphibians like salamanders and frogs, or are they more sensitive than fish?

A. For some chemical classes, amphibians seem to be more sensitive. But my goal is not to translate my findings directly to amphibians. Many of the biochemical pathways that we've discovered certainly exist in amphibians, and I will let the amphibian experts translate my results to their studies. Because I work with zebrafish, people think I am a "fish guy," but I really am not. Part of my research focus is to understand chemicals—how they modify a biological target or pathway. I want to discover the structural attributes of the chemicals so that we can identify their targets. If we do this well, we can eventually predict the activity of all chemicals. As you can imagine, there would be tremendous societal value in reaching that goal. We do not do one chemical at a time or one pathway at a time but rather systems approaches to tackle bigger problems.

Q. Do you usually treat zebrafish embryos with chemicals and watch for developmental defects?

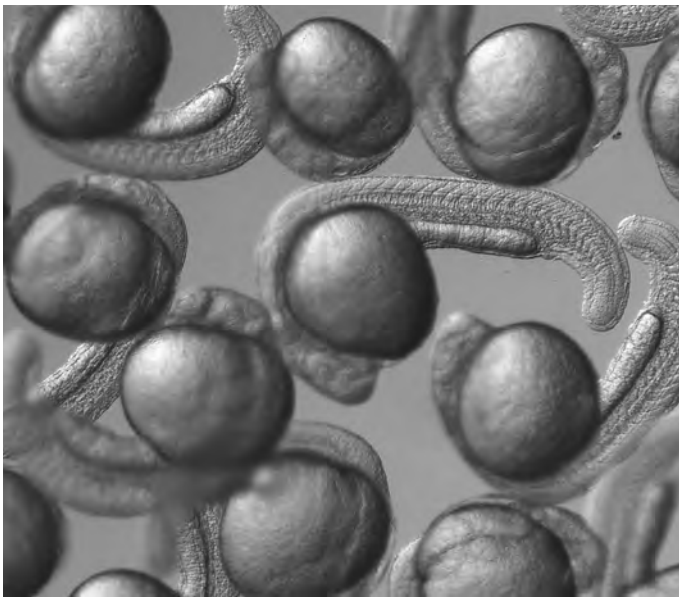
A. We actually ask the embryos, in as many ways as we can imagine, to tell us if a chemical has biological activity. Does it cause changes in heart development, eye development, hearing, swimming behavior, learning and memory, anxiety, social behavior, fear responses, skeletal problems, gene expression (mRNAs, non-coding RNAs, and proteins), or metabolism.

Q. How long do you follow these embryos?

A. For the early life stage studies we typically follow them for five days. In that five-day period, the embryo goes from a single cell to an animal that has all of the functioning organs—the heart, the brain, the kidneys, the liver, etc. We do that because I'm interested in finding compounds that affect development. By definition, chemicals that affect development have the ability to interact with and alter evolutionarily conserved molecular pathways. These chemicals may pose risks to humans, or they may have therapeutic value. We use zebrafish as the ultimate biological sensor.

Q. What kind of defects do you typically observe in embryos that are treated with pesticides or herbicides?

A. We've spent almost 20 years doing this, trying to identify anything that's different. That may be the length of the animal, the diameter of the eye, the axis, the spinal cord, vascular development, heart rate, or complex behaviors. All these can be measured. Since we have studied over 100,000 compounds, we know that many chemicals produce the same effects. What that means is that development is so integrated and dynamic that there are almost countless ways that you can disrupt it to lead to the same problem. For example, in order for dioxin to cause cardiovascular failure, you need to have a certain



24-hour-old zebrafish embryos

cellular receptor that is directly activated by dioxin. If you delete that receptor in fish or other animals, dioxin doesn't cause problems.

Q. Do you test these toxins in adult fish?

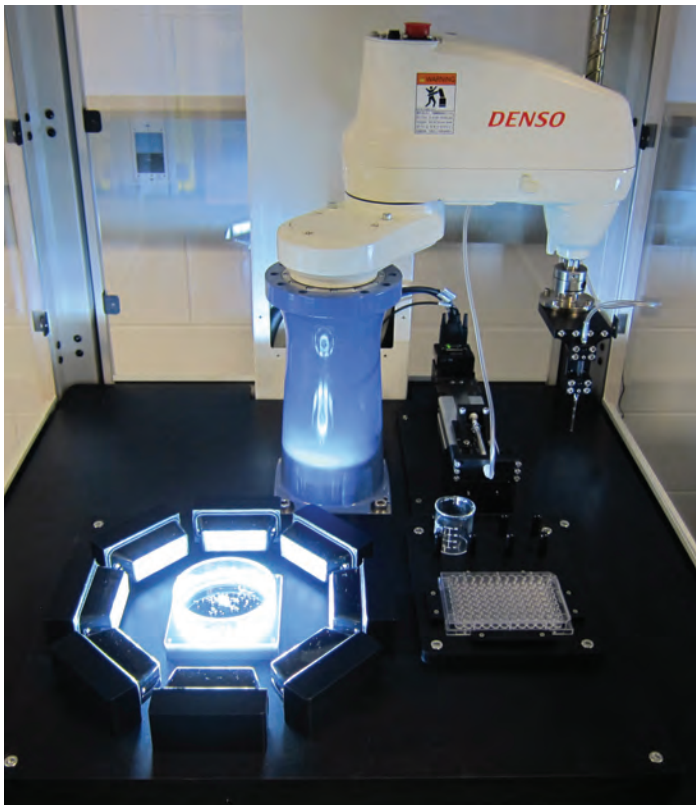
A. We certainly do some exposures in adult fish—over three months old—but you can't do rapid screening in adults. With the genetic tools at our disposal we are increasingly doing more adult studies, but the true advantages of zebrafish are the early life stages. Stay with the strengths of your model is something I preach to my students and other scientists.

Q. How long do the fish live?

A. Zebrafish can live up to three years. They are typically in our facility about a year.

Q. *You also investigated the effect of embryonic exposure to alcohol and various flame retardants that are ubiquitous these days. What did you find in those experiments?*

A. The ethanol work started when I was a post-doctoral fellow. I wanted to see whether or not I could use zebrafish to study fetal alcohol syndrome. Amazingly, we still don't fully understand how ethanol produces the typical cranial facial malformations and, more importantly, the learning and memory deficits. And we don't understand why there is such a variation of responses between people exposed to similar doses of ethanol. We published a number of papers demonstrating that the zebrafish model is appropriate to study the developmental responses to ethanol exposure. In particular, we are looking at the involvement of some microRNAs that are regulated in response to ethanol exposure. MicroRNAs are small, non-coding pieces of RNA that regulate gene expression. They were discovered about 20 years ago. These 22-nucleotide modulators can bind recognition sequences through the genome and affect gene expression in any number of interesting ways.



Automated embryo plating system

Q. *So they are regulators of protein synthesis?*

A. Absolutely, they are. It's amazing what these can do. And they are incredibly well conserved evolutionarily across species. In fact, some of the microRNAs that we discovered are 100% identical between humans and zebrafish.

Q. *How many microRNAs are known?*

A. In zebrafish, there are under 400. In humans, it's more like 800. But discovering them is one thing; identifying what they do is another. What they do varies by tissue and life stage. We have all the tools necessary to do much more microRNA work, which I really enjoy as this gets me back to my scientific roots in post-transcriptional regulation of gene expression.

Q. *Do the behavioral effects caused by alcohol persist into adulthood?*

A. We haven't done the behavioral analysis in adults that other collaborators have. In early development we typically identify either hyper- or hypo-activity in swimming behavior. When we raise these fish to the juvenile and young adult stage, we see hypo-activity in swimming that persists in adults. We developed a whole roomful of new instruments to assess higher orders of learning and memory. We can evaluate the effect of chemicals on decision-making in fish.

Q. *What about flame retardants?*

A. The study of flame retardants is a very active area in my lab right now. We just completed the world's largest evaluation of broad classes of flame retardants in zebrafish. We looked at 44 different flame retardants in four different chemical classes. Flame retardants are required by law to be in many consumer products, and there are hundreds of millions of tons used worldwide. But some of these chemicals are not as bad as others. There are hundreds of different flame retardant compounds used commercially, but many people group them all together based on their commercial use. This is equivalent to suggesting that all the food we consume is equally healthy because it is all food. I maintain that some flame retardants are safer than others. I want to identify which of these chemicals are intrinsically bioactive and which ones aren't and then advise the industry so that they can make the safe and effective ones.

Q. *What environmental pollutants or carcinogens have you studied using zebrafish?*

48-hour-old zebrafish embryo

A. We have looked at hundreds of different compounds—polycyclic aromatic hydrocarbons (PAHs), dioxins, and PCBs. There is much to be learned. Not all carcinogenic compounds are mutagenic, so we are not looking for mutagenicity in our model. Can we use this model to identify mechanisms and relate those to the chemical structure of the compound? If we test 50 compounds that produce a similar response—for instance, molecular networks are perturbed in a similar way—then we may be able to predict the biological effects of many other chemicals with similar structures. Again, we think predictively, not one chemical at a time.

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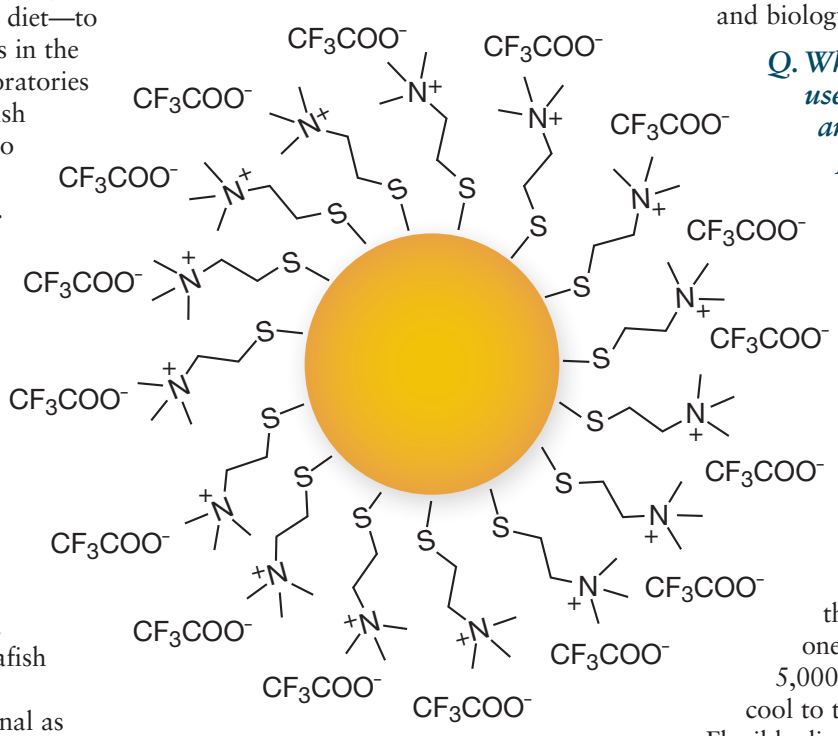
Q. With Maret Traber's lab you published a number of papers on induced vitamin E deficiency in zebrafish embryos. What did you find?

A. The first challenge was to generate a fully defined zebrafish diet—to control exactly what is in the food. Worldwide, laboratories feed billions of zebrafish many different diets, so the lab-to-lab dietary variation is significant. So we first had to develop a defined diet to identify the constituents that we should use. We leaned heavily on the substantial OSU rainbow trout work with well-developed defined diets. With Dr. Traber's help we developed a defined diet and demonstrated that feeding it to zebrafish allowed them to be reproductively functional as adults. That was the first big hurdle. Together, we found that when we fed the zebrafish parents the vitamin E-deficient diets, they produced embryos that had poor viability. So there were developmental defects in the embryos—the lack of vitamin E prevented normal vertebrate development.



Functionalized gold nanoparticle

Q. What were the effects in these embryos from parents that were depleted of vitamin E?

A. There was clear evidence that vitamin E was necessary to accomplish normal vertebrate development. This had been hypothesized in the literature on mammals, but it was always difficult to separate out the effects of vitamin E on the mother and the effects *in utero*.

Q. Since vitamin E mainly functions as an antioxidant, oxidative stress probably played a role in those defects that you observed.

A. More than likely, but that doesn't necessarily mean that oxidative damage is the only factor. We now understand oxidative signaling, and since there are developmentally important genes, it's my hypothesis that by changing the oxidative environment you change signaling in subtle ways that affect gene expression. Additionally, changes in membrane integrity or function may be main drivers.

Q. You've also had a long-term interest in the toxicology of nanoparticles, including fullerenes. What are nanoparticles?

A. Nanoparticles are touted as the foundation for the next technological revolution. Nanomaterials are less than 100 nanometers, or about 100 billionths of a meter, in one dimension. Nanoparticles called fullerenes are perfect spheres made from 60 atoms of carbon. We proposed in 2005 that we could use the zebrafish embryo to test interactions between nanoparticles and biology.

Q. Why are nanoparticles used in research, medicine, and technology?

A. It really depends on the chemical class and market advantage or improvement. For example, space shuttles had thick heat shields for protection upon re-entry. It may become possible to have a thin layer of nanomaterial, as thin as a piece of paper, that could be wrapped around the space shuttle structure that only conducts heat in one direction. So it could be 5,000 degrees on one side and cool to the touch on the other side. Flexible displays, next-generation computers, batteries, solar cells, pesticides, food packaging, diagnostic imaging, LED lighting, building materials, and catalysts all use nanoparticles. In medicine, one of the huge advantages of nanomaterials is the surface area that you gain. You can take a sphere that is a millimeter in diameter and decorate it with functional drugs. Now if you can do this in nanoscale, the surface area and opportunities for multi-functional, drug delivery devices become fantastic.

Q. Your work is focused on gold and silver nanoparticles, sometimes coated with various chemicals. What does the chemical treatment achieve, and do you find that some chemical treatments of these nanoparticles might attenuate toxicity?

A. This is relevant to our "green" chemistry focus and "green" nanotechnology with my collaborator, Jim Hutchison, at the University of Oregon. The gold nanoparticles are a good model. We want to learn something about the feature of a particle that might predict what it can do. That knowledge will help us to develop better performing products that are also safer. For example, we can put different functional chemical groups on nanoparticles and ask what impact does this substitution have on bioactivity? Is it the core, the size, or the actual chemical group? We've learned that the functionality of the surfaces of gold nanoparticles, in

particular, is the most important. We can now play around with those functionalities. If you are manufacturing a compound for industrial purposes or for clinical use, understanding these rules is very important. We are trying to come up with the general rules for building safe but functional particles.

Q. You found that the treatment of zebrafish with gold nanoparticles causes eye and neuronal problems, as well as defects in adult swimming behavior. Did you figure out why the gold nanoparticles caused these effects?

A. Not yet, but we are still working on it. We know that gold nanoparticles induce changes in neuronal development and apoptosis, or cellular death.

Q. With apoptosis, you might be losing cells that are critical to development.

A. Exactly.

Q. Are zebrafish the best model to screen for nanoparticle toxicology?

A. I would say zebrafish is the best model by far for many of the reasons I've discussed. The work we are doing absolutely cannot be done in laboratory rodent models. Again, stay with the strength of your model.

Q. How do you treat the embryos with nanoparticles? Are they injected?

A. Typically, we put nanoparticles in their water or inject them. We can modulate the aqueous environment in such a way that it affects the dispersion of nanomaterials, which is a powerful advantage. When you put nanoparticles in an ion-containing medium, they sometimes aggregate, which affects bioavailability. Some of the data from mammalian cell-based models are complicated by the media in which the cells are grown. And we don't have that problem. We can run our assays under various ionic conditions to confirm the dispersion of the particles before we do the studies.

Q. Your major focus has been on the disruption of development in zebrafish embryos treated with nanoparticles and the persistence of those defects into adulthood. Have you checked nanoparticle toxicity in adult fish?

A. The strength of our model is in the developmental stage. We have done a little bit of work with adult exposures, but the cost of materials is huge because you have to use tanks of water instead of miniscule amounts needed for embryos. We only need 50 or 100 microliter volumes with just a few micrograms of particles to do a full bioactivity profile with embryos. With our new instruments we can measure learning and memory, social behavior, respiratory function, and cardiovascular function, as well as pathology.

Q. Are results from zebrafish experiments likely to be applicable to humans?

A. Absolutely. We have dozens and dozens of papers to indicate that. The zebrafish field in general has put this question to rest, which explains the exponential rise in funded zebrafish research and a decline in funding for other models.

Q. Are people exposed environmentally to nanoparticles, or would exposure be limited to a certain context like manufacturing?

A. Right now, there is limited environmental exposure to nanomaterials. I'm part of a large grant at Arizona State University where we're trying to assess the likely routes of exposure. For example, you might make a tennis racket with carbon nanotubes, but the likelihood of a carbon nanotube being liberated during a tennis game is pretty small. However, if you worked at an aircraft-manufacturing facility and part of the fuselage is a carbon nanotube material, sanding surfaces would create a fair amount of dust. The good news is that research has demonstrated that personal protective equipment can be very effective in the work place.

Q. Have nanoparticles found their way into commercial products that people commonly use?

A. There are hundreds of commercial applications of nanomaterials already in the cosmetics industry in consumer products like creams and sunscreens, and also in paints, coatings, building materials, airplanes, fabrics, and food.

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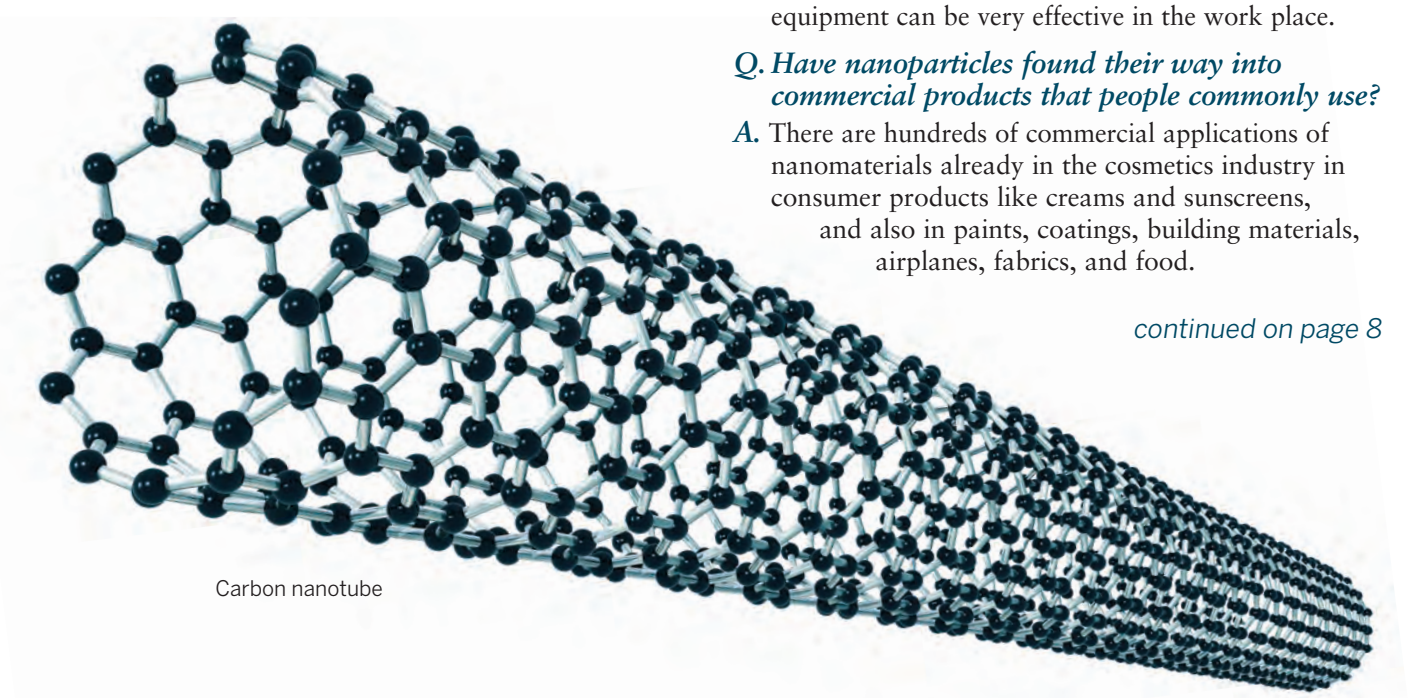


Carbon nanotube

Continued from page 7 — Fish Toxicology

Q. Would nanoparticles be absorbed through the skin, ingested, or inhaled?

A. Absolutely. Some applications in medicine, food, and cosmetics are designed to do so. Most of the exposure, however, I suspect will be from manufacturing or release into the environment at the end of product life.

Q. There is a possibility that nanoparticles will find their way into the water supply. Are common water-treatment processes adequate to remove nanoparticles?

A. No, they're not completely adequate. There are a lot of arguments about this in the field. The bulk of nanomaterials don't dissolve. Silver nanoparticles are the most well-studied and widely used. Silver nanoparticles have antimicrobial uses in clothing, wound dressing, band aids, and the linings of washing machines. There is a very slow release of ionic silver, and ionic silver is antimicrobial. If the silver nanoparticle gets into the environment, one of three things will happen. It's either going to completely dissolve into ionic silver, in which case it's not a nanoparticle any longer. It may stay in the silver form and get chemically modified by bacteria, which changes the dissolution rate. Or third, it's just going to sediment with other stuff in water treatment as sludge. Sludge is removed from our drinking water but sometimes dispersed back into the environment.

Q. What do you think are the important lessons to apply to future work with nanoparticles?

A. I think getting a strong focus on green chemistry and green nanotechnology in the beginning to avoid mistakes is important—trying to identify hazardous nano features prior to releasing 100,000,000 metric tons of a product. And that is working. We've tested hundreds of different nanomaterials, and most appear biologically benign.

Q. People are concerned about safety and are willing to apply some guidelines to the development and production of some of these compounds. Historically, many chemicals were synthesized and released into the environment without establishing an adequate safety profile.

A. I totally agree. We've been fortunate none of the nanomaterials are likely to be really nasty. The biggest application of nanotechnology has been in diagnostics—special chips, diagnostic tools, and biomarker detection.

Q. You were recently invited to join LPI as an adjunct professor. Why does this position interest you?

A. I have so many collaborations on this campus, and many of them are with LPI investigators. As I mentioned earlier, I've been working with Maret Traber for about eight years now, and I have strong collaborations with LPI's Dave Williams and Emily Ho.

STATE OF OREGON

PROCLAMATION

OFFICE OF THE GOVERNOR

WHEREAS: Linus Carl Pauling, a native son of Oregon, is the only person to have been awarded two unshared Nobel Prizes (Chemistry, 1954; and Peace, 1962); and

WHEREAS: Dr. Pauling's courageous efforts for world peace and to ban the testing of nuclear weapons, carried out with great personal sacrifice and despite the disapproval of his own government, established a new basis for world peace negotiations; and

WHEREAS: Dr. Pauling's genius in chemistry established the basis of modern chemistry and set the stage for major discoveries that benefited humankind; and

WHEREAS: Dr. Pauling discovered the cause of sickle-cell anemia, the first disease to be described as a molecular disease, and is acknowledged as a major founder of the science of molecular biology; and

WHEREAS: Dr. Pauling was unafraid of controversy in his pursuit of truth and devoted his life to the good of humanity; and

WHEREAS: February 28, 2015, is the birthday of Linus Carl Pauling and a time to recognize the life and work of this great Oregonian and American.

NOW, THEREFORE: I, John A. Kitzhaber, M.D., Governor of the State of Oregon, hereby proclaim **February 28, 2015** to be

LINUS CARL PAULING DAY

in Oregon and encourage all Oregonians to join in this observance.

IN WITNESS WHEREOF, I hereunto set my hand and cause the Great Seal of the State of Oregon to be affixed. Done at the Capitol in the City of Salem in the State of Oregon on this day, January 27, 2015.

John A. Kitzhaber, M.D., Governor

Kate Brown, Secretary of State

Linus Pauling Day

As has been done in prior years, the Governor of Oregon issued a proclamation that declared Linus Pauling's birthday, February 28, "Linus Pauling Day."

Pauling was lauded for his two Nobel Prizes and his "courageous efforts for world peace and to ban the testing of nuclear weapons," as well as for his seminal and momentous scientific accomplishments that included establishing the basis of modern chemistry; the discovery of the cause of sickle-cell anemia—the first disease to be described as a molecular disease; and setting the stage for the science of molecular biology with his discovery of the alpha helix—a central structural theme of proteins—and advancing the concept of biological specificity.

As an essay in *Nature*, one of the world's pre-eminent scientific journals, opined in 2000, "Linus Pauling ranks with Galileo, Da Vinci, Shakespeare, Newton, Bach, Faraday, Freud and Einstein as one of the great thinkers and visionaries of the millennium. Truly he was not of this age, but for all time."

Q. *What is the nature of those collaborations?*

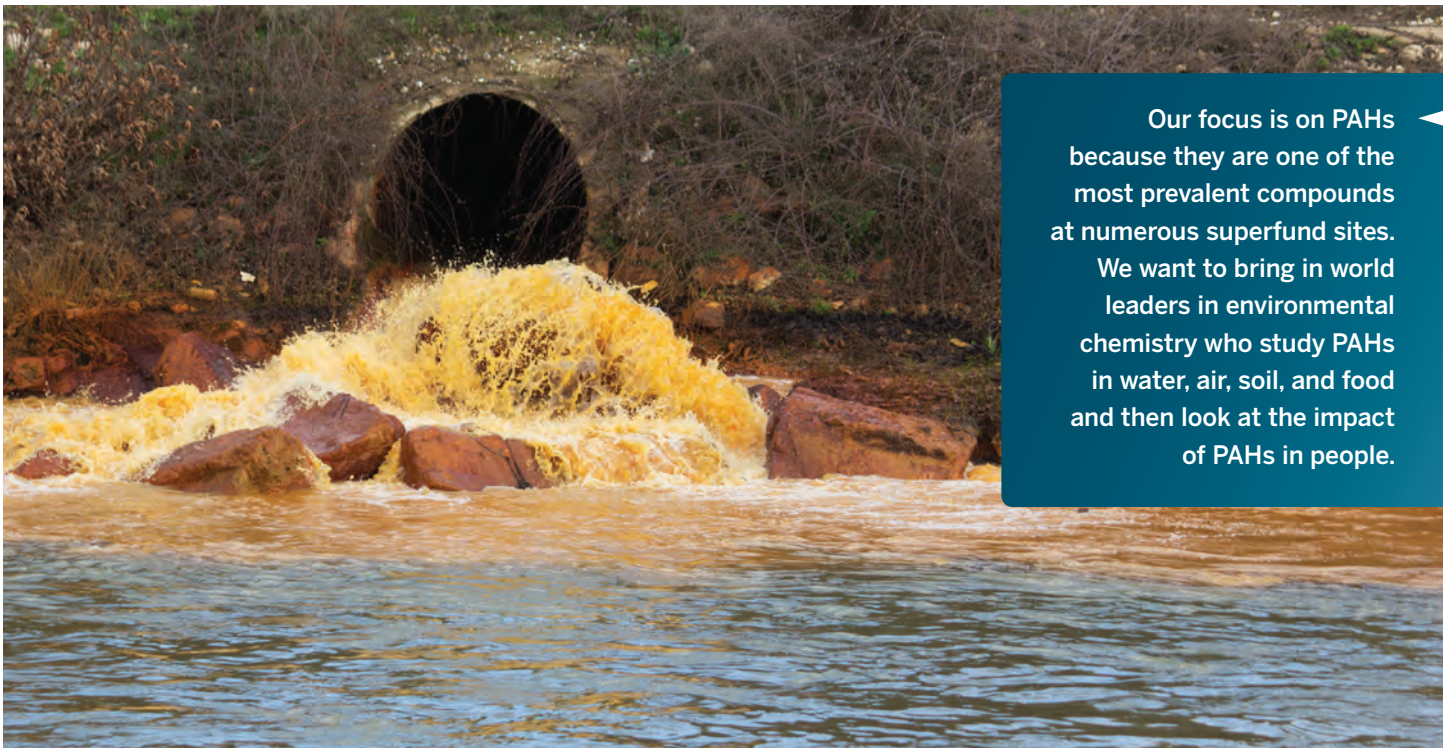
A. Dave and I co-directed the Superfund Research Center, and we published one paper together. Emily has a long-standing interest in trying to understand the role of zinc in early development and also in susceptibility to disease. We have an NIH grant that was recently funded to support this collaboration. I think our zebrafish model is ideal to identify how zinc deficiency affects disease and susceptibility to other environmental stressors. We can use our model and unique infrastructure to produce tens of thousands of zinc-deficient embryos and then ask global questions that nobody else can answer.

Q. *What are the objectives of the Superfund Research Program?*

A. It's funded by the National Institute of Environmental Health Sciences. I became the director at the end of 2014. The main goal is to use a perfect blend of environmental and human health-focused research to identify polycyclic aromatic hydrocarbons (PAHs) in the environment, to characterize their toxicity, and to determine the levels of those chemicals in the environment below which they pose no threat to human health.

Q. *These are sites that have a lot of industrial contamination?*

A. Yes. A Superfund site is a heavily contaminated area where the responsible companies are out of business and have left it to the government to clean up. The EPA's goal is to try to find the resources to make these sites clean enough for humans and other organisms. Our focus is on PAHs because they are one of the most prevalent compounds at numerous Superfund sites. We want to bring in world leaders in environmental chemistry who study PAHs in water, air, soil, and food and then look at the impact of PAHs in people. The main strength of our center is our interactions—we have five projects and six cores that work incredibly well together and are centrally focused on identifying the structural characteristics of PAHs that adversely affect human health. Currently, most of the research in the field is focused on less than two dozen PAHs and their role in cancer. Growing evidence suggests that PAHs affect early development, learning and memory, and cardiovascular disease later in life. Kim Anderson and Stacy Simonich at OSU are discovering, for the first time, abundant PAHs in our environment that may be the real causes of human disease. And Dave Williams and Rick Corley at Pacific Northwest National Labatory are defining, for the first time, how these chemicals move through the human body. Together with our zebrafish work, our center is poised to have a substantial positive impact on human health.



Our focus is on PAHs because they are one of the most prevalent compounds at numerous superfund sites. We want to bring in world leaders in environmental chemistry who study PAHs in water, air, soil, and food and then look at the impact of PAHs in people.

Q. *What do you like to do in your free time?*

A. I don't have a lot of free time, but when I do, I spend it with my family—my wife Shari and daughter Lilli. I have a huge lab and am really fortunate to have the opportunity to interact with students and scientists from different backgrounds every day—I guess that's my most consistent social life. Managing this complex group and guiding career development are more fun to me than having a dinner party or playing poker with the guys.

LPI

New Technologies Track Human Metabolism of Polycyclic Aromatic Hydrocarbons, an Important Class of Environmental Pollutants

Erin Madeen, LPI Graduate Student

David Williams

Dr. David Williams' laboratory is applying novel technology to track human absorption, metabolism, and excretion of polycyclic aromatic hydrocarbons (PAHs), universal dietary pollutants and carcinogens. PAHs are products of combustion found in essentially all foods but present at highest levels in smoked and charcoal-grilled foods, due to environmental contamination from sources like burning of coal, wood, and fossil fuels. Previous work in Williams' and other laboratories has demonstrated that some of these PAHs induce cancer in laboratory rodents through binding to DNA (adduction) and generation of reactive oxygen species. The most well studied of these PAHs is benzo(a)pyrene (BaP); 95% or more of our exposure (non-smokers) to BaP is through our diet.

In December 2014, colleagues from OSU, the Pacific Northwest National Laboratory (PNNL), the Lawrence Livermore National Laboratory (LLNL), and I published the first phase of these studies. Human volunteers were given a capsule of the PAH dibenzo(def,p)chrysene (DBC) containing a 29-nanogram dose, which was chosen to represent the dosage of PAHs that the average person consumes daily in food. This dose was calculated to contribute no appreciable disease risk to volunteers.

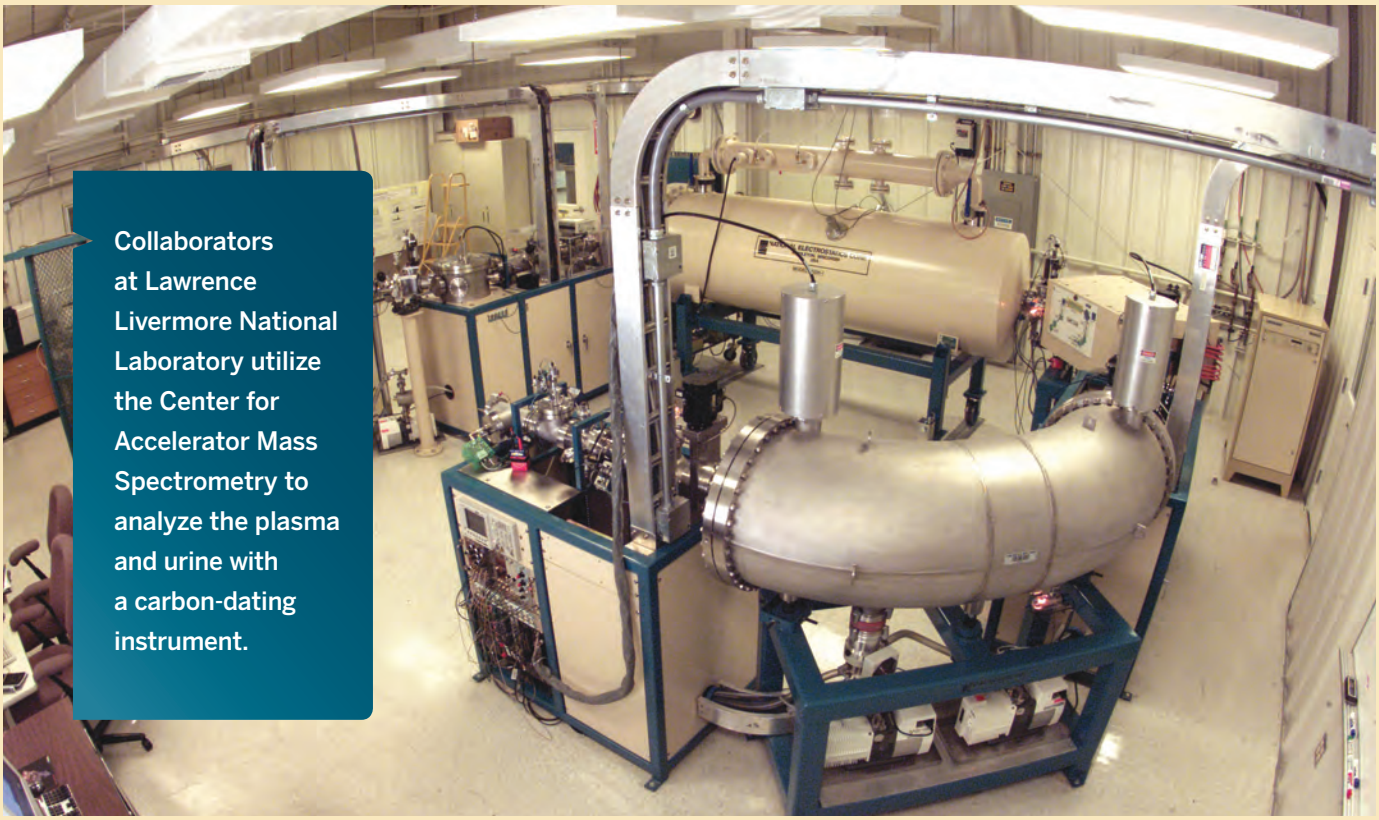
To assess the range of human metabolism, healthy, nonsmoking male and female volunteers were included in a range of BMI and ages. Volunteers donated blood and urine over four days to be analyzed for the presence and elimination of DBC. To track the DBC that was administered, it was labeled with the radioactive isotope ^{14}C. Collaborators at LLNL utilize the Center for Accelerator Mass Spectrometry to analyze the plasma and urine with a carbon-dating instrument (an accelerator mass spectrometer or AMS) that has been modified to detect carbon isotopes in biological samples. Because this spectrometer is so sensitive, we can safely give very small quantities of radioactive DBC. This sensitivity permits detection of environmentally relevant doses of PAHs. The AMS is capable of the detection of DBC and metabolites in blood or urine at concentrations equivalent to one drop of water in 4,000 Olympic-size swimming pools. From the collected data, a collaborator at PNNL and I calculated the maximum concentration of DBC in plasma and its elimination in urine. These results are used by risk assessors and risk modelers who generate data used in policy decisions related to pollutant emissions and acceptable exposure recommendations.

Phase II of this work is focused on determining which of several enzymes carry out the specific metabolism of DBC. DBC and other PAHs are pro-carcinogens, meaning that they are not capable of DNA adduction or generating reactive oxygen species without first being modified to a reactive form by a cytochrome P450 enzyme. Two additional reactions generate the ultimate carcinogen, a dihydrodiol-epoxide. Understanding DBC metabolite generation will provide a better understanding of risk from ingestion of these compounds. LLNL has developed a novel coupling of ultrahigh-performance liquid chromatography (UHPLC) to AMS for detection of biological samples containing ^{14}C. The phase II samples are the first to be analyzed

with this new technology. Preliminary tests are able to detect the diol, tetrol, and DBC for calculation of pharmacodynamics (dose-response effects in the body) of DBC metabolism. Phase II is expected to be completed by June 2015.

Building on the success of applying the sensitivity and selectivity of AMS to PAH metabolism, the Williams lab has begun recruitment of healthy nonsmoking volunteers for a new project to analyze the metabolism in humans of a dietarily relevant dose of another PAH, benzo(a)pyrene (BaP), alone or in combination with smoked salmon from the Confederated Tribe of the Umatilla Indian Reservation in Washington. Mixtures of various PAHs form on food during the traditional smoking process. The risk from ingestion of any PAH mixture is estimated by adding the concentration multiplied by the carcinogenicity of each compound in the mixture, dubbed the Relative Potency Factor, which assumes additivity of individual PAHs in complex mixtures. The accuracy of this approach has been challenged because not all PAHs are carcinogenic. Non-carcinogenic and weakly carcinogenic PAH fractions in a mixture compete for the same enzyme systems, potentially reducing the activation of carcinogenic PAHs in the mixture. Volunteers agree to exclude smoked meats, cheese, and charcoal-grilled meats from their diet during the study. Study participants ingest 46 nanograms of BaP, labeled with ^{14}C, for UHPLC-AMS assessment of pharmacokinetics (absorption, metabolism, and excretion) and pharmacodynamics of BaP. This dose is about 10-fold less than the estimated amount of BaP consumed by the average non-smoking American every day, so the study is being done at an environmentally relevant dose. A subset of volunteers will be given a serving of smoked salmon in addition to the ^{14}C-BaP dose. If the pharmacodynamics of ^{14}C-BaP metabolites are altered by the addition of the salmon, the Relative Potency Factor approach will not be supported. Conversely, if the detected ^{14}C-BaP metabolites remain the same, this approach will be supported. Results are expected to affect calculations of risk from exposure to PAHs and regulations concerning allowable limits of PAH emissions. LPI

Traditional salmon smoking



Collaborators at Lawrence Livermore National Laboratory utilize the Center for Accelerator Mass Spectrometry to analyze the plasma and urine with a carbon-dating instrument.

Diet and Optimum Health

September 9 - 12, 2015 Corvallis, Oregon

Wednesday, September 9, 2015

12:00 PM Registration begins

1:45–2:00 **Welcome and Opening Remarks**
Balz Frei, PhD, Linus Pauling Institute, Oregon State University

VITAMIN E: A CRITICAL NUTRIENT FOR DEVELOPMENT AND HEALTH

Chair: Maret G. Traber, PhD
Linus Pauling Institute, Oregon State University

2:00–2:30 **Vitamin E, necessary nutrient for the brain**
Maret G. Traber, PhD
Linus Pauling Institute
Oregon State University, Corvallis, OR

2:30–3:00 **Vitamin E deficiency: An unfolding public health concern in poor rural societies in South Asia**
Keith West, PhD
Johns Hopkins Bloomberg School of Public Health
Baltimore, MD

3:00–3:30 **Stroke and vitamin E**
Chandan Sen, PhD
The Ohio State University Medical Center
Columbus, OH

3:30–4:00 **Vitamin E requirements in metabolic syndrome**
Rich Bruno, PhD, RD
The Ohio State University, Columbus, OH

4:00–4:30 **Interactions of vitamins E and C in diabetics**
Mark Levine, MD
National Institutes of Health, Bethesda, MD

6:30 Welcome Reception

Thursday, September 10, 2015

6:00 AM Organized Walk/Run

7:30–8:30 Breakfast provided

EXTENDING HEALTHSPAN: BASIC CONCEPTS, UNDERLYING MECHANISMS, AND ROLE OF DIET AND LIFESTYLE

SESSION 1

Risk Factors that Limit Healthspan

Chairs: Tory Hagen, PhD and Viviana Pérez, PhD
Linus Pauling Institute, Oregon State University

8:30–9:00 **Basic biology of aging, healthspan, and geroscience**
Felipe Sierra, PhD
National Institute on Aging
National Institutes of Health, Bethesda, MD

9:00–9:30 **Global burden of disease**
Mohammad Forouzanfar, MD, PhD
University of Washington, Seattle, WA

9:30–10:00 **What comparative biology can tell us about healthspan**
Steven Austad, PhD
University of Alabama, Birmingham, AL

10:00–10:30 Coffee/Tea Break

Inflammation, Redox Biology, and Healthy Aging

Chairs: Fritz Gombart, PhD and Kathy Magnusson, DVM, PhD
Linus Pauling Institute, Oregon State University

10:30–11:00 **Metagenomics and metabolomics in centenarians and their offspring: Two new pieces of the inflamm-aging puzzle**
Claudio Franceschi, MD
University of Bologna, Italy

11:00–11:30 **Chronic inflammation and cell senescence**
James Kirkland, MD, PhD
Mayo Clinic, Rochester, MN

11:30–12:00 **The redox code: Toward an understanding of genome–exposome interactions and healthy aging**
Dean Jones, PhD
Emory University, Atlanta, GA

12:00–1:30 PM Lunch provided

Oral Abstracts

Chair: TBA

1:30–2:50 Talks

2:50–3:15 Coffee/Tea Break

3:15–4:55 Talks

5:00–7:00 Poster Session
(hors d'oeuvres and beverages provided)

Friday, September 11, 2015

6:00 AM Organized Walk/Run

7:30–8:30 Breakfast provided

EXTENDING HEALTHSPAN: BASIC CONCEPTS, UNDERLYING MECHANISMS, AND ROLE OF DIET AND LIFESTYLE

SESSION 2

Strategies to Maintain Cognitive Function

Chairs: Kathy Magnusson, DVM, PhD and Fritz Gombart, PhD
Linus Pauling Institute, Oregon State University

8:30–9:00 **This is your brain on aging**
Kathy Magnusson, DVM, PhD
Linus Pauling Institute
Oregon State University, Corvallis, OR

9:00–9:30 **Calorie restriction mimetics: Promise and pitfalls**
Donald Ingram, PhD
Pennington Biomedical Research Center
Louisiana State University System
Baton Rouge, LA

9:30–10:00 **Nutraceutical interventions for cognitive functions throughout life**
Andrew Scholey, PhD
Swinburne University of Technology
Melbourne, Australia

10:00–10:30 Coffee/Tea Break

Sarcopenia, Frailty, and Exercise

Chairs: Viviana Pérez, PhD and Tory Hagen, PhD
Linus Pauling Institute, Oregon State University

10:30–11:00 **Cardiovascular risk and kidney function in older adults: The role of frailty**
Michelle Odden, PhD
Oregon State University, Corvallis, OR

11:00–11:30 **TBA**

11:30–12:00 **Risk factors and preventative strategies for accidental falls and bone fractures in older adults**
Lynn Marshall, Sc.D.
Oregon Health & Science University
Portland, OR

12:00–1:00 Lunch provided

CELEBRATING THE LIFE AND WORK OF GEORGE BAILEY

SESSION 1

Chairs: Rod Dashwood, PhD
Texas A&M Health Science Center and
Tom Kensler, PhD, *University of Pittsburgh*

1:00–1:30 **George Bailey's career at Oregon State University**
Richard Scanlan, PhD
Emeritus Dean of Research
Oregon State University, Corvallis, OR

1:30–2:00 **Contamination of rainbow trout diet and development of rainbow trout as a cancer model**
Roger Coulombe, PhD
Utah State University, Logan, UT

2:00–2:30 **Indole-3-carbinol: Its mode(s) of action as a chemopreventative agent—and why it may cause drug interactions**
David Stresser, PhD
Corning Incorporated, Woburn, MA

2:30–3:00 **Large trout studies (ED001) and application for risk assessment**
Ashok Reddy, PhD
Oregon Health & Science University, Portland, OR

3:00–3:30 Coffee/Tea Break

CELEBRATING THE LIFE AND WORK OF GEORGE BAILEY

SESSION 2

Chairs: Sharon Krueger, PhD and Emily Ho, PhD,
Linus Pauling Institute, Oregon State University

3:30–4:00 **Mechanistic and preclinical studies with natural and synthetic chlorophylls**
Rod Dashwood, PhD,
Texas A&M Health Science Center, Houston, TX

4:00–4:30 **"Green" chemoprevention in humans: Chlorophyllin leads the way**
Tom Kensler, PhD
University of Pittsburgh, Pittsburgh, PA

4:30–5:00 **Low-dose accelerator mass spectrometry studies of carcinogens and anti-carcinogens in human volunteers**
David Williams, PhD
Linus Pauling Institute
Oregon State University, Corvallis, OR

5:00–5:30 **International impact of George Bailey's scientific body of work**
John Groopman, PhD
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

6:30 Reception

7:30 Banquet Dinner

Saturday, September 12, 2015

PUBLIC SYMPOSIUM

9:00–11:00 AM **LPI's Rx for Health: The role of diet, micronutrients, and supplements in human health and disease**

Undergraduate Students in the Lab

Learning While Advancing Science

Photo by Clint Shock

Fagonia plant

Toftner is working on a project with Stevens to determine the properties of fagonia, a plant native to Pakistan that is used by people in the region to treat breast cancer.

Stevens' lab is part of the Linus Pauling Institute's Cardiometabolic Disease Prevention program.

Jackilyn Toftner

In addition to the faculty researchers and graduate students who are determining the impact of supplements on health and disease and the relevant cellular mechanisms, the Linus Pauling Institute has many undergraduate students working alongside them each term.

One of these students is Jackilyn Toftner, a junior who is majoring in chemistry with a biochemistry emphasis. Toftner decided early on a medical career in disease prevention and treatment and is strongly motivated to help individuals and their families stay healthy to avoid the suffering that accompanies chronic diseases. She chose OSU because it is known for strong science and engineering programs and opportunities for undergraduates to work in a research lab.

Last year Toftner reached out to a number of faculty at the university to see if any were accepting undergraduate students in their laboratories. She was especially interested in working with Professor Fred Stevens in the Linus Pauling Institute, "because I am very passionate and interested in the potential applications of chemistry to other disciplines. I want to use my ability to learn and my thirst for knowledge and lab techniques to help others in any way I can."

Fred Stevens

Toftner is working on a project with Stevens to determine the properties of fagonia, a plant native to Pakistan that is used by people in the region to treat breast cancer.

"In America, there has been controversy about the medicinal properties of this plant," explains Toftner. "My project is focused on extracting the bioactive components out of a dried, ground sample of the plant to make a chemical profile. The goal is to then test the bioactive components individually on breast cancer cells to see if they trigger apoptosis, or cell death, in the cancer cells."

Stevens' lab is part of the Linus Pauling Institute's Cardiometabolic Disease Prevention program, which is focused on a better understanding of the molecular and cellular mechanisms of cardiometabolic diseases and the role of oxidative stress and inflammation. Scientists in this area also are investigating the protective effects of micronutrients and other dietary factors or supplements, such as vitamins C and E, lipoic acid, fish oil, and flavonoids.

In addition to Stevens, Toftner works with graduate student Elizabeth Axton. She notes that both have been helpful with not only her lab techniques but also in brainstorming solutions to problems that she encounters in her experiments.

"Working in a lab is a lot different from being in chemistry lab class where you learn how to use the general applications from class. Working in a research lab not only applies those classroom techniques to real life experiments but requires the ability to think independently and to make adjustments to those experiments when problems arise," Toftner adds. "It's a great experience for students, and even if you aren't considering a career in research, the practical experience is invaluable." LPI

A Lasting Legacy of Nutritional Science

When the Linus Pauling Institute moved from Palo Alto, California, to Corvallis, Oregon, in 1996, it focused on advancing knowledge in areas of interest to Dr. Linus Pauling, with an emphasis on the role of nutritional factors in health and disease prevention. Almost 20 years later, the Institute has 12 principal investigators and is nationally recognized for its work in helping us all live longer, better lives.

Researchers in the Institute are driven to understand not only the causes of diseases, but also how we can minimize our risk through eating right and supplementing a healthy diet with vitamins and minerals. Research spans a number of disciplines: from the impact of environmental factors like pollution and toxic chemicals, to the ways in which our bodies absorb (or don't, in some cases) the nutrients that we need, to determining the beneficial effects of flavonoids in plants.

Although the Linus Pauling Institute is located in a small town in Oregon, the Institute's reach is global. The Micronutrient Information Center website gets visitors from around the world, and this year we have received gifts from 45 states and four countries outside the US.

Since its inception, the Institute's research on vitamins and other nutritional factors has been supported by many, many individuals, including more than 100 who have made gifts to support our research through their estate plans. We are so grateful to all of you who have chosen to make a gift to the Linus Pauling Institute—your generosity enables all that we do.

If you are interested in supporting the Linus Pauling Institute in a way that wouldn't affect your current lifestyle or your family's security, you can make the Institute part of your estate plans by including special language in your will or trust. If this is something you are considering or have already done, please let us know of your intentions so that we can better steward your gift according to your wishes.

I enjoy talking with supporters of the Linus Pauling Institute and would love to hear how you came to know of the Institute and its work. Many of you had a chance to meet Dr. Pauling, as well—some at Caltech or Stanford, some through professional societies, and some who worked with him. If you have a story about Dr. Pauling that you'd like to share with us, please don't hesitate to call (541-737-0055) or email me at marlys.amundson@oregonstate.edu.

If you're online, the Linus Pauling Institute's Facebook site is a great way to keep up with news from the Institute: https://www.facebook.com/LinusPaulingInstitute. LPI

Marlys Amundson
Director of Development

Photo by Jim Carroll Photography

LPI is grateful for the bequests we have received from the following friends this past year:

Sylvia Arenson
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Linus Pauling Institute

Stephen Lawson, Research Newsletter Editor
Oregon State University
307 Linus Pauling Science Center
Corvallis, Oregon 97331

phone: 541-737-5075
fax: 541-737-5077
email: lpi@oregonstate.edu
website: lpi.oregonstate.edu

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Look for these informative articles inside!

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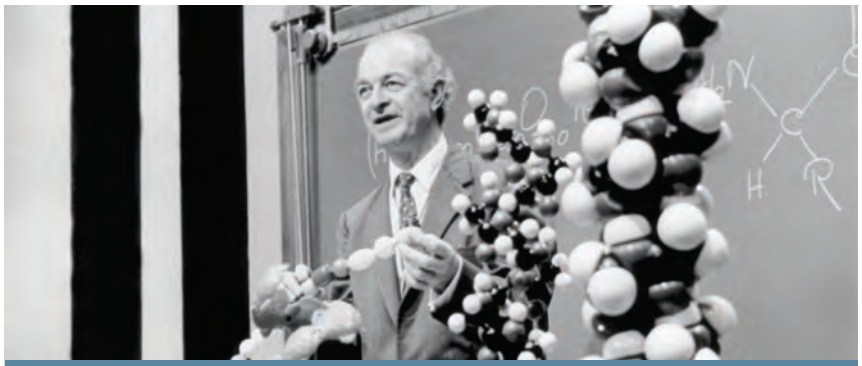

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Including the Linus Pauling Institute in your will or trust is an easy way to make a big difference for future generations.

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Special thanks to *Barbara McVicar for editorial assistance and photographs and to authors of signed articles.*