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Pathology in Ecological Research with Implications for One Health: Session Summary.

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Abstract

This session explored the effects of pollutants on One Health at the ecosystem level that included microbes, insects, fish and humans. The concept of One Health seeks to synergize medical, veterinary, and other health science disciplines to more effectively advance human and animal health. Presentations explored the interactions of pesticides, pathogens, phytochemicals and xenobiotic biotransformation in bee colony losses critical for food security (bees have been recently listed under the 2017 US FDA veterinary feed directive); the role of pathology in identifying the effects of pollutants on fish as sentinels for human health; the effects in rats of per- and polyflouroalkyl substances (PFAS) that can persist in the environment and contaminate drinking water; harmful algal blooms and toxin production leading to animal and human disease; and the processing of environmental carcinogens by intestinal microbiota.

Keywords

toxicologic pathology; ecotoxicology; one health; pollutants; bees; fish; PFAS; hazardous algal blooms; cyanotoxins; microbiome; arsenic; sucralose; Environmental toxicology

Introduction

Ecological toxicologic pathology is a relatively new field that builds on the science of environmental toxicologic pathology to study the effects of toxic substances and physical agents, especially pollutants, at the population, community, and ecosystem levels. The

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objective of this session was to illustrate the wide-ranging aspects of this field and its contributions of toxicologic pathology.

Honey bees and environmental stress—toxicologic pathology of a superorganism

The first presentation was given by May Berenbaum who is Professor and Head of the Department of Entomology at the University of Illinois and holds the Swanlund Chair of Entomology. She is a member of the National Academy of Sciences and has chaired two National Research Council committees, the Committee on the Future of Pesticides in U.S. Agriculture (2000) and the Committee on the Status of Pollinators in North America (2007). In 2011 she received the Tyler Prize for Environmental Achievement and in 2014 she was awarded the National Medal of Science. She is known for elucidating chemical mechanisms underlying interactions between insects and their host plants. Her research, supported primarily by NSF and USDA, has produced over 300 refereed scientific publications and 35 book chapters. She is the current editor of PNAS.

As a eusocial species, A. mellifera is effectively a superorganism—a group of genetically related individuals functioning as a collective unit. Because the unit of selection is the colony and not the individual, standard methods for assessing toxicologic pathology can miss colony-level responses to stress. For over a decade, U.S. populations of honey bees have experienced severe annual losses attributed to a variety of environmental stressors varying temporally and geographically; differentiating among those stressors is accordingly a high priority. Social interactions among individuals in this social species, however, mean that the “footprint” of stressors such as pesticides, phytochemicals, pathogens, and parasites may be most discernible in individuals that did not themselves directly encounter the stressor. For example, neurotoxic effects of pesticides on nurse bees may impair their behavioral responses to queen-destined larvae, which may then emerge as adults with altered anatomy or physiology. Similarly, pesticide-induced size alterations of nurse hypopharyngeal glands, which produce royal jelly, the exclusive food of larval and adult queens, may disproportionately affect queen (and thus colony) health. Thus, evaluating toxicologic pathology in the honey bee requires a new perspective and development of assays that preserve the social context that ultimately determines colony health.

The full article by Berenbaum and Liao can be found in this issue.

Integration of Pathology in the Assessment of Adaptation to Polycyclic Aromatic Hydrocarbons (PAHs) in Atlantic Killifish (*Fundulus heteroclitus*)

The second presentation was given by David E. Hinton, Nicholas Professor of Environmental Quality at Duke University. The Hinton laboratory focuses on mechanistic toxicity in all life stages of small, aquarium model fishes and in selected marine and freshwater species with particular environmental relevance. Hinton has authored a total of 266 publications in refereed journals, and was the Editor of Aquatic Toxicology.

This presentation explored how resident organisms adapt over multiple generations to environmental pollution representing significant selection pressure potentially driving evolution.[1] Populations improve their abilities to survive (i.e., become resistant) in these ecosystems but there is an associated cost of this resistance (e.g., higher rates of cancers).

Such pollution-driven adaptation is often associated with highly polluted sites, including those listed by the USEPA as Superfund sites and has important ramifications for environmental science and management including conservation biology, environmental risk assessment, and evaluation of remediation efforts. Atlantic killifish (*Fundulus heteroclitus*) inhabiting the Elizabeth River in the Tidewater region of Virginia provide an excellent "natural experiment."[2] The historic use of creosote, at wood treatment facilities contaminated the river, resulting in one of the highest reported accumulations of a complex and persistent mixture of polycyclic aromatic hydrocarbons (PAHs) in river sediment (100–500 μg/g).[1] Porewater was extracted from this sediment and chemically characterized for use in laboratory experiments.[3] Multi-year, ongoing investigations of adapted and non-adapted populations of killifish revealed mechanisms of toxicity at multiple levels of biological organization. Killifish are amenable to the laboratory, enabling cultivation of colonies and precise, environmentally relevant exposure studies at multiple life stages.[4] Embryos were exposed to various concentrations of extract and grown out under clean conditions. Embryonic and adult life stages were processed for histology. Reference population fish, not adapted fish, responded to exposure. However, adapted fish showed significantly more background rates of change. Responses consisted of liver alterations, including neoplastic lesions and microvesicular vacuolation, heart valvulopathy, effusion of blood into the pericardial cavity, and thyroid hyperplasia. Evaluation of these fish at an embryonic life stage has shown PAH induced teratogenicity including pericardial edema and tube heart formation.[5] Combining toxicologic pathology with *in vivo* and molecular analyses provides a more complete story of morphologic change and adaptation.

Biochemical and hematologic changes in 28-day rat studies of seven per- and polyfluoroalkyl substances (PFAS) - beyond PFOA and PFOS

The third presentation was by Michelle Cora, who is at the National Toxicology Program. She provides clinical pathology oversight and preparation of technical reports and publications for NTP studies. In addition, she provides collaborative and consultative support to investigators at the National Institute of Environmental Health Sciences (NIEHS). She has authored numerous publications and book chapters and served on numerous committees and editorial boards.

Dr. Cora discussed seven perfluorinated substances (PFAS) administered by oral gavage to male and female Sprague-Dawley rats for 28-days to understand relative toxicity in relation to functional group and chain length: perfluorobutane sulfonic acid (PFBS), perfluorohexane sulfonic acid salt (PFHxSK), perfluorooctanesulfonic acid (PFOS), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), or perfluorodecanoic acid (PFDA).[6–8] These chemicals have been used to make products more resistant to stains, grease and water (e.g., non-stick cookware, stain resistant carpets), and have been used as a lubricant and in firefighting foams.[9] PFAS persist in soil, water, living tissue and some bioaccumulate.[9] Clinical pathology changes and comparison of results across the chemicals, as well as correlation to histopathologic findings were presented. With some exceptions, clinical pathology results included increased liver enzyme activities; increased bilirubin and bile acids concentrations; and decreased globulin, cholesterol and triglyceride concentrations.[10] In PFHxA, a dose-dependent regenerative decrease in the

erythron (regenerative anemia) was observed. Histopathological changes included hepatocyte hypertrophy and necrosis, and bone marrow hypocellularity. In general, total T3, total T4 and free T4 were decreased with no compensatory increase in TSH.[11,12] PPARα and CAR receptor activation was assessed by measuring Cyp2b1, Cyp2b2, Acox1, and Cyp4a1 gene expression; in general gene expression was increased.

The Occurrence and Toxicological Effects of Freshwater Cyanobacterial Toxins

The fourth presentation was delivered jointly by Neil Chernoff, from the USEPA, Research Triangle Park and Gregory Travlos, NIEHS. Neil Chernoff's research is currently concentrated on mammalian toxicology associated with exposures to freshwater cyanobacterial toxins including anatoxin-a, cylindrospermopsin, and a variety of microcystin congeners. He has received ten Scientific and Technical Achievement Awards for his published papers. His copresenter, Gregory Travlos leads the Clinical Pathology Group within the Cellular and Molecular Pathology Branch at the National Institute of Environmental Health Sciences and is the Clinical Pathology Discipline Leader for the National Toxicology Program. He has authored/coauthored numerous articles, book chapters and technical reports, and served on editorial boards.

Chernoff and Travlos discussed cyanobacteria, photosynthetic organisms that inhabit all ecosystems where there is sufficient light to support them, and their toxins. The growth of cyanobacteria has been linked to increased water temperature and the presence of excess nutrients, notably increased nitrogen and phosphorus levels in bodies of water experiencing eutrophication. Periodically, cyanobacteria undergo extremely rapid growth (i.e. blooms) and the occurrence of blooms appears to be rising globally, possibly linked to increased water temperatures and incidence of eutrophication.[13] During blooms, numerous cyanobacterial species produce bioactive compounds that are released into the water, either directly or with cell death, and many of these have proven to be toxic to vertebrates. The concentration of these toxins during periods of rapid growth can reach dangerous levels, producing Hazardous Algal Blooms (HABs) and are well known to have adversely affected fish, birds, and mammals including wildlife, livestock, pets, and people. Isolation, purification, and structural characterization of toxins is difficult, but a series of different toxins that occur globally have been identified. The most common toxins of freshwater cyanobacteria are the alkaloids, cylindrospermopsin (CYN) and anatoxin (A-a), and the peptides, microcystins (MCs). CYN affects a variety of systems including the liver and vascular system, A-a is a neurotoxin, and the MCs produce hepatic effects. Reported toxicities have been evaluated by gross changes in intact animals and affected organs, clinical chemistries indicating cell death and/or loss of organ function; and histopathological changes.[14,15] The histopathological changes induced by toxins in HABs are, as expected, compound-specific. One of the most common toxins in U.S. waters is CYN. The effects of CYN on the hepatic and renal systems have been studied in mice after oral dosing for 90 consecutive days. This exposure regimen resulted in hepatocyte hypertrophy and cell death, and inflammation, resulting in sinusoid ectasia/hemorrhage. Renal effects include cortical lesions consisting of tubule dilation, basophilia, and the deposition of intraluminal protein. Lesions also occurred in the renal medulla and include thinning of the outer stripe and the presence of intraluminal protein in the inner stripe and inner medulla.[16]

Processing of environmental carcinogens by the intestinal microbiota

The final presentation was by Chih-Wei Liu, a Postdoctoral Associate, in the laboratory of Professor Kun Lu at the University of North Carolina. He is working on DNA adductomics, DNA-protein crosslinks, metabolomics, and metaproteomics.

The intestinal microbiota exert multifactorial effects on physiology and alter the metabolism of many endo- and xenobiotic molecules. Included in the latter are a variety of therapeutic compounds, nutrients and environmental pollutants.

In this presentation, Dr. Liu demonstrated the three-way interaction between host, gut microbiome and chemicals using arsenic and sucralose as examples. Multiple omics strategies including meta-genomics, metabolomics, proteomics and the emerging meta-proteomics, have been implemented for studying this unique interaction. For arsenic, the gut microbiome plays a positive role in arsenic excretion in feces and detoxification in liver by affecting one carbon metabolism mediated arsenic methylation.[17] Moreover, a new meta-proteomics approach, using isobaric labeling quantification, revealed arsenic induced gut microbiome perturbations with several key microbiome proteins and pathways affected by arsenic exposure.[18] On the other hand, recent data caused concern regarding artificial sweetener uptake, especially for the roles of the microbiome in our gut environment.[19] Sucralose is the most commonly used artificial sweetener, and it cannot be directly absorbed or metabolized by the human body. Using a mouse model, it was found that sucralose consumption alters the gut microbiome and its functions, with higher expressions of pro-inflammatory genes and metabolites observed in the gut microbiome.[20]

Furthermore, the traditional proteomics approach to profile the mouse liver proteome affected by sucralose, suggested that treatment with sucralose, down-regulated ribosomal proteins and translation-related pathways cause ribosomal inactivation which further induces cytokine-mediated inflammation in mouse liver.[21]

Conclusion

The Society of Toxicologic Pathology embraces “One Health” in principle and practice. [22] The 2019 Annual Symposium focused on Environmental Toxicologic Pathology and One Health. As can be seen from these presentations, scientists from many different fields can contribute concepts and data which can ultimately be used to advance human and animal health.

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