HHS Public Access

Author manuscript

Toxicol Pathol. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

Toxicol Pathol. 2017 January ; 45(1): 52–56. doi:10.1177/0192623316675765.

A Brief Overview of the STP 35th Annual Symposium on the Basis and Relevance of Variation in Toxicologic Responses

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Keywords

variation; toxicologic responses; clinical pathology; toxicologic pathology; toxicology; annual symposium; nonclinical toxicity studies

Introduction

Toxicologic responses elicited by chemical, pharmacological, or environmental agents can vary considerably among and within species and studies. Variation in toxicologic responses can confound results, complicate interpretation of data, interfere with reproducibility, and make extrapolation to humans problematic. Many factors can influence or be the source of significant variation in toxicologic responses. The STP 35th Annual Symposium focused on the basis and relevance of variation in toxicologic responses. The plenary sessions and many of the posters described key factors that introduce variation and impact the interpretation of toxicologic responses. There were six scientific sessions that kicked off with “Real World Toxicology Outcomes: Impact of Species and Strain Selection on Drug Development Programs, followed by sessions on Deciphering Variability in Clinical Pathology, Influence of Experimental Design and Environmental Conditions, Influence of Epigenetics, Genetics and Immunology (Part A & B) and Influence of Age, Hormones and the Microbiome. The keynote address was dynamic and interactive and focused on the cornerstones and basis of toxicology with a historical perspective. The co-chairs of the scientific sessions, continuing education courses, and other sessions assembled a diverse group of speakers from academia, biopharmaceutical industry, contract research organizations, government (research institutions and regulatory organizations), and independent consultants. These individual speakers were very well versed in their area of expertise and covered a wide range of variables that can influence outcomes in toxicologic responses from genetic composition of the animal and epigenetic influences, to country of origin of test species to vendor sources. Many of the speakers emphasized real-life examples to demonstrate how to identify

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underlying causes of variability in nonclinical studies and to provide the background to place variability into context for translation of animal data sets to human hazard identification and risk assessment.

The General Scientific Symposium on the Basis and Relevance of Variation in Toxicologic Responses: Monday–Thursday, June 27–30

The Symposium began on Monday morning with an enthusiastic and interactive keynote address, “Cornerstones of Toxicology,” presented by A. Wallace Hayes. Dr. Hayes, a leading authority in toxicology with more than 35 years of experience in industry and academia, is a member of the Academy of Toxicological Sciences and has over 250 publications and numerous books including *Hayes’ Principles and Methods of Toxicology*. Dr. Hayes reviewed the basic principles of toxicology beginning with a historical perspective highlighting historic figures including Philippus Paracelsus, the proposed “Father” of modern toxicology and the concept of the dose determines the poison. He also gave many examples of chronicled or alleged poison cases throughout the ages, such as the Borgia Family (including Lucrezia) of Italy that understood the concept that the dose makes the poison and maleficently used it to increase their wealth and power by serving poison-laced wine to their wealthy guests, family, members of the papal court, and suitors. Through many science-based graphs, data and real-life examples, Dr. Hayes emphasized 4 basic principles of toxicology that should be remembered and summarized his presentation by stating that 1) dose matters; 2) people are different; 3) things change (related to metabolism), and hinted at the concept of 4) timing is critical (see Hayes Symposium Issue).

The first scientific session, titled “Real World Toxicology Outcomes: Impact of Species and Strain Selection on Drug Development Programs,” was introduced by Diane Gunson, and was co-chaired by Emily Meseck. The overview set the stage for several presentations that addressed the impact of variation of rodent strain and non-rodent species on pharmacologic and toxicity testing in drug development programs. The first presentation in this session, given by Klaus Weber, reviewed excellent comparative data on the incidence and types of neoplastic lesions observed in toxicology studies in two commonly used outbred rat strains, the Wistar Han and Sprague-Dawley rat (see Weber, Symposium Issue). This was followed by three detailed “real world” case studies, the first, presented by Peter Hoffmann, highlighted the differences of sensitivity between cynomolgus monkeys of Mauritian or Asian origin following oral administration of vildagliptin (LAF237), a dipeptidyl peptidase-4 inhibitor (see Hoffman, Symposium Issue); a second, presented by Ellen W. Evans, on the impact of origin of cynomolgus monkeys on immunologic responses, and the importance of identifying the influence of species on results from nonclinical studies; and the last, presented by John Sargartz and Sherry Morgan, on skin toxicity noted in dogs when unrelated compounds were administered with copovidone amorphous solid dispersion (ASD) as a vehicle (see Morgan, Symposium Issue). The session summarized the importance of species and strain selection on variation in toxicologic responses and how variation can impact drug development programs.

The second scientific session, titled “Deciphering Sources of Variability in Clinical Pathology – It’s Not Just About the Numbers,” was organized and co-chaired by Adam

Aulbach, Anne Provencher, and Niraj Tripathi (see Tripathi, Symposium Issue). This session provided attendees with a comprehensive overview of the sources of variability in clinical pathology data and how this variability can influence the interpretation of data from nonclinical toxicity studies. After an introduction by Adam Aulbach, Nancy Everds reviewed sources of pre-analytical variation drawing from her extensive experience and deep understanding of the literature. Complementing Nancy's review, A. Eric Schultze provided case-based examples of variability originating in the analytical phase of laboratory analyses. The cases were drawn from real-life situations that impacted study outcomes. The presentation was given by Armando R. Irizarry because Dr. Schultze was unable to attend. The design of a study is a key factor in our ability to properly interpret data from toxicity studies. Drawing from the extensive collective experience of Adam, Anne, and Niraj. Adam reviewed how study design could influence clinical pathology data in nonclinical toxicity studies. After a short break Robert Hall discussed the use of statistical analyses, reference intervals, and qualifiers in the interpretation data from nonclinical toxicity studies.

The third scientific was titled "Influence of Experimental Design and Environmental Conditions," was introduced by Theresa Boulineau and was co-chaired by Sherry Morgan. Five presentations highlighted the impact that experimental design decisions (choice of species/strain differences on anatomic and clinical pathology endpoints, effects of the choice of vehicles/formulations on study outcomes, a new paradigm for developmental and reproductive toxicology study design, and leveraging bioinformatics to improve study design and data interpretation) have on study outcomes. Leading off the session was Karyn Colman's presentation on the significant genetic diversity of rats and cynomolgus macaque monkeys which, when considered with differences in housing, nutrition, pathogen exposure, etc., can have very significant effects on toxicologic responses (see Colman, Symposium Issue). Kristin Barnhart followed with her presentation on how not only can the choice of species/strain have an impact on clinical pathology parameters but even the country of origin and supplier can impact variability and determination of reference intervals. After the morning break, Brian Enright, Katharine Whitney, and Michael Logan reviewed a multi-functional approach to the effects of vehicles/formulations on study outcomes, in particular the advantages and disadvantages of cyclodextrins. Paul Foster presented a new approach to the design of developmental and reproductive toxicology studies which increased robustness while decreasing the total numbers of animals used (see Foster, Symposium Issue). Finally, Elizabeth Skuba reviewed the creation of a user interface for a comprehensive non-clinical data warehouse application allowing for easy retrieval and visualization of study data.

The fourth scientific session was divided into sessions A and B, and was titled "Influence of Epigenetics, Genetics and Immunology." Session 4A, the morning session, was introduced by co-chairs Robert Johnson and Michael Leach and focused on the role of genetic and epigenetic events on variability in nonclinical studies. In the first presentation, genotype-phenotype relationships were characterized and case studies were presented that described the functional impact of genetic variation associated with toxic phenotypes and gave an example of drug- induced fulminant liver failure in non-human primates (see Bhoumik, Symposium Issue). The presentation introduced the concept of enhanced genetic characterization of species used in toxicity studies to understand the genetic basis of drug-associated toxicity and carcinogenicity. Next, the importance of genetic variability in

cynomolgus monkeys and its potential impact on drug development was covered by Karissa Adkins, and a case study on immune-mediated drug hypersensitivity reactions (IDHRs) and the association with major histocompatibility complex (MHC) alleles in cynomolgus monkeys was discussed (see Adkins, Symposium Issue). The session also explored the use of diversity outbred (DO) mice in toxicity testing and for predicting adverse drug reactions in a presentation by Alison Harrill, with the aim of proposing the use of DO mice for improving the estimation of human safety risk (see Harrill, Symposium Issue). Also, a Student Presentation by Elizabeth Clark highlighted the effects of loss of TGF-beta Receptors Type-1 and -2 in acute polymeric graft remodeling. Following the morning break, Jonathan Moggs gave an overview of epigenetics in toxicology and covered the utility of integrated genome-wide epigenomic and transcriptomic profiling of tissues from animal models. The session concluded with an interesting presentation by Hellmut Augustin on the contribution of epigenetic modifications on the regulation of endothelial cell phenotype and function during blood vessel maturation. Session 4B continued in the afternoon with the theme of the Influence of Epigenetics, Genetics and Immunology with more of a focus on the role of the immune response and immune genotypes on variation in toxicologic responses. The session was introduced and co-chaired by Cory Brayton and Paul Snyder. The first presentation by Rani Sellers reviewed pertinent differences between the mouse and human immune systems, and between inbred strains of mice (see Sellers, Symposium Issue). Additionally, the presentation covered differences in strain-related genetics and its impact on the immune response, the role of confounding variables associated with source of animal acquisition, breeding background and genetic drift. Dr. Sellers also gave examples of how these differences impact reproducibility and translatability of rodent models in efficacy testing. The second presentation in the session by Gary Burleson gave examples of how immunological variation in the genetic composition of different strains of mice as well as the age of mice can largely influence the susceptibility of mice to influenza virus and suggested this variation may be related to inflammatory single nucleotide polymorphisms (SNPs) (see Burleson, Symposium Issue). Following the afternoon break, a Student Presenter, Fuyuan Wang, discussed the effects of morphine on the microbiome and showed data on the potentiation of *Citrobacter rodentium* virulence and dissemination in mice following opioid administration (see Wang, Symposium Issue). The session concluded with two talks. The first by Nicholas Maness covered immunologic variation in macaques used in nonclinical studies with emphasis on MHC alleles associated with variable immunologic control and viral infections. In his talk he stressed the importance of careful selection of groups of animals to minimize biologic variation and to improve the use of these animals in testing and as models of human disease (see Maness, Symposium Issue). The last presentation of the session by Jack Harkema, looked at the interaction between genetic and environmental factors through a series of experiments outlining mouse strain-related differences in allergy-induced responses to common environmental allergens, and stressed the implications of gene-environmental interactions for public and precision health (see Harkema, Symposium Issue).

The fifth scientific session, titled “Influence of Age, Hormones, and the Microbiome,” was well-attended and was co-chaired by Dinesh J. Stanislaus, and Justin D. Vidal. Dinesh introduced this informative session and paved the way for a thought-provoking presentation

by Michael Gochfeld. Michael presented a series of examples from both human and animal studies showing how sex can have a significant impact on how we interpret toxicity data. Remarkably, sex is one of the most influential variables on study outcomes yet it is often ignored (see Gochfeld, Symposium Issue). In the second presentation of the session, Ellen Kovner Silbergeld convincingly articulated the importance of the microbiome in the physiology of animals and on how animals respond to xenobiotics. She challenged the audience to consider how to take into account the microbiome in toxicity studies and left us with the thought that perhaps we live in the microbiome's universe rather than the microbiome living in us (see Silbergeld, Symposium Issue). In the third presentation Paul Foster drew from his extensive experience with the reproductive effects of phthalates to demonstrate the influence of time of exposure on the outcome of toxicity studies (see Foster, Symposium Issue). Following Paul's presentation, Amera Remick discussed a pathologist's perspective on the impact of age on the male reproductive system and highlighted differences between nonclinical species (see Remick, Symposium Issue). Justin Vidal utilized his extensive experience to wrap up the session and the symposium with a pathologist's perspective on the impact of age on the assessment of the female reproductive system. As part of his presentation Dr. Vidal highlighted the difficulties encountered by pathologists during the evaluation of the reproductive system in nonhuman primates and provided his recommendations on the appropriate lexicon for documenting sexual maturity in females (see Vidal, Symposium Issue).

Additional Symposium Educational Opportunities: Saturday–Wednesday, June 25–29

Pre-Symposium activities began on Saturday with the "National Toxicology Program (NTP) Satellite Symposium: Pathology Potpourri" chaired by Susan Elmore. This was a continuing education (CE) symposium that allowed audience members to interact with the presenters through wireless keypads used for selecting a diagnosis of the lesions presented by the speaker, and active discussion of the results, once all votes are tabulated. Topics and cases included various organ systems and a wide-range of neoplastic and nonneoplastic lesions (see 2016 NTP Satellite Symposium, Symposium Issue).

On Sunday morning, in addition to two half-day morning CE courses, there was a half-day Career Development Workshop titled "Toxicity Testing in the 21st Century: Will *In Vivo* Studies Become Obsolete?," co-chaired by Erin Quist and Kyathanahalli Janardhan, and sponsored by the Career Development and Outreach Committee (CDOC). There were several talks that addressed the pros and cons of high throughput assays and high content screening and its impact on animal testing and implications for future regulatory paradigms. The presentations were given from the perspective of pathologists, toxicologists, basic researchers and regulatory scientists.

The CE courses on Sunday consisted of 2 half-day morning courses and 2 half-day afternoon courses. CE course 1, titled "The Respiratory System As a Target for Drug-Induced Toxicity: Pathology and Investigational Techniques," was co-chaired by Nicholas Macri and Kumar Changani. In the first presentation Alison Rowles provided participants with an introduction to the methods used to deliver drugs by inhalation and the appropriate processing of tissues. Nicholas followed Alison's presentation with a review of common

findings encountered by pathologists in inhalation studies conducted in dogs and rodents. Visanthi Mowat discussed differences in sensitivity between laboratory animal species (see Mowat, Symposium Issue). The second half of the session started with Kristen Nikula reviewing the role of pulmonary macrophages in health and disease. Following Dr. Nikula's presentation, Ronald Wolff provided an expert review of how to assess and interpret respiratory functional endpoints in toxicity studies. The session culminated with Kumar's discussion on the use of imaging techniques that can be utilized to assess pulmonary function and injury.

CE course 2 was co-sponsored by the American College of Toxicology and was titled "Interpreting and Integrating Clinical and Anatomic Pathology Results: Pulling It All Together." Mary Jane Hinrichs and Lila Ramaiah co-chaired this informative session. After an introduction by Dr. Hinrichs, Dr. Ramaiah leveraged her experience in the evaluation of nonclinical toxicity studies to outline her recommendations for correlating anatomic and clinical pathology data (see Ramaiah, Symposium Issue). Drawing from their own experiences in drug development, Elizabeth Skuba and William Iverson provided complementary presentations with real case examples of how they combined and correlated clinical and anatomic pathology data to appropriately interpret nonclinical toxicity data of small and large molecule xenobiotics. Daniella Ennulat wrapped up the course by sharing with the audience her deep knowledge and experience in the evaluation and implementation of non-traditional/novel biomarkers of liver and kidney injury.

CE course 3, titled "Hematotoxicity and Immunotoxicity Assessment: Essential Principles and Emerging Modalities," complemented CE course 2 and was co-chaired by Bill Siska and Denise Bounous. The course started with Anne Provencher discussing principles and case examples of how to correlate data from bone marrow smears, histopathology, and hematological analysis to assess hematotoxicity in nonclinical studies. Cindy Zhang demonstrated how to complement the traditional methods to evaluate hematotoxicity in rodent bone marrow with flow cytometric techniques. *In vitro* assays are increasingly used to assess hematotoxicity and Jacqueline Tarrant shared a case study of the utilization of a megakaryocyte colony-forming assay as an *in vitro* model of drug-induced thrombocytopenia. The assessment of potential immunotoxicity is an important part of the development of xenobiotics, particularly those that have intended or unintended effects on the immune system. Appropriately, the course offered participants an opportunity to learn more about immunotoxicity from Florence G. Burleson and Ellen W. Evans. Dr. Burleson focused on T-cell-Dependent Antibody Response (TDAR) testing and its limitations, and Dr. Evans delineated why in immunotoxicity assessments "one size does not fit all".

CE course 4 was co-chaired by Thomas Steinbach and Arun Pandiri and offered participants information and case studies to help them deal with the question "Is It Adverse, Adaptive, Artifact?" (see Pandiri, Symposium Issue). Roy Kerlin kicked-off the session by addressing the question of "What is an adverse effect in toxicologic pathology?". In this presentation Dr. Kerlin covered the recently published recommendations from the SRPC regarding determining, communicating, and using adverse effect data. Peter Mann followed with an informative presentation sharing examples of artifacts that could be confused with adverse or with adaptive effects. After the session break Nancy Everds discussed how clinical

pathology data influences the determination of the NOAEL and Alok Sharma reviewed adaptive, non-adverse, and adverse responses in nonclinical studies. The session concluded with a regulatory perspective on adverse versus adaptive responses presented by Peyton Myers.

On Monday afternoon, there was another CDOC-sponsored event that was part of the Career Development Lunchtime Series, entitled “Interacting with Our MD Colleagues” that consisted of a panel discussion between toxicologic pathologists, physicians, and / or MD pathologists that explored the ways to enhance communication between the toxicologic pathologist and their medical colleagues in the use of preclinical data to inform clinical trial design, interpretation of clinical adverse events, regulatory submissions and other means to foster dialogue and collaborations between toxicologic pathologists and our physician colleagues.

On Wednesday afternoon, the International Academy of Toxicologic Pathologists (IATP) and STP co-sponsored a lecture titled “Postnatal Organ Development As a Complicating Factor in Juvenile Toxicity Studies,” presented by George A. Parker and Catherine Picut. With the advent of toxicologic studies being conducted in juvenile animals, there were two timely lectures that covered the histology of postnatal organ development in young male and female rats. The lectures consisted of amazing histomorphologic images that emphasized developmental features of organs of the reproductive, digestive, and immune systems and showed normal physiologic changes that occur during development in the rat that can be misinterpreted as pathologic alterations. The authors emphasized the importance of age-appropriate controls when evaluating juvenile toxicity studies (see Picut and Parker, Symposium Issue).

Variation among and within strains or studies in response to chemical, pharmacological or environmental agents can confound results, complicate interpretation of data, interfere with reproducibility, and make extrapolation to humans problematic. The STP 35th Annual Symposium focused on the basis and relevance of variation in toxicologic responses and the session topics thoroughly covered many aspects of variation and how it influences toxicologic outcomes. Outstanding speakers provided case studies or real-life examples of what many of us grapple with on a daily basis in understanding the impact of variation and its relevance to nonclinical studies. The articles published in this issue reflect the extraordinary efforts of the scientific session co-chairs, the CE course chair and co-chair, and the excellent speakers that all contributed to the success of the 2016 STP Symposium. The high quality articles published in this issue will demonstrate how to identify the underlying causes of variability in nonclinical studies and provide the background to place variability into context for translation of animal data sets to human hazard identification and risk assessment.