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Juvenile Toxicology: Relevance and Challenges for Toxicologists and Pathologists

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Abstract

The Society of Toxicologic Pathology (STP) Education Committee and the STP Reproductive Special Interest Group held a North Carolina regional meeting entitled, “Juvenile Toxicology: Relevance and Challenges for Toxicologists and Pathologists” on March 13, 2015, at the National Institute of Environmental Health Sciences/National Toxicology Program in Research Triangle Park, North Carolina. The purpose of this regional meeting was to familiarize attendees with the topic of juvenile toxicity testing and discuss its relevance to clinical pediatric medicine, regulatory perspectives, challenges of appropriate study design confronted by toxicologists, and challenges of histopathologic examination and interpretation of juvenile tissues faced by pathologists. The 1-day meeting was a success with over 60 attendees representing industry, government, research organizations, and academia.

Keywords

developmental pathology; pediatrics; pharmaceutical development/products; preclinical safety assessment/risk management; toxicologic pathology; drug development

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Juvenile toxicity testing has become a hot topic in recent years with the institution of the Best Pharmaceuticals for Children Act (BCPA) and the Pediatric Research Equity Act (PREA) in 2002 and 2003, respectively. These acts resulted in the need for pharmaceutical safety assessment in pediatric or juvenile populations (U.S. Congress 2001; U.S. Congress 2003). The purpose of this regional meeting was to familiarize attendees with the topic of juvenile toxicity testing and discuss relevance to clinical pediatric medicine, regulatory perspectives, challenges of appropriate study design, and histopathologic interpretation of juvenile tissues. The meeting provided a public forum in which scientists from various fields could discuss strategies for addressing the many complexities of juvenile toxicology. The meeting organizing committee was composed of Thomas J. Steinbach, DVM, DACVP, DABT, director of the North Carolina Laboratory of Experimental Pathology Laboratories, Inc.; Darlene Dixon, DVM, PhD, DACVP, group leader of the Molecular Pathogenesis Group of the National Toxicology Program Laboratory (NTPL) and National Institute of Environmental Health Sciences (NIEHS); and Amera K. Remick, DVM, DACVP, DABT, assistant director of pathology at WIL Research. The organizing committee divided the meeting into 2 sessions: (1) clinical, regulatory, and toxicology perspectives and (2) pathology perspectives; and identified and invited 8 skilled professional speakers to address these diverse aspects of juvenile toxicology. A brief postmeeting synopsis was provided in the National Toxicology Program (NTP) Update Newsletter (Catlin and Quist 2015).

Opening Remarks and Clinical, Regulatory, and Toxicology Perspectives

Opening remarks for the meeting were provided by John R. Bucher, PhD, associate director of the NTP and director of the NTP Division. Dr. Bucher discussed the NTP's activities surrounding assessment of juvenile toxicity. A conceptual shift has occurred in the last decade in which the NTP has begun to study hormonally active compounds that exhibit juvenile effects derived from gestational exposures. The NTP has also begun to replace reproductive assessment by continuous breeding (RACB) studies with modified one-generation (MOG) studies, which capture the early life exposure period through perinatal dosing (Foster 2014). The MOG studies provide an efficient design that consolidates multiple studies into 1 study and assigns pups to several testing cohorts in order to maximize information gained. This paradigm shift at the NTP has inspired new testing strategies that encourage pathologists to examine early life exposures and interpret new data sets from juvenile animals.

The clinical, regulatory, and toxicology perspectives session commenced with a brief introduction by Dr. Steinbach and a lively presentation on the clinical relevance of juvenile toxicity studies by David B. Peden, MD, MS, University of North Carolina School of Medicine, director of the Center for Environmental Medicine, Asthma and Lung Biology; chief of the Division of Pediatric Allergy, Immunology, and Rheumatology; and Andrews Distinguished professor of Pediatrics, Medicine and Microbiology/Immunology and Toxicology. Dr. Peden discussed the challenges faced by pediatricians in extrapolating drug safety information to children. This extrapolation occurs when drugs are approved for use in adults and used off-label in children. Even when preclinical juvenile animal studies are performed, clinicians must frequently make assumptions based on data collected from different or inappropriate life stages where the true toxic potential of a compound might be

missed (Soellner and Olejniczak 2013). These pediatric drug safety concerns are not limited to pharmaceuticals, but can also be critical when food or environmental toxicants are "unintended drugs" in developing children (Donohue et al. 2013; Du Toit et al. 2015; Gascon et al. 2015; Midoro-Horiuti et al. 2010; Miller and Peden 2014; Petzold et al. 2014; Strobel and Ferguson 1984). Another consideration for pediatric safety assessment and study design is the increasing use of long-term pharmaceutical interventions, where continuous exposure may result in differential toxicities over the lifetime of the individual (Belvisi et al. 2005; Pedersen et al. 2010; Pruteanu et al. 2014; Zhang, Prietsch, and Duchame 2014).

From a historical perspective, as highlighted by both Drs. Peden and Elayan, pharmaceutical treatment in children based on extrapolation from adult data has been the standard, and concerns regarding the ethics or feasibility of clinical trials in children have hindered forward movement in pediatric safety assessment. Unfortunately, many drug safety incidents negatively impacting children could have been prevented with appropriate regulatory measures and testing. Ikram Elayan, PhD, is a senior pharmacology/toxicology reviewer for the Division of Psychiatry Products at the Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER). Dr. Elayan presented a background on pediatric drug regulation and a regulatory perspective on the importance of performing well-designed juvenile animal toxicity studies with appropriate endpoints. In recent years, as regulatory agencies have begun to encourage pediatric safety assessments for pharmaceuticals, a shift has occurred from voluntary testing with the BPCA in 2002, which offered an additional 6 months of marketing exclusivity in exchange for performing pediatric safety studies, to mandatory testing with the PREA in 2003 (U.S. Congress 2001; U.S. Congress 2003). Both acts were reauthorized with the FDA Amendments Act (FDAAA) in 2007 and made permanent with the FDA Safety and Innovation Act (FDASIA) in 2012. Thus, sponsors must submit a pediatric study plan within 60 days of the end-of-phase 2 FDA meeting for any pharmaceutical intended for use in humans ≤12 years of age (U.S. Congress 2007; U.S. Congress 2012). Nonclinical juvenile animal studies are a vital component of the pediatric study plan and are used to support data for the age-group between weaning and adulthood. These studies target pediatric safety concerns that are not adequately addressed in general toxicity studies and cannot be adequately or ethically studied in all instances in pediatric clinical trials (Tassinari et al. 2011). Nonclinical juvenile animal toxicity studies should be scientifically justified and well designed with appropriate endpoints (Bolon et al. 2013; R. M. Parker 2014b; Rao et al. 2011).

The intricacies and challenges of creating well-designed nonclinical juvenile toxicity studies were addressed by Robert M. Parker, PhD, DABT, director of Developmental and Reproductive Toxicology at Huntingdon Life Sciences. Dr. Robert Parker's seemingly simple statements of "Children are not small adults. Rats are not humans. Each pediatric age group is different" quickly developed into a detailed discussion of the logistical complexities of juvenile animal studies (R. M. Parker 2014a, 2014b). Juvenile toxicity studies involve a large number of animals, numerous and diverse study endpoints, and often multiple cohorts of animals in order to adequately address these endpoints. Factors such as latency of effects, litter effect (best controlled with a cross-fostering method), dosing of pups as young as postnatal day (PND) 1, pooled blood collections, dosing adjustments based on daily body weights due to rapid growth rate, and pharmacokinetic and pharmacodynamic differences in

young animals must all be considered and handled with appropriate scientific and technical expertise and resources. With the many factors involved, it is difficult to have a standardized juvenile toxicity study design; thus, each study must be designed and performed on a case-by-case basis. Multiple references were provided for additional information on comparative organ system development (Beckman and Feuston 2003; Hew and Keller 2003; Holsapple, West, and Landreth 2003; Marty et al. 2003; Walthall et al. 2005; Watson et al. 2006; Wood, Beyer, and Cappon 2003; Zoetis and Hurtt 2003a, 2003b; Zoetis et al. 2003), developmental and reproductive toxicology (Bailey et al. 2009; Greaves 2012; Haschek, Rousseaux, and Wallig 2013; Hoberman and Lewis 2012; Hood and Parker 2008; Lerman et al. 2009; R. M. Parker 2014a, 2012; R. M. Parker and York 2013; Wise et al. 2009; York et al. 2014), and juvenile toxicology (Bailey and Mariën 2011; Cappon 2011; Cappon et al. 2009; Carleer and Karres 2011; Hurtt 2011; Leconte et al. 2011; R. M. Parker 2014b; Rose 2011; Shimomura 2011; Tassinari et al. 2011).

LaRonda L. Morford, PhD, director of Juvenile Toxicology at WIL Research, supplemented Dr. Robert Parker's discussion of juvenile toxicity studies with special considerations in bio-pharmaceutical drug development. Biopharmaceuticals have unique characteristics that can change traditional study designs and have specific challenges such as structural and biologic diversity, species specificity, and immunogenic potential (Morford et al. 2011). Pharmacological relevance must be established in the test species and species specificity often limits applicability of on- and off-target effects to the nonhuman primate (NHP). Immunogenicity can significantly impact the design and interpretation of animal studies but does not reliably predict immunogenicity in humans (Morford et al. 2011). Dr. Morford discussed the advantages and disadvantages of using surrogate molecules, genetically engineered mouse (GEM) models, and various species including NHPs, rodents, dogs, and minipigs in juvenile toxicity studies for biopharmaceuticals. While the rat remains the preferred species in nonclinical evaluation for pediatric safety assessment of small molecules, NHPs are often the only applicable species for biopharmaceuticals. However, variation in biopharmaceutical properties drives the need to find the most relevant animal model on a case-by-case basis, if a relevant model is available.

Pathology Perspectives

In the afternoon session, the meeting's focus shifted to the pathology perspectives of juvenile toxicity testing. George A. Parker, DVM, PhD, DACVP, DABT, vice president of Global Pathology at WIL Research, reviewed the major challenges faced by pathologists in the assessment and interpretation of histopathological changes in tissues from juvenile animals. Dr. George Parker discussed the dynamics of postnatal development, using the immune system as the primary example (G. A. Parker et al. in press). Significant tissue changes occur during the course of a juvenile toxicity study. The biggest challenge faced by pathologists in assessing juvenile toxicity studies is the absence of concurrent controls when animals die or are euthanized prior to the scheduled study endpoint. This issue highlights the importance of the pathologist's need to understand normal tissue development at different ages in light of the typical relative lack of experience with juvenile tissues and the lack of concurrent controls for comparison. To address this point, Dr. George Parker proposed adding more non-dosed control animals to a given study to serve as age and sex-matched

controls for unscheduled deaths. This would also enable pathologists to accumulate a database representing the different time points that could be used as a reference for future studies.

Catherine A. Picut, VMD, JD, DACVP, DABT, senior pathologist with WIL Research, presented the beginnings of a project to publish a detailed atlas of juvenile histology. Dr. Picut's presentation of this project served as an appropriate and relevant illustration of the database of information that could be accumulated on juvenile tissues and used as a reference for pathologists. Dr. Picut reminded the audience that while we generally refer to "juvenile" toxicology, this subject truly encompasses multiple early life stages from neonatal, to early and late infantile, juvenile, and peripubertal. In the evaluation of juvenile toxicity endpoints, it is important for pathologists to recognize normal development from abnormal development through all these life stages. The testis, ovary, thyroid, brain, and lung were used as example tissues to demonstrate the dynamic fluctuations and salient histological developmental landmarks that occur throughout postnatal development (Bandeira, Lent, and Herculano-Houzel 2009; Bayer 1982; Bayer et al. 1993; Downes and Mullins 2014; O'Reilly and Thebaud 2014; Picut et al. 2014; Picut et al. 2015a; Picut et al. 2015b; Schittny and Burri 2008).

Focusing on the kidney, John C. Seely, DVM, DACVP, senior pathologist at Experimental Pathology Laboratories, Inc., described the toxicologic and pathologic issues surrounding juvenile renal studies. The importance of not only morphologic development but also functional development of the kidney was highlighted. Completion of morphologic nephrogenesis occurs around PND 8 to 11 while full functional renal maturation occurs by 6 to 7 weeks of age in the rat (Zoetis and Hurtt 2003a). The current stage of nephrogenesis and functional maturation of the kidney with variations in parameters such as renal blood flow, drug transport systems, and clearance mechanisms will result in striking differences in drug sensitivity (Schreuder et al. 2011). In most cases, the developing kidney appears to be more resistant than the adult to toxic agents; thus, juvenile nephrotoxicity cannot be extrapolated from adult data (Cappon and Hurtt 2010). The pathologist must be acutely aware of these potential differences for accurate interpretation of study data.

In addition to understanding differences in organ development within a species, the pathologist must be aware of differences in development between species. Using the NHP as an animal model in juvenile studies can add some significant challenges. However, as Dr. Morford indicated in her earlier discussion, in certain situations the NHP is the only relevant animal model. J. Mark Cline, DVM, PhD, DACVP, professor of pathology/comparative medicine and head of the section on comparative medicine at Wake Forest School of Medicine, delved into the pros and cons of using NHPs in toxicity studies and how one can manage some of the challenges. While NHPs have genetic and physiological similarities to humans, the ethical concerns, expense, biohazard potential, and high individual variability make sound scientific justification and appropriate handling of this species a must. With careful planning and design of studies using NHPs, and objective documentation of age and sexual maturation status especially when using juvenile animals, the interindividual animal variability can be partially controlled. Controlling this variability will aid in more accurate interpretation of subtle test article–related effects and yield more reliable results needed for

extrapolation of NHP data to humans (Chellman et al. 2009; Cline and Wood 2008; Mansfield and Kemnitz 2008; Mattison et al. 2011; Morton et al. 2008; Rodriguez et al. 2010; Sasseville and Diters 2008; Schoeb et al. 2008; Stute et al. 2012; Tharp et al. 2012; Uckun et al. 1997; Yasuda et al. 2005).

The meeting was concluded with a summary of key points from each presenter given by Dr. Remick. Final closing remarks by Dr. Dixon thanked the participating speakers and audience members for a successful and highly informative regional meeting on the relevance and challenges of juvenile toxicology for toxicologists and pathologists.

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Abbreviations

BCPA	Best Pharmaceuticals for Children Act
CDER	Center for Drug Evaluation and Research
DABT	Diplomate of the American Board of Toxicology
DACVP	Diplomate of the American College of Veterinary Pathologists
DVM	Doctor of Veterinary Medicine
FDA	Food and Drug Administration
FDAAA	FDA Amendments Act
FDASIA	FDA Safety and Innovation Act
GEM	genetically engineered mouse
JD	Juris Doctor
MOG	modified one-generation
NHP	nonhuman primate
NIEHS	National Institute of Environmental Health Sciences
NTP	National Toxicology Program
NTPL	National Toxicology Program Laboratory
PND	postnatal day
PREA	Pediatric Research Equity Act
RACB	Reproductive Assessment by Continuous Breeding
STP	Society of Toxicologic Pathology
VMD	Veterinariae Medicinae Doctoris (equivalent to DVM)

References

Bailey GP, Mariën D. The value of juvenile animal studies "What have we learned from preclinical juvenile toxicity studies? II. Birth Defects Res B Dev Reprod Toxicol. 2011; 92:273–91.10.1002/bdrb.20328 [PubMed: 22623019]

Bailey GP, Wise LD, Bushmann J, Hurtt M, Fisher E. Pre- and postnatal developmental toxicity study design for pharmaceuticals. Birth Defects Res B Dev Reprod Toxicol. 2009; 86:437–45.10.1002/bdrb.20217 [PubMed: 20025040]

Bandeira F, Lent R, Herculano-Houzel S. Changing numbers of neuronal and non-neuronal cells underlie postnatal brain growth in the rat. Proc Natl Acad Sci USA. 2009; 106:14108–13.10.1073/pnas.0804650106 [PubMed: 19666520]

Bayer SA. Changes in the total number of dentate granule cells in juvenile and adult rats: A correlated volumetric and 3H-thymidine autoradiographic study. Exp Brain Res. 1982; 46:315–23.10.1007/BF00238626 [PubMed: 7095040]

Bayer SA, Altman J, Russo RJ, Zhang X. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. Neurotoxicology. 1993; 14:83–144. [PubMed: 8361683]

Beckman DA, Feuston M. Landmarks in the development of the female reproductive system. Birth Defects Res B Dev Reprod Toxicol. 2003; 68:137–43.10.1002/bdrb.10016 [PubMed: 12866705]

Belvisi MG, Bundschuh DS, Stoeck M, Wicks S, Underwood S, Battram CH, Haddad E, Webber SE, Foster ML. Preclinical profile of ciclesonide: A novel corticosteroid for the treatment of asthma. J Pharmacol Exp Ther. 2005; 314:568–74.10.1124/jpet.105.085217 [PubMed: 15878996]

Bolon B, Garman RH, Pardo ID, Hensen K, Sills RC, Roulois A, Radovsky A, Bradley A, Andrews-Jones L, Butt M, Gumprecht L. STP position paper: Recommended practices for sampling and processing the nervous system (brain, spinal cord, nerve, and eye) during nonclinical general toxicity studies. Toxicol Pathol. 2013; 41:1028–48.10.1177/0192623312474865 [PubMed: 23475559]

Cappon GD. Nonclinical support of pediatric drug development in a global context: An industry perspective. Birth Defects Res B Dev Reprod Toxicol. 2011; 92:269–72.10.1002/bdrb.20303 [PubMed: 21594976]

Cappon GD, Bailey GP, Bushmann J, Feuston MH, Fisher JE, Hew KW, Hoberman AM, Ooshima Y, Stump DG, Hurtt ME. Juvenile animal toxicity study designs to support pediatric drug development. Birth Defects Res B Dev Reprod Toxicol. 2009; 86:463–69.10.1002/bdrb.20220 [PubMed: 20025047]

Cappon, GD.; Hurtt, ME. Developmental toxicity of the kidney. In: Kapp, RW., Jr; Tyl, RW., editors. Reproductive Toxicology. 3. Informa Healthcare; New York, NY: 2010. p. 193-204.

Carleer J, Karres J. Juvenile animal studies and pediatric drug development: A European regulatory perspective. Birth Defects Res B Dev Reprod Toxicol. 2011; 92:254–60.10.1002/bdrb.20310 [PubMed: 21638754]

Catlin, N.; Quist, E. NTP hosts regional meeting to highlight juvenile toxicology. UPDATE Newsletter. Apr. 2015 Retrieved on July 07, 2015 from U.S. Department of Health and Human Services, National Toxicology Program Web site: http://ntp.niehs.nih.gov/go/749028

Chellman GJ, Bussiere JL, Makori N, Martin PL, Ooshima Y, Weinbauer GF. Developmental and reproductive toxicology studies in nonhuman primates. Birth Defects Res B Dev Reprod Toxicol. 2009; 86:446–62.10.1002/bdrb.20216 [PubMed: 20025046]

Cline JM, Wood CE. The mammary glands of macaques. Toxicol Pathol. 2008; 36:134s–41s. 10.1177/0192623308327411 [PubMed: 21475638]

Donohue KM, Miller RL, Perzanowski MS, Just AC, Hoepner LA, Arunajadai S, Canfield S, Resnick D, Calafat AM, Perera FP, Whyatt RM. Prenatal and postnatal bisphenol A exposure and asthma development among inner-city children. J Allergy Clin Immunol. 2013; 131:736–42.10.1016/j.jaci.2012.12.1573 [PubMed: 23452902]

Downes N, Mullins P. The development of myelin in the brain of the juvenile rat. Toxicol Pathol. 2014; 42:913–22.10.1177/0192623313503518 [PubMed: 24129760]

Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, Brough HA, Phippard D, Basting M, Feeney M, Turcanu V, Sever ML, Gomez Lorenzo M, Plaut M, Lack G. LEAP Study Team. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med. 2015; 372:803–13.10.1056/NEJMoa1414850 [PubMed: 25705822]

Foster PMD. Regulatory forum opinion piece: New testing paradigms for reproductive and developmental toxicity—The NTP modified one generation study and OECD 443. Toxicol Pathol. 2014; 42:1165–67.10.1177/0192623314534920 [PubMed: 24862797]

Gascon M, Casas M, Morales E, Valvi D, Ballesteros-Gomez A, Luque N, Rubio S, Monfort N, Ventura R, Martinez D, Sunyer J, Vrijheid M. Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. J Allergy Clin Immunol. 2015; 135:370–78.10.1016/j.jaci.2014.09.030 [PubMed: 25445825]

Greaves, P., editor. Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation. 4. Academic Press, Elsevier; San Diego, CA: 2012.

Haschek, M.; Rousseaux, CG.; Wallig, MA., editors. Haschek and Rousseaux's Handbook of Toxicologic Pathology. Elsevier; San Diego, CA: 2013.

Hew KW, Keller KA. Postnatal anatomical and functional development of the heart: A species comparison. Birth Defects Res B Dev Reprod Toxicol. 2003; 68:309–20.10.1002/bdrb.10034 [PubMed: 14666994]

Hoberman, AM.; Lewis, EM., editors. Pediatric Non-clinical Drug Testing: Principles, Requirements, and Practices. John Wiley; Hoboken, NJ: 2012.

Holsapple MP, West LJ, Landreth KS. Species comparison of anatomical and functional immune system development. Birth Defects Res B Dev Reprod Toxicol. 2003; 68:321–34.10.1002/bdrb.10035 [PubMed: 14666995]

Hood, RD.; Parker, RM. Reproductive and developmental toxicology. In: Gad, SC., editor. Preclinical Development Handbook. John Wiley; Hoboken, NJ: 2008. p. 363-422.

Hurtt ME. Introduction to special issue on the value of juvenile animal studies. Birth Defects Res B Dev Reprod Toxicol. 2011; 92:251.10.1002/bdrb.20297 [PubMed: 21770022]

Leconte I, Bailey G, Davis-Bruno K, Hew KW, Kim J, Lima BS, Liminga U, Moffit J, Schaepdrijver LD, Schmitt G, Tassinari M, Thompson K, Hurtt M. Value of juvenile animal studies. Birth Defects Res B Dev Reprod Toxicol. 2011; 92:292–303.10.1002/bdrb.20331 [PubMed: 22623020]

Lerman SA, Hew KW, Stewart J, Stump DG, Wise LD. The nonclinical fertility study design for pharmaceuticals. Birth Defects Res B Dev Reprod Toxicol. 2009; 86:429–36.10.1002/bdrb.2022 [PubMed: 20025039]

Mansfield KG, Kemnitz JW. Introduction: Challenges in microbial quality control for nonhuman primate. ILAR J. 2008; 49:133–36.10.1093/ilar.49.2.133 [PubMed: 18323575]

Marty MS, Chapin RE, Parks LG, Thorsrud BA. Development and maturation of the male reproductive system. Birth Defects Res B Dev Reprod Toxicol. 2003; 68:125–36.10.1002/bdrb.10015 [PubMed: 12866704]

Mattison DR, Plant TM, Lin HM, Chen HC, Chen JJ, Twaddle NC, Doerge D, Slikker W Jr, Patton RE, Hotchkiss CE, Callicott RJ, Schrader SM, Turner TW, Kenser JS, Vitiello B, Petibone DM, Morris SM. Pubertal delay in male nonhuman primates (*Macaca mulatta*) treated with methylphenidate. Proc Natl Acad Sci USA. 2011; 108:16301–6.10.1073/pnas.1102187108 [PubMed: 21930929]

Midoro-Horiuti T, Tiwari R, Watson CS, Goldblum RM. Maternal bisphenol A exposure promotes the development of experimental asthma in mouse pups. Environ Health Perspect. 2010; 118:273–77.10.1289/ehp.0901259 [PubMed: 20123615]

Miller RL, Peden DB. Environmental effects on immune responses in patients with atopy and asthma. J Allergy Clin Immunol. 2014; 134:1001–8.10.1016/j.jaci.2014.07.064 [PubMed: 25439226]

Morford LL, Bowman CJ, Blanset DL, Bøgh IB, Chellman GJ, Halpern WG, Weinbauer GF, Coogan TP. Preclinical safety evaluations supporting pediatric drug development with biopharmaceuticals: Strategy, challenges, current practices. Birth Defects Res B Dev Reprod Toxicol. 2011; 92:359–80.10.1002/bdrb.20305 [PubMed: 21770023]

Morton WR, Agy MB, Capuano SV, Grant RF. Specific pathogen-free macaques: Definition, history, and current production. ILAR J. 2008; 49:137–41.10.1093/ilar.49.2.137 [PubMed: 18323576]

Pediatric Research Equity Act of 2003 (Public Law No. 108–155); One Hundred Eighth Congress of the United States of America (108th U.S. Congress); January 7 2003; Retrieved on July 07, 2015 from United States Department of Health and Human Services (U.S. DHHS), Food and Drug Administration (FDA), Pediatric Product Development Web site: http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/UCM077853.pdf

Best Pharmaceuticals for Children Act (Public Law No. 107–109); One Hundred Seventh Congress of the United States of America (107th U.S. Congress); January 3 2001; Retrieved on July 07, 2015 from United States Department of Health and Human Services (U.S. DHHS), Food and Drug Administration (FDA), Pediatric Product Development Web site: http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/UCM049874.pdf

Food and Drug Administration Amendments Act of 2007; One Hundred Tenth Congress of the United States of America (110th U.S. Congress); January 4 2007; Retrieved on July 07, 2015 from United States Department of Health and Human Services (U.S. DHHS), Food and Drug Administration (FDA), Pediatric Product Development Web site: http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/UCM049870.pdf

Food and Drug Administration Safety and Innovation Act; One Hundred Twelfth Congress of the United States of America (112th U.S. Congress); 2012 January 3; Retrieved on July 07, 2015 from United States Department of Health and Human Services (U.S. DHHS), Food and Drug Administration (FDA), Pediatric Product Development Web site: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm049867.htm; http://www.gpo.gov/fdsys/pkg/BILLS-112s3187enr/pdf/BILLS-112s3187enr.pdf

O'Reilly M, Thebaud B. Animal models of bronchopulmonary dysplasia: The term rat models. Am J Physiol Lung Cell Mol Physiol. 2014; 307:L948–58.10.1152/ajplung.00160.2014 [PubMed: 25305248]

Parker GA, Picut CA, Swanson C, Toot JD. Histologic features of postnatal development of immune system organs in the Sprague-Dawley rat. Toxicol Pathol. (In press). Epub ahead of print pii: 0192623315578720.

Parker, RM. Reproductive toxicity testing—Methodology. In: Hood, RD., editor. Developmental and Reproductive Toxicology, a Practical Approach. 3. Informa Healthcare; New York, NY: 2012. p. 184-228.

Parker, RM. Developmental and reproductive toxicology. In: Derelanko, MJ.; Auletta, CS., editors. Handbook of Toxicology. 3. Taylor & Francis Group; Boca Raton, FL: 2014a. p. 355-98.

Parker, RM. Juvenile toxicology. In: Derelanko, MJ.; Auletta, CS., editors. Handbook of Toxicology. 3. CRC Press, Taylor & Francis Group; Boca Raton, FL: 2014b. p. 400-52.

Parker, RM.; York, RG. Developmental and reproductive toxicology. In: Brock, W.; Hastings, K.; McGowan, KM., editors. Nonclinical Safety Assessment: A Guide to International Pharmaceutical Regulations. John Wiley; Hoboken, NJ: 2013. p. 265-96.

Pedersen S, Potter P, Dachev S, Bosheva M, Kaczmarek J, Springer E, Dunkely J, Engelstatter R. Efficacy and safety of three ciclesonide doses vs placebo in children with asthma: The RAINBOW study. Respir Med. 2010; 104:1618–28.10.1016/j.rmed.2010.06.012 [PubMed: 20619624]

Petzold S, Averbeck M, Simon JC, Lehmann I, Polte T. Lifetime-dependent effects of bisphenol A on asthma development in an experimental mouse model. PloS One. 2014; 9:e100468.10.1371/journal.pone.0100468 [PubMed: 24950052]

Picut CA, Dixon D, Simons ML, Stump DG, Parker GA, Remick AK. Postnatal ovary development in the rat: Morphologic study and correlation of morphology to neuroendocrine parameters. Toxicol Pathol. 2015a; 43:343–53.10.1177/0192623314544380 [PubMed: 25107574]

Picut CA, Remick AK, Asakawa MG, Simons ML, Parker GA. Histologic features of prepubertal and pubertal reproductive development in female Sprague-Dawley rats. Toxicol Pathol. 2014; 42:403–13.10.1177/0192623313484832 [PubMed: 23599412]

Picut CA, Remick AK, de Rijk EP, Simons ML, Stump DG, Parker GA. Postnatal development of the testis in the rat: Morphologic study and correlation of morphology to neuroendocrine parameters. Toxicol Pathol. 2015; 43:326–42.10.1177/0192623314547279 [PubMed: 25217330]

Pruteanu AI, Chauhan BF, Zhang L, Prietsch SO, Duchame FM. Inhaled corticosteroids in children with persistent asthma: Dose-response effects on growth. Evidence-based Child Health. 2014; 9:931–1046.10.1002/ebch.1989 [PubMed: 25504973]

Rao DB, Little PB, Malarkey DE, Herbert RA, Sills RC. Histopathological evaluation of the nervous system in National Toxicology Program rodent studies: A modified approach. Toxicol Pathol. 2011; 39:463–70.10.1177/0192623311401044 [PubMed: 21430177]

Rodriguez JS, Morris SM, Hotchkiss CE, Doerge DR, Allen RR, Mattison DR, Paule MG. The effects of chronic methylphenidate administration on operant test battery performance in juvenile rhesus monkeys. Neurotoxicol Teratol. 2010; 32:142–51.10.1016/j.ntt.2009.08.011 [PubMed: 19737611]

Rose K. The value of juvenile animal studies: A pediatric clinical perspective. Birth Defects Res B Dev Reprod Toxicol. 2011; 92:252–53.10.1002/bdrb.20306 [PubMed: 21594978]

Sasseville VG, Diters RW. Impact of infections and normal flora in nonhuman primates on drug development. ILAR J. 2008; 49:179–90.10.1093/ilar.49.2.170 [PubMed: 18323580]

Schittny, JC.; Burri, PH. Development and growth of the lung. In: Fishman, AP.; Elias, JA.; Fishman, JA.; Grippi, MA.; Senior, RM.; Pack, AI., editors. Fishman's Pulmonary Diseases and Disorders. 4. McGraw-Hill Medical; New York, NY: 2008. p. 91-114.

Schoeb TR, Eberle R, Black DH, Parker RF, Cartner SC. Diagnostic exercise: Papulovesicular dermatitis in rhesus macaques (*Macaca mulatta*). Vet Pathol. 2008; 45:592–94.10.1354/vp.45-4-592 [PubMed: 18587109]

Schreuder MF, Bueters RR, Huigen MC, Russel FG, Masereeuw R, van den Heuvel LP. Effect of drugs on renal development. Clin J Am Soc Nephrol. 2011; 6:212–17.10.2215/CJN.04740510 [PubMed: 21071516]

Shimomura K. The value of juvenile animal studies: A Japanese industry perspective. Birth Defects Res B Dev Reprod Toxicol. 2011; 92:266–68.10.1002/bdrb.20300 [PubMed: 21594974]

Soellner L, Olejniczak K. The need for juvenile animal studies: A critical review. Regul Toxicol Pharmacol. 2013; 65:87–99.10.1016/j.yrtph.2012.10.010 [PubMed: 23108189]

Strobel S, Ferguson A. Immune responses to fed protein antigens in mice. 3. Systemic tolerance or priming is related to age at which antigen is first encountered. Pediatr Res. 1984; 18:588–94.10.1203/00006450-198407000-00004 [PubMed: 6332297]

Stute P, Sielker S, Wood CE, Register TC, Lees CJ, Dewi FN, Williams JK, Wagner JD, Stefenelli U, Cline JM. Life stage differences in mammary gland gene expression profile in non-human primates. Breast Cancer Res Treat. 2012; 133:617–34.10.1007/s10549-011-1811-9 [PubMed: 22037779]

Tassinari MS, Benson K, Elayan I, Espandiari P, Davis-Bruno K. Juvenile animal studies and pediatric drug development retrospective review: Use in regulatory decisions and labeling. Birth Defects Res B Dev Reprod Toxicol. 2011; 92:261–65.10.1002/bdrb.20304 [PubMed: 21594977]

Tharp AP, Maffini MV, Hunt PA, VandeVoort CA, Sonnenschein C, Soto AM. Bisphenol A alters the development of the rhesus monkey mammary gland. Proc Natl Acad Sci USA. 2012; 109:8190–95.10.1073/pnas.1120488109 [PubMed: 22566636]

Uckun FM, Yanishevski Y, Tumer N, Waurzyniak B, Messinger Y, Chelstrom LM, Lisowski EA, Ek O, Zeren T, Wendorf H, Langlie MC, Irvin JD, Myers DE, Fuller GB, Evans W, Gunther R. Pharmacokinetic features, immunogenicity, and toxicity of B43(anti-CD19)-Pokeweed antiviral protein immunotoxin in cynomolgus monkeys. Clin Cancer Res. 1997; 3:325–37. [PubMed: 9815689]

Walthall K, Cappon GD, Hurtt ME, Zoetis T. Postnatal development of the gastrointestinal system: A species comparison. Birth Defects Res B Dev Reprod Toxicol. 2005; 74:132–56.10.1002/bdrb.20040 [PubMed: 15834902]

Watson RE, Desesso JM, Hurtt ME, Cappon GD. Post-natal growth and morphological development of the brain: A species comparison. Birth Defects Res B Dev Reprod Toxicol. 2006; 77:471–84.10.1002/bdrb.20090 [PubMed: 17066419]

Wise LD, Buschmann J, Feuston MH, Fisher JE, Hew KW, Hoberman AM, Lerman SA, Ooshima Y, Stump DG. Embryo-fetal developmental toxicity study design for pharmaceuticals. Birth Defects Res B Dev Reprod Toxicol. 2009; 86:418–28.10.1002/bdrb.20214 [PubMed: 20025038]

Wood SL, Beyer BK, Cappon GD. Species comparison of postnatal CNS development: Functional measures. Birth Defects Res B Dev Reprod Toxicol. 2003; 68:391–407.10.1002/bdrb.10037 [PubMed: 14745989]

Yasuda I, Yasuda M, Sumida H, Tsusaki H, Arima A, Ihara T, Kubota S, Asaoka K, Tsuga K, Akagawa Y. In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affects tooth development in rhesus monkeys. Reprod Toxicol. 2005; 20:21–30.10.1016/j.reprotox.2004.12.016 [PubMed: 15808782]

York, RG.; Parker, RM.; Haber, LT. Test methods for assessing female reproductive and developmental toxicology. In: Hayes, EW.; Kruger, CL., editors. Hayes' Principles and Methods of Toxicology. 6. CRC Press, Taylor & Francis Group; Boca Raton, FL: 2014. p. 1637-722.

Zhang L, Prietsch SO, Duchame FM. Inhaled corticosteroids in children with persistent asthma: Effects on growth. Evidence-based Child Health. 2014; 9:829–930.10.1002/ebch.1988 [PubMed: 25504972]

Zoetis T, Hurtt ME. Species comparison of anatomical and functional renal development. Birth Defects Res B Dev Reprod Toxicol. 2003a; 68:111–20.10.1002/bdrb.10013 [PubMed: 12866702]

Zoetis T, Hurtt ME. Species comparison of lung development. Birth Defects Res B Dev Reprod Toxicol. 2003b; 68:121–24.10.1002/bdrb.10014 [PubMed: 12866703]

Zoetis T, Tassinari MS, Bagi C, Walthal K, Hurtt ME. Species comparison of postnatal bone growth and development. Birth Defects Res B Dev Reprod Toxicol. 2003; 68:86–110.10.1002/bdrb.10012 [PubMed: 12866701]