Reproduction, Development, and Pathology: Finding Common Ground

Points to Consider for the Inclusion of Reproductive and Pathology Endpoints for Assessment of Reproductive and Developmental Toxicity in Pharmaceutical Drug Development

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Integration of results from General Toxicity and DART studies, including Pathology, is necessary to identify Hazards to Development and Reproduction

Clinical and Anatomic Pathology

- General Toxicity Studies
  - Acute Toxicity
  - Subacute Toxicity
  - Chronic Toxicity
- Special Toxicity Studies
  - *Immunotoxicity*
  - *Local Tolerance*
  - *Phototoxicity*
  - *Carcinogenicity*
  - *Juvenile Toxicity*

Developmental and Reproductive Toxicity

- Reproductive Studies
  - Fertility studies
  - Embryofetal Development
  - Pre-and Postnatal development
- Developmental Studies
  - Embryofetal Development
  - Pre-and Postnatal development
  - *Juvenile toxicity*

Guidance for Pathology and DART Assessments

- *'Generally…toxicity studies should be designed to evaluate hematology, clinical chemistry, necropsy and histopathology data'* (ICH M3)
- STP Best Practices for Pathology Assessments in General Toxicity Studies have been established *(Bregman et al. 2003, Sellers et al. 2007)*
- Reproductive tract and function are discussed in guidelines for:
  - Development of drugs (ICH M3)
  - Drugs for use in advanced cancer (ICH S9)
  - Biopharmaceuticals (ICH S6)
- **Specific DART Guidance (ICH S5) is currently under revision**
- **Development of a new guideline for nonclinical testing in support of pediatrics (ICH S11) is underway**

Considerable flexibility in existing guidance…

- ***Ultimate goal is to evaluate any potential risk to human patients based on the available information, and communicate this risk to physicians & patients***

- When should DART endpoints be added to general toxicity studies?
- When should pathology endpoints be added to DART studies?
- What are the current practices?
- What are the limitations and concerns?

- STP commissioned an Expert Working Group (EWG) composed of Anatomic Pathologists, Clinical Pathologists, and Reproductive Toxicologists
- EWG conducted a survey through the STP, but distributed broadly to both toxicologic pathology and reproductive toxicology communities

2014 Survey of Pathology and DART Endpoints

- Survey Respondents included Academic, Industry and Contract Research Organizations
  - Global Distribution (North America, Europe, Africa, Asia)
  - Individuals and Groups (One response submitted per organization)
  - Pathology, Clinical Pathology and Reproductive Toxicity Groups
  - No enumeration of total individuals or of the total number of studies
- Several opportunities for respondents to add comments
- Survey Results enabled identification of:
  - Areas of common practice
  - Areas of variable/inconsistent practice
  - Areas of limited experience
- Integrated Assessment
  - Existing Guidance
  - Survey Responses
  - Review of Literature
  - EWG Member Experience

2016 Reference

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Original Article

Scientific and Regulatory Policy Committee Points to Consider Review: Inclusion of Reproductive and Pathology End Points for Assessment of Reproductive and Developmental Toxicity in Pharmaceutical Drug Development

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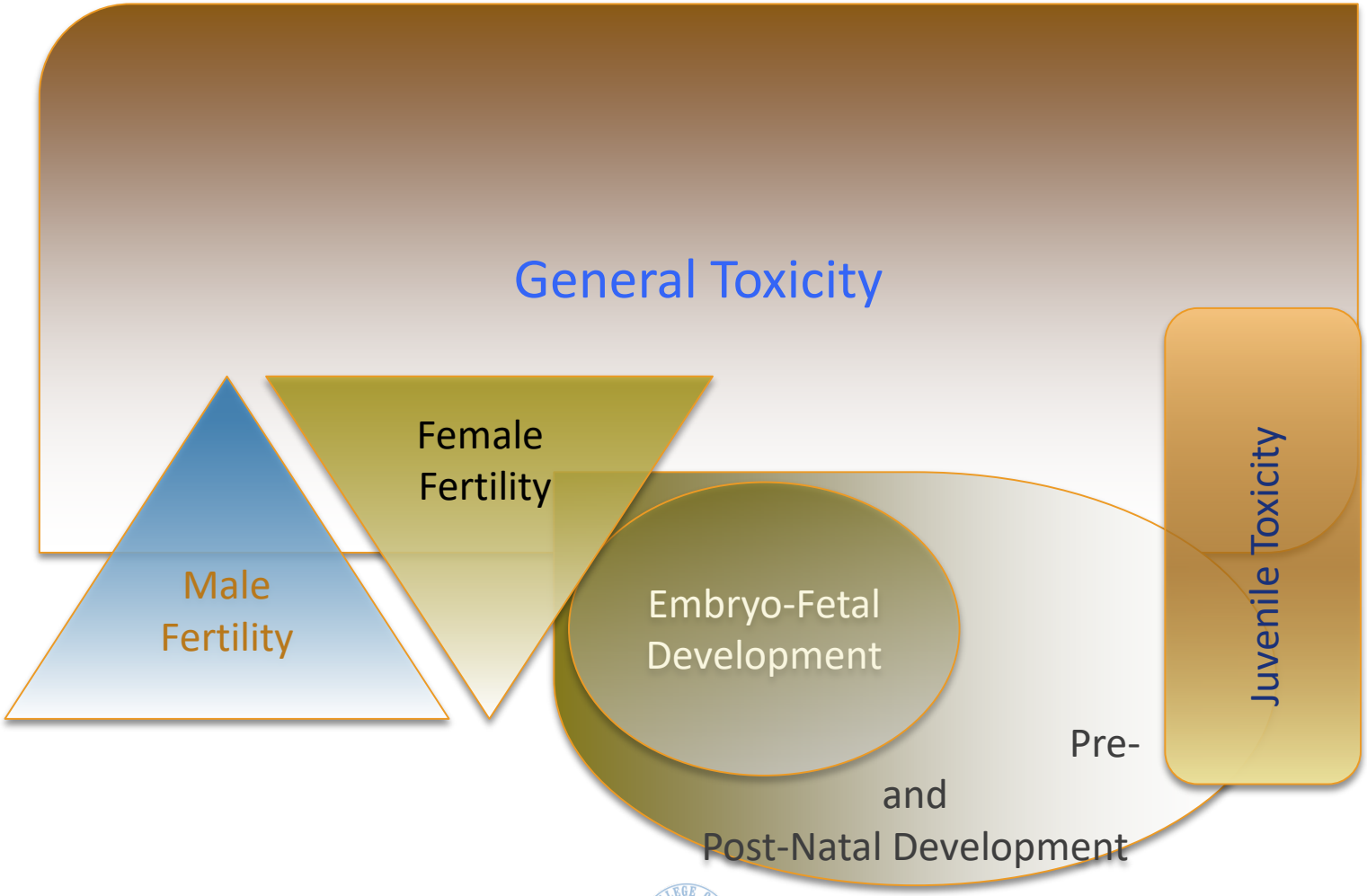
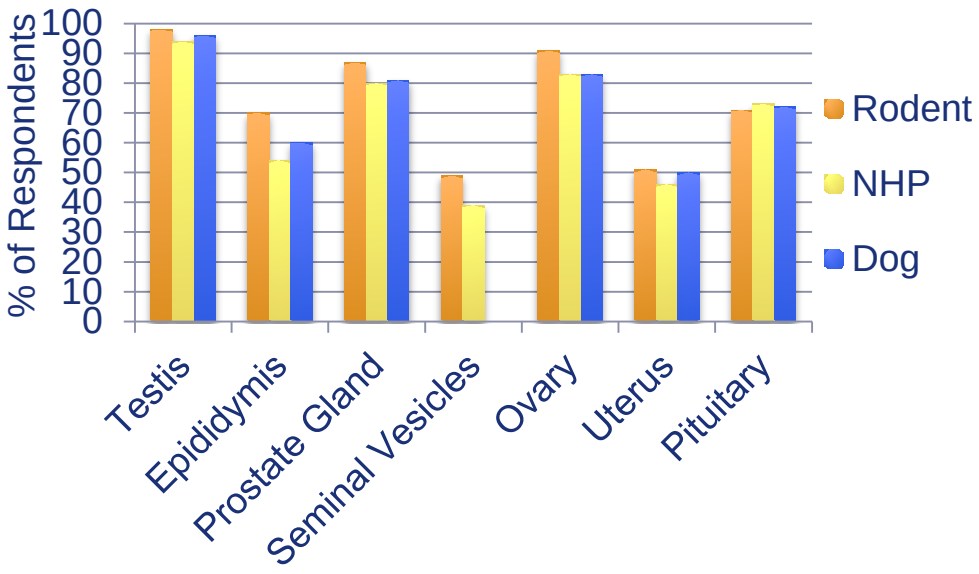
DART Studies Overlap with General Toxicity Testing



Routine Pathology Endpoints in General Toxicity Studies that Contribute to DART Assessment

Organ Weights Collected Routinely



Stage-aware histologic evaluation of reproductive tissues:

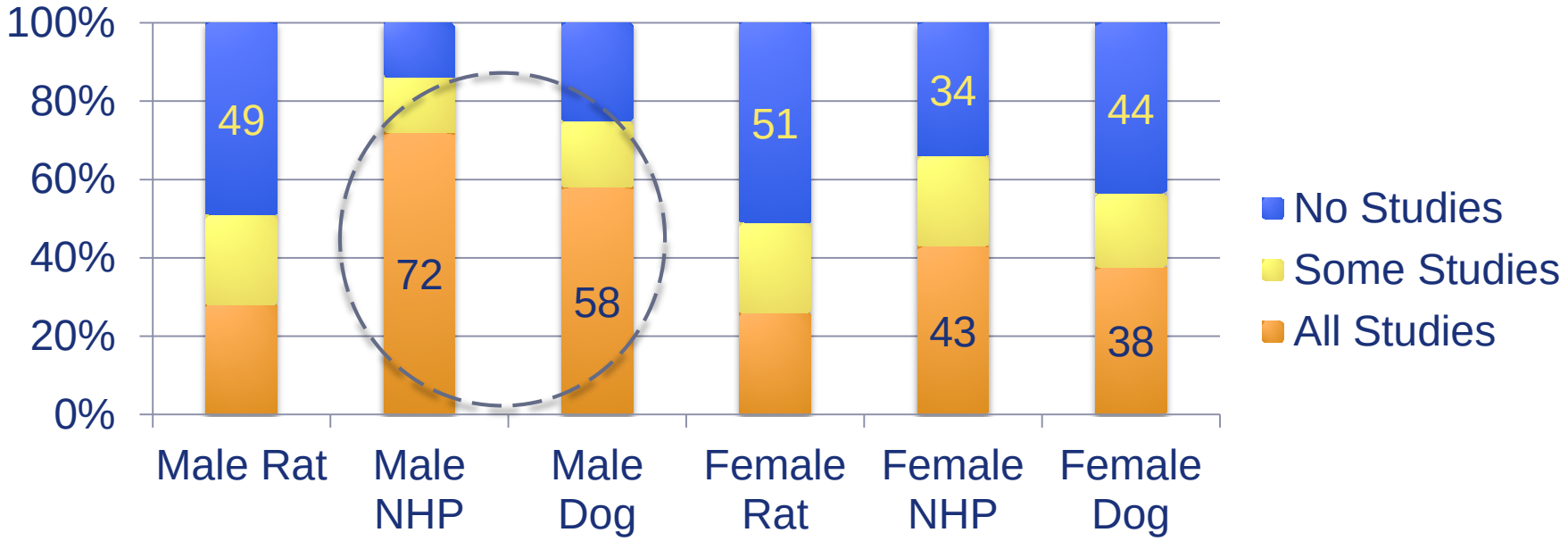
- Spermatogenic progression and transit in mature males
- Cycle stage in mature females

- Clinical Pathology
  - Hematology, Clinical Chemistry, Coagulation
  - Hormones only ‘for cause’
- Anatomic Pathology
  - Pituitary Gland
  - Testes
  - Epididymides
  - Prostate Gland
  - Seminal Vesicles
  - Ovaries
  - Uterus
  - Vagina

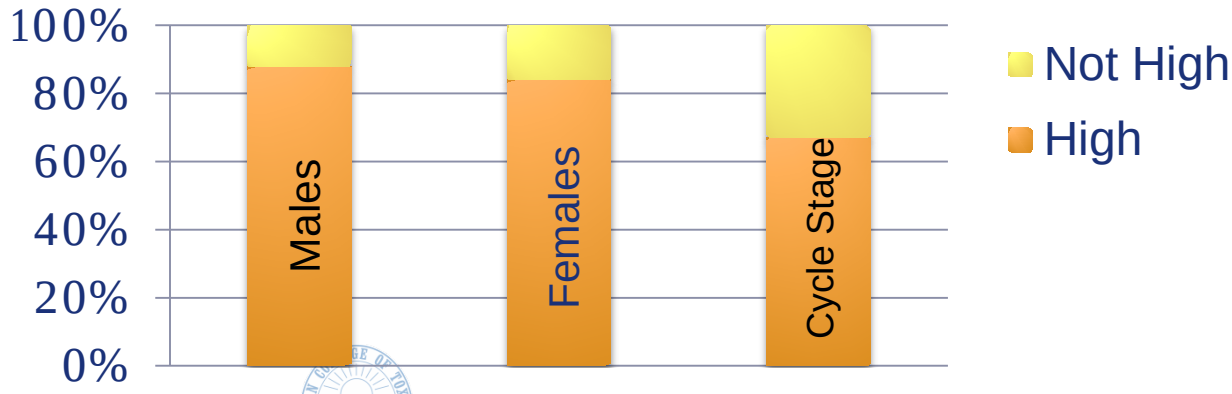
Assessment of Maturity

- Can be important for non-rodents as part of test-system characterization
- Often, initial nonclinical studies supporting FIH are conducted with pre- or peripubertal dogs or monkeys
  - Normal for the individual animal, so should not be recorded as a microscopic pathology finding
  - BUT nice to know if study could have identified effects on the mature reproductive tissues in that species
  - Typically, at least 1 study of at least 3 months duration should be conducted in mature males to detect potential testicular toxicity

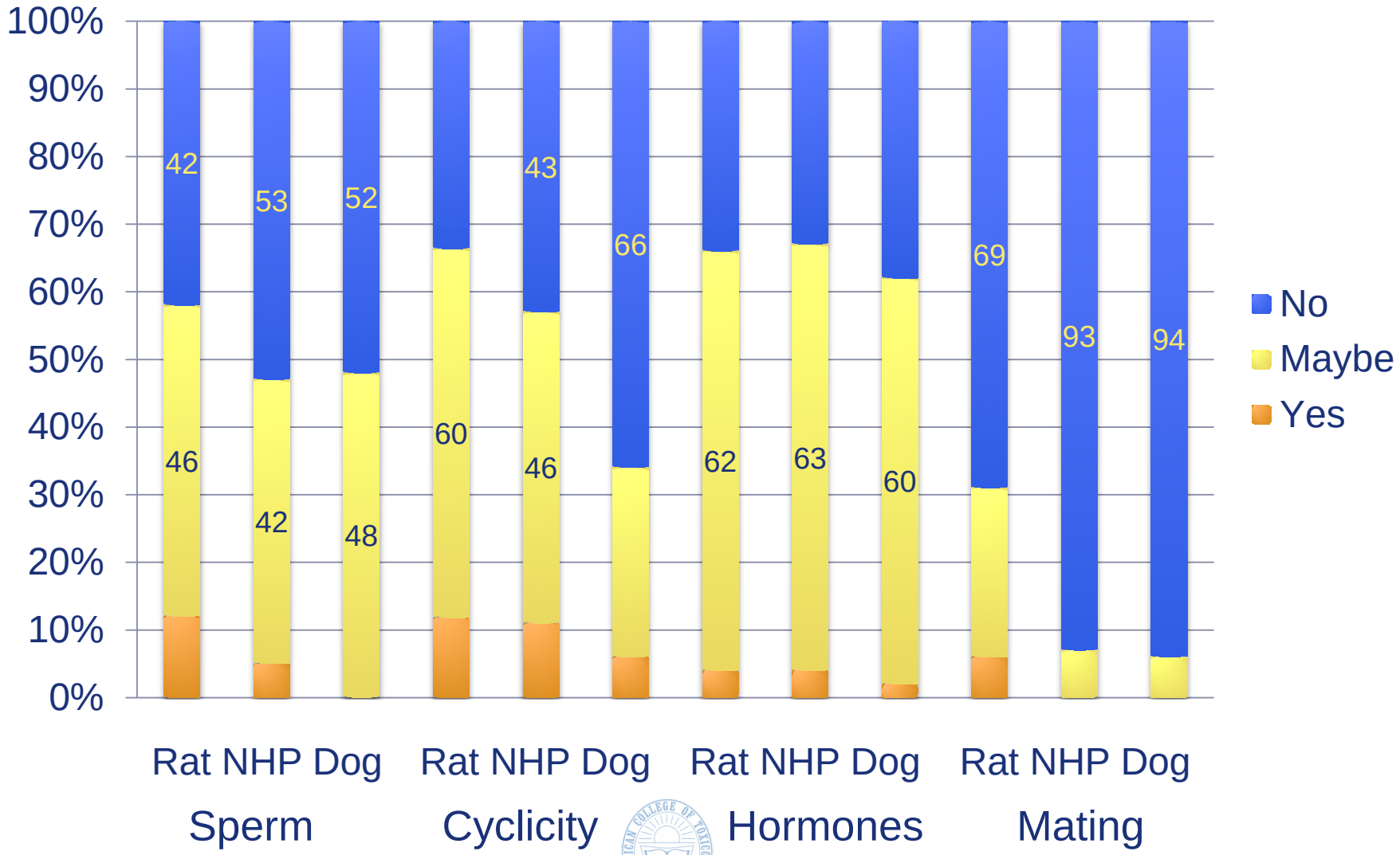
Recording of Sexual Maturity in General Toxicity Studies



Confidence of Pathologist Group (Majority) in Histologic Evaluation of Sexual Maturity



Inclusion of Dedicated DART Endpoints in General Toxicity Studies



Opportunities and 'Watch Outs' for Reproductive Endpoints in General Toxicity Studies

- Most repeat dose toxicity studies include a gross and histologic assessment of the male and female reproductive tract
  - Mammary gland assessment can also be useful, especially in cycling females
- Specialized reproductive assessments, are infrequently included, but may be added for cause
  - May include sperm assessments, monitoring cyclicity, hormone evaluations, and/or mating trials
- 'Stage aware' assessment of male and female reproductive tissues in mature animals
  - Documentation of sexual maturity should be considered
- Use caution in interpretation of findings in the reproductive tract
  - Easily confounded by stress or body weight loss
  - Endpoints should not be interpreted in isolation

Additional Considerations

- Discussion of **NHP** Test Systems
  - Challenges in determining maturity
  - Challenges of environmental stress and social hierarchy
  - Challenges of high variability and low ‘n’
- Evaluation of the **Mammary Gland** in General Toxicity Studies (all species)
  - Not a standard/required tissue, but typically evaluated
  - Characteristic changes during cycle in mature females
  - Sensitive to hormonal disruption
  - First tissue to mature during puberty in NHP
- Tiered approach to assessment of **Ovaries**
  - Initial assessment of single section, both ovaries
  - Step sectioning, with follicle counts, as a second tier for cause
    - May require additional investigative study

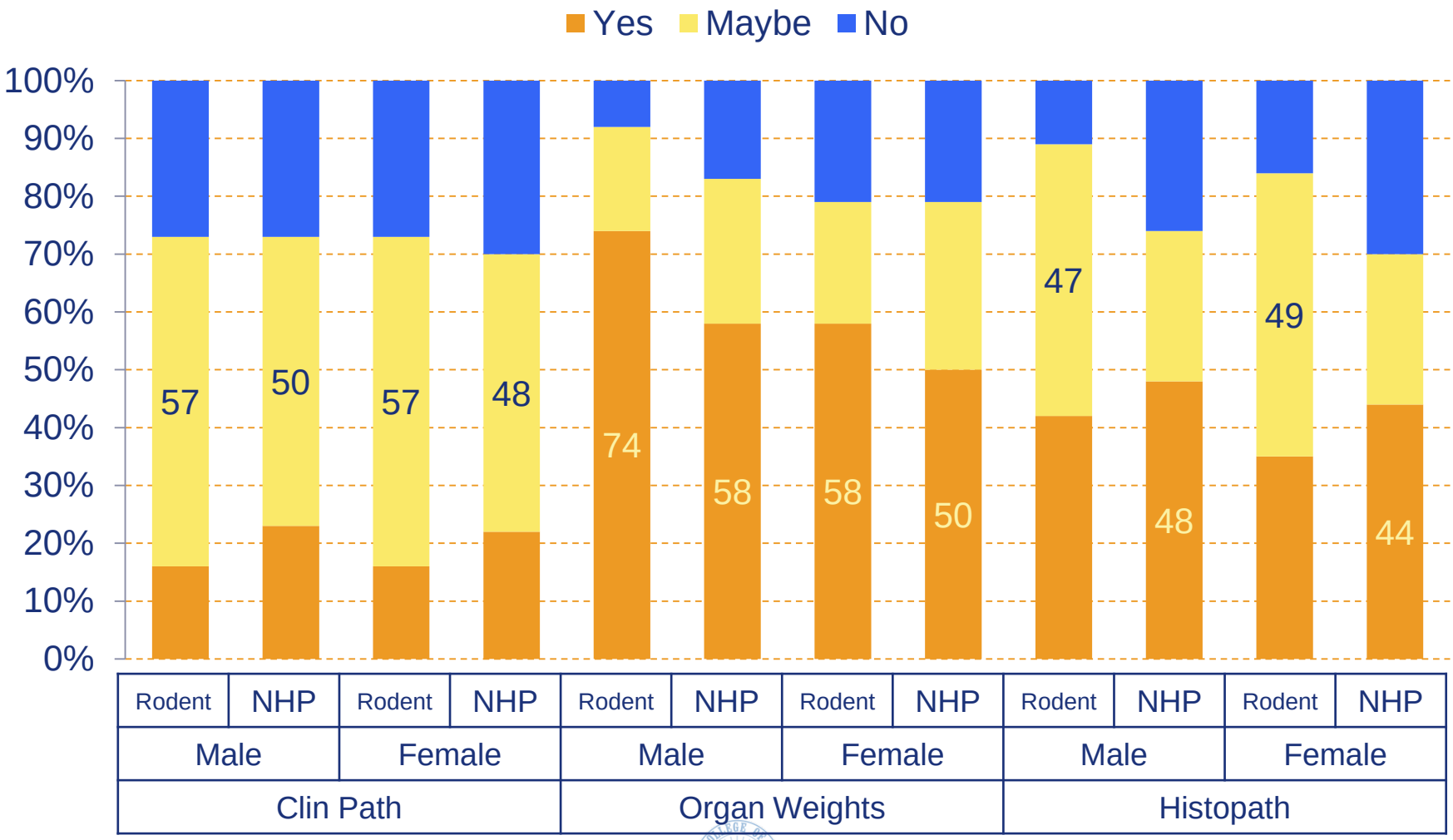
Integrated Assessment-General Toxicity Studies

Subset	Species	Fertility	Clinical Pathology, Organ Weights, Gross and Histopathology
Males	Rodent	Sperm at necropsy and/or hormones for cause	Complete (Standard Endpoints) Record Maturity
	Dog	Semen and/or hormones for cause	
	NHP		
Females	Rodent	Cycling (vaginal smears) and/or hormones for cause	Complete (Standard Endpoints) Record Maturity Record estrous/menstrual cycle stage if needed to clarify or interpret results
	Dog	Rarely Assessed	
	NHP	Cycling for cause; hormones rare	

Pathology endpoints in DART studies

- Flexibility in available guidelines
- ICH S5(R2)
  - All DART studies: Tissues with macroscopic findings for possible histology
  - Fertility study: preserve testes, epididymides, ovaries, & uteri for possible histology (then discard)
  - Organ weights not specifically required
- ICH S6(R1)
  - NHP-only programs may rely on reproductive tissue weights & pathology from repeat dose study in mature animals in lieu of fertility study, with additional endpoints added for cause
- Other
  - FDA Guidance (2015) Testicular Toxicity
    - Recommends histopathology in fertility study if adverse findings in repeat-dose toxicity study
  - FDA Guidance (2011) Integrating study results to assess concerns

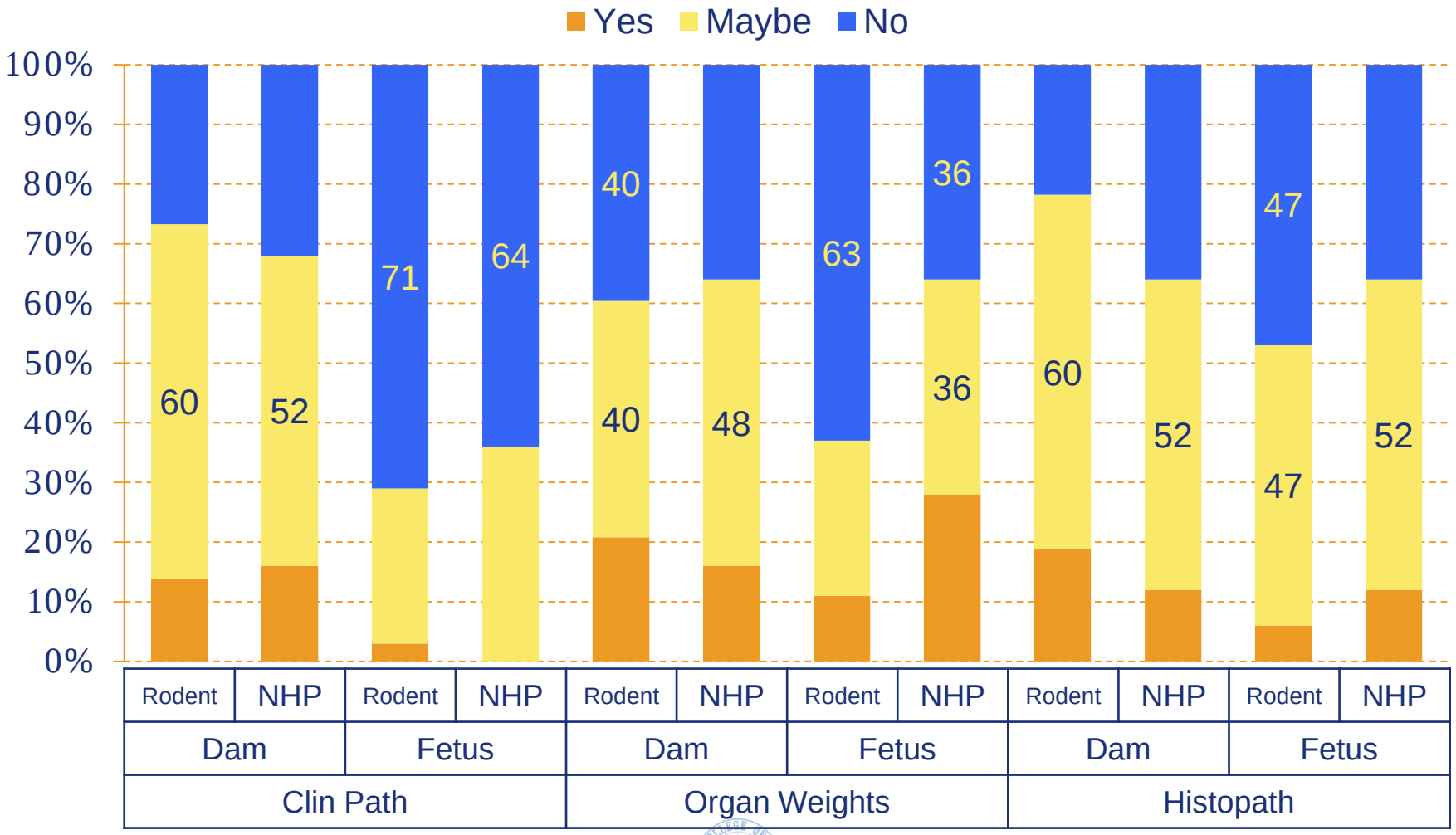
Survey: Path Endpoints in Fertility Studies



Pathology Endpoints in Fertility Studies

- Limited experience with NHP fertility studies
- **Clinical pathology** not typically added without scientific justification, but majority of respondents would include if useful based on previous findings or pharmacological relevance
- **Organ weights** routinely collected by a majority of respondents
- **Histopathology** done either routinely or conducted for cause by a majority of respondents
- Experience indicates that tissues are typically collected for possible future examination but NOT routinely evaluated if no effects or pathology data already available from repeat-dose studies in that species and dose range
- Overall, general practice based on the survey results appear consistent with current regulatory guidance

Survey: Path Endpoints in EFD Studies



Pathology Endpoints in EFD Studies

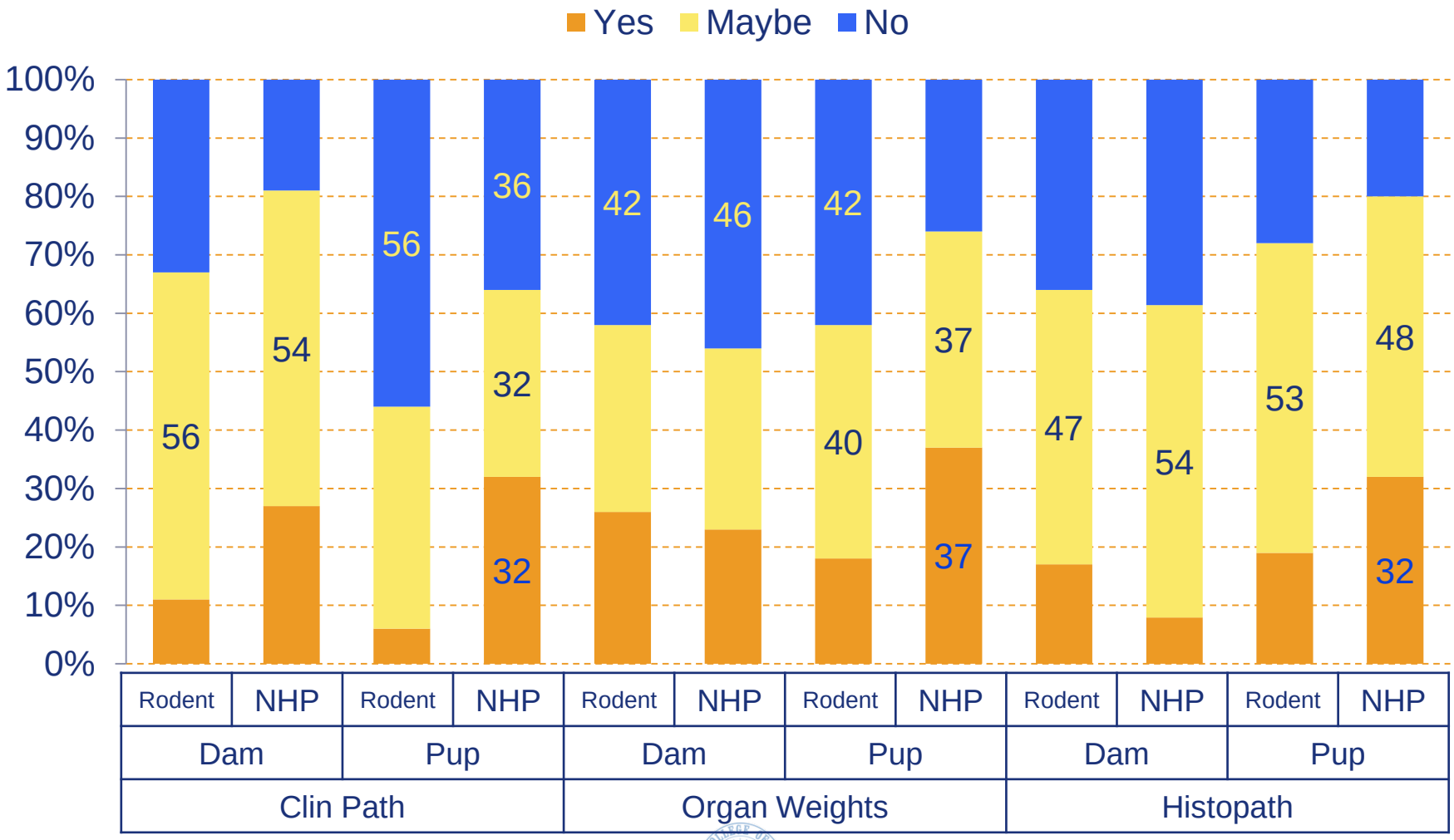
- Limited experience with NHP EFD studies
- Few respondents routinely add path endpoints (clin path, organ weight, histopath) to EFD studies
- Addition of path endpoints to define maternal toxicity

  (slightly different question)
  - 30% respondents yes
  - 48% respondents if no other signs were anticipated
- Biologics (S6R1) may use path endpoints to justify high dose
- Most respondents indicated potential challenges of including path endpoints during gestation or development did not impede interpretation
  - Lack of historical data/familiarity
  - Changes with pregnancy/development
  - Effects and/or NOAEL may be different than tox studies

Pathology Endpoints in EFD Studies

- **Clinical pathology** not routinely evaluated (dams or fetuses), but majority of respondents would include for dams if useful based on previous findings or pharmacological relevance
- **Organ weights** from dams not routinely collected (40%) or triggered on case-by-case basis (40%), although acknowledged that gravid uterine weights collected per guideline
- Fetal **organ weights** not routinely collected in rodents (63%) but collection is variable in NHP (36% No, 36% Maybe, 28% Yes)
- Only 6-19% respondents routinely include maternal or fetal **histopathology** but 47-60% respondents would trigger histopathology ….however, it is unclear how often this would actually happen across studies or programs

Survey: Pathology Endpoints in Pre- and Post-Natal Development (PPND) Studies



Pathology Endpoints in PPND Studies

- Similar to EFD studies, few respondents routinely add path endpoints (clin path, organ weight, histopath) to PPND studies but they can be incorporated into the maternal or pup aspects when deemed appropriate based on previous findings or pharmacological relevance
  - 47% respondents add path endpoints case-by-case (ie, pharmacodynamic effects on development)
  - 37% respondents do not add these path endpoints in this way
- In general, it appeared slightly more respondents would consider path endpoints on NHP studies compared to rodent
  - Different dose rationale for NHP studies (often biologics)?
  - Maximize use of NHP tissues?
- Most respondents indicated potential challenges of including path endpoints during gestation or development did not impede interpretation
  - Lack of historical data/familiarity
  - Changes with pregnancy/development
  - Effects and/or NOAEL may be different than tox studies

Pathology Endpoints in PPND Studies

- Like EFD studies, **clinical pathology** not routinely evaluated (dams or pups), but majority of respondents would include for dams if useful based on previous findings or pharmacological relevance
- **Organ weights** from dams not routinely collected (42-46%) but 31-32% indicated would trigger based on cause
- Fetal **organ weights** not routinely collected in rodents (42%) but may be triggered (37%) or routinely collected (37%) in NHP
- 47-54% respondents would trigger maternal or fetal **histopathology** ….however, it is unclear how often this would actually happen across studies or programs
- Relative to rodent, slightly more respondents would consider endpoints in NHP
  - Maternal & offspring clin path
  - Offspring organ weight & histopath

Integrated Assessment-Fertility

Species	Subset	Clinical Pathology	Organ Weights	Histopathology
Rodent	Male	Targeted[1]	Yes, typically limited to reproductive organs	Yes, if reproductive organs are not already characterized. Otherwise preserve for possible future evaluation
	Female			
NHP	Male	Targeted[1]	Yes, typically limited to reproductive organs[2]	Routinely evaluated[2] but not driven by guidance
	Female			

[1] limited to endpoints to evaluate efficacy markers, pharmacologic endpoints or specific toxicity concerns
[2] when part of general toxicity study a standard panel of tissues including reproductive tissues

Integrated Assessment-EFD

Species	Subset	Clinical Pathology	Organ Weights	Histopathology
Rodent	Dam	Targeted[1]	Targeted[1] *(uterine weights routine)*	Targeted[1]
	Fetus	Not typical	Targeted[1] *(not typical)*	No
NHP	Dam	Targeted[1]	Targeted[1] *(uterine weights routine)*	Targeted[1]
	Fetus	Not typical	Yes	No

[1] limited to endpoints to evaluate efficacy markers, pharmacologic endpoints or specific toxicity concerns that may contribute to defining maternal toxicity when no other signs of maternal tox are anticipated

Integrated Assessment-PPND

Species	Subset	Clinical Pathology	Organ Weights	Histopathology
Rodent	Dam	Targeted[1]	Targeted[1] *(Not typical)*	Targeted[1] *(Not typical)*
	Pup	Targeted[1] *(Not typical)*	Targeted[1] *(Not typical)*	Targeted[1]
NHP	Dam	Targeted[1]	Targeted[1] *(Not typical)*	Targeted[1]
	Offspring	Yes/targeted[1] *(may depend on age/blood volume)*	Targeted[1]	Routinely evaluated but not driven by guidance

[1] limited to endpoints to evaluate efficacy markers, pharmacologic endpoints or specific toxicity concerns

Nonclinical Studies Supporting Pediatrics

- Currently regional juvenile toxicity testing guidance is in effect in the US, EU and Japan, and there is an active ICH working group to develop harmonized guidance for nonclinical safety testing in support of development of pediatric medicines (ICH S11)

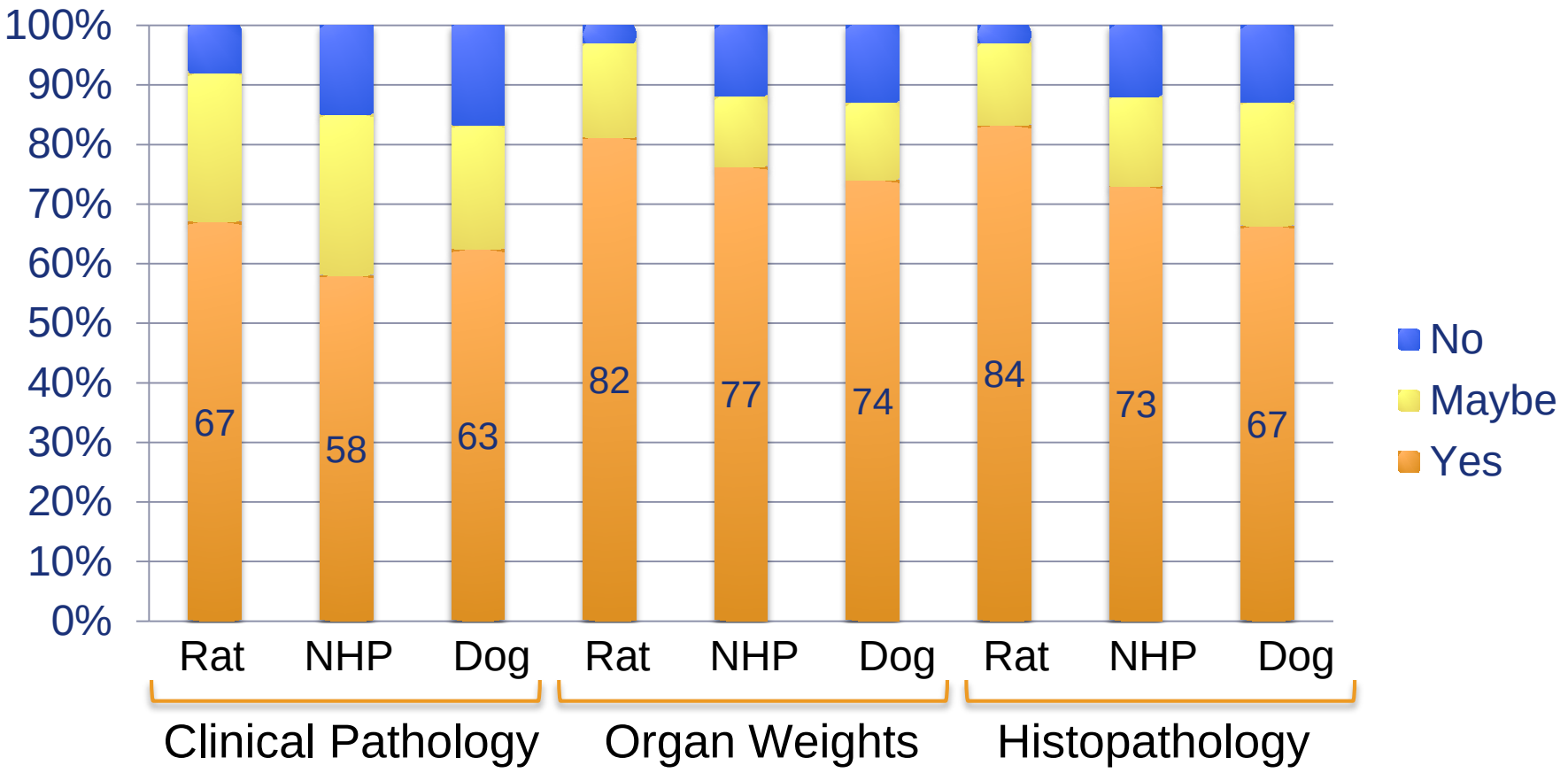
- Goal is to assess potential effects on development
  - Focus on organ systems that undergo postnatal development
  - Studies tend to be complex and logistically challenging

- Each species grows and develops at a different rate
  - …and each organ system can mature at a different rate
  - May be difficult to discriminate between a cumulative toxicity vs exposure during a developmental window of susceptibility

Experience with Juvenile Toxicity Studies

- …is still somewhat limited
  - Relatively low survey response rate (only 24-38 responding organizations out of the ~100 participating organizations)
  - Comments indicated that some responding organizations lacked direct experience
- General willingness of survey respondents to include anatomic and clinical pathology endpoints
- Generally positive experience with inclusion of pharmacodynamic endpoints on a ‘case-by-case’ basis
- Some concern with availability of historical control data
  - Importance of concurrent controls
  - Challenges of unscheduled necropsies

Current Practices for Pathology Endpoints in Juvenile Toxicity Studies



What pediatric-relevant information is already available?

..from General Toxicity Studies?

- General effects on growth
  - Body Weight Gain
  - Histology of bone, including growth plate (if open)
- Effects on tissues of developmental concern
  - Nonrodents are often sexually immature or pubertal

…from DART studies?

- Fertility and EFD studies are not directly supportive
  - May identify target tissues of developmental concern
- PPND studies can be useful
  - Late gestation and lactation period exposure of offspring
  - May provide ‘worst-case’ scenario for youngest patients
  - Must understand exposure of offspring
  - Consider inclusion of pathology endpoints in offspring

Integrated Assessment-Juvenile Toxicity

Species	Subset	Fertility	Clin Path, Organ Wt, Gross and Histopath	Record Maturity
Rodent	Pre-Weaning	Not Applicable	Complete/Standard (typically after post-natal day 70)	Yes, both sexes
	Post-weaning to Mature	• Sperm at necropsy • Hormones for cause once mature • Mating trials possible		
NHP	Immature	Not Applicable	Complete (typically after 6-12 months)	
Dog	Immature	Not Applicable	Complete (typically after 3-6 months)	

Key aspects of integrating pathology and reproductive endpoints in safety assessment

- General toxicity studies/endpoints provide baseline safety assessment
  - May inform fertility assessment (repro tissues)
  - May inform potential risks in pediatrics
  - Do not adequately inform other life stages
- DART studies/endpoints
  - Functional effects on fertility, pregnancy, lactation
  - Developmental toxicity (pre/postnatal)
- Juvenile toxicity studies
  - As needed to complement available data

Summary

- Across general toxicity and DART studies should integrate findings from all available information (including general toxicity and DART studies) to identify any hazards to development and reproduction
- Ultimate goal is to evaluate any potential risk to human patients based on the available information, and communicate this risk to physicians & patients

Acknowledgements

- Working Group Member Affiliations
  - **Government** (NIEHS/National Toxicology Program, Food Safety Commission of Japan)
  - **Biopharmaceutical Industry** (Bristol-Myers Squibb, Genentech, GlaxoSmithKline, Incyte, Pfizer)
  - **Contract Research and Consulting** (Covance, Charles River, Regan Path/Tox)
  - *Includes diplomates or members of the STP, ABT, ACT, ACVP, ASVCP, SOT, Teratology Society, and the ILSI/HESI DART Technical Committee*
- Survey Participants
  - Broadly distributed to DART and Toxicologic Pathology Community
  - Approximately 100 responding organizations
- Reviewers
  - *Executive Committee of the STP*
  - *Scientific and Regulatory Policy Committee of the SRPC*
  - *Reproductive Pathology Interest Group of the STP*
  - *Regulatory Affairs Committee of the American College of Veterinary Clinical Pathologists*
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Presenter Bio

- Wendy G Halpern, DVM, PhD, DACVP
  - Principal Scientist/Pathologist, Safety Assessment
    Genentech, South san Francisco, CA

Dr. Halpern is a veterinary pathologist with more than 17 years of drug development experience in the biopharmaceutical industry. She earned her DVM from the Ohio State University, and also completed an MSc at Ohio State in Veterinary Pathobiology. This was followed by a PhD in Biomedical Sciences from the University of New Mexico focusing on molecular characterization of myeloid leukemias, after which she joined Human Genome Sciences in 2000, and then Genentech in 2007. Wendy is a Diplomate of the American College of Veterinary Pathologists, and is also a member of the Society of Toxicologic Pathology, the Safety Pharmacology Society, the Teratology Society, and the ILSI/HESI DART Technical Committee. She has contributed to 24 publications, 4 book chapters, and has presented at more than 30 national and international scientific meetings. She is the current Chair of the Society of Toxicologic Pathology Scientific and Regulatory Policy Committee, is a member of the BioSafe Leadership committee, and represents BIO on the ICH S11 Working Group. She also supports nonclinical strategy and safety assessment for pediatric oncology drug development at Genentech, and leads internal Working Groups for reproductive and developmental toxicology.

Presenter Bio (EXAMPLE—CB and WH will be added)

- Christopher J Bowman, PhD, DABT
  - Associate Research Fellow, Pfizer, Groton, CT

Chris completed his PhD in 2001 from the University of Florida. For the next 2 years as a postdoc at CIIT, Centers for Health Research (Research Triangle Park, NC) he worked on anti-androgens. After his post-doc, Chris worked at WIL Research Laboratories (Ashland, OH) for ~5 years serving as study director of developmental & reproductive toxicity (DART) studies. Since 2008 Chris has been part of the senior staff in the Pfizer DART group (Groton, CT) being actively involved in regulatory & investigative strategies evaluating DART, nonclinical support of pediatric development, & serving as a drug safety representative. Chris has also co-mentored a post-doc in DART. Over the past 15 years Chris has chaired sessions, presented, & participated at meetings of the Society of Toxicology (SOT), Teratology Society, European Teratology Society, BioSafe (part of BIO), ILSI-HESI (DART), & several regional meetings including lecturing at University of Rhode Island. He is a member of the BioSafe Leadership Committee and was recently President of the Reproductive & Developmental Toxicology Specialty Section of SOT. Chris has been a Diplomate of the American Board of Toxicology since 2005. Chris has over 35 publications in peer-reviewed journals & numerous book chapters. He has been & continues to be a leader & member of several working groups tackling various challenges associated with DART study strategy, design, interpretation and risk assessment.