Finding A Common Ground: Translation of the Principles of Teratology in Today's Regulatory Climate

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Charles River Laboratories, Inc.
Josef Warkany Lecture

June 2022

Objectives

- The Legacy of Josef Warkany: The First and Second Workshops on Teratology (1964 and 1965)
- The Principles of Teratology
- The Era of Regulatory Teratology: Development of ICH and OECD Testing Guidelines
- Those Who Have Guided Us
- The State of Regulatory Reproductive Toxicology: Examples of Issues We Have Created
- The Future of Testing: Short and Long Term

Dr. Josef Warkany

We are all part of a great legacy

The First and Second Workshops on Teratology

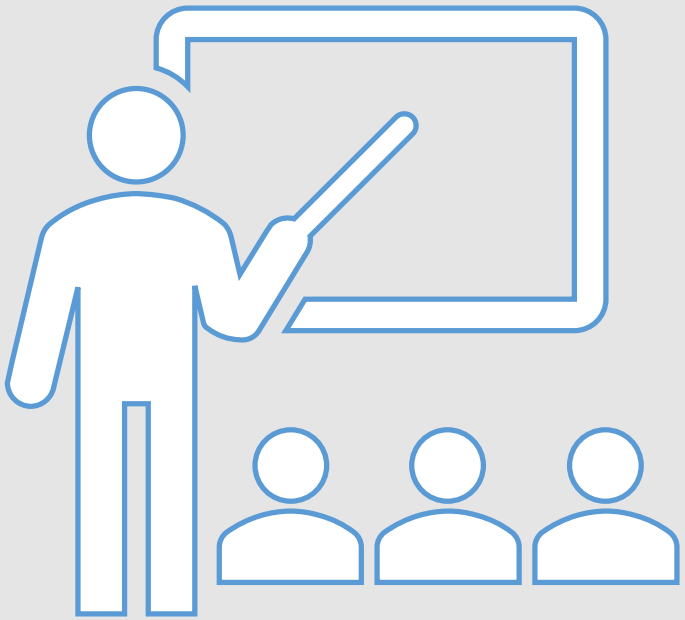
1964 and 1965

Commission on Drug Safety – August 1962 – funded by Pharmaceutical Manufacturing Association (PMA)

- Charged to "*seek new knowledge of the predictability of action of drugs in man*"
- 17 subcommittees
- 1 of 17 subcommittees - Commission on Teratology, chaired by Josef Warkany
- Conference on Prenatal Effects of Drugs
  - Proceedings published Dec 1963
- Recommended workshops in teratology to allow for an exchange of information and techniques with scientists of related disciplines
- Dr. Warkany extended an invitation to Dr. James Wilson to chair the first workshop
  - Held in Florida, February 2 to 8, 1964
  - 41 participants, 18 observers, and 11 faculty
  - The lectures and demonstrations were published as Teratology – Principles and Techniques – edited by Drs. Wilson and Warkany
- Second Workshop
  - Berkeley, California, January 25 to 30, 1965

Faculty of the Two Workshops

** denotes President of BDRP (Teratology Society)*



- Robert Brent*
- F. Clarke Fraser*
- E. Marshall Johnson*
- Harold Kalter*
- David A. Karnofsky*
- Norman A. Klein
- M. Louis Murphy
- Meredith N. Runner*
- Daphine G. Trasler*
- Josef Warkany*
- James G. Wilson*
- Ursula K. Abbott
- C. Willet Asling
- Charles S. Delahunt
- Charles R. Grau
- Lucille S. Hurley*
- Ian Monie*

Workshop Lectures

1964 and 1965

- Factors Influencing Teratogenic Response to Drugs
  - Presented by Lois Murphy
- Effects of Proteins, Antibodies, and Autoimmune Phenomena upon Conception and Embryogenesis
  - Presented by Robert Brent
- Some Genetic Aspects of Teratology
  - Presented by F. Clarke Fraser
- Nutritional Factors in Mammalian Teratology
  - Presented by E. Marshall Johnson
- Embryological Considerations in Teratology
  - Presented by James Wilson
- Dosage and Developmental Stage in Teratogenesis
  - Presented by James Wilson
- Teratogenic Effects of Thalidomide in the Rabbit, Monkey, and Man
  - Presented by C.S. Delahunt

Workshop Demonstrations

1964 and 1965

- Methods for Administering Agents and Detecting Malformations in Experimental Animals
  - Presented by James Wilson
- Electrophoretic and Histochemical Analyses or Embryonic Tissues
  - Presented by E. Marshall Johnson
- Dose-Response Relationships in Growth-inhibiting Drugs in the Rat: Time of Treatment as a Teratological Determinant
  - Presented by M. Lois Murphy
- Chick Embryo Explanation
  - Presented by Norman Klein
- Chick Embryo in Drug Screening
  - Presented by David Karnofsky
- Alizarin Staining of Bone
  - Presented by Lucille Hurley

Principles of Teratology

Any combination of tests and models that accounts for Wilson's 6 Principles of Teratology should allow us to continue to prevent another thalidomide tragedy.

Underlying Principles *Wilson*

Susceptibility to Teratogenesis Depends on the Genotype of the Conceptus and the Manor in Which this Interacts with Adverse Environmental Factors

Suscepibility to Teratogenesis Varies with the Developmental Stage at the Time of Exposure to an Adverse Influence

Teratogenic Agents Act in Specific Ways (Mechanism) on Developing Cells and Tissues to Initiate Sequences of Abnormal Developmental Events

The Access of Adverse Influences to Developing Tissues Depends on the Nature of the Influence (Agent)

The Four Manifestations of Deviant Development are Death, Malformation, Growth Retardation, and Functional Deficit

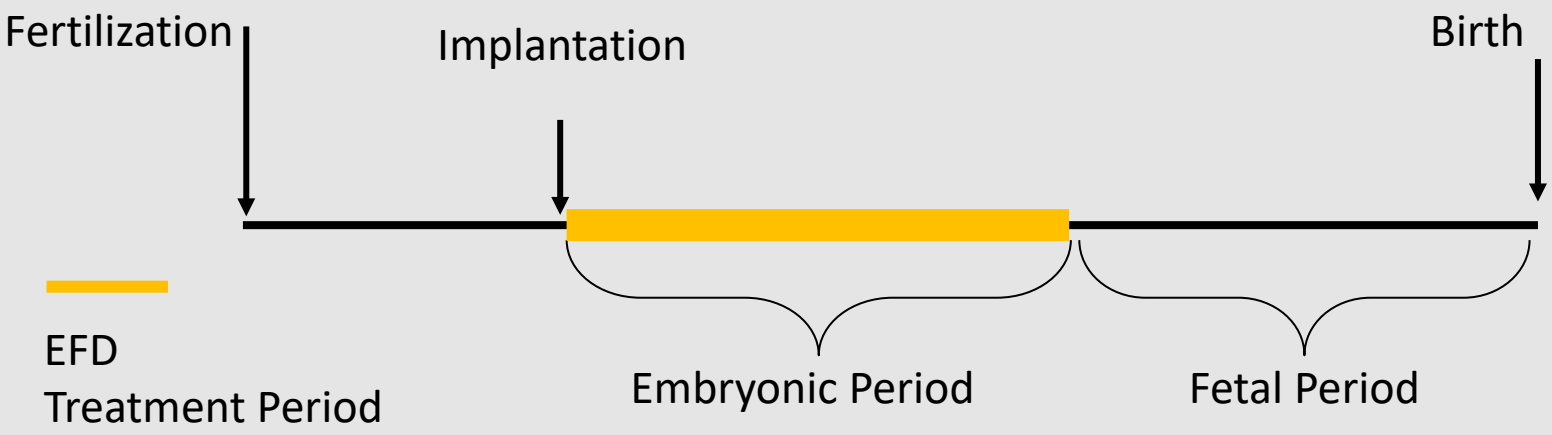
Manifestations of Deviant Development Increase in Frequency and Degree as Dosage Increases, from the No-Effect to the Totally Lethal Level

Stages of *In Utero* Development

EFD: Embryo-fetal development

Embryonic Period: Establishment of body form, organogenesis

Fetal Period: Growth/ differentiation of organ systems

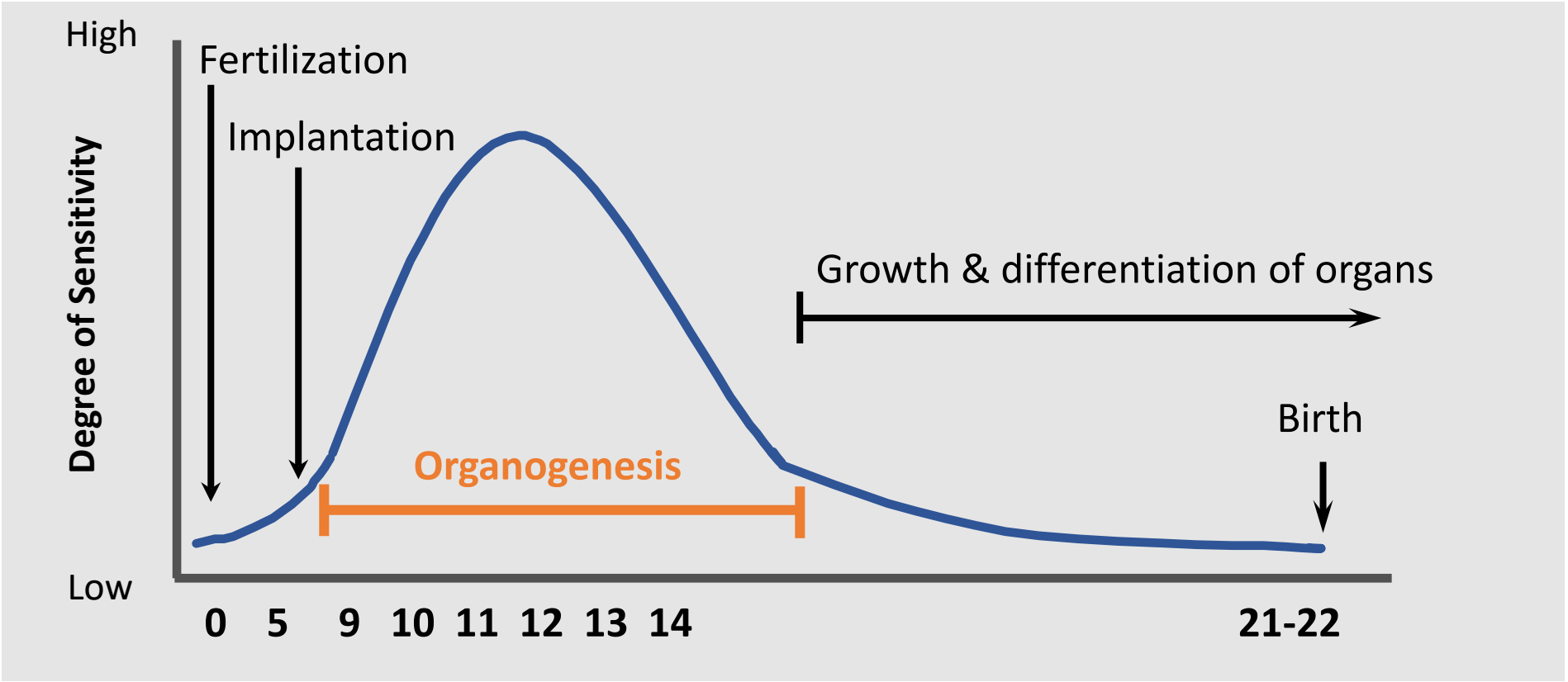


Species	Implantation*	Organogenesis Ends*	Birth*
Mouse	5	15	19-20
Rat	5-6	16	21-22
Rabbit	6-7	19	30-32
Monkey	9**	44-45	166
Human	6-7	50-56	266

* *Days from fertilization*

** *Dosing typically starts on GD 20 due to the need to confirm pregnancy by ultrasound on GD 18-20*

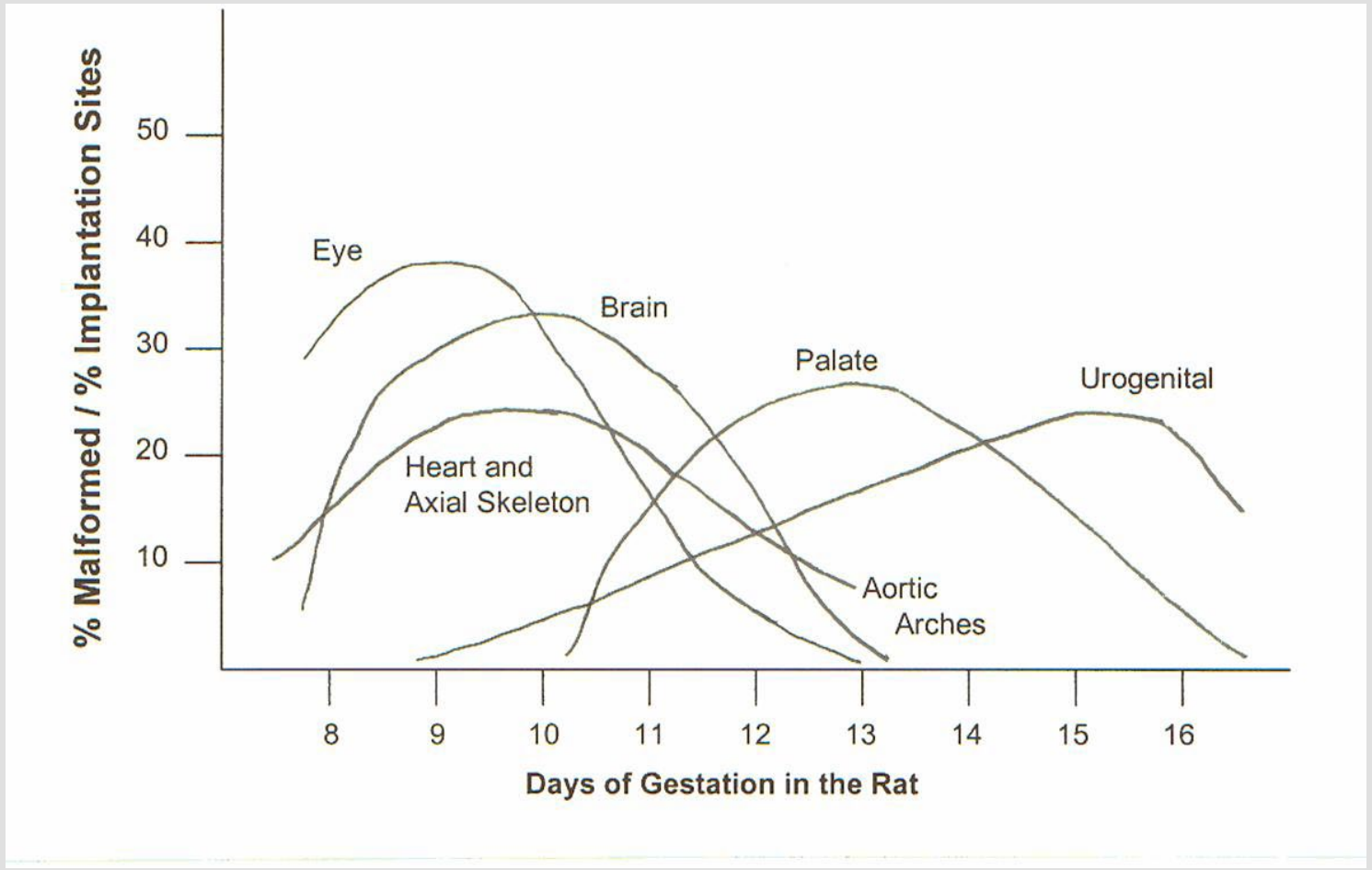
Window of Sensitivity Greatest During Organogenesis



Modified from Wilson (1973)

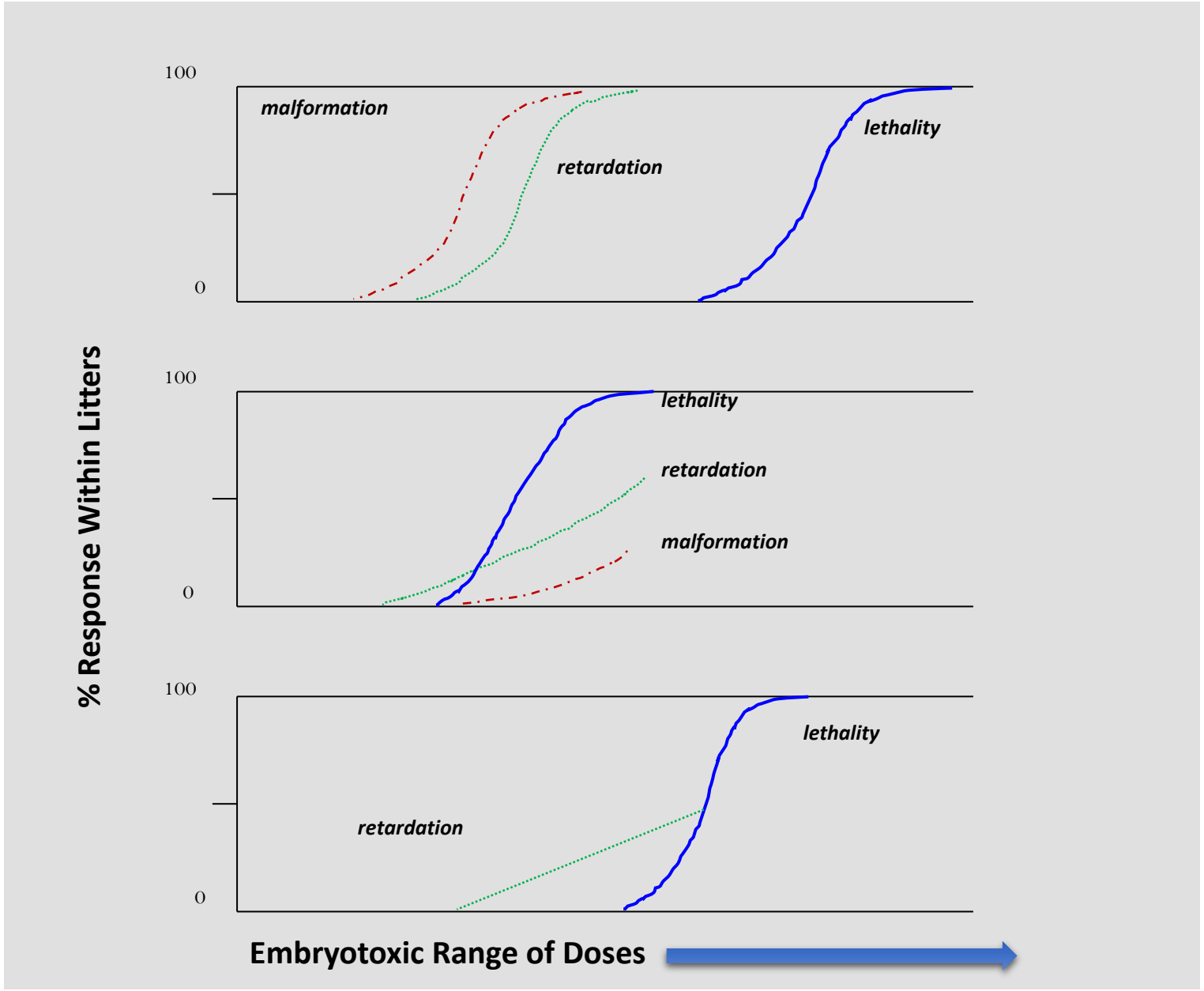
Period of Organogenesis = Treatment Period for EFD Study

Susceptibility of Fetal Organs to Alterations as a Function of Time



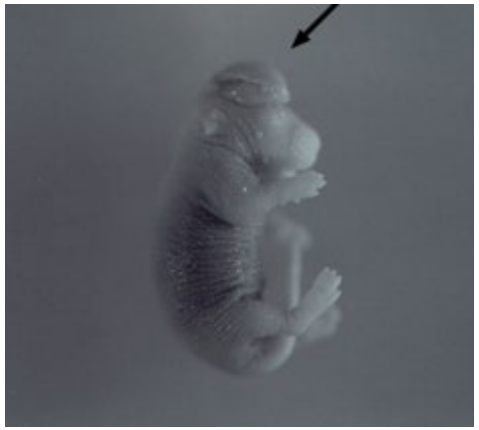
Adapted from Wilson, 1965, p.256

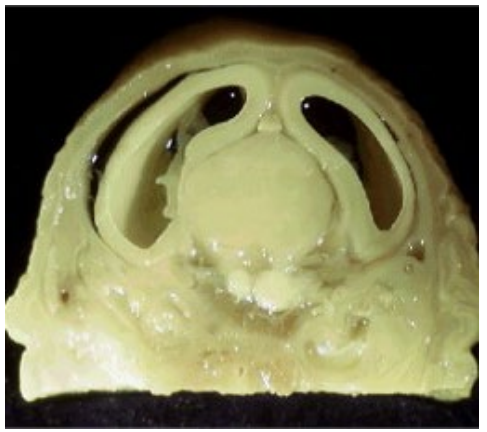
Susceptibility of Fetal Organs to Alterations as a Function of Time

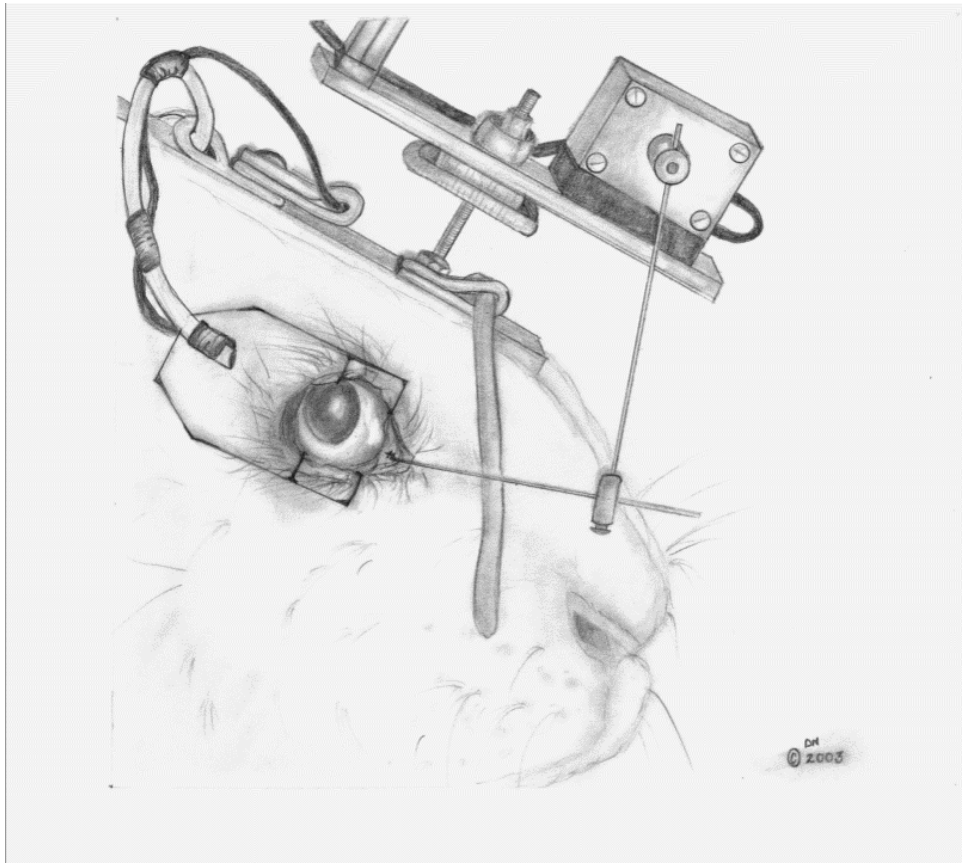


Adapted from Neubert, D. et al. (1980) as shown in Christian, M.S. (2008). Test Methods for Assessing Female Reproductive and Developmental Toxicology. In: Principles and Methods of Toxicology, 5th Ed. edited by Hayes, A.W. CRC Press, Boca Raton, FL

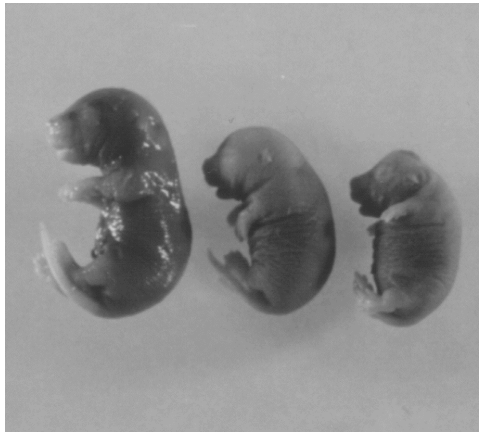
Underlying Principles







- Death
- Malformations
- Growth Retardation
- Functional Deficits



The Era of Regulatory Teratology

What came from the first two workshops?

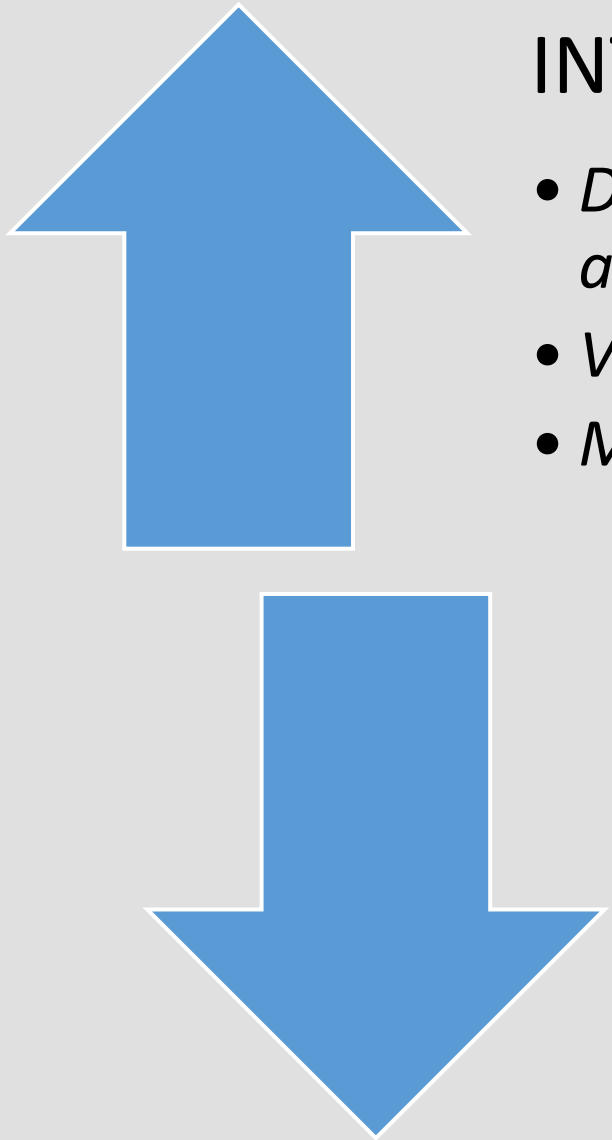
Whole animal models used as the basis for hazard assessments

Testing Guidelines – Intentional Exposure

- 1966 FDA "Goldenthal Letter"
  - Three segment testing
  - Goldenthal concluded his letter by stating "*it must be realized that even…improved guidelines reflect merely the 'state of the art' at the present time, and undoubtedly further modifications will be needed in the future as additional knowledge in this area is developed*."
- 1984 British Guidelines
- 1988 Japanese Guidelines
- 1994 International Conference of Harmonisation (ICH) S5
- 2020 International Council for Harmonisation (ICH) S5(R3)

Testing Guidelines - Unintentional Exposure

- Environmental Protection Agency (EPA) Guidelines
  - Federal Insecticide Fungicide and Rodenticide Act (FIFRA)
  - Toxic Substance Control Act (TSCA)
  - Harmonized as the EPA 870 test guidelines in late 1990s
  - Harmonized with Organization Economic Co-operation and Development (OECD)
- OECD Guidelines
  - Now the standard for chemicals



INTENTIONAL

- *Drugs, including small and large molecules*
- *Vaccines*
- *Medical Devices*

UNINTENTIONAL

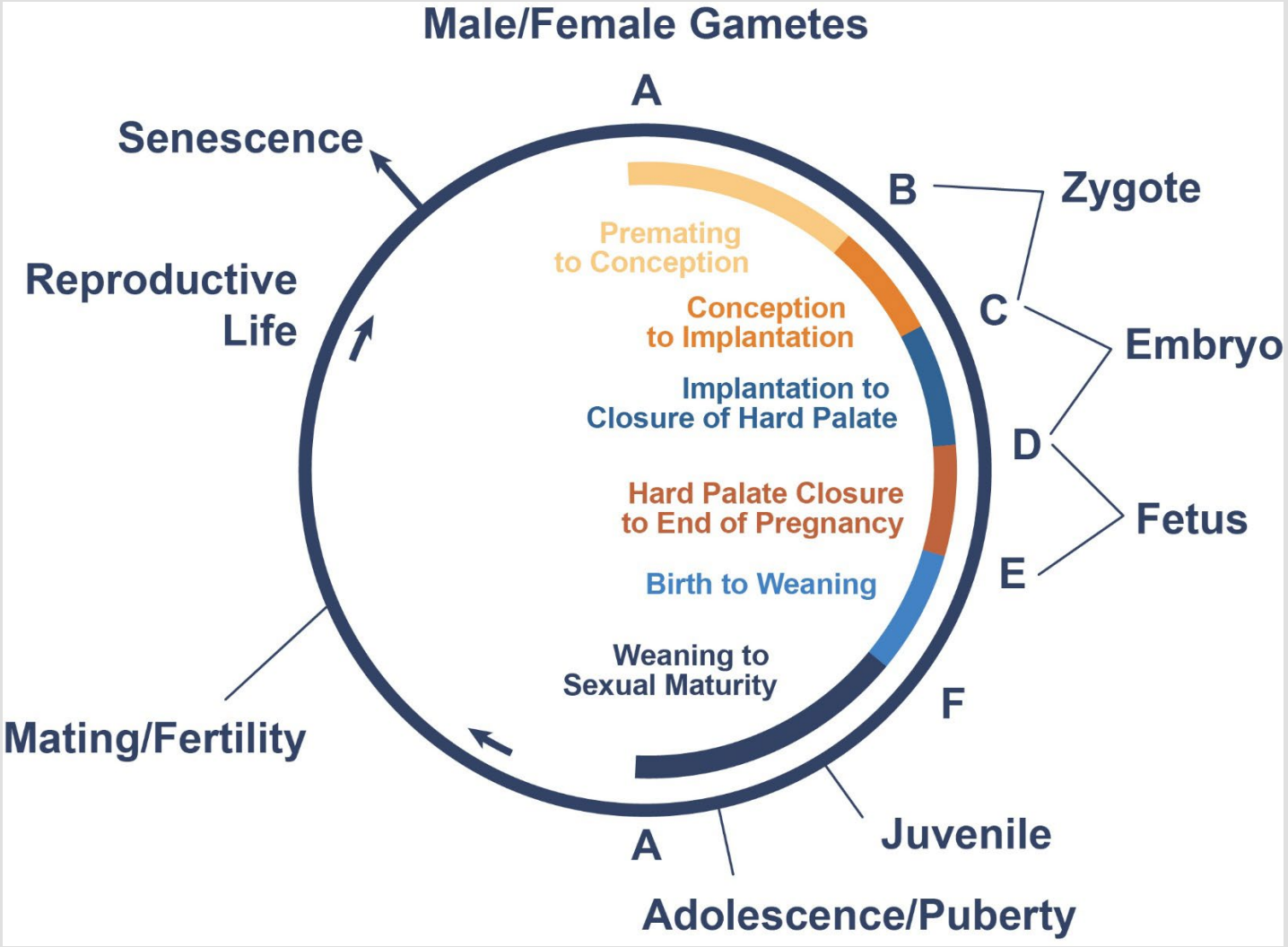
- *Chemicals*
- *Consumer Products*
- *Foods*
- *Food Additives*

Guidance for Testing

Today's guidelines can be divided into those for **intentional** and **unintentional** exposure

Guidance for Intentional Exposure
(Pharmaceuticals)

Segmented study designs recommended



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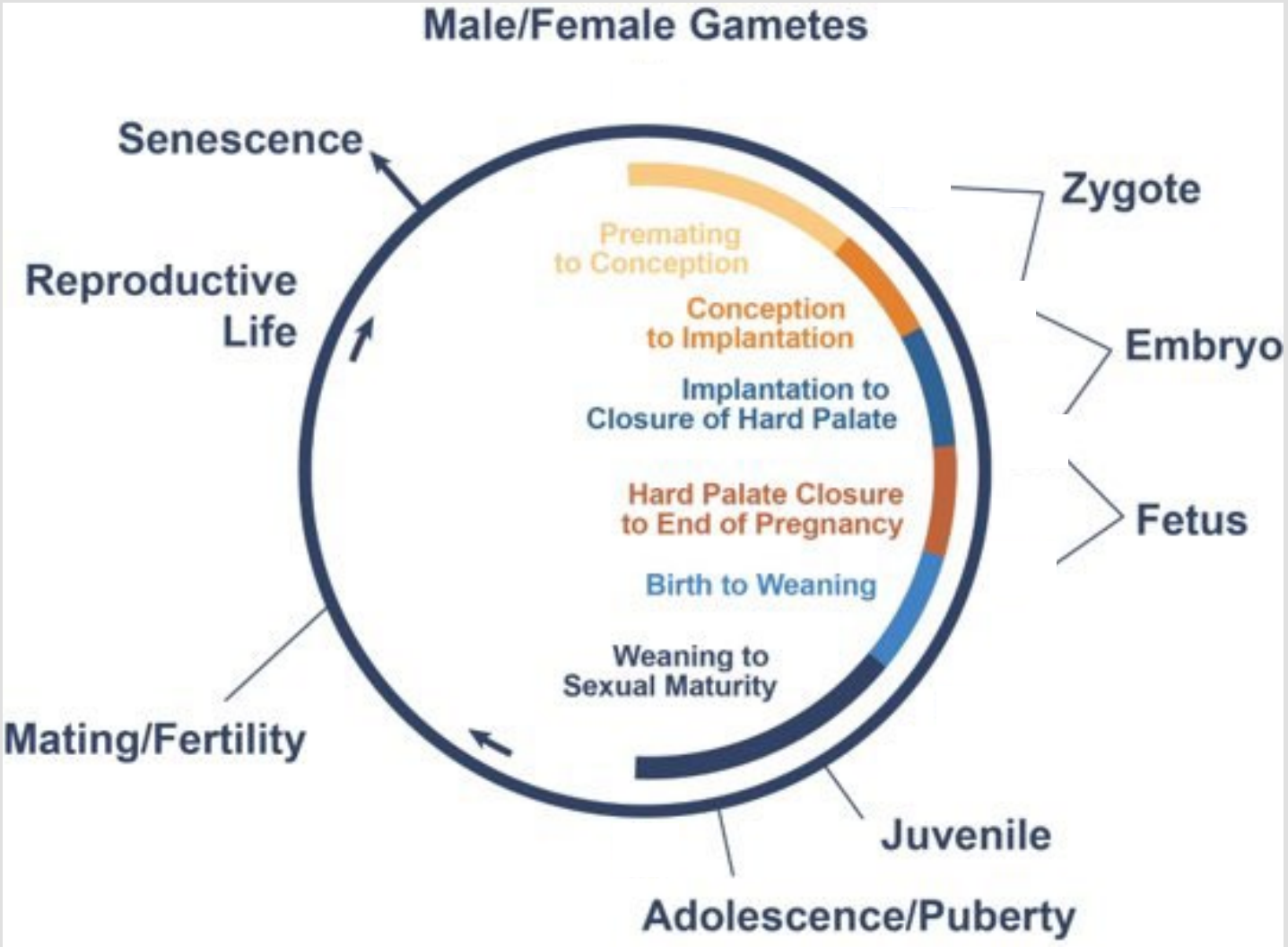
Guidance for Unintentional Exposure
(Chemicals)



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Those Who Guided Us

Pioneers in the use of animal testing models for hazard assessment

THOSE WHO GUIDED US

- Mildred S. Christian, Teratology Society
- Frank Sullivan, European Teratology Society
- Tony Palmer, European Teratology Society

Those Who Guided Us

- Picture to left: Mineo Yasuda, Robert L. Brent, Takasi Tanimura
- Picture to right: Godfrey Oakley, Mildred Christian, Nigel Brown

Those Who Guided Us

- Picture to left: Richard Hoar, Mildred Christian, Rita Hoar
- Picture to right: Robert (Bob) Staples

Those Who Guided Us

John and Shelia Tesh

We All Have to Start Somewhere

From Luggage Handler to BDRP President

State of Regulatory Reproductive Toxicology Today

Limitations of Animal Testing – never have been a perfect model for humans

Issues with data interpretations – “maternal toxicity caused it”

Desire for easy answers – an outcome from an *in vitro* test explains what happens *in vivo*

What Do the Animal Studies Do For Us (Or Not)

- **Substitute for Human Exposure**
  - Mimic human exposure in terms of absorption, distribution, metabolism, and excretion (ADME)
  - Testing in gestating animals allows inclusion of the placenta and its potential role in mediating the developmental toxicity that may result from exposure
- **Or Not**
  - Exposure in terms of ADME may not be identical to that in humans
  - Placental structure and function varies from species to species
  - Testing in “normal” animals does not mimic special susceptible or compromised populations

Interpretation issues/ dilemmas we created with our latest guidelines

Intentional Exposure

- Enhanced dose-range studies to allow women of childbearing potential (WOCBP) into clinical trials

Unintentional Exposure

- European Chemical Health Agency's (ECHA) need to see toxicity, but do they understand the difference between general and reproductive toxicity
- Alternative tests for reproductive and developmental toxicity
- With the desire to replace animals what will our testing look like in the future

ICH S5 (R3) Enhanced Dose Range Study

- Purpose was to allow a drug to move into Phase 2 clinical trials in WOCBP
- Eliminate further testing when the hazard is clear
  - Example of oncology drugs
- This design was never intended to provide a definitive answer especially when no hazard is observed - often get equivocal answer due to limited group size and background incidence of spontaneous abnormalities

Enhanced Dose Range Embryo Fetal Developmental (pEFD) Study Design

- Animal Numbers: Uses 6 to 8 animals/group verses 20/group for a definitive EFD
- Dose period: Same as full EFD
- Endpoints: Evaluation limited to maternal toxicity, uterine contents, fetal weight, gender, external and visceral examinations
- Normal ranges for dose range studies are wider than full studies
- Single or few malformations in the fetuses in one litter in a high dose group may lead to an equivocal conclusion

Parameter		Definitive Studies		Dose Range Studies	
		Min	Max	Min	Max
Litter Size	Mean/Litter	13.3	15.0	12.8	15.7
Resorptions	Mean/Litter	0.1	1.1	0.5	2.0
Postimplantation Loss	%	1.4	8.8	0.0	16.2
Dams with Resorptions	%	15.0	62.5	38.2	75.0

Parameter		Definitive Studies	Dose Range Studies
Eye Bulge Depressed	Mean Litter/Fetal (% range/% range)	1/1 (0 to 5.6/0 to 0.4)	1/1 (0 to 25/0 to 1.9)

ECHA's need to see toxicity BUT do they understand the difference between general and reproductive toxicity?

Neonatal Growth, Survival, and Maternal Test Article Administration

Neonatal Growth

- Adverse effects on neonatal growth manifest as reduced birth weights caused by growth retardation *in utero* and/or by reduced body weight gain following birth
- PND 1*: Male Mean = 7.1 g ± 0.24 (N = 199 studies)
- PND 21*: Male Mean = 49.2 g ± 4.66 (N = 126 studies)
- Evaluation of the neonatal growth curve, in conjunction with litter size, is important to control for the confounding effects of within-litter competition
- Mean pup body weight differences of ≥5% will typically show statistical significance

* Charles River Ashland Historical Control Data

Offspring Survival (% per Litter)

Group	Control	Low	Mid	High	HCD#
Birth–PND 4 (pre-selection)	97.2	97.0	96.9	96.4	95.9
PND 4–7 (post-selection)	100	100	98.7	99.5	99.0
PND 7–14	100	100	99.6	91.3	99.3
PND 14–21	100	100	99.6	**72.6****	95.9
PND 4–21 (post-selection)	100	100	97.8	**69.2****	97.7

What is going on during PND 14–21?

PND: Postnatal Day
HCD: Historical Control Data
Charles River Ashland Historical Control Data
* $p<0.05$
** $p<0.01$

Offspring Survival

- Neonatal survival, in conjunction with pup body weights, is often used to gauge disturbances in postnatal health, growth, and development
- Most frequently, adverse effects on pup survival occur during the period prior to litter standardization (culling) on PND 4
- In the US, litters are standardized on PND 4 to 4/sex
- In our laboratory, less than 1% of control dams that deliver have total litter loss
  - Therefore, a treatment group (20–30 animals) with just two total litter losses would be considered a strong signal
    - Birth to PND 4 (pre-selection)*: Mean = 98.3% ± 2.14 (N = 194 studies)
    - PND 4 (post-selection) to PND 21*: Mean = 97.9% ± 2.18 (N = 119 studies)

- Charles River Ashland Historical Control Data
- PND: Postnatal Day

Pup Pre-weaning Body Weights

Group	Control	Low	Mid	High	HCD#
PND 1 (M)	6.8 g	6.9 g	6.8 g	6.9 g	7.1 g
PND 4 (M)	9.7 g	9.8 g	9.7 g	9.9	10.0 g
PND 7 (M)	15.8 g	15.9 g	15.9 g	15.6	15.2 g
PND 14 (M)	31.6 g	31.6 g	30.9 g	**16.7**** (47.1%)	30.5 g
PND 21 (M)	49.7 g	49.7 g	48.9 g	**23.4**** (52.9%)	47.9 g

PND: Postnatal Day
HCD: Historical Control Data
M: Males
Charles River Ashland Historical Control Data
* p<0.05
**p<0.01

Clearly, there is an effect on pup body weights and body weight gain at the high dose level.

Maternal Test Article Consumption (mg/kg/day)

Calculated Value (mg/kg/day)	Control	Low	Mid	High
Premating	0	4	43	414
GD 0–21	0	2	34	323
LD 1–4	0	6	**59**	**535**
LD 4–7	0	8	**78**	**581**
LD 7–14	0	9	**90**	**678**
LD 14–21	0	8	**78**	**610**

GD: Gestation Day
LD: Lactation Day
Charles River Ashland Historical Control Data
* p<0.05
**p<0.01

Dams, and hence the offspring, received a much higher than intended dose during lactation (compared with premating and gestation periods).

Data Interpretation

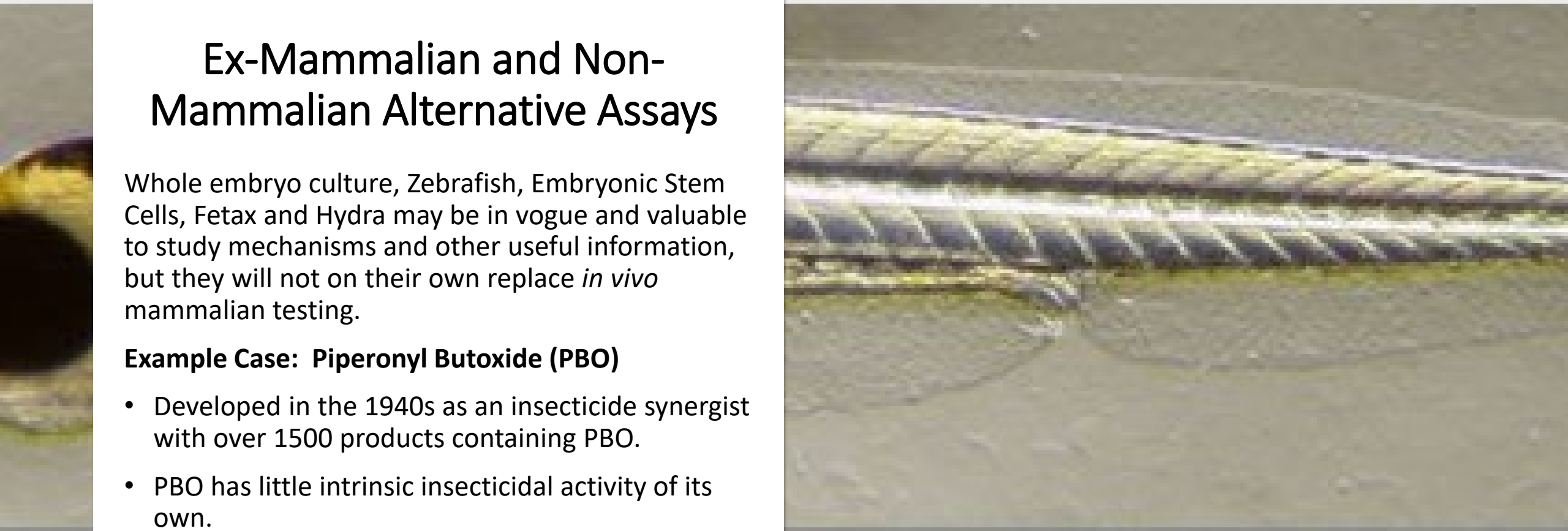
- Test substance consumption was high during PND 1–21 because of the increased maternal diet consumption during lactation.
- Pups generally start consuming diet prior to the last week prior to weaning.
- In studies using a fixed concentration of the test article in the diet, the mg/kg exposure of pups during the week prior to weaning can often be greater than that of the dam, causing effects on body weight. Direct toxicity of the test substance not reproductive toxicity.
- Postweaning - Not surprising to see an effect on sexual maturation in the high dose and overall general lower body weights post weaning.
- ECHA has classified some of these test substance as a reproductive toxicant.
    - Certain reproductive hazard levels lead to banning of the use of a chemical.
- EPA recognizes that “the dose makes the poison” and would establish a NOAEL for neonatal toxicity and conduct a risk assessment.

Ex-Mammalian and Non-Mammalian Alternative Assays

Whole embryo culture, Zebrafish, Embryonic Stem Cells, Fetax and Hydra may be in vogue and valuable to study mechanisms and other useful information, but they will not on their own replace *in vivo* mammalian testing.

Example Case: Piperonyl Butoxide (PBO)

- Developed in the 1940s as an insecticide synergist with over 1500 products containing PBO.
- PBO has little intrinsic insecticidal activity of its own.
- PBO increases the effectiveness of pyrethrin insecticides.



In vivo Data

Study Type	Species	Doses (mg/kg)	Doses (PPM)	General Toxicity NOAEL	Reproductive NOAEL	Reference
Developmental Toxicity	Rat	0, 200, 500, 1000	NA	200 mg/kg	1000 mg/kg	Chun, J.S, and Neeper-Bradley, T.L. (1991)
	Mouse	0, 1065, 1385, 1800 GD 9 only	NA	1065 mg/kg	1065 mg/kg	Tanaka Fujitani, Takahashi, Oishi (1994)
	Rabbit	0, 50, 100, 200	NA	50 mg/kg	200 mg/kg	Schardein, J.L. (1986)
Multigenerational	Rat	43 to 14, 151 to 44, 856 to 241	0, 300, 1000, 5000	1000 ppm	5000 ppm	Robinson, K., Pinsonneault, L., Procter, B.G. (1986)

EPA Review June 2006

No developmental toxic effects were noted in guideline studies using rats and rabbits.

A few developmental studies in the open literature reported limb deformities, increased resorption and decreased number of viable fetuses in rodents at doses close to or higher than the highest dose tested in the guideline studies.

Epidemiology Study
Horton 2011

- Observed a significant inverse association between prenatal exposure to PBO and 36-month neurodevelopment.
- Study lacks any measurement of internal exposure to PBO, and the authors noted "*These findings should be considered preliminary and may be useful in generating future hypotheses*".

Transgenic Mouse, Non Mammalian and *In Vitro Data*

- The Insecticide Synergist Piperonyl Butoxide Inhibits Hedgehog Signaling: Assessing Chemical Risks
  - Wang et al., 2012
  - PBO was identified as a Hedgehog/Smoothened antagonist capable of inhibiting Hedgehog signaling
  - Hedgehog/Smoothened signaling is critical in neurological development
  - PBO disrupted zebrafish development
- Developmental Toxicity Assessment of Piperonyl Butoxide Exposure Targeting Sonic Hedgehog Signaling and Forebrain and Face Morphogenesis in the Mouse: An *In Vitro* and *In Vivo* Study
  - Everson, 2019
  - PBO attenuated Shh signaling *in vitro* through a mechanism similar to that of the known teratogen cyclopamine

How Do We Handle Data As It Arises From New Methodologies?

- Do we ignore the mammalian testing results?
- Do we immediately revise our hazard risk and assessments?
- We need short- and long-term approaches that will differ.
- In the near or short-term we need to act based on the type of exposure.
  - Intentional verses Unintentional

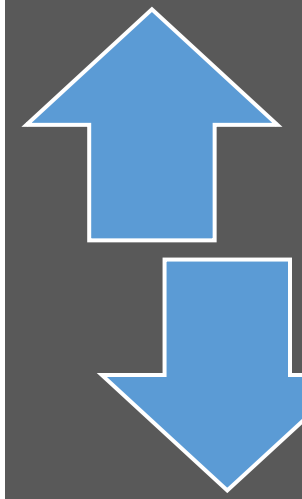
Intentional Exposure

- For pharmaceuticals, we use the data from all sources to better advise the patient and physician. Pharmaceuticals are meant to be given at exposures that will cause an effect.
- When we have equivocal results concerning toxicity, we need to be clear about what we have observed and what it might mean.

Unintentional Exposure

- For chemicals, we must put the data in context.
- No animal model exactly mimics humans.
- No single *in vitro* test will totally define a hazard in humans, except for (maybe) a genotoxicant.
- We need to look at the weight of the evidence and make a risk assessment.

Short Term Guidance For Testing



INTENTIONAL

- *Drugs, including small and large molecules*
- *Vaccines*
- *Medical Devices*

UNINTENTIONAL

- *Chemicals*
- *Consumer Products*
- *Foods*
- *Food Additives*

The Future
(Longer Term)

"Animal testing may be around for awhile but it's not the future. It's the interim as we gain a better understanding of developmental toxicity at the molecular level and relate exposure to the real-life situation."

Tox 21 New Dimensions of Toxicity Testing – Schmidt, 2009

Tox 21 – New Dimensions of Toxicity Testing
Richard Schmidt, 2009

"Right now, we're prioritizing chemicals on the basis of other criteria, such as production volume, the likelihood for human exposure, or their structural similarity to other chemicals with known liabilities. By incorporating more biology into prioritization, we think we can do a better job selecting the right chemicals for animal testing."

Robert Kavlock, Former BDRP President, when he headed the National Center for Computational Toxicology

Tox 21 – New Dimensions of Toxicity Testing

- *"Going forward, Tox21 offers the opportunity to confer the advantages of high throughput research on toxicology and risk assessment. But its promise is tempered by the vast research challenges that lie ahead. Scientists are aiming for nothing less than a complete map of the cell circuits that dictate toxicity, assembled from untold millions of data points, converted somehow into something useful."* Richard Schmidt

- *"Regulatory officials will have to devise ways to replace decisions made on traditional end points with ones made on cell-based findings."* Melvin Andersen, Hamner Institute

- Officials will also have to craft new strategies to explain those findings to the public. "*Your average person on the street understands that when something causes birth defects in a rat, that's something for humans to be concerned about. But when you base policies on perturbations of thyroid hormone homeostasis, well, it's going to be harder for the public to know what to think about that*." says Gina Solomon National Resources Defense Council.

Dr. Warkany's legacy is alive and well here at BDRP!

Two examples of what makes us relevant and brings our work from the bench to the bedside. We are addressing the immediate issues while working on the future of hazard assessment.

Can Experimental Animal Studies Be Used in Counseling?

- Chairpersons: Sarah G. Običan, Anthony R. Scialli
  - What Experimental Animal Testing Is Used to Evaluate the Developmental Toxicity of Drugs
    - Lori A. Dostal
  - How Animal Test Results Are Used to Inform Product Approval and Labeling
    - Melissa S. Tassinari
  - Can Experimental Animal Results Be Used in Counseling Patients?
    - Sarah G. Običan
  - Can Experimental Animal Results Be Used in Counseling Patients?
    - Anthony R. Scialli

The DARTable Genome: Bringing Molecular and Developmental Biology to DART

- Chairpersons: Thomas B. Knudsen, Richard A. Currie
- Overview of the DARTable Genome
  - Richard A. Currie
- Retinoic Acid Signaling in Developmental and Reproductive Toxicology: In Vitro and In Silico Approaches to Assess Toxicity
  - Joshua F. Robinson
- Thalidomide: Historical Perspective and New Insights into Molecular Mechanisms of Teratogenicity
  - David G. Belair
- Valproic Acid: Linking In Vitro and In Silico Techniques to Understand and Predict Developmental Toxicity
  - Nicole Churchill Kleinstreuer

CONCLUSION

BDRP plays and needs to continue to play an active role as we transition away from animal models to more relevant models that consider exposure combined with adverse outcome pathways, down to the molecular level.