CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
REPRODUCTIVE SYSTEM

ORGANIZED BY SOCIETY FOR TOXICOLOGIC PATHOLOGY IN INDIA (STPI)

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Design and Conduct of Developmental and Reproductive Toxicology Studies

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PART I
• Thalidomide as catalyst for regulatory requirements of Pre-clinical testing
• Introduction to Developmental & Reproductive Toxicology
• Guidelines and Study Designs:
  • Fertility and Early Embryonic Toxicity Study (Segment 1)
  • Embryo-fetal Development Study (Segment II)
  • Pre-and Postnatal Development Study (Segment III)
Thalidomide Tragedy

- The modern history of teratology started with the introduction of a drug known as thalidomide in the late 1950’s under the name of Contergen® by Chemie Grunenthal, a German pharmaceutical company.

- It was used as a potent and apparently safe non-barbiturate sedative hypnotic, which was prescribed to women during pregnancy for treatment of the symptoms of morning sickness.

- In November 1961, Lenz suggested that these deformities resulted from the mothers exposed to thalidomide during pregnancy (Diggle, 2001).

- The same suggestion was made at much the same time by McBride in Australia.
Thalidomide Tragedy-cont

- Thalidomide is believed to be the catalyst that initiated regulatory requirements for new drugs to be thoroughly tested on animals prior to arrival in the market place.

- In 1962, President John F. Kennedy signed into law the Kefauver-Harris drug amendment to ensure drug efficacy and greater drug safety. Key provisions of the amendment were:
  - Drug companies must show a proof-of-efficacy
  - Establishment of new safety testing procedures

- No new drug can be marketed in the USA until the FDA has determined that the drug is both safe and effective for its intended use.
Development of Guidelines

- Testing requirements for food additives, color additives, and animal drugs administered to food producing animals were extended to include multigeneration reproduction studies with a teratology phase incorporated into the design.

- Similar guidelines were then adopted by other regulatory agencies in the U.S. and the western world including Japan, which were applied not only to pharmaceutical drugs, but to all classes of chemicals with significant human exposure potential.
Development of Guidelines

• As most new pharmaceuticals are developed in the United States, the European Union, and Japan; and because the rate of attrition for new drug development is estimated to be as high as 1:5,000-10,000 (Lumley and Walker 1992); this has caused dramatic increases in both the time and cost needed for drug development

• Efforts to harmonize various elements of drug regulatory activities were initiated by various inter-governmen tal organizations at regional and interregional levels in the past 20 years
Development of Guidelines

- In 1966, the Guideline for Reproductive Studies for Safety Evaluation of Drugs for Human Use was finalized (Goldenthal, 1966)

- This consisted of a series of studies divided into three segments, each of which would pertain to a specific phase of the reproductive process.

- The Segment I design addresses fertility and general reproductive performance, the Segment II design requires classic teratology animal testing, and the Segment III design limits treatment to the peri- and postnatal period.
ICH S5(R2) Guideline

• The ICH S5(R2) document entitled “Detection of Toxicity to Reproduction for Medicinal Products”:

• Describes that developmental and reproductive toxicity studies be performed to evaluate the potential adverse effects of a drug product on different segments of the reproductive cycle, defined as stages A-F.
ICH S5(R2) Guideline

- Study of Fertility and Early Embryonic Development to Implantation (ICH 4.1.1.)
- Study for Effects on Pre- and Postnatal Development, including Maternal Function (ICH 4.1.2.)
- Study for Effects on Embryo-fetal Development (ICH 4.1.3.)
- The studies are designed to identify the effects of drugs on mammalian reproduction and include exposure of mature adults, as well as all stages of development from conception to sexual maturity.
Table 19.4  ICH treated and evaluated stages

<table>
<thead>
<tr>
<th></th>
<th>FDA</th>
<th>UK</th>
<th>EEC</th>
<th>JAPAN</th>
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<td>A–E&lt;sup&gt;a&lt;/sup&gt;</td>
<td>A–E</td>
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<td>A–F</td>
<td>A–F</td>
<td>A–D</td>
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<td>C</td>
<td>C</td>
<td>C, ½ D</td>
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<tr>
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<td>C, D</td>
<td>C, D</td>
<td>C–F</td>
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<td>D, E</td>
<td>D, E</td>
<td>D, E</td>
<td>½ D, E, F</td>
</tr>
</tbody>
</table>

<sup>a</sup>  A = premating to conception, B = conception to implantation, C = implantation to closure of the hard palate, D = closure of the hard palate to the end of pregnancy, E = birth to weaning, F = weaning to sexual maturity.
Introduction to DART

- Evaluation of developmental and reproductive toxicology endpoints is an integral part of the safety assessment process for compounds with:

  - Potential use in women of childbearing age or females that might be exposed during pregnancy

  - Men of reproductive potential.

- Developmental toxicity is defined as:
  - The study of adverse effects on the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation.
• We currently rely on animal testing to predict the potential for drugs or chemicals to cause developmental toxicity in humans.

• Rodents (rats & mice) and rabbits are the most relevant species used in developmental toxicity testing.

• Dogs & minipigs are rarely used.

• Use of nonhuman primates is increasing due to increase in biologics.
Developmental & Reproductive Toxicity Manifestations

Manifestation of DART toxicity may include adverse effects on:

– onset of puberty
– gamete production and transport
– reproductive cycle normality
– sexual behavior
– fertility, gestation, parturition, lactation
– developmental toxicity
– premature reproductive senescence
– modifications of other functions that are dependent on the integrity of the reproductive systems
ICH 4.1.1. Rat Fertility and Early Embryonic Toxicity Study (Segment I)
ICH 4.1.1. Rat Fertility and Early Embryonic Toxicity Study (Segment I): Study Design

Pre-mating Mating GD 7 C-section GD 13 Necropsy of males

Male organ Weights Sperm analyses: Motility, Morphology and concentration

= Dosing males and females

= Dosing males
Fig. 1—Ways to holding the female rat for the collection of the vaginal lavage (a-c), individual tail marks of five female rats from the same cage (d) and way to distribute the drops of vaginal lavage from each female rats in a glass slide (e). During a month, every morning between 8:00 and 9:00 a.m., vaginal secretion was collected from female rats. It was done by using a plastic pipette filled with 10 μL of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina, but not deeply (1a). If the animals were aggressive ways 1b and 1c were used. Vaginal fluid was placed on glass slides and a different glass slide was used for each cage of animals (1e). One drop was collected with a clean tip from each rat (1d, 1e).
Estrus Cycle

• Pro-estrus is characterized predominately by nucleated epithelial cells
• Estrus primarily consists of anucleated cornified cells
• Metestrus is characterized by similar proportions of leukocytes, cornified and nucleated epithelial cells
• Diestrus primarily consists predominantly of leukocytes
Comparison of Human and Rat Reproductive Cycle

Human Menstrual Cycle

Rat Estrous Cycle

Mating

• A mating ratio of 1:1 is recommended when both sexes are being dosed and this should be equally considered in separate male and female studies.

• If cycling normally, most of the females will mate within the first 5 days of cohabitation.

• Mating index:
  number of males with confirmed mating/number of males cohabited x 100
Parameters

• In females, uterine data including implantation sites and resorption sites are recorded.
• In males, determination is made to assess
  • Sperm parameters including:
    • motility, morphology, and concentration
  • Sexual organ weights including testes, epididymis, prostate, and seminal vesicle
Gestation Day 13 Parameters

- Mean number of corpora lutea
- Implantation sites
- Pre-implantation loss
- Viable/nonviable embryos
- Post-implantation loss
- Resorptions
- Time to mating
- Mating, Pregnancy, and Fertility indices
SPERMATOGENESIS

1. Primordial germ cell
2. Spermatogonium (diploid)
3. Mitotic division
4. Primary spermatocyte (diploid) (in prophase of meiosis I)
5. First meiotic division
6. Secondary spermatocyte (haploid)
7. Second meiotic division
8. Spermatids (haploid)
9. Spermatids (at two stages of differentiation)
10. Sperm cells (haploid)
Fertility Assessments

- Increased abnormal sperm is considered evidence that the agent has gained access to the germinal cells.

- Abnormal sperm may not reach the oviduct or participate in the fertilization.

- The greater the number of abnormal sperm in the ejaculate, the greater the probability of reduced fertility.
Fertility Assessments

• In rats, fertility assessments are limited by their insensitivity as measures of reproductive injury

• A reduction of up to 90% of normal sperm production in rats and mice was reported not to compromise the fertility

• Subfertility defined as increased time to conception, i.e. number of days to mating or copulatory interval.

• Statistical significance vs. biological significance
Follicular Count

- Histological processing of the ovary involves taking 5 serial sections, each 100 μm apart, from the central 1/3 of each ovary (left and right), resulting in 10 sections per female.

- These sections are stained with a PCNA stain, which clearly delineates the presence of oocyte nuclei from ovarian background and enhances the ability to identify granulosa cells, zona pellucida, basal lamina and thecal layers.
Follicular count

According to the Society of Toxicology Pathology (2005):

• Properly conducted follicular counts can supplement qualitative ovarian assessment to characterize ovarian toxicants.

However, data generated from follicle counts can be:

• Highly variable with larger SD both between animals and between groups making interpretations difficult

• Follicle counts can be highly time consuming
Ovarian Weight

- Ovarian weight in rats does not fluctuate during the estrous cycle, like uterus weight.

- Ovarian weight and histopathology can provide very useful information about the effects of toxicants on the female reproductive system.

- Ovarian weights can be reduced by either depletion of oocyte or disruption of HPG axis.
Ovarian Weight

- A survey conducted by the Pharma industry on the relevance of organ weights in toxicity testing (Bindhu et al., 2007) found that:
  - Ovarian weight was considered valuable by
    - 35% of pharmaceutical companies
    - 33% of veterinary
    - 25% of chemical industry respondents
  - Ovarian weights were considered of little value by
    - 19% of pharmaceutical companies
    - 50% of chemical industry
    - 100% of food/nutritional
    - 67% of consumer product industry respondents

Factors diminishing the value of ovarian weight included:
- Weight variability due to its small size
- Technical issues (such as trimming)
Site of Action for Female Reproductive Toxicants

Measure estrous cyclicity → Mate → Sacrifice Count pups

- Normal

- Decreased

implantation

- Normal

- Decreased

Count corpora lutea

- Decreased

Site of action is developmental and/or altered corpora lutea function

Site of action is fertilization or maintenance of implantation

Site of action is ovary or hypothalamus/pituitary

Adapted from Foster, P & Gray J in Casarett & Doull's, 2008; pp. 774

PART III
ICH 4.1.3. Embryo-fetal Development (Segment II)
ICH 4.1.3. Embryo-fetal Development (Segment II)

- Time-mated GD 0
- GD 6
- GD 17 in rats
  GD 15 in mice
  GD 18 in rabbits
- GD17 mice
- GD 20 rat
- GD29 rabbit

C-section

Fetal Evaluation:
External, Visceral, and Skeletal

= Dosing period
Embryo-fetal Development in Primates: Study design

2 months cycle check

GD 0
Mating

GD 18-20
Ultrasound To confirm pregnancy

GD 50
End of treatment

GD 100
C-section

Fetal Exam
- External
- Visceral
- Skeletal

Biologics: Immunologic testing
Immunoglobulins (mother)
Immunohistochemistry (fetus)
Dosing optional if treatment-related preimplantation loss is demonstrated
Dose Selection

- At least three dose levels and a concurrent control should be used.
- Unless limited by the physical or chemical properties, the high dose is selected to produce:
  - Minimal maternal toxicity, including marginal, but significant reduction of body weight, decreased weight gain, or specific organ toxicity, and at the most produces no more than 10% mortality.
- The mid dose should produce minimal observable toxic effects.
- The low dose is expected to generally produce a No Observed Adverse Effect Level (NOAEL) for maternal and developmental effects.
- Information on developmental effects may be of limited value or difficult to interpret if doses that cause excessive maternal toxicity are employed.
Hypothetical pattern of susceptibility of embryonic injury to teratogenic insult
A Brief Pulse of Teratogenic Treatment on the 10th Day of Gestation Would Result in the Following Incidence of Malformations:

- 35% Brain Defects
- 33% Eye Defects
- 24% Heart Defects
- 18% Skeletal Defects
- 6% Urogenital Defects
- 0% Palate Defects
Gestation Day 20 rat uterus

Left uterine horn

Right uterine horn

Implant site #1

#2

#3

#4

#5

#6

#7

#8

#9

#10

#11

Cervix
EXTERNAL EVALUATION

Cleft Lip
EXTERNAL VERSUS SKELETAL
GROSS EXTERNAL EVALUATION

SPINA BIFIDA
TAIL ABNORMALITIES

NORMAL

KINKED

BENT

ABSENT
HYDROCEPHALY

- Dilated Lateral Ventricle
- Slight Dilation of Third Ventricle
- Doomed Head
ABSENT KIDNEY(S)

ADRENAL

URINARY BLADDER
HEART CUT

1. **Ascending Aorta**
2. **Pulmonary Trunk**
3. **Approximate position of IV septum**

[Diagram showing heart cut with labeled structures]
HEART CUT

RIGHT VENTRICLE

- Tricuspid valve
- Interventricular Septum
- Papillary muscle

LEFT VENTRICLE

- Tricuspid valve
- Papillary muscle
1. Premaxilla
2. Nasal
3. Frontal
4. Parietal
5. Interparietal
6. Supraoccipital
7. (7) Cervical vertebrae
8. (13) Thoracic vertebrae
9. (6) Lumbar vertebrae
10. Ilium
11. Pubis
12. Ischium
13. Metatarsals
14. Fibula
15. Tibia
16. Femur
17. (13) full pair of thoracic ribs
18. (6) Sternebrae
19. Metacarpals
20. Hyoid
Common Skeletal Malformations/Variations

- Absent
- Accessory skull bone
- Additional ossification center
- Bent
- Branched
- Discontinuous
- Extra
- Supernumerary
- Fused
- Incomplete Ossification
- Unossified
- Misaligned
- Misshapen
- Rudimentary
- Unilateral Full
Definition: Malformation and Variation\(^1\)

- Malformation is defined as a permanent (or irreversible) change in the species under investigation that is likely to affect survival or health.

- Variation is defined as a change that occurs within the normal population under investigation and is unlikely to adversely affect survival or health. This change might include a delay in growth or morphogenesis that has otherwise followed a normal pattern of development.

\(^1\)Chahoud et al. 1999
Skeletal Abnormalities

- The interpretation of several skeletal abnormalities, namely delayed ossification, supernumerary number, and wavy ribs poses some problems. These abnormalities occur at a high background in control animals and are observed frequently in a dose-related manner (Daston and Seed, 2007).

- Delay in ossification is often related to maternal toxicity; whereas wavy ribs seem to be caused by a few agents with little or no maternal toxicity (Carney and Kimmel, 2007).

- The delay in ossification is considered to be indicative of a delay in development; this may be an adverse effect, but is probably not a predictor of teratogenic potential (Daston and Seed, 2007).
Key Factors to Consider in a Weight of Evidence Approach for Interpreting Delayed Ossification

Examples of insignificant findings:
- Isolated statistical increases in a few variations, but no consistent pattern indicative of delayed ossification
- Incidences are within recent historical control ranges

Examples of low significance findings (may not be adverse):
- Pattern of ossification is consistent with a slight generalized delayed (e.g., limited to effects on bones such as phalanges, sternebrae 5/6, cervical, thoracic, sacral, and caudal vertebral centra, and/or calvarium)
- Normal cartilage is present
- Delayed ossification associated with maternal toxicity

Findings that MUST warrant increased attention:
- Usually patterns of ossification that do not follow normal sequence
- Specific delays involving bones that normally are well ossified in term fetus (e.g. ribs, clavicle, long bones of the limbs, lumbar vertebral centra)
- Abnormally-shaped cartilage template
- Delayed ossification without decreases in fetal body weight
- Delayed ossification in the absence of maternal toxicity
- Delayed ossification associated with teratogenesis

Adapted from Carney and Kimmel, (2007); Birth Defects Res. (Part B); 80: 473-496
Prediction of Developmental Effects in Human

• There is no appropriate species that can predict the type of developmental effects observed in human.

• The types of developmental effects seen in animal studies are not necessarily the same as those produced in humans.

• A multi-company database of 131 pharmaceutical agents showed a true positive prediction rate of animal models for human toxicity of 69%.

• It should be assumed that humans are more sensitive than the most sensitive animal species tested, if no pharmacokinetic/toxicokinetic data are available.
The FDA established a rating system based upon their safety for use in pregnancy:

- **Category A**: Drugs with no fetal risk in well-controlled clinical studies in women.
- **Category B**: Drugs for which there are no human data available and animal studies show no fetal risk or Animal studies show a risk but human studies do not show fetal risk.
- **Category C**: Drugs with adverse effects in animal studies, but for which well-controlled clinical studies in humans are lacking, and drugs for which studies in women and animals are not available.
- **Category D**: Drugs in which human use show fetal risk, but whose potential benefits may be acceptable despite known risks.
- **Category X**: Drugs in which fetal abnormalities have been demonstrated in animals or human studies or both, and risk to fetus clearly outweighs any benefits for use of these drugs during pregnancy.

Hect, 1979
ICH 4.1.2. Pre- and postnatal Developmental Toxicity Study (Segment III)
Diethylstilbestrol

- Diethylstilbestrol (DES) is a "synthetic estrogen."

- During 1938-1971, U.S. physicians prescribed DES to pregnant women to prevent miscarriages and avoid other pregnancy problems because at the time physicians believed that some pregnant women did not produce enough estrogen naturally.

- An estimated 5-10 million pregnant women and the children born of these pregnancies were exposed to DES.

- In 1971, the FDA issued a Drug Bulletin advising physicians to stop prescribing DES to pregnant women.

- The warning was based on a study published in 1971 that identified DES as a cause of a rare vaginal cancer in girls and young women who had been exposed to DES before birth (in the womb).
In-utero and Lactational Exposure

- The reproductive system is under complex integrative control by the central nervous system (CNS), pituitary, gonads, and the genital tract, all of which are potential targets of transplacental chemical exposure.

- Postnatal reproductive development can also be altered by changes in synthesis, metabolism, and control of gonadal hormones.

- In the postnatal period, these changes can be manifested as alterations in sexual differentiation and sexual maturation that could lead to fertility effect.
ICH 4.1.2. Pre- and postnatal Developmental Toxicity Study (Segment III) Study Outline:

- Examine the effect of the test material on late development through parturition and to weaning
- Typical assessments includes:
  - Developmental Landmarks
  - Sexual maturation:
  - Motor activity
  - Learning and Memory
  - Fertility Assessment

GD 6
Day of Parturition
Lactation Period Developmental landmarks
Weaning PND 22

= Dosing period
Enhanced Pre- and Postnatal Development in Cynomolgus Monkey: Study Design

Menstrual cycle observations for at least 2-3 months

Mating

Ultrasound to confirm pregnancy, BC

Dosing period (between GD 20 and birth)

Ultrasound conducted monthly during treatment period, through parturition

Parturition ~GD 160

BC

Postnatal development period

Infant Necropsy/Histopath?

Postpartum 3-12 months?

BC: Blood collection for clinical pathology analyses

*Infant evaluations may include clinical observations, BW, TK, clinical pathology, necropsy/histo, immunoglobulin, TDAR, NK cell, immunophenotyping, and neurobehavioral assessments.
## F1 Generation Survival and Growth Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham control</th>
<th>150</th>
<th>300</th>
<th>600</th>
<th>Cage control</th>
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<td><strong>Survival</strong></td>
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<tr>
<td>Birth, pups alive/litter</td>
<td>10 ± 3.0</td>
<td>11 ± 2.5</td>
<td>10 ± 3.3</td>
<td>12 ± 2.6</td>
<td>12 ± 2.9</td>
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<td>Day 0, pups dead/litter</td>
<td>0 ± 0.3</td>
<td>0 ± 0.8</td>
<td>0 ± 0.0</td>
<td>1 ± 1.0*</td>
<td>0 ± 0.6</td>
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<td>Number found dead/cannibalized</td>
<td>2</td>
<td>9</td>
<td>0</td>
<td>18</td>
<td>9</td>
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<tr>
<td>Live birth index (%)</td>
<td>99 ± 3.0</td>
<td>96 ± 7.7</td>
<td>100 ± 0.0</td>
<td>93 ± 8.9*</td>
<td>97 ± 5.3</td>
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<td>PND 4, pups dead/litter</td>
<td>1 ± 2.7</td>
<td>2 ± 3.8</td>
<td>0 ± 0.3</td>
<td>2 ± 3.4</td>
<td>1 ± 1.3</td>
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<tr>
<td>Viability index (%)</td>
<td>92 ± 21</td>
<td>82 ± 37.4</td>
<td>98 ± 4.7</td>
<td>80 ± 32.2</td>
<td>95 ± 13.1</td>
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<td>PND 21, pups dead/litter</td>
<td>0 ± 0.0</td>
<td>0 ± 0.2</td>
<td>0 ± 1.4</td>
<td>0 ± 0.0</td>
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<tr>
<td>Lactation index (%)</td>
<td>100 ± 0.0</td>
<td>99 ± 3.4</td>
<td>95 ± 22.9</td>
<td>100 ± 0.0</td>
<td>100 ± 0.0</td>
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<td><strong>Body weight (g)</strong></td>
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<td>PND 0</td>
<td>6.79 ± 0.54</td>
<td>6.34 ± 0.39*</td>
<td>6.40 ± 0.64*</td>
<td>5.58 ± 0.52*</td>
<td>6.87 ± 0.45</td>
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<td>PND 4</td>
<td>10.67 ± 1.58</td>
<td>10.16 ± 1.89</td>
<td>10.60 ± 1.32</td>
<td>9.15 ± 1.16*</td>
<td>11.23 ± 1.16</td>
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<td>PND 21</td>
<td>55.80 ± 6.14</td>
<td>53.42 ± 6.87</td>
<td>52.85 ± 4.73</td>
<td>45.99 ± 4.84*</td>
<td>65.04 ± 4.97</td>
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<td>Mean body weight gain, PND 0–21</td>
<td>49.14</td>
<td>47.15</td>
<td>46.63</td>
<td>40.50</td>
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<td>Male, PND 28</td>
<td>103 ± 7.7</td>
<td>100 ± 11.5</td>
<td>97 ± 7.6*</td>
<td>89 ± 6.8*</td>
<td>111 ± 8.1</td>
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<tr>
<td>Male, PND 63</td>
<td>339 ± 23.0</td>
<td>336 ± 24.6</td>
<td>329 ± 22.6</td>
<td>303 ± 24.7*</td>
<td>353 ± 29.4</td>
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<td>Mean body weight gain, PND 28–63, male</td>
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<td>236</td>
<td>232</td>
<td>214</td>
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<td>Female, PND 28</td>
<td>95 ± 7.5</td>
<td>91 ± 8.3</td>
<td>87 ± 5.7*</td>
<td>81 ± 6.6*</td>
<td>103 ± 9.3</td>
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<tr>
<td>Female, PND 63</td>
<td>230 ± 19.2</td>
<td>220 ± 16.3</td>
<td>215 ± 13.0*</td>
<td>200 ± 14.4*</td>
<td>219 ± 17.7</td>
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<tr>
<td>Mean body weight gain, PND 28–63, female</td>
<td>135</td>
<td>129</td>
<td>128</td>
<td>119</td>
<td>116</td>
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</table>

*Note. Survival values represent mean ± SD, n = 23 to 26. Live birth index = (No. viable pups born/litter) × 100; viability index = (No. viable pups at day 4/no. viable pups born) × 100; lactation index = (No. viable pups at day 21/No. viable pups at day 4) × 100. Body weight data for PND 0 to 21 are on a per-litter basis with the values representing mean ± SD unless noted, n = 16 to 31. Relative organ weight values represent the organ weight/terminal body weight × 100, n = 10. PND, postnatal day.

*Significantly different from sham control (p ≤ 0.05).
Sexual Maturation

- Age at vaginal opening
- Age at preputial separation
  - The mean age at vaginal opening and preputial separation of Wistar rats in Japan was calculated to be 30.3 and 42.4 days, respectively (Aoyama, 2002).
  - The mean age range at vaginal opening and preputial separation for Sprague-Dawley rats CD® [Crl:CD®(SD)] in our lab is 33.3-34.2 and 45-46.3 days, respectively.
- Timing of puberty is related to attainment of a critical level of body weight.
  - Body weight should be collected on the confirmed day of sexual maturation
Learning and Memory: Passive avoidance
Learning and Memory: Cincinnati Water Maze
A  The watermaze

B  Paths and latency during place navigation

C  Post-training probe tests (no platform)
   - control
   - hippocampus lesioned
   - subiculum lesioned
   - hippocampus & subiculum lesioned

D  Overtraining
   - hippocampus lesioned
Water maze: Learning and Memory

Time to Platform (Females)

Time to Platform (Males)
Scopolamine - Male

Animal 111, Day 5

Vehicle - Male

Animal 129, Day 5
Vehicle - Female

Animal 165, Day 5
Acoustic Startle Response
Acoustic Startle Response

• The acoustic startle response (ASR) is a transient motor response to an unexpected intensive stimulus.

• The response is determined by stimulus parameters such as its intensity, rise time and duration.

• Behaviorally, the startle response consists of rapid contraction of head, neck, trunk and legs muscles in addition to the arrest of ongoing activity.
### TABLE 9
F<sub>1</sub> Generation Acoustic Startle Response Assessment Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham control</th>
<th>150</th>
<th>300</th>
<th>600</th>
<th>Cage control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PND 22, 80 dB</td>
<td>8 ± 1.9</td>
<td>7 ± 1.6*</td>
<td>7 ± 2.0</td>
<td>6 ± 1.7*</td>
<td>7 ± 1.8</td>
</tr>
<tr>
<td>PND 22, 120 dB</td>
<td>36 ± 13.0</td>
<td>28 ± 11.9</td>
<td>36 ± 14.6</td>
<td>32 ± 17.5</td>
<td>43 ± 13.9</td>
</tr>
<tr>
<td>PND 61, 80 dB</td>
<td>26 ± 8.3</td>
<td>23 ± 6.8</td>
<td>20 ± 6.6*</td>
<td>20 ± 5.7*</td>
<td>23 ± 6.6</td>
</tr>
<tr>
<td>PND 61, 120 dB</td>
<td>88 ± 64.1</td>
<td>88 ± 58.0</td>
<td>87 ± 46.1</td>
<td>84 ± 83.8</td>
<td>75 ± 36.2</td>
</tr>
<tr>
<td><strong>Latency (ms)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PND 22, 80 dB</td>
<td>86 ± 11.8</td>
<td>84 ± 10.8</td>
<td>79 ± 13.0</td>
<td>82 ± 12.1</td>
<td>87 ± 10.3</td>
</tr>
<tr>
<td>PND 22, 120 dB</td>
<td>43 ± 12.0</td>
<td>50 ± 14.2</td>
<td>48 ± 11.1</td>
<td>46 ± 11.3</td>
<td>38 ± 9.5</td>
</tr>
<tr>
<td>PND 61, 80 dB</td>
<td>91 ± 13.4</td>
<td>94 ± 15.0</td>
<td>92 ± 17.1</td>
<td>92 ± 18.2</td>
<td>89 ± 14.6</td>
</tr>
<tr>
<td>PND 61, 120 dB</td>
<td>46 ± 11.7</td>
<td>53 ± 18.1</td>
<td>42 ± 11.3</td>
<td>43 ± 11.5</td>
<td>46 ± 14.2</td>
</tr>
</tbody>
</table>

*Note. Peak acoustic startle response values represent the mean ± SD of grams displaced by the animals responding to either 80 or 120 dB of sound, n = 19 to 20 (males and females combined). PND, postnatal day.*

*Significantly different from sham control (p ≤ 0.05).
Motor Activity

• Animals are placed individually into a Plexiglas chamber containing two equally sized compartments.

• The chamber is surrounded by a PC-interfaced horizontal photobeam frame (SmartFrame® Cage Rack System; Hamilton/Kinder), consisting of photocells (8Lx4W) that continuously tracts the animal’s movement in each compartment.

• A 10-minute test is typically used to determine baseline exploration or the effect of a drug.

• Data are collected in the form of photobeam breaks in each compartment using MotorMonitor® software (Hamilton/Kinder).
Procedure

- The data are subsequently reduced to the following parameters for each area:
  - Basic movements (beam breaks)
  - Distance traveled (cm)
  - Time spent (sec)
  - Number of repetitive beam breaks (i.e. stereotypic movement).

- Data may be collected and analyzed in time bins (e.g. every minute) or as a total over the course of collection.
Questions?
References


