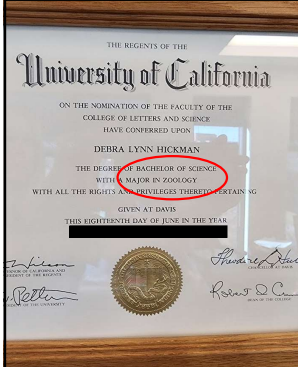


**LIGHTS, VIBRATION,  
AND MORE: HOW  
EXTRINSIC FACTORS  
AFFECT THE ANIMALS  
IN THE RESEARCH  
FACILITY**

**DEBRA L. HICKMAN, DVM,  
MS, DACLAM, DCAW**

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**DISCLAIMERS**

- This talk is going to be very rodent centric (as they are the most commonly used species)
- I have no conflict of interest to declare
- The opinions in this presentation are mine alone.
- They do not represent the opinion of AAALAC International
- I am an animal welfare scientist

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**WHEN A BEHAVIORIST  
IS ASKED TO EXPLAIN  
PHYSICS. . . .**

3

**AGENDA**

- Why do we care?
  - Animal welfare
  - Reproducibility
- Intrinsic versus extrinsic factors
  - Deep dive on extrinsic

4

**WHY DO WE  
CARE?**

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**WHY DO WE CARE?**

- Good welfare = good science
- Reproducibility
- Translatability

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# ANIMAL WELFARE ASSESSMENT

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## WELFARE DEFINITION

**Animal welfare** refers to the state of the *individual animal*.

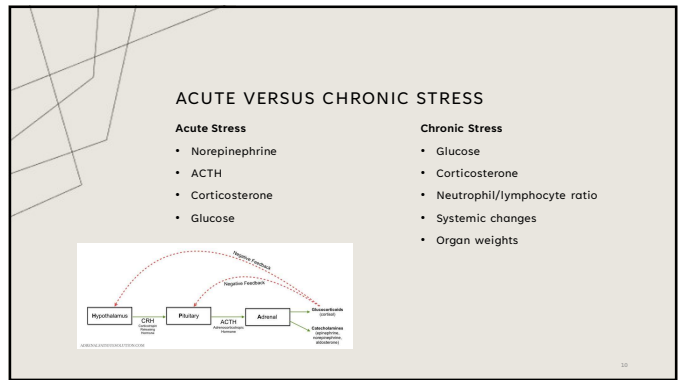
Protecting an animal's welfare means providing for its physical and mental needs.

We assess welfare in groups to guide our strategies, but recall that welfare is truly individual.

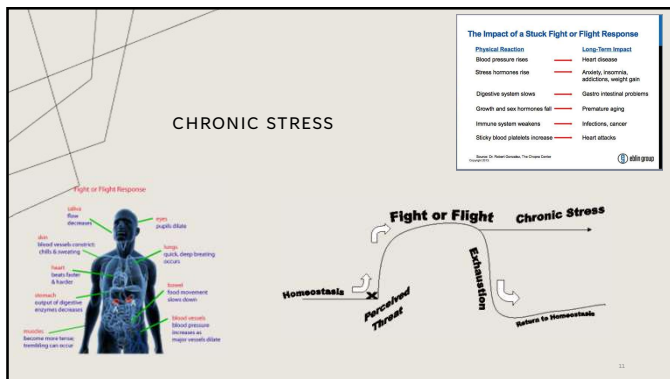
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- ## TYPICAL WELFARE ASSESSMENT
1. Corticosterone
  2. Basic health
  3. Physiologic changes
    - a. Cardiovascular
    - b. Reproductive
  4. Behavioral changes
    - a. Stereotypies
    - b. Compare to normal ethogram
    - c. Perform active behavioral assessment
- 

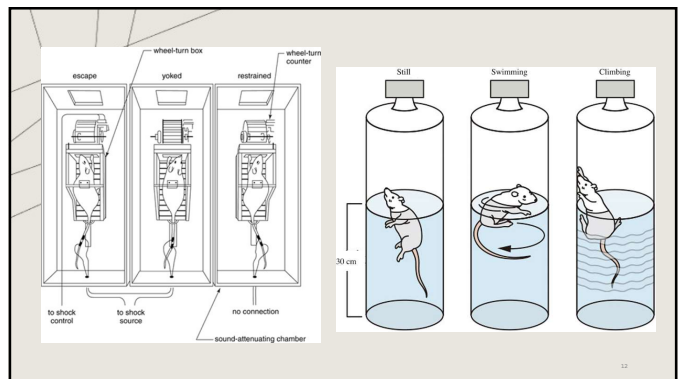
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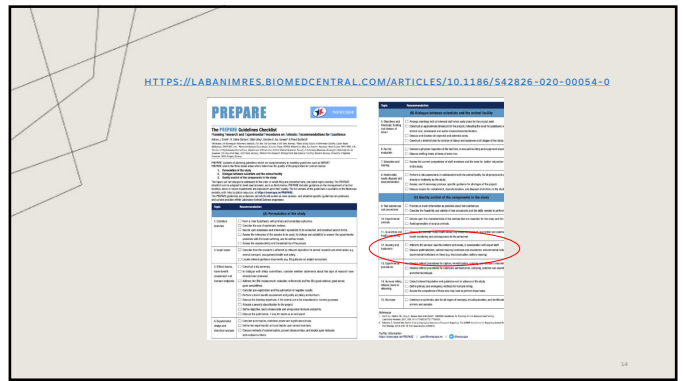
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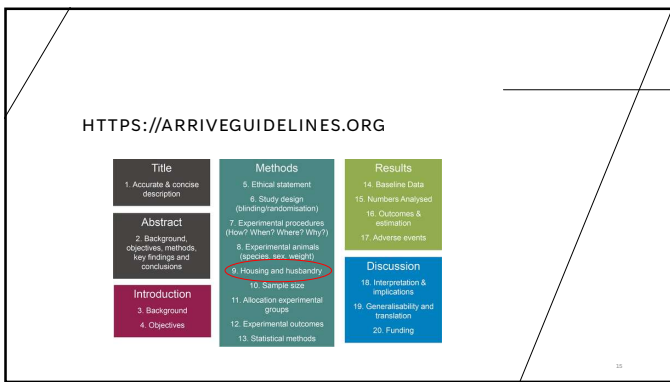
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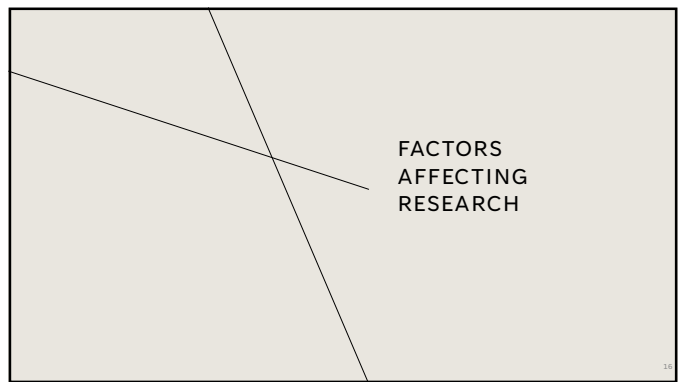
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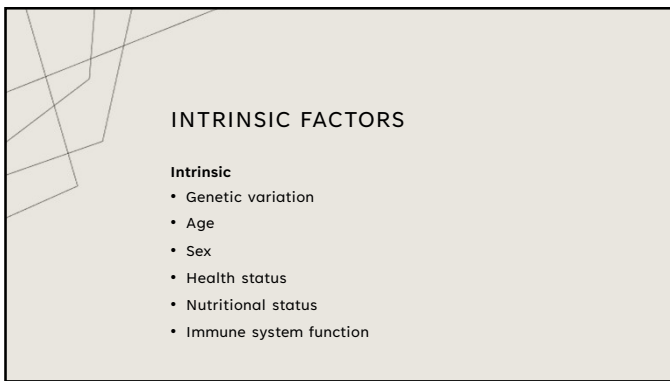
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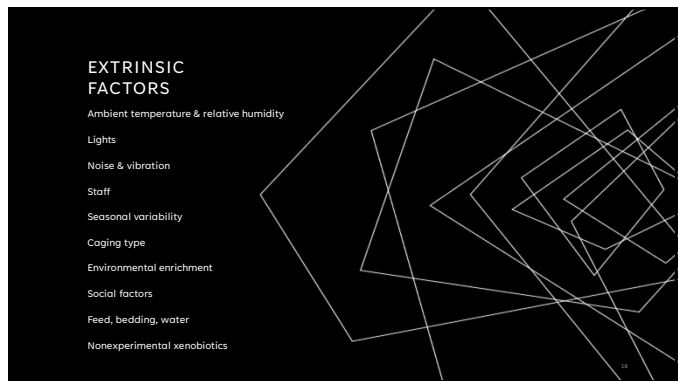
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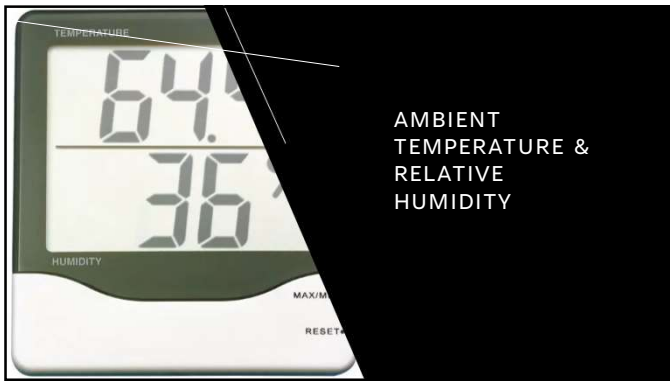
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### TEMPERATURE & HUMIDITY

**Temperature**

- Thermoneutral zone
- Lower and upper critical temperatures
- Minimal fluctuation

**Humidity**

- 30 to 70% relative humidity for most mammalian species

| Animal                                   | Dry-Bulb Temperature |       |
|--|----------------------|-------|
|  | °C                   | °F    |
| Mouse, rat, hamster, gerbil, guinea pig* | 20-26                | 68-79 |
| Zebrafish                                | 16-22                | 61-72 |
| Cat, dog, nonhuman primate               | 18-29                | 64-84 |
| Farm animals, poultry                    | 16-27                | 61-81 |

\*Dry-bulb room temperature settings for rodents are typically set below the animal's LCT to avoid heat stress, and should reflect different species-specific LCT values. Animals should be provided with adequate resources for thermoregulation (nesting material, shelter) to avoid cold stress.

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### LIGHT

midcage (NASA 1988). Rats and mice generally prefer cages with low light intensity (Blom et al. 1996), and albino rats prefer areas with a light intensity of less than 25 lux (Schlingmann et al. 1993a). Young mice prefer much lower illumination than adults (Wax 1977). For animals that have been shown to be susceptible to phototoxic retinopathy, light should be between 130 and 325 lux in the room at cage level.

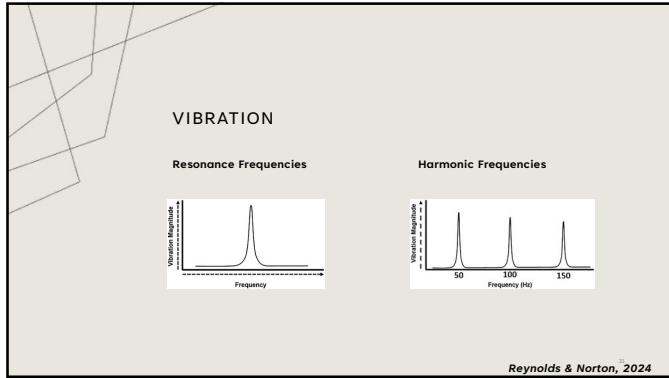
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### LIGHT

- Light cycle
- Light intensity
- Spectrum

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### NOISE

**Hearing frequencies**

1. Mice do not hear well below 1,000 to 2,000 Hz (which is human communication)
2. Ultrasonic (greater than 20,000 Hz) is more critical for them

**Sources of Ultrasonic Contamination**

- Computers
- Lights
- Motors
- Carts
- Ultrasonic motion sensors

*Turner & Manker, 2024*

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WE ARE STILL LEARNING. . . BUT DO HAVE SOME RECOMMENDATIONS

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### SOURCES OF VIBRATION

- Equipment**
- Housing location**
- Husbandry procedures**
- Transportation**

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### CONSTRUCTION

- Administrative controls**
- Procedural controls**
- Equipment used**
- Engineering Controls**

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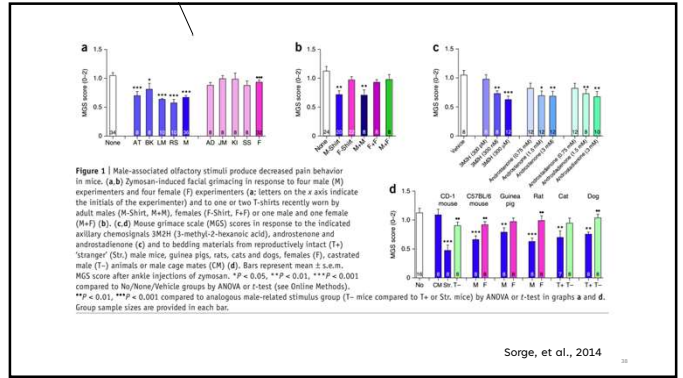
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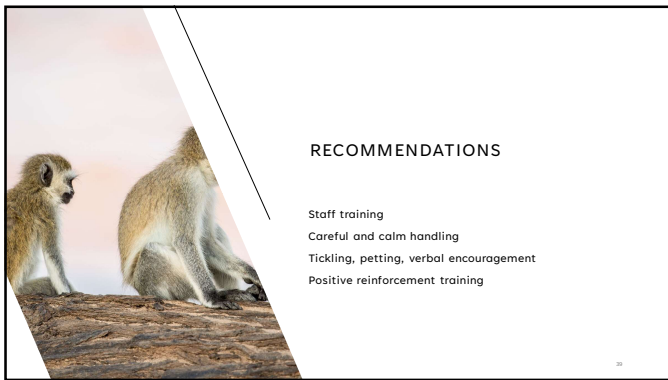
### HUMAN-ANIMAL INTERACTIONS



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### RECOMMENDATIONS

- Staff training
- Careful and calm handling
- Tickling, petting, verbal encouragement
- Positive reinforcement training

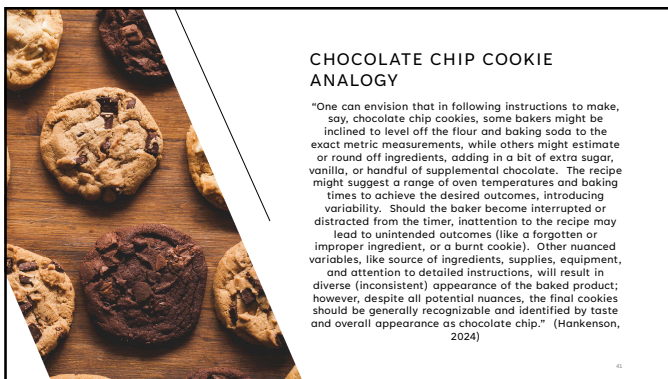
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### NON AVERSIVE HANDLING

<https://nc3rs.org.uk/3rs-resource-library/mouse-handling>

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### CHOCOLATE CHIP COOKIE ANALOGY


"One can envision that in following instructions to make, say, chocolate chip cookies, some bakers might be inclined to level off the flour and baking soda to the exact metric measurements, while others might estimate or round off ingredients, adding in a bit of extra sugar, vanilla, or handful of supplemental chocolate. The recipe might suggest a range of oven temperatures and baking times to achieve the desired outcomes, introducing variability. Should the baker become interrupted or distracted from the timer, inattention to the recipe may lead to unintended outcomes (like a forgotten or improper ingredient, or a burnt cookie). Other nuanced variables, like source of ingredients, supplies, equipment, and attention to detailed instructions, will result in diverse (inconsistent) appearance of the baked product; however, despite all potential nuances, the final cookies should be generally recognizable and identified by taste and overall appearance as chocolate chip." (Hankenson, 2024)

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### SEASONAL VARIABILITY

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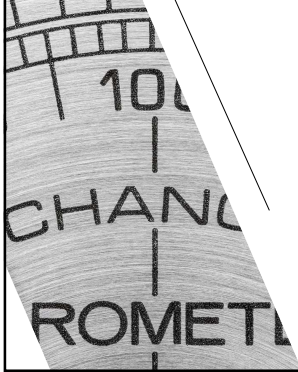
### SEASONAL VARIATIONS

Confer evolutionary advantages:

- Ambient temperature and precipitation
- Food availability
- Exposure to allergens
- Infectious pathogens
- Reproduction

Suckow & Tirado-Muniz, 2023

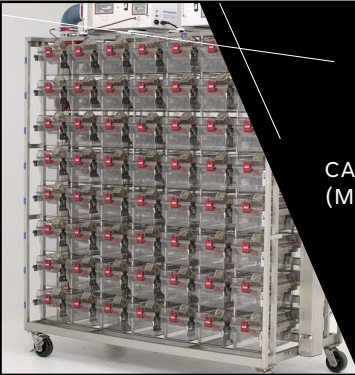
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### SEASONAL VARIATION CONTROL

- Internal clock?
- Photoperiod?
- Vibration & noise?
- Personnel?
- Barometric pressures?




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### CAGING TYPE (MICROENVIRONMENT)

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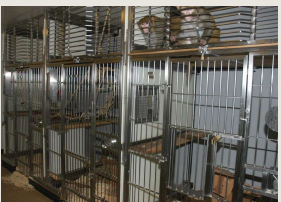

### FACTORS THAT INFLUENCE TEMPERATURE & HUMIDITY



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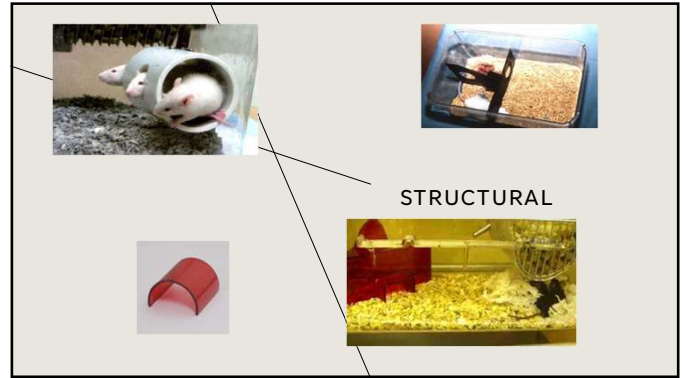


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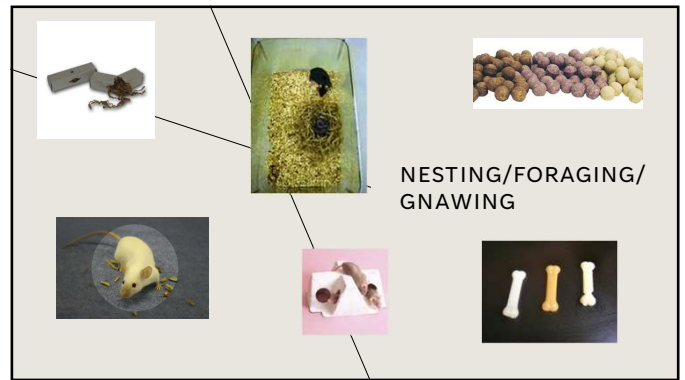
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### FEED

**General Considerations**

1. Clean, non-contaminated, quality controlled
2. Appropriate for the species
3. Nutritionally complete

**Natural Ingredient Diets**

Open formula, natural ingredient diets

- Complete formulation is reported and can be reproduced by any feed manufacturer

Closed formula diets

- Proprietary mixture of components
- Can be least-cost, fixed, or variable formulations

Kurtz & Feeney, 2019 56

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### FEED

**Chemically Defined Diets**

1. Contain specific amino acids, sugars, essential fatty acids, and purified vitamins and minerals.
2. Very high cost
3. Rarely used

**Purified or Semi-purified Diets**

Open formula, natural ingredient diets

- Complete formulation is reported and can be reproduced by any feed manufacturer

Closed formula diets

- Proprietary mixture of components
- Can be least-cost, fixed, or variable formulations

Kurtz & Feeney, 2019 57

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### FEED

**Sources of Experimental Variation:**

- Diet formulation (e.g. phytoestrogens)
- Contaminants (e.g. pesticides, microbial, PFASs, mycotoxins)
- Sterilization methods (e.g. steam sterilization versus irradiation)
- Diet harness
- Manufacturing errors or discrepancies
- Feed storage
- Ad libitum feeding versus restricted feeding

Kurtz & Feeney, 2019 58

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### BEDDING

1. Clean, non-contaminated, quality controlled
2. Appropriate for the species



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### WATER

**Recommendations**


1. Clean, non-contaminated, quality controlled
2. Appropriate for the species
3. Nutritionally complete

**Treatments**

Filtration or UV irradiation

Deionization or Reverse osmosis

Chlorination or acidification



Kurtz & Feeney, 2019 60

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## NONEXPERIMENTAL XENOBIOTICS

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## NONEXPERIMENTAL XENOBIOTICS

- Administered as part of routine conditioning or experimental procedure
- May cause physiologic alternations (intended or not) in the animal
- Examples can include:
  - Anesthetics, analgesics, tranquilizers, sedatives
  - Anti-infectives
  - Drugs to activate a promoter
  - Topical treatments (e.g. TABO, lanolin, NAIR, surgical scrub)

Perkins & Hankenson, 2019

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## REFERENCES & OTHER PAPERS OF INTEREST

Brenes-Soto, A. et al. (2019). The role of feed in aquatic laboratory animal nutrition and the potential impact on animal models and study reproducibility. *ILAR Journal*, 60(2):197.

Dauchy, R. T., Hanifin, J. L., Brainard, G. C. & Blask, D. E. (2024). Light: an extrinsic factor influencing animal-based research. *JAALAS*, 63(2): 116.

Franklin, C. L. & Ericsson, A. C. (2019). Complex microbiota in laboratory rodents: management considerations. *ILAR Journal*, 60(2):289.

Hanifen, J. P., et al. (2019). Relevance of electrical light on circadian, neuroendocrine, and neurobehavioral regulation in laboratory animal facilities. *ILAR Journal*, 60(2): 150

Hankenson, F. C. (2024). The elephant in the room: recognition and documentation of personnel practices that confound reproducibility. *JAALAS*, 63(3): 232.

Haseneau, J. J. (2019). Reproducibility and comparative aspects of terrestrial housing systems and husbandry procedures in animal research facilities on study data. *ILAR Journal*, 60(2): 228.

Kurtz, D. M. & Feeney, W. P. (2019). The influence of feed and drinking water on terrestrial animal research and study replicability. *ILAR Journal*, 60(2): 175.

Lee, V., et al. (2019). Micro- and macroenvironmental conditions and stability of terrestrial models. *ILAR Journal*, 60(2): 120.

Lieggi, C. et al. (2019). The influence of behavioral, social, and environmental factors on reproducibility and replicability in aquatic animal models. *ILAR Journal*, 60(2): 270.

63

## REFERENCES & OTHER PAPERS OF INTEREST

Perkins, S. E. & Hankenson, F.C. (2019). Nonexperimental xenobiotics: unintended consequences of intentionally administered substances in terrestrial animal models. *ILAR Journal*, 60(2): 214.

Pritchett-Corning, K. R. (2019). Environmental complexity and research outcomes. *ILAR Journal*, 60(2): 239.

Reynolds, R. et al. (2019). Sound and vibration as research variables in terrestrial vertebrate models. *ILAR Journal*, 60(2): 159.

Reynolds, R. P. & Norton, J. N. (2024). Evaluation of vibration as an extrinsic variable in in vivo research. *JAALAS*, 63(2):107.

Sanders, E. & Farmer, S.C. (2019). Aquatic models: water quality and stability and other environmental factors. *ILAR Journal*, 60(2): 141.

Sorge, R. E., et al. (2014). Olfactory exposure to males, including men, causes stress and related analgesia in rodents. *Nature Methods*, 11: 629

Suckow, M. A. & Tirado-Muniz, N. (2023). Seasonal variation of laboratory animals as a consideration for research reproducibility. *Comp Med*, 73(4):255.

Swan, M., Horvath, A., Pritchett, R. K., Barabas, A. J., Hickman, D., & Gaskill, B. N. (2024). The future is not bright: evaluation of rat preferences for color and intensity of light. *Animals*, 14(14): 2045

Turner, J. G. & Manker, J. R. (2024). Noise as an extrinsic variable in the animal research facility. *JAALAS*, 63(3):209.

Whittaker, A. L. & Hickman, D. L. (2019). The impact of social and behavioral factors on reproducibility in terrestrial vertebrate models. *ILAR Journal*, 60(2):252.

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## THANK YOU

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