Ethical considerations in experimental animal's research

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احاديث عن الرفق بالحيوان

جاء في الحديث الذي رواه البخاري ومسلم عن أبي هريرة ((ﷺ عن رسول الله (ﷺ) قال: ((بينما رجل يمشي فاشتد عليه العطش فنزل بئر فشرب منها، ثم خرج فإذا هو بكلب يلهث، يأكل الثرى من شدة العطش قال: لقد بلغ هذا الكلب مثل الذي بلغ بي، فملأ خفه، ثم أمسكه بفيه، ثم رقي، فسقى الكلب ! فشكر الله له، فغفر له)) قالوا يا رسول الله! وإن لنا في البهائم أجرا؟ قال: ((في كل كبد رطبة أجر)).

عن أبي هريرة ((ﷺ قال: [قال رسول الله (ﷺ) بينما كلب يطيف بركية كاد يقتله العطش، إذ رأته بغي من بغايا بني إسرائيل فنزعت موقها فسقته فغفر لها به]. ومعنى الركية: البئر.

روى البخاري وغيره عَنْ عَبْدِ اللَّهِ بْنِ عُمَرَ رَضِيَ اللَّهُ عَنْهُمَا أَنَّ رَسُولَ اللَّهِ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ قَالَ: [عُذِّبَتْ امْرَأَةٌ فِي هِرَّةٍ سَجَنَتْهَا حَتَّى مَاتَتْ فَدَخَلَتْ فِيهَا النَّارَ لَا هِيَ أَطْعَمَتْهَا وَلَا سَقَتْهَا إِذْ حَبَسَتْهَا وَلَا هِيَ تَرَكَتْهَا تَأْكُلُ مِنْ خَشَاشِ الْأَرْضِ]. أخرجه البخاري.

روى أحمد وأبو داود عن عبد الله بن جعفر ( ) أن رسول الله (ﷺ) دخل حائط لرجل من الأنصار، فإذا فيه جمل، فلما رأى النبي (ﷺ) حن وذرفت عيناه، فأتاه رسول الله (ﷺ) فمسح ذفراه فسكت فقال: ((من رب هذا الجمل؟ لمن هذا الجمل؟)) فجاء فتى من الأنصار فقال: لي يا رسول الله، فقال: ((أفلا تتقى الله في هذه البهيمة التي ملكك الله إياها؟ فإنه شكا إلي أنك تجيعه وتدئبه)). ومعنى ذفراه: مؤخرة رأسه، ومعنى تدئبه: تتعبه.

وروى أبو داود وأحمد وابن حبان وابن خزيمة عن سهل بن الحنظلية –((ﷺ قال مر الرسول (ﷺ) ببعير قد لصق ظهره ببطنه، فقال: ((اتقوا الله في هذه البهائم المعجمة، فاركبوها صالحة، وكلوها صالحة)).

Outline

- Is it Ethical to use animal in research?
- the 3 R's rule
- The 5 rights
- Humane end points
- International laws to protect animal rights
- Bahrain act- 52
- Animal ethical applications

Is it Ethical to use animal in research?

Why not replace animal studies with studies solely on humans?

- ✓ Its not only about medicines for humans, but many medicines also developed on animals are used to treat animals.
- ✓ Almost 9 out of 10 medicines that are developed for humans are the same or very similar to those used by vets.
- ✓ If we do replace animals with humans, are all humans equal, do we all have equal rights. Should we replace the use of animals with the mentally/physically disadvantaged as occurred in the Nazi era or use criminals or those soon to be executed (as is suggested to occur in China). What about volunteers?
- ✓ Do you really know what you are letting yourself in for and the potential consequences of acting as a human volunteer (remind them of the recent TGN1412 trial), particularly the long-term consequences of acting as a subject.

- ✓ Is the person in the developing world who uses the money gained from volunteering to prevent their family from starving truly giving “informed consent”?

- ✓ Is it right to “export” our clinical trials to those less fortunate than ourselves?

Is it Ethical to use animal in research?

- There are, however, other views of the moral value (or status) of animals that influence the question of if and how animals are used in research.
- Animal rights theorists equate the lives of animals (or some animals more than others) with those of human beings and believe that humans do not have the right to use animals solely as a means to a human end. Others have taken political and social action to draw attention to their views.

Can animal models truly replicate human diseases?

- We have similar genes (mice share >90% of their genes with humans) and vital organs (in the same places doing the same things).
- Humans and animals get similar diseases e.g. dogs and cats get diabetes and asthma whilst ferrets develop a flu-like condition. In addition, the advent of genetic technologies has enabled us to create better models of human diseases
- There are some differences between species that result in differences in the ways in which individual species handle or metabolize compounds e.g. thalidomide.
- As we become more aware of these, we can design our studies accordingly.

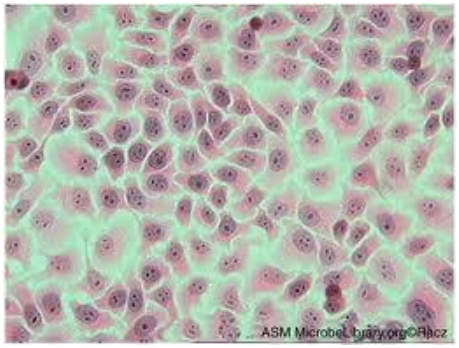
The Three R's of using animals in research

- The Three R's are principles of good science that scientists must adhere to when conducting animal-based research.
- First R- Replacement Using non-animal alternative wherever they exist in order that the only research done using animals is that which can be done no other way.

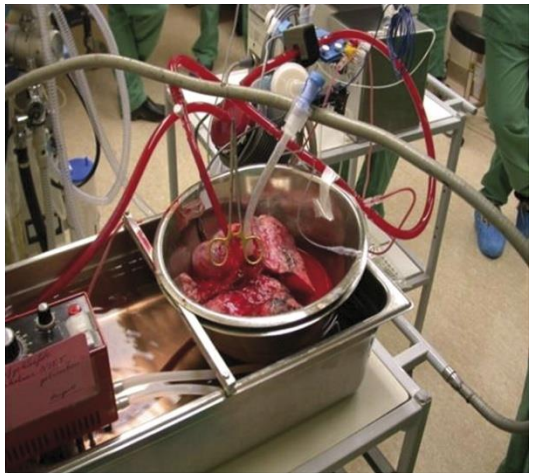
This is synthetic skin. It can be used in some research situations.

Difference between *in vitro*, and *in* studies

Available in vitro systems:



Cell line;
Isolated Haptocytes
Primary renal epithelium
Hematopoietic stem cells
3 D ,Co-culture



Isolated organs;
Lung, Skin, Intestine

No matter how good individual organ model is, they will not replace animal testing unless they are organized into a predictive integrated testing system to mimic the kinetics of a chemical in humans.

NOT YET POSSIBLE

Advantages and Challenges of *In Vitro* Testing for Nanoparticles Toxicology

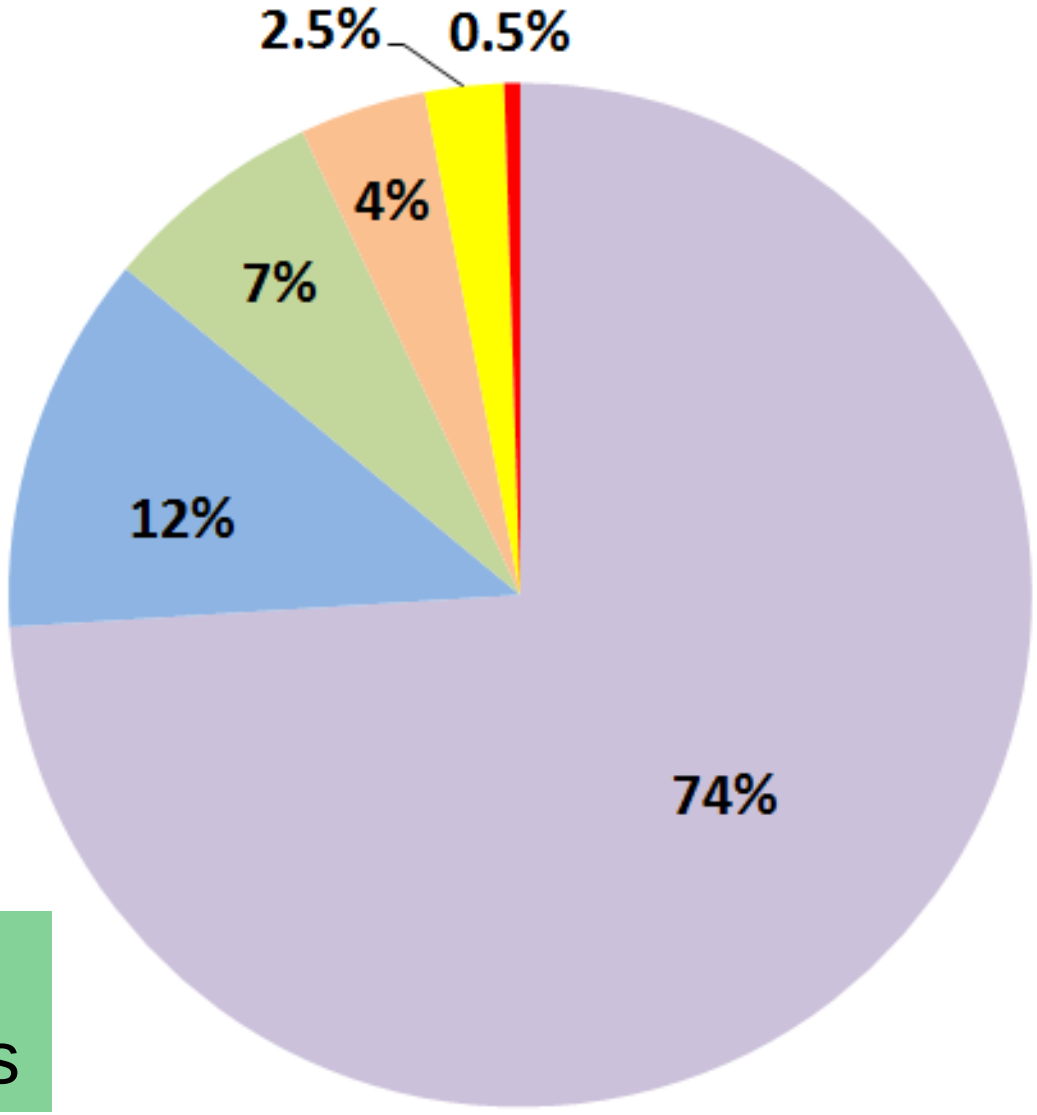
- Save animals
- Cost effective
- Efficient
- Homogeneous and stable
- Eliminate secondary effects caused by inflammation
- Easy to delineate the mechanism of toxicity

- *In vitro* environment lacks complete pattern of stimuli (cytokines, growth factors, extracellular matrix, other cells)
- Cultured cells do not represent *in vivo* phenotype
- Monocultures do not replicate *in vivo* responses
- Cell culture experiments do not replicate chronic exposure
- Luck of standardized methods of cell line maintenance and experimental protocols

Second R- Reduction

- Using as few animals as possible to attain statistically significant results, as well as finding ways to cut down on the number of animals used for any specific part of research.
- This is done mostly through experimental design and the use of statistics.

Which Percentage Applies To Which Animals?



Fish

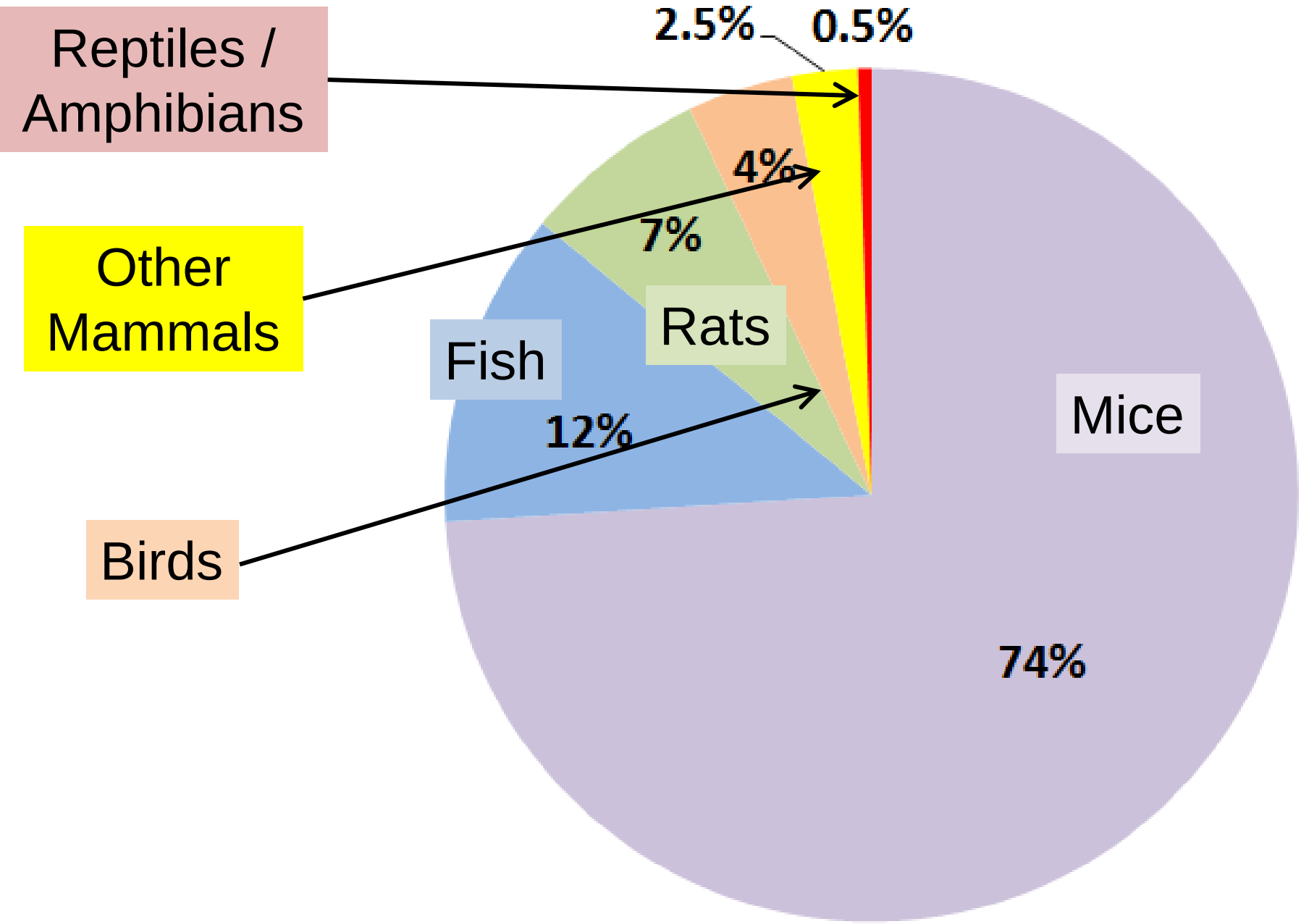
Birds

Rats

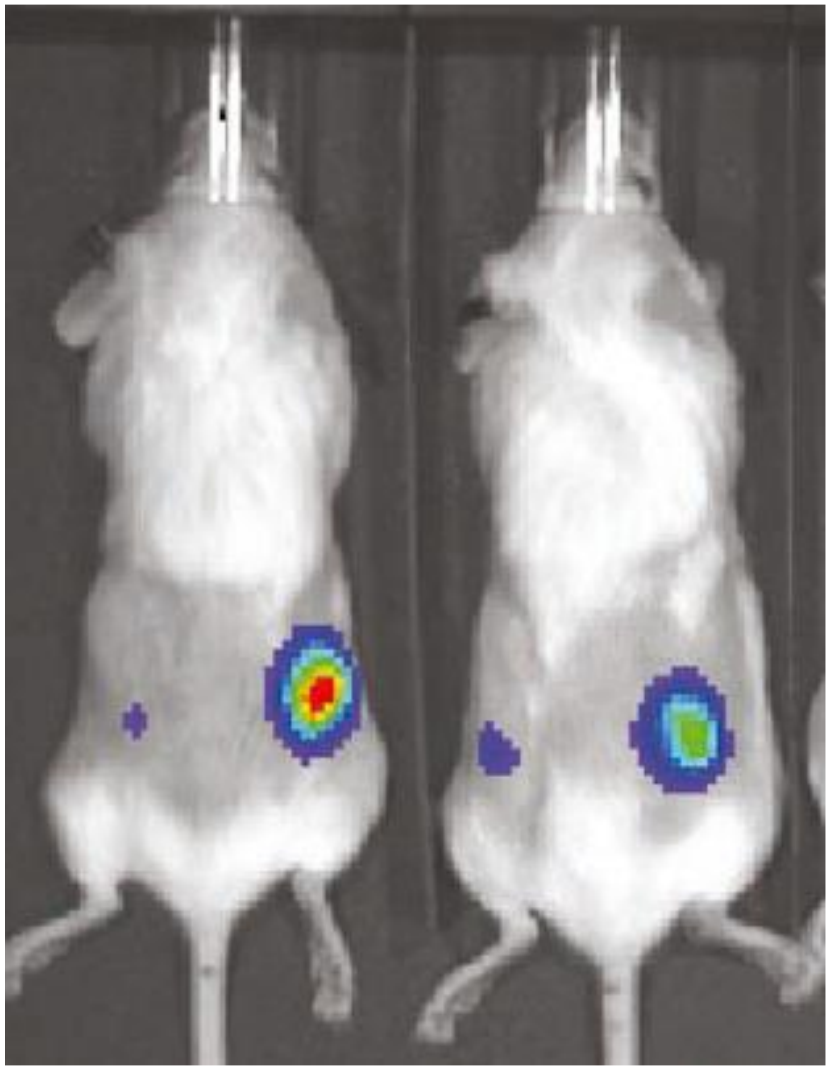
Mice

Reptiles / Amphibians

Other Mammals


Reptiles / Amphibians
Other Mammals
Fish
Birds
Rats
Mice
2.5%
0.5%
4%
7%
12%
74%

Second R- Reduction; Examples



Second R- Reduction; Examples 3

Synthetic cannabinoids nano-micelles for the management of triple negative breast cancer

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ABSTRACT

Triple-negative breast cancer (TNBC) is a highly heterogeneous disease with poor prognosis and inadequate therapeutic outcome. This contribution reports the use of a cannabinoid derivative, WIN55,212-2 (WIN) on the growth of TNBC in a 4T1 syngeneic mouse model. To reduce the well-known psychoactive side effects of cannabinoids, we prepared a nanomicellar formulation of WIN (SMA-WIN). *In vivo* biodistribution, *in silico* ADME predictions, anticancer activity, and psychoactive effect of WIN and SMA-WIN studies suggest that SMA-WIN formulation can reduce to greater extent tumor growth with milder psychoactive side effects when compared to free drug. Finally, the effects of WIN and SMA-WIN in combination with doxorubicin (Doxo), an established chemotherapeutic agent for the treatment of TNBC, were investigated *in vitro* and *in vivo*. SMA-WIN in combination with Doxo showed therapeutic efficacy and was able to reduce the tumor volume of TNBC murine model drastically. Moreover, SMA-WIN, while favoring drug tumor accumulation, minimized the adverse psychoactive effects that have impeded the use of this agent in the clinic. To our knowledge, this is the first report for the assessment of cannabinoid nanoparticles *in vivo* for the treatment of TNBC and its enhanced anticancer effect at low doses with Doxo. These findings suggest a new therapeutic strategy in the management of TNBC.

When applicable use the same animals for multiple experiments to obtain more/different set of data

Second R- Reduction; Examples 4

Input Data

Confidence Interval (2-sided)	90%
Power	90%
Ratio of sample size (Group 2/Group 1)	1

	Group 1	Group 2	Difference*
Mean	100	80	20
Standard deviation	15	10	
Variance	225	100	

Sample size of Group 1	10
Sample size of Group 2	10
Total sample size	20

*Difference between the means

•OpenEpi, Version 3, open source calculator SSMean (http://www.openepi.com/SampleSize/SSMean.htm) accessed Jan , 2019

OpenEpi development was supported in part by a grant from the Bill and Melinda Gates Foundation to Emory University, Rollins School of Public Health.

Reduction; Summary

- ✓ Refer to methods which minimize animal use and enable researchers to obtain comparable levels of information from fewer animals or to obtain more information from the same number of animals, thereby reducing future use of animals.
- ✓ Improved experimental design and statistical analysis
- ✓ Techniques, such as imaging , which require smaller numbers of animals
- ✓ Genetically similar animals
- ✓ Sharing of animals/animal tissues (e.g. whiteboards indicating future use in animal facilities)
- ✓ Use of new techniques/technologies
- ✓ Recording multiple parameters in single animal
- ✓ Pilot studies

To use animals at Animal Care Facility you must:

- Have an approved and up to date (active) animal use protocol
- Be certified by the Animal care unit to use animals in research or teaching
- Complete any additional training needed
- Ensure the humane care and treatment of animals

3rd R, Refinement

- to alleviate or minimize the pain, distress and other adverse effects suffered by the animals involved or enhance animal well-being.

The 5 rights

1. **Freedom from Hunger and Thirst**
   by ready access to fresh water and diet to maintain health and vigor.
2. **Freedom from Discomfort**
   by providing an appropriate environment including shelter and a comfortable resting area.
3. **Freedom from Pain, Injury or Disease**
   by prevention or rapid diagnosis and treatment.
4. **Freedom to Express Normal Behavior**
   by providing sufficient space, proper facilities and company of the animal's own kind.
5. **Freedom from Fear and Distress**
   by ensuring conditions and treatment which avoid mental suffering.

The 5 rights

First Freedom:

https://www.youtube.com/watch?v=9AUt2Zp3QZk

Second Freedom:

https://www.youtube.com/watch?v=2yirs78kWh4

Third Freedom:

https://www.youtube.com/watch?v=lPldWooY8-o

Fourth Freedom:

https://www.youtube.com/watch?v=USbBBPqcorQ

Fifth Freedom:

https://www.youtube.com/watch?v=C7YhrDukBeM

Quiz

How many animals are used to test cosmetics each year?

A. None
B. 50,000
C. 1.2 million

Humane end points and Euthanasia

- **Animal death is no longer accepted as an end point!!**
- **In AGU animal facility CO_2 is the accepted method of Euthanasia**
- **Training and certification are required.**

Selecting a Humane Endpoint

- Considering the experimental goal and what might be the earliest observable indicators of that effect prior to signs of animal pain and/or distress
- Considering that it may not be necessary to reproduce a condition or effect in detail (and potential severity) as may be seen in humans (for example, using animals to study arthritis may not actually require chronic painful joint disease to be manifested, just an increase or decrease in urinary excretion of cartilage breakdown products to be measured)
- Developing specific endpoints for each experiment after consulting references on humane endpoints and a clinical veterinarian, to determine likely adverse effects and clinical signs
- Making observations of the animals (e.g., behavior, physiology) to determine which are the most significant indicators of pain and/or distress in the specific circumstances of the research
- Determining which observations are the most significant predictors of further deterioration in the animal's condition, and identify the earliest point at which those signs appear, monitoring and recording the use of humane endpoints during the experiment using pilot studies with small numbers of animals to determine the onset and progress of adverse effects and identify early scientific and humane endpoints
- Including all information about humane endpoints used when publishing the results of the study
- ensuring appropriate training for staff who are monitoring animals for signs of adverse effects

Example: Humane Endpoint in tumor models

- Animal losing more than 20% of weight
- Weight loss of 10% over 24hr
- Tumors greater than 10% of body weight
- Animals showing signs of pain
- Self mutilation
- Bleeding
- Dehydration

International laws to protect animal rights

- https://en.wikipedia.org/wiki/Cruelty_to_animals

Bahrain act- 52

قانون رقم (٥٢) لسنة ٢٠١٤
بالموافقة على قانون (نظام) الرفق بالحيوان
لدول مجلس التعاون لدول الخليج العربية

نحن حمد بن عيسى آل خليفة ملك مملكة البحرين.

بعد الاطلاع على الدستور،

وعلى القانون رقم (٣) لسنة ١٩٧٥ بشأن الصحة العامة، وتعديلاته،

وعلى قانون العقوبات الصادر بالمرسوم بقانون رقم (١٥) لسنة ١٩٧٦، وتعديلاته،

وعلى المرسوم بقانون رقم (١٨) لسنة ١٩٩٧ في شأن تنظيم مهنة الصيدلة والمراكز الصيدلية،

وعلى قانون البلديات الصادر بالمرسوم بقانون رقم (٣٥) لسنة ٢٠٠١، المعدل بالقانون رقم (٣٨) لسنة ٢٠٠٦،

وعلى القانون رقم (٨) لسنة ٢٠٠٣ بالموافقة على نظام الحجر البيطري في دول مجلس التعاون لدول الخليج العربية،

وعلى قانون (نظام) الرفق بالحيوان لدول مجلس التعاون لدول الخليج العربية، الصادر باعتماده بشكل إلزامي قرار من المجلس الأعلى لمجلس التعاون لدول الخليج العربية في دورته الثانية والثلاثين المنعقدة في الرياض بالمملكة العربية السعودية يومي ٢٤ و٢٥ محرم ١٤٣٣هـ، الموافق ١٩ و٢٠ ديسمبر ٢٠١١،

أقر مجلس الشورى ومجلس النواب القانون الآتي نصه، وقد صدقنا عليه وأصدرناه:

Animal ethical applications AGU

- Word file