Using Laboratory Animals

The moral approach to working with animals, knowledge of the laws and special procedures, operating procedures, submitting ethical applications for experiments to IACUC

Virtual THERACAT meeting

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Tel Aviv University

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The Veterinary Service Center : Link: https://med.tau.ac.il/The-Veterinary-Service-Center

- Contact
- Information for researchers
- Forms
- Biosafety
- Health examinations

Performing experiments on animals – For and Against

- In many scientific and medical experiments it is mandatory to use animals, and some object to these experiments under the claim that they are not moral.
- Thanks to thousands of studies - **drugs, surgeries and medical technologies have been developed** and have saved millions of lives. Alongside the compassion we feel for animals and the need to prevent them from suffering, it is necessary to develop medical treatments based on animal experiments.
- Opponents of animal experiments argue that there are **alternatives in the form of simulators or models** that eliminate the need to test the drug, device or theory on an animal, but in fact no technology has yet been developed to mimic complex physiology and anatomy and no method has been developed to simulate blood flow analysis, heart rate, live tissue response and body with millions of complex actions occurring simultaneously.

Performing experiments on animals – For and Against

- **Are we allowed from the moral point of view to use animals for our needs?**
- Scientists tend to see the expected benefit of animal research as a key consideration for evaluating research ethics, while the main consideration for animal welfare advocates is the degree of suffering expected for the animal during the experiments.
- Almost all advocates of animal research agree that suffering of animals should be avoided as far as possible during the experiments.

Claims requiring animal research

- Advocates of animal research claims that humanity has achieved its medical achievements thanks to these experiments. Examples: vaccines against diseases (polio, measles), drugs, disease research and organ transplantation.
- For the past 40 years, all Nobel Laureates for Physiology or Medicine have based their research on animal experiments.
- In October 2014, six Israeli Nobel laureates and university presidents wrote a letter to the Prime Minister highlighting the importance of animal experiments to research.

Claims requiring animal research

- Importance of the **Complete Animal** - Without experiments on animals, medicine will not progress due to the fact that alternatives available for animal use are not full alternatives.
- **Animals are complex** and there are interactions between the various animal body systems, which cannot be traced their effects but to the entire animal body: for example, a new hypertensive drug test or the regulation of heart rate on isolated tissue culture cannot teach On the effect of the metabolic decomposition products of the same drugs on the body, after liver decomposition.
- The need for **training and gaining experience** - Surgeons become skilled in their work with accumulated surgery practice. Many doctors claim that practice surgery on corpse and living animal is necessary for the experience of the trainee.
- The need of animals for **basic research** - Many scientists believe that a absolute ban on animal experiments will greatly delay our efforts to add and acquire basic knowledge about life processes. Medicine relies on biology hence basic biomedical research is a prerequisite for its advancement.

Arguments for negating animal experiments

- Animals and humans are **not comparable** - the differences between animals reactions to a given substance are so great that it makes no sense to draw conclusions about the potential effect of a drug on humans according to its tested effect on animals
- **Animal disease models are not reliable** - in many studies these models produces similar syndromes to human disease but different disease factors.
- **Existing Alternatives** - Today we have all the means to promote both basic biological research and animal-free medicine, including cell cultures, computer simulations, non-invasive imaging methods, epidemiological research etc

Animals in Research – Pros & Cons

- Following the complex arguments for and against animal research, the animal welfare institutional committees were established under the amendment to the 1985 Animal Welfare Law
- In Israel, animal research was regulated legally by means of the Israeli law (Animal Experiments 1994). According to section 2 of the Law, an animal experimentation council was established.
- Handling and treatment of the animals are in the responsibility of the researchers themselves. Only proper education and training can strengthen their sensitivity and promote humane treatment of animals.

Contribution of research in animals to science and medicine

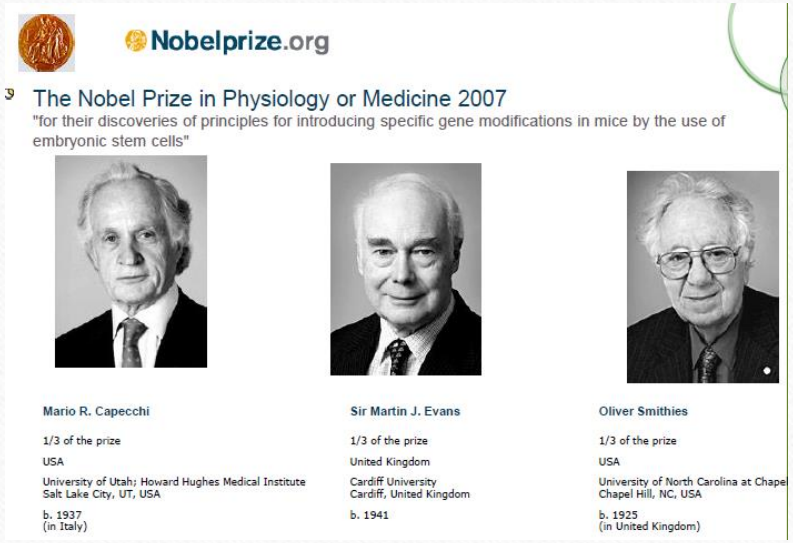
- Israeli examples
  - ✓ Copaxon
  - ✓ Drug coated stents
  - ✓ Cardiac valve for catheterization.
  - ✓ Pegunigalsidase alfa
- FDA
- Nobel Prizes

https://www.youtube.com/watch?v=X1Uk9Jvbut0

The attached video is showing a breakthrough procedure developed using small ruminants

Nobel prizes following animal research

- http://www.animalresearch.info/en/medical-advances/nobel-prizes/



https://www.fda.gov/drugs/resourcesforyou/consumers/ucm143534.htm

Information for Consumers (Drugs)

Educational Resources: Free Drug-Related Publications

JumpStarting Drug Review

Questions & Answers

Buying & Using Medicine Safely

Tips for Seniors

Tips for Parents

The FDA's Drug Review Process: Ensuring Drugs Are Safe and Effective

The path a drug travels from a lab to your medicine cabinet is usually long, and every drug takes a unique route. Often, a drug is developed to treat a specific disease. An important use of a drug may also be discovered by accident.

For example, Retrovir (zidovudine, also known as AZT) was first studied as an anti-cancer drug in the 1960s with disappointing results. Twenty years later, researchers discovered the drug could treat AIDS, and Food and Drug

Topics on this Page

- Protecting America's Health Through Human Drugs
- FDA Drug Approval Process Infographic (Horizontal)
- FDA Drug Approval Process Infographic (Vertical)

drug--from the design of clinical trials to the severity of side effects to the conditions under which the drug is manufactured.

- The Quality of Clinical Trials

Stages of Drug Development and Review

Investigational New Drug Application (IND)--The pharmaceutical industry sometimes seeks advice from the FDA prior to submission of an IND.

Sponsors--companies, research institutions, and other organizations that take responsibility for developing a drug. They must show the FDA results of preclinical testing in laboratory animals and what they propose to do for human testing. At this stage, the FDA decides whether it is reasonably safe for the company to move forward with testing the drug in humans.

Clinical Trials--Drug studies in humans can begin only after an IND is reviewed by the FDA and a local institutional review board (IRB). The board is a panel of scientists and non-scientists in hospitals and research institutions that oversees clinical research.

IRBs approve the clinical trial protocols, which describe the type of people who may participate in the clinical trial, the schedule of tests and procedures, the medications and dosages to be studied, the length of the study, the study's objectives, and other details. IRBs make sure the study is acceptable, that participants have given consent and are fully informed of their risks, and that researchers take appropriate steps to protect patients from harm.

Phase 1 studies are usually conducted in healthy volunteers. The goal here is to

The 3Rs

- Replacement
- Reduction
- Refinement

Advancement of Humane Techniques

- The 3Rs terminology refers to a study published in 1959
- William Russell & Rex Burch
- Conducted a systematic study of the ethical aspects and set norms for working with laboratory animals

William Russell & Rex Burch

Humanity vs. Inhumanity

- Russell and Birch based their research on a philosophical term of humanity versus inhumanity in the context of animal research. They noted that true humanity, which distinguishes humans of all other species, was the ability for social cooperation, close bonding, and compassionate and empathic attitude toward other species.
- While they assumed that biologists treated their animals as humanely as possible within the boundaries of experimental protocols, they did note that some procedures were inhuman

Humanity vs. Inhumanity

- By analyzing and documenting "humanity" or "inhumanity" in context of biological experiments, the authors hoped to promote the development of human experimentation techniques and reduce the pain and stress of laboratory animals.
- Russell and Birch limited their definitions of "humane" and "non-humane" to experimental procedures only; With no criticism or moral judgment on the experimenters involved.

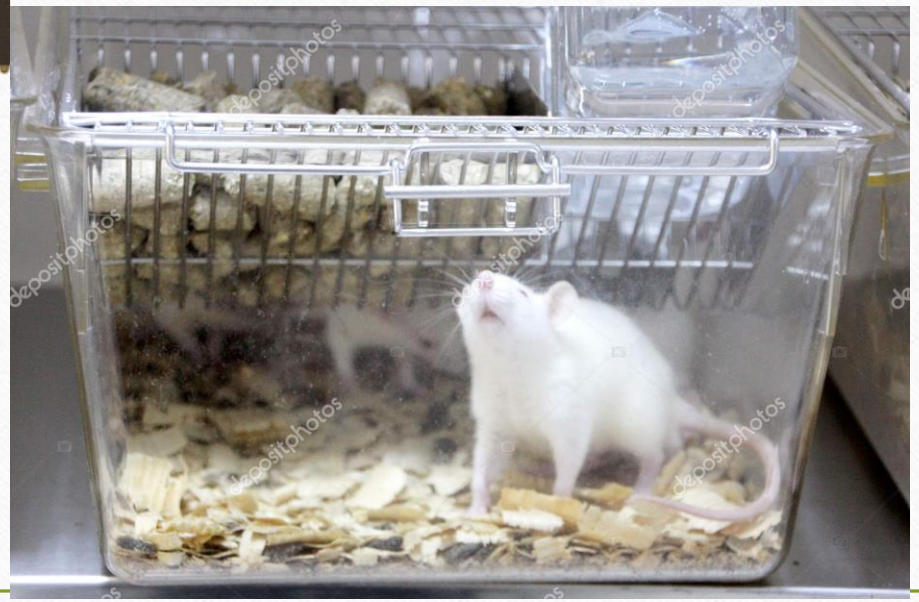
Stressors criteria

- How does "inhumanity" measure? Russell and Burch used the criteria of pain or "distress" experienced by the animals.
- Physiological and endocrine parameters suggested as an objective measurements of stress.
- Each experiment that included using negative reinforcement (punishment) as a response motive was defined as a source of stress and fear.
- The behavior of the animals towards the experimenters served as another indicator of well-being or distress (e.g., incidents of non-social behavior such as bites and scratches, the need for restraint during procedures with working with a peaceful animal, etc.).

Animals surrounding

- Another criteria examined by Russell and Burch was the environment, both social and physical of the experiments in mice.
- Laboratory environment planning is often done with an emphasis on the experimenter's needs and convenience and less on the animal's needs (temperature at the facility).

Home cage



Standard animal house cage

Metabolic cage

The research

- Russell and Bırch's first step in research was to collect data on animal experiments: their sources included a survey from 1952 (data analysis by the British Medical Research Council) which provided the following information: Animals used, Lab type, Research type and number of animals.

The research

- The researchers then analyzed each humanity learning procedure.
- They examined the prevalence of inhumanity and rated the severity of stress experienced by the animals from moderate to severe. Particularly severe forms of stress such as potentially fatal or postoperative pain procedures, were classified separately.
- This data analysis formed the basis for humane guidelines for biological experiments, known as **the three Rs**.

Replacement

- Replacement was the most radical proposal: replacing animal use with experiments using non-sensing organisms.

- Absolute Replacement: Use of microorganisms, and plants have been suggested as possible alternatives. This is a complete replacement as higher animals are not required at any stage.
- Relative Replacement : Use of non-vertebrate cells or animals (such as molluscs or flies), In vitro-cell culture techniques from animal tissues have been experimentally defined as replacement since the experiments themselves have been conducted with non-sensory material, but still use is made.

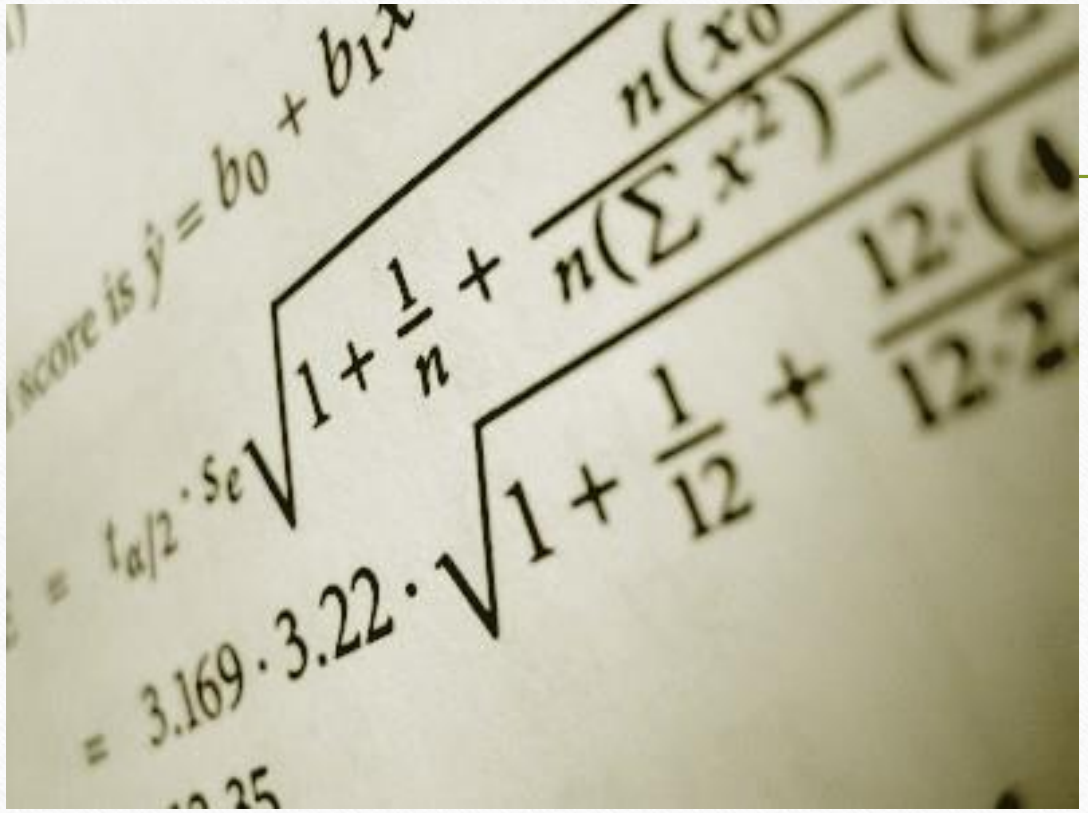
Who will suffer more?

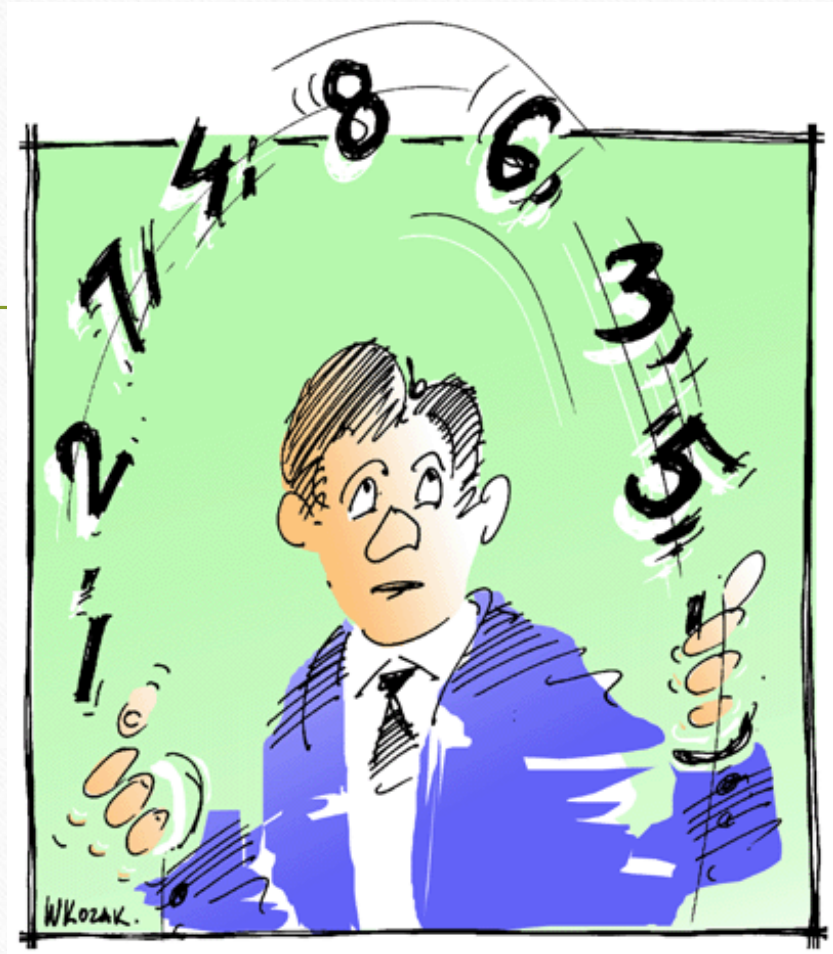
Reduction

- Reduction of the number of animals used to obtain a certain amount of knowledge with the requisite precision.
- Reduction comprises instances where a smaller number of animals can be used in a given situation in a specific experimental model than previously. Consequently, reduction must always be a target for lowering the number of animals used for generating a certain amount of knowledge and not as a target for whether a given organization, state or company has reduced the overall number of animals within a given time frame compared to corresponding previous periods of time.

Reduction

- The development of animal models with a view to enhancing the scientific results achievable from each individual animal is therefore also considered reduction. Reduction can be obtained using measures such as screening with animal-free models or technologies prior to animal testing, by using animals with the exact characteristics that one is interested in or by designing more systematic experiments.


score is $\hat{y} = b_0 + b_1 x$
$= t_{\alpha/2} \cdot s_e \sqrt{1 + \frac{1}{n} + \frac{n(x_0}{n(\sum x^2) - (}}$
$= 3.169 \cdot 3.22 \cdot \sqrt{1 + \frac{1}{12} + \frac{12 \cdot (}{12 \cdot 2}}$


WKozak.

Refinement

- Experiments should be refined as far as possible by changes in protocols that reduce the incidence and severity of stress for laboratory animals. (Handling, analgesia, supportive care, sterile environment)

Refinement - Enrichment

Rodents' cage with ground paper enrichment

https://www.nc3rs.org.uk

A website dedicated to the 3 Rs

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

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The 3Rs | Our science | 3Rs resources | Funding | News & blogs | Events | About us

NC3Rs resources

ARRIVE

FDA Experimental Design Assistant

SYRF SYSTEMATIC Review Facility

Experimental design

Resources to help you improve the design and reporting of your research using animals.

Tickling rats: an enrichment to improve rodent welfare

Re-use of needles: is this an

Reference

- Russell, W.M.S. and Burch, R.L., The Principles of Humane Experimental Technique. Methuen, London, 1959.
  Reprinted by UFAW, 1992: 8 Hamilton Close, South Mimms, Potters Bar, Herts EN6 3QD England. ISBN 0 900767 78 2

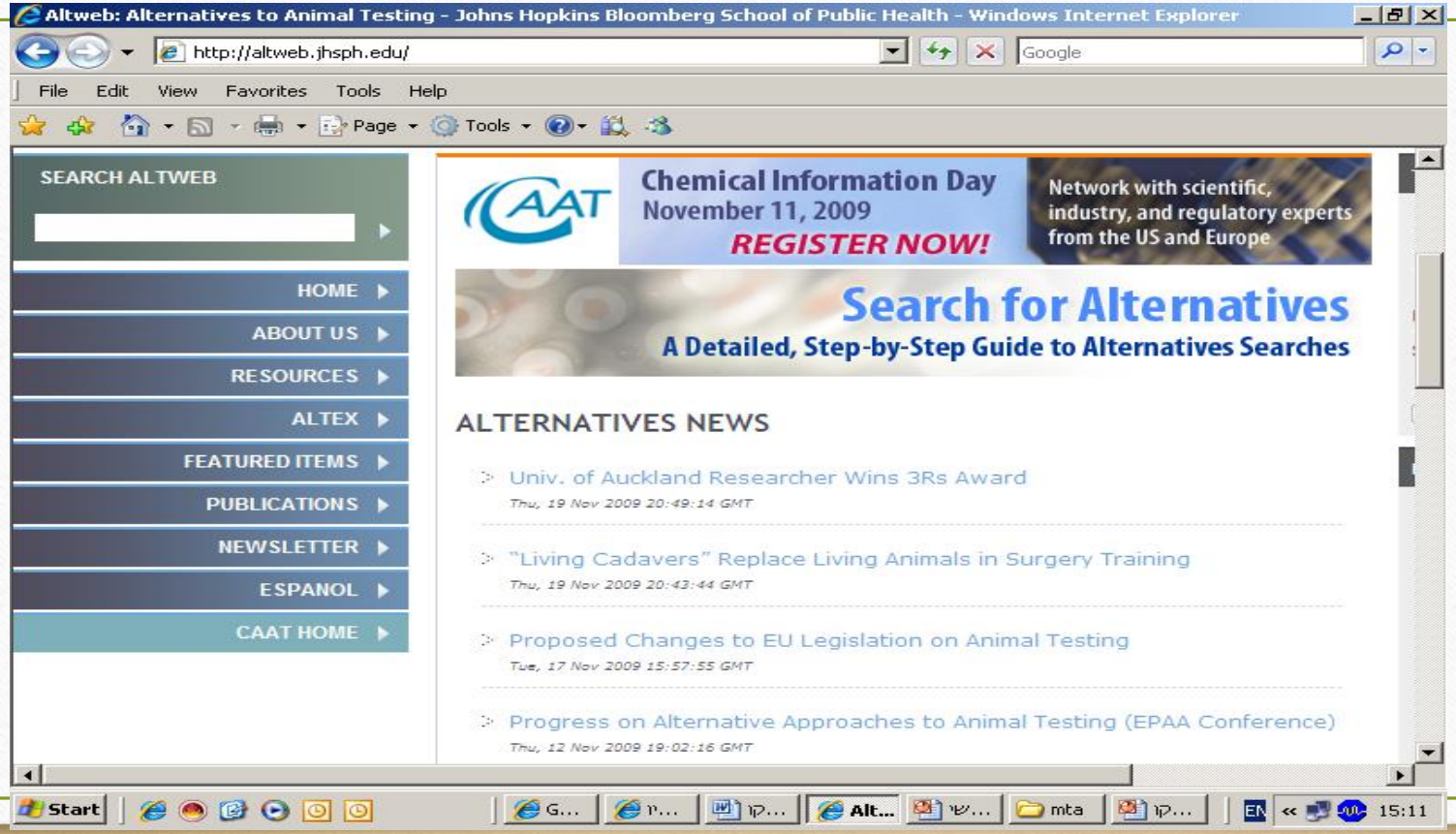
Responsibility

- Added as the fourth R and emphasizes the importance of the researcher's responsibility towards the animals.

The importance of conducting ethical research for the results of the study

- Replacement - decrease in the phylogenetic scale will allow greater uniformity
- Refinement- sterile environment, easier protocol, analgesia.
- Reduction - Proper design of the experiment.

It is mandatory to look for replacements in the following websites before asking for lab animals



Annex I—Summary status of the adoption of Test Guidelines based on alternative methods in the OECD TG programme (2012-2015)

Table 1 summarises the status of adoption of OECD test guidelines on alternative methods from 2012 to 2015. It should be noted that beside TGs, also Guidance Documents and new projects on alternative methods were respectively adopted and included on the OECD Work programme during that period. For additional information, please consult the OECD website of the Test Guideline Programme: http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicalsandrelateddocuments.htm

Table 1. Status of adoption of OECD Test Guidelines based on alternative methods 2012-2015

Nr.	Toxicity area	Test method description	Acceptance status
1	Skin corrosion	Reconstructed human Epidermis (RhE) test methods as included in OECD TG 431/EU TM B.40 bis	Adopted in 2004; updated version (sub-categorisation, inclusion of performance standards, inclusion of SkinEthic™ RHE and epiCS®) adopted in 2013. Revised version including sub-categorisation with the epiCS® test method adopted in 2014. Updated in 2015 for the deletion of the performance standards (published separately on the Series on Testing and Assessment No. 219), inclusion of paragraphs referring to the IATA for Skin Corrosion and Irritation (OECD GD No. 203) and inclusion of the use of HPLC/UPLC-spectrophotometry as an alternative procedure to measure tissue viability (increasing the applicability domain of the test methods to coloured substances interfering with the measurement of MTT-formazan)
2	Skin corrosion	Transcutaneous Electrical Resistance (TER) test method as included in OECD TG 430/EU TM B.40	Adopted in 2004; updated version (inclusion of performance standards) adopted in 2013 Updated in 2015 for the deletion of the performance standards (published separately on the Series on Testing and Assessment No. 218) and the inclusion of paragraphs

European Commission

JRC SCIENCE AND POLICY REPORT

EURL ECVAM Status Report on the Development, Validation and Regulatory Acceptance of Alternative Methods and Approaches (2015)

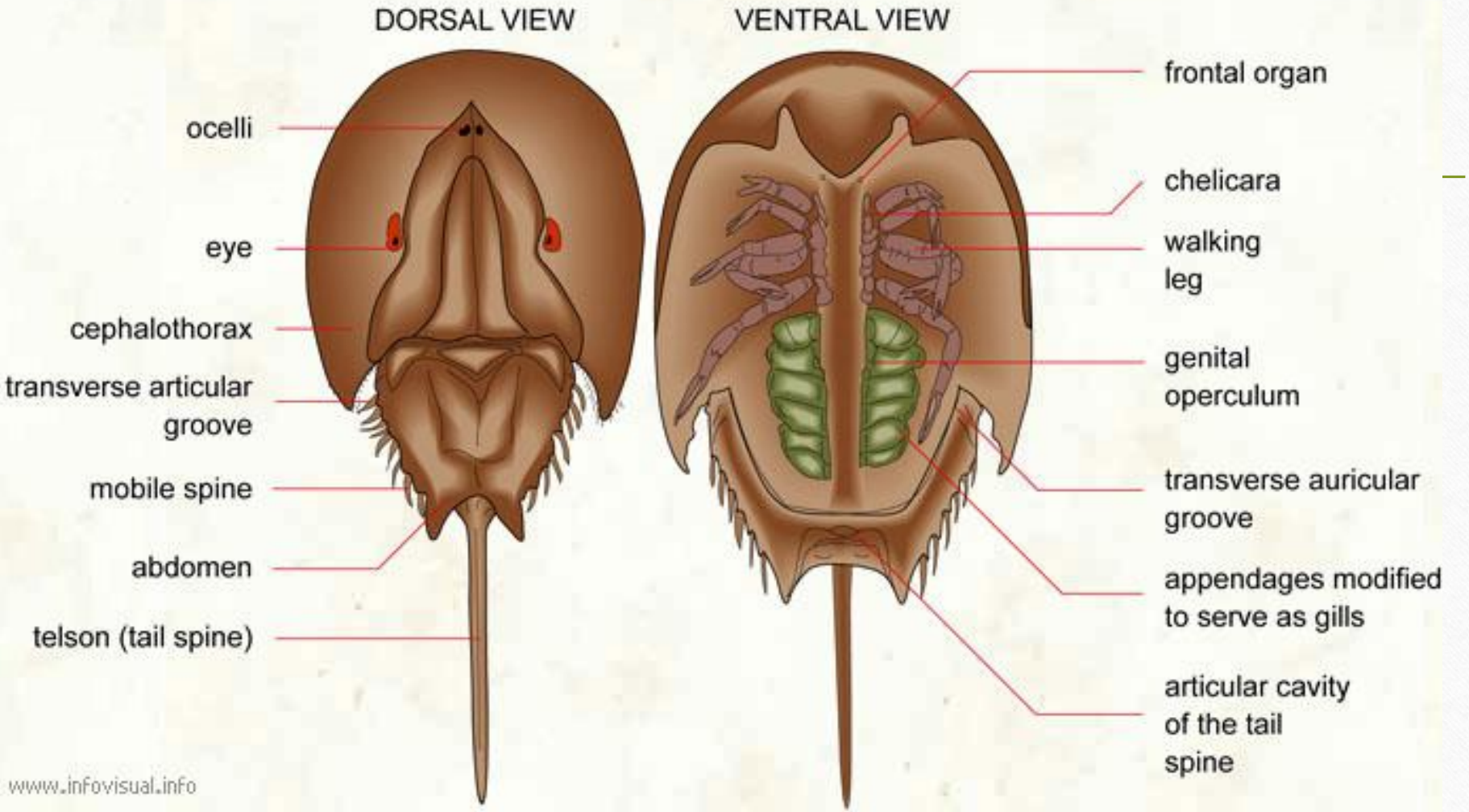
Valérie Zuang, Bertrand Desprez, João Barroso, Susanne Belz, Elisabet Berggren, Camilla Bernasconi, Jos Bessems, Stephanie Bopp, Silvia Casati, Sandra Coecke, Raffaella Corvi, Coralie Dumont, Varvara Gouliarmou, Claudius Griesinger, Marlies Halder, Annett Janusch-Roi, Aude Kienzler, Brigitte Landesmann, Federica Madia, Anne Milcamps, Sharon Munn, Anna Price, Pilar Prieto, Michael Schäffer, Jutta Triebe, Clemens Wittwehr, Andrew Worth and Maurice Whelan

September 2015

Replacement examples

- Imaging
- In Vitro Alternatives: LAL- Limulus Amebocyte Lysate (endotoxin), 3D organoids, monoclonal antibody.
- Insects, shellfish and other non-invertebrates.
- Use of dead animals
- Teaching

MORPHOLOGY OF A LIMULUS (horseshoe crab)



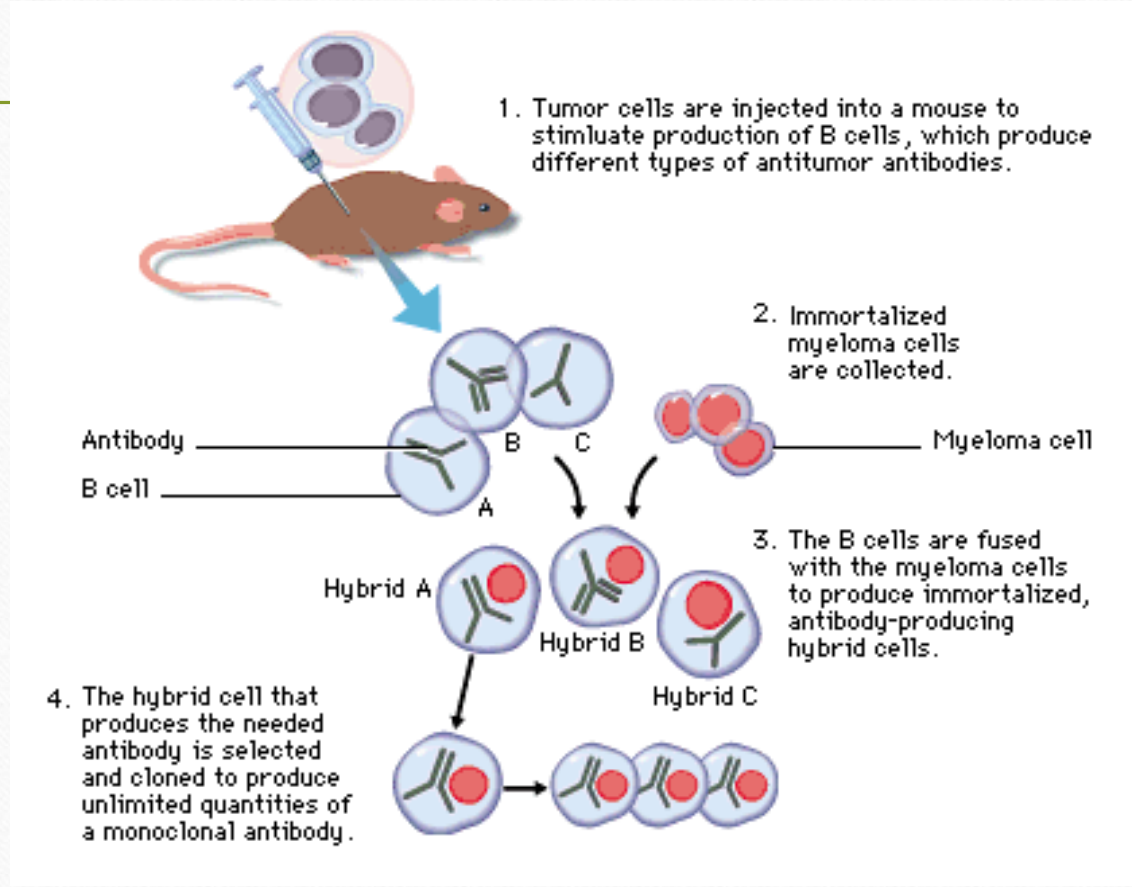
Limulus Amebocyte Lysate LAL

- LAL is a test for identification of endotoxins (substances that build the cell walls in negative gram bacteria). Endotoxins are pyrogenic substances, which increase the body's temperature and are dangerous to the person upon entry into the bloodstream. Therefore it is important to check their concentration in the pharmaceutical industry and medical products that come into contact with the bloodstream.
- Limulus Amebocyte Lysate is the most sensitive and specific test available for this purpose. The reagents provide a wide sensitivity range, great repeatability, and excellent durability.

Advanced Imaging Methods - Imaging Center

- The complete animal imaging system includes fluorescence imaging, bioluminescence imaging, an internal confocal fiber imaging device with minimal invasiveness, as well as ultrasound and micro-CT devices at the imaging center.

Monoclonal antibody production



Alternatives - Monoclonal antibody production

- Production of monoclonal antibodies by bioreactors: quality control and validation strategies for the manufacturing industry

ELSEVIER

Enzyme and Microbial Technology
Volume 17, Issue 3, March 1995, Pages 225-230

Paper

Monoclonal antibody production in hollow-fiber bioreactors: Process control and validation strategies for manufacturing industry

A. Handa-Corrigan *, S. Nikolay *, D. Fletcher †, S. Mistry †, A. Young †, C. Ferguson †

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https://doi.org/10.1016/0141-0229(94)00012-G