Translation and experimental animal use:
How can we improve?
17.1.2019

Standards in the mouse house:
Quality and publication practise

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„ A mouse´s house may ruin studies: Environmental factors lie behind many irreproducible rodent experiments“

(Reardon, Nature **2016** (530): 254)

Standardizing surrounding conditions is the basis of a scientific, reproducable experiment, but:

- Is the standard high enough?
- Is the standard too high?

Or, perhaps ...

- Applied to the wrong situation?

Is the standard as high as it should be?
As high as we state?
A few examples:

Parameter	Standardisation ?
room temperature	yes (too low?)
light / dark period	yes
light intensity (lx)	no, only recommendations
light quality	no
hygiene	pathogen and opportunistic germs, parasites: predominatly yes microbiome: no
water decontamination	no; can be autoclaved, UV irradiated, acidified
cage dimensions	yes, in Europe (within a certain range)
cage microenvironment	European standard: minimum of litter and nesting material
group housing	recommended; we still don´t know, what to do with male mice….
olfaction, odor	no

Is the standard too high/applied in the wrong place?

Do the standard conditions meet the animals‘ needs?
Do the standard conditions support the comparability of laboratory mice and human patients?

Environmental standardization: cure or cause of poor reproducibility in animal experiments?

Richter, Garner, Würbel, Nature Methods 2009, Vol 6(4): 257-261

Süddeutsche.de Wissen

19. April 2016, 18:50 Labor

Labormäusen ist es viel zu kalt

Die Tiere fühlen sich unwohl. Forscher fürchten, dass dadurch Versuchsergebnisse verfälscht werden.

Von Christian Endt

In vielen medizinischen Labors ist es zu kalt, sodass die dort lebenden Mäuse frieren müssen. Das könnte die Ergebnisse von Studien verfälschen, warnen Ärzte nun in einem Meinungsbeitrag in der Fachzeitschrift *Trends in Cancer*. Bonnie Hylander und Elizabeth Repasky vom Roswell Park Cancer Institute im US-Bundesstaat New York verweisen darin auf eine ganze Menge von Untersuchungen, die das Leiden der Mäuse bei Kälte belegen. Richtig wohl fühlen sich die Nagetiere erst bei 30 bis 32 Grad Celsius.

„Laboratory mice are freezing“

Mice prefer temperatures between 20-30°C when active,
30 - 32°C when resting (3 mice/cage, no nesting material)

→ is the standard room temperature with 20-24°C to low?

Hylander BL, Repasky EA: Thermoneutrality, mice and cancer, Trends in Cancer **2016** Apr 2(4): 166-175
Gaskill BN et al: Some like it hot: Mouse temperature preferences in laboratory housing. Appl Anim Behav Sci 2009, 116:279-285
Council of Europe, European Convention 123, appendix A, 2006

Individually Ventilated Cages Impose Cold Stress on Laboratory Mice: A Source of Systemic Experimental Variability

David LM et al: Journal of the American Association for Laboratory Animal Science, 2013, 52(6): 738-744

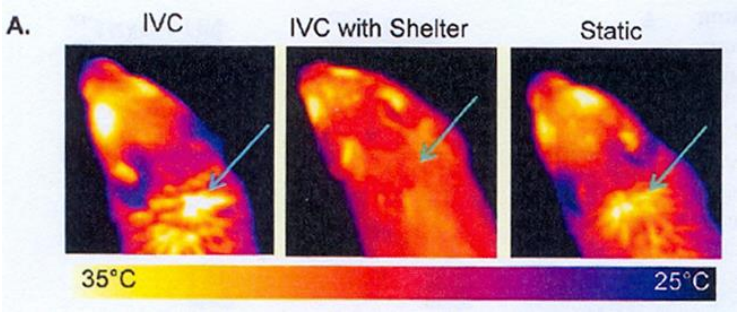


Fig. 1(A): Thermography: activated brown fat tissue

Results:

IVC housing leads to chronic cold stress, cold-induced thermogenesis, enlarged adrenal glands and reduced tumor growth

→ Influence on experimental data, systemic mistakes, reduced comparability of results from open housing

→ Would it be better not use IVC housing in research facilities?

Material and Methods:

Animals: male mice, strains CB17/lcr-Prdc^{scid} and Crl:Nu-Foxn1^{Nu}
Single housing
Room temperature: 20-21^{o}C
3 experimental groups:

1. IVC with red mouse igloo, no nesting material
2. IVC without igloo or nesting material
3. Static/ open cages without igloo or nesting material

Cage type: Inno Vive

homepage Fa. Inno vive, San Diego, CA

Is the standard cage sufficient?

or

Is this an impoverished environment and the mice, in general, live under non-physiological, unhealthy conditions?

Today we are still far away from the demands of Directive 2010/63/EU, appendix III, part A3.3b):

All animals...

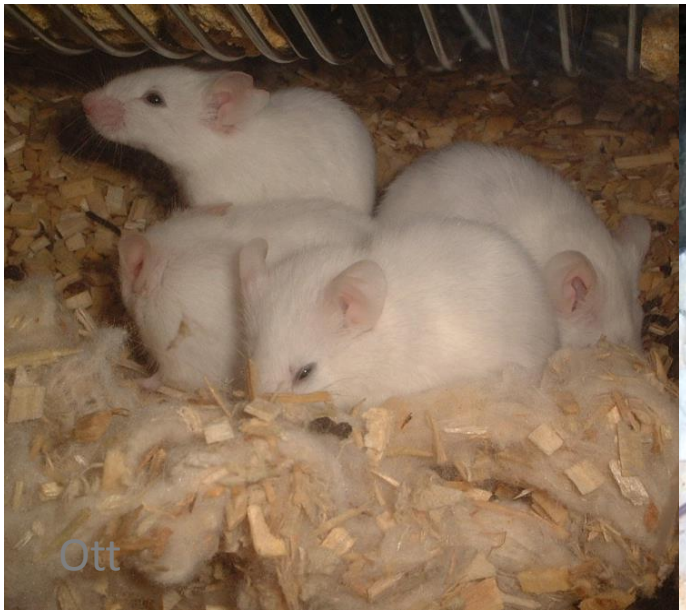
- should be provided with a complex environment
- should be allowed to display species-specific cognitive, manipulative and locomotor behaviour
- should have some control over their environment
- individual requirements should be considered

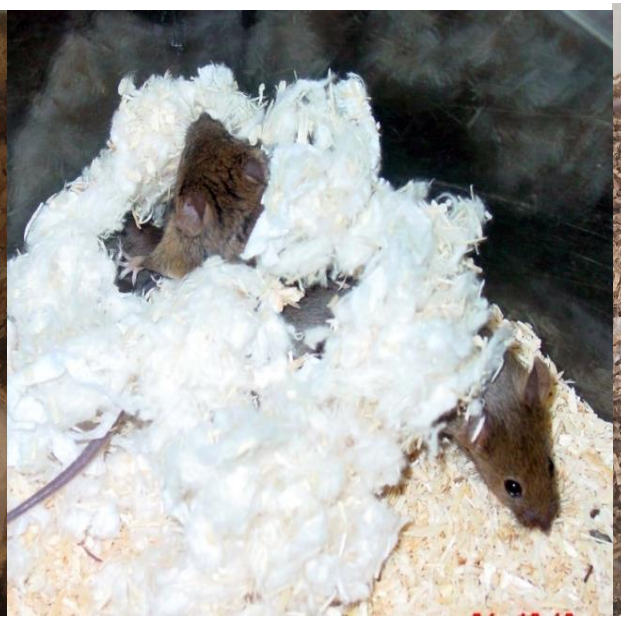
Stereotypic reaction in a mouse triggered by changing cages

But it should not be overlooked that:

- Streotypies in mice are strain-specific; ICR mice are used in certain studies *because* they easily display stereotypies
- In some publications the „standard cage“ is a still a cage without nesting material; this is not in accordance with European legislation
- Some cage designs are not used in Europe

European standard cages have to include enough nesting material to allow the mice to build a **complete, covered nest,** and the mice should be kept in **groups**





Nesting material →

- •micro climate (≥ 34°C inside the nest)
- •choosing between temperature zones
- •provides for other acitivities, such as building a nest, manipulating, cuddling, hiding and avoiding, …

Room temperature better above 20°C, at least 21°-22°C?

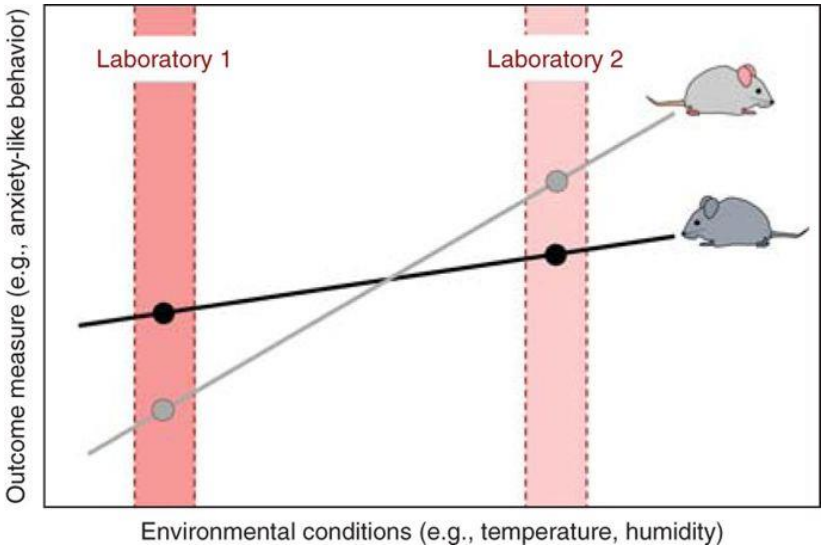
Conclusions:

- World-wide standardization is inadequate in many respects
- the European standard meets the animals‘ needs in some basic respects, but according to Directive 2010/63/EU a greater degrees of environmental enrichment is required
- More enrichment, for example supporting curious behaviour, will lead to less standardization and higher heterogeneity
- Many aspects such as handling, operating schedules, noise, odor ... can hardly be standardized

Is there a way out?

It is possible that controlled, systematic heterogenization (different enrichment measures, different locations/labs,…) will be a solution

But: This would require larger numbers of animals (double? four-fold??) !!!



Richter SH: Systematic heterogenization for better reproducibility in animal experimentation. Lab Anim (NY, 2017, Aug 31; 46(9): 343-349

Publication of methods:
What is specified about mice, mouse housing, caretaking and hygiene levels?

73 of the 200 most recent articles on mouse experiments listed in PubMed were analyzed for this purpose*

		yes[+]	no[+]
Mice	sex	68	5
	single- or group housed	22	51
Cage	static or IVC	8	65
	cage dimensions	6	67
	enrichment/nesting material,…	4	69
Room	light/ dark period	43	30
Hygiene/ microbiology			
	SPF or other statement	15	58

*Key words „laboratory mouse AND animal experiment“: 7606 total, the 200 most recent from Februrary to June where surveyed, only free-online-access, only experiments (no reviews, cell culture reports,…)

[+] mentioned/not mentioned, regardless of quality

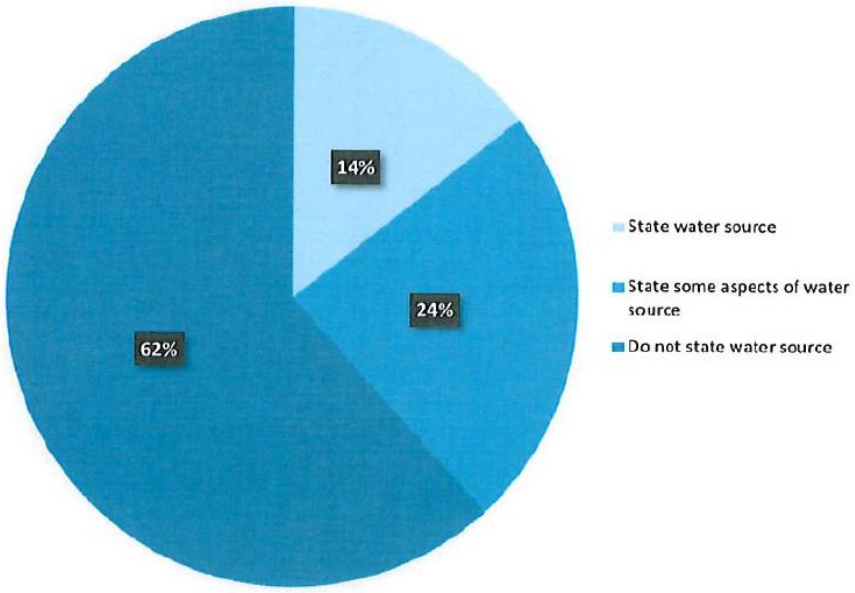


Figure 1. The majority of microbiome research literature does not report the complete details of water source. Of the 76 primary research articles surveyed for the current commentary, 62% of the articles did not state any information regarding the water source provided to their *in vivo* animals (47 articles.) While 24% stated some aspect of their water source, but not enough to be repeated by another researcher (18 articles.) Only 14% of the articles listed what the current authors would consider adequate detail with regards to the water source being provided to experimental animals (11 articles.).

Barnett JA, Gibson L: H_2Oh No! The importance of reporting your water source in your *in vivo* microbiome studies, Gut microbes 2018, https://doi.org/10.1080/19490976.2018.1539599

Thank you for your attention!