Your review report

Manuscript

How many rats and mice are used in American laboratories: a reproducible evidence-based estimate

Confidential feedback for the Editor

Your recommendation	• *Revise*
Is the study design appropriate to answer the research question (including the use of appropriate controls), and are the conclusions supported by the evidence presented?	• *No, but these points can be addressed with revisions*
Comments	*I thank the author very much for this important article. The absence of reliable statistics on mice and rat usage in animal research in the USA makes the assessment of the impact of 3R measures virtually impossible. It is therefore very laudable that the author tried to shed additional light on this issue by providing an estimate of his own. There are two issues that should be addressed, however: 1) A minor error: There is a discrepancy between the total MR numbers reported in the methods section and the total MR numbers reported in table one. It is probably a copy-past-error, as the total number indiciated in methods section corresponds to the number of RM used in institution 13 alone. 2) The author's extrapolation of the animal numbers from a subset of NIH-funded institutions to all the institutions in the USA hinges on the assumption that the ratio of RM numbers to AWA-covered animal numbers of the selected NIH-funded institutions is representative of all institutions that use RM and AWA-covered animals in reserach. In the absence of other information, such an assumption might be justified. However, the nature of regulations involving animal research in the preclinical setting and available data*

from Europe cast some doubt on it: Much of publicly funded research is basic research where there usually aren't regulatory restrictions with regard to the animal species that *must* be used for certain experiments. On the other hand, a lot of translation, preclinical and safety studies underly regulatory rules as to which animal species must be used. For example, for drug research most regulatory bodies demand studies in at least one non-rodent species before the drugs can be tested in humans. This type of reserach is often conducted by research institutions who are privately funded (e.g. by pharmaceutical companies) and thus were not covered by the author's data. Hence, it is to be expected that the number of AWA-covered species is relatively higher in privately-funded research institutions than in NIH-funded institutions due to the mentioned regulations. The author's extrapolation would therefore overestimate the total number of rats and mice that are used in the USA. This point is partially supported by empirical data from Europe. For example, the Swiss animal reserach statistics allows a relatively fine-grained assessment of which animal species are predominantly used in which type of research institutions (https://www.tv-statistik.ch/de/erweiterte-statistik/, only in French and German, unfortunately). It can be seen, for example, that the number of rabbits, dogs, cats and primates used in industry is alomst double the number used at universities and public research hospitals. Similarly, data from Germany shows that non-RM species are overrepresented in translational and applied research and in regulatory use compared to their use in basic research (https://www.bmel.de/SharedDocs/Downloads/DE/_Tiere/Tierschutz/Tierversuche/Ve __blob=publicationFile&v=1, page seven). Similar issues apply with regard to the extrapolation of the severity degrees. These potential limitations should be addressed in the text - ideally by providing an additional estimate incorporating the available data from Europe regarding the distribution of animals across different categories of institutions.

Are the methods sufficiently described to allow the study to be repeated?	• *Yes*
Is the use of statistics and treatment of uncertainties appropriate?	• *No*

| Comments | *see remarks above regarding assumptions of equal ratio of AWA-covered animals to RM in NIH-funded institutions and other research institutions.* |

| Is the presentation of the work clear? | • *Yes* |

| Are the images in this manuscript (including electrophoretic gels and blots) free from apparent manipulation? | • *Not applicable* |

| Comments | *no images provided* |

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Feedback for the author(s)

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