# Reporting checklist for study using laboratory animals.

Based on the ARRIVE guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the ARRIVEreporting guidelines, and cite them as:

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen O, Rawle F, Peynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T and Wurbel H. The ARRIVE Guidelines 2.0: updated guidelines for reporting animal research.

| | | Reporting Item | Page Number |
|---|---|---|---|
| **Essential 10** | | | |
| Study design | #1a | Give details of the groups being compared, including control groups. If no control group has been used, the rationale should be stated. | Methods/line 120-128; Results/line 208-228. |
| Study design | #1b | Give details of the experimental unit (e.g., a single animal, litter, or cage of animals). | Methods/line 111-113. |
| Sample size | #2a | Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. | Methods/line 120-121; Results/line 185-188, 208-227. |
| Sample size | #2b | Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done. | N/A. The sample size calculation is not conducted. |
| Inclusion and exclusion criteria | #3a | Describe any criteria used for including or excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established a priori. If no criteria were set, state this explicitly. | Methods/line 120-128.No criteria are set for including or excluding animals. |
| Inclusion and exclusion criteria | #3b | For each experimental group, report any animals, experimental units, or data points not included in the analysis and explain why. If | No experiments units or data points is excluded. |

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| | | there were no exclusions, state so. | |
| Inclusion and exclusion criteria | #3c | For each analysis, report the exact value of n in each experimental group. | Results/line 187-188, 208-228. |
| Randomisation | #4a | State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. | Methods/line 120-121; Results/line 208-212. |
| Randomisation | #4b | Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly. | Methods/line 111-113. |
| Blinding | #5 | Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis). | N/A. This study does not involve blinding. |
| Outcome measures | #6a | Clearly define all outcome measures assessed (e.g., cell death, molecular markers, or behavioural changes). | Methods/line 129-131. |
| Outcome measures | #6b | For hypothesis-testing studies, specify the primary outcome measure, i.e., the outcome measure that was used to determine the sample size. | N/A. This study does not involve sample size determination. |
| Statistical methods | #7a | Provide details of the statistical methods used for each analysis, including software used. | Methods/line 178-182. |
| Statistical methods | #7b | Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met. | Methods/line 178-182. |
| Experimental animals | #8a | Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. | Methods/line 111-113；Discussion/line 364-385. |
| Experimental animals | #8b | Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. | Methods/line 111-113. |
| Experimental procedures | #9a | For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate what was done, how it was done, and what was used. | Methods/line 110-176. |
| Experimental procedures | #9b | Timing and frequency of procedures | Methods/line 110-176. |
| Experimental procedures | #9c | Where procedures were carried out (including detail of any acclimatisation periods). | Methods/line 110-176. |
| Experimental procedures | #9d | Rationale for procedures | Methods/line 110-176. |
| Results | #10a | For each experiment conducted, including independent replications, report summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g., mean and SD, or median and range). | Methods/line 178-182. |
| Results | #10b | If applicable, for each experiment conducted, including independent replications, report the effect size with a confidence interval. | N/A. It's not applicable. |

| **Recommended set** | | | |
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| Abstract | #11 | Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions. | Abstract/line 20-47. |
| Background | #12a | Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. | Introduction/line 63-90. |
| Background | #12b | Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology. | Introduction/line 91-107. |
| Objectives | #13 | Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested. | Introduction/line 91-107. |
| Ethical statement | #14 | Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification. | Methods/line 116-118; Statement of Ethics /line 470-474. |
| Housing and husbandry | #15 | Provide details of housing and husbandry conditions, including any environmental enrichment. | Methods/line 111-113. |
| Animal care and monitoring | #16a | Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering, and distress. | N/A. No relevant interventions are conducted. |
| Animal care and monitoring | #16b | Report any expected or unexpected adverse events. | N/A. No relevant interventions are conducted. |
| Animal care and monitoring | #16c | Describe the humane endpoints established for the study, the signs that were monitored, and the frequency of monitoring. If the study did not set humane endpoints, state this. | N/A. No humane endpoints are established in this study. |
| Interpretation/scientific implications | #17a | Interpret the results, taking into account the study objectives and hypotheses, current theory, and other relevant studies in the literature. | Discussion/line 348-443. |
| Interpretation/scientific implications | #17b | Comment on the study limitations, including potential sources of bias, limitations of the animal model, and imprecision associated with the results. | Discussion/line 444-448. |
| Generalisability/translation | #18 | Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate). | Discussion/line 348-443. |
| Protocol registration | #19 | Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered. | A protocol was prepared before the study without registration |
| Data access | #20 | Provide a statement describing if and where study data are available. | N/A |
| Declaration of interests | #21a | Declare any potential conflicts of interest, including financial and nonfinancial. If none | Conflict of interest/line, 482. |

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| | | exist, this should be stated. | |
| Declaration of interests | #21b | List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis, and reporting of the study. | Funding Sources/line, 476-480. |