

| Item | Item No | RECOMMENDATION | Reported on Page Number/Line Number | Reported on Section/Paragraph |
|-------------------------|---------|---|-------------------------------------|-------------------------------|
| Title | 1 | Provide as accurate and concise a description of the content of the article as possible. | Page 1/Line 1-3 | Title |
| Abstract | 2 | Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study. | Page 2/Line 30-56 | Abstract |
| INTRODUCTION | | | | |
| Background | 3 | a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology. | Page 3-4/Line 59-100 | Introduction/Paragraph 1-3 |
| Objectives | 4 | Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. | Page 4/Line 101-104 | Introduction/Paragraph 4 |
| METHODS | | | | |
| Ethical statement | 5 | Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research. | Page 6/Line 178-183 | Methods/Paragraph 9 |
| Study design | 6 | For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out. | Page 6-7/Line 184-193 | Methods/Paragraph 9 |
| Experimental procedures | 7 | For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used). | Page 4-7/Line 107-215 | Methods/Paragraph 1-13 |

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|---|----|--|-----------------------|----------------------|
| Experimental animals | 8 | <p>a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range).</p> <p>b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.</p> | Page 6-7/Line 184-193 | Methods/Paragraph 9 |
| Housing and husbandry | 9 | <p>Provide details of:</p> <p>a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish).</p> <p>b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment).</p> <p>c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.</p> | Page 6/Line 184-187 | Methods/Paragraph 9 |
| Sample size | 10 | <p>a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group.</p> <p>b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used.</p> <p>c. Indicate the number of independent replications of each experiment, if relevant.</p> | Page 6-7/Line 184-193 | Methods/Paragraph 9 |
| Allocating animals to experimental groups | 11 | <p>a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done.</p> <p>b. Describe the order in which the animals in the different experimental groups were treated and assessed.</p> | Page 6-7/Line 184-193 | Methods/Paragraph 9 |
| Experimental outcomes | 12 | Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes). | Page 6-7/Line 184-193 | Methods/Paragraph 9 |
| Statistical methods | 13 | <p>a. Provide details of the statistical methods used for each analysis.</p> <p>b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron).</p> <p>c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.</p> | Page 7/Line 210-215 | Methods/Paragraph 13 |
| RESULTS | | | | |
| Baseline data | 14 | For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated). | Page 8-9/Line 247-259 | Results/Paragraph 3 |
| Numbers analysed | 15 | <p>a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%).</p> <p>b. If any animals or data were not included in the analysis, explain why.</p> | Page 8-9/Line 247-259 | Results/Paragraph 3 |
| Outcomes and estimation | 16 | Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval). | Page 8-9/Line 247-259 | Results/Paragraph 3 |
| Adverse events | 17 | <p>a. Give details of all important adverse events in each experimental group.</p> <p>b. Describe any modifications to the experimental protocols made to reduce adverse events.</p> | Page 8-9/Line 247-259 | Results/Paragraph 3 |

| DISCUSSION | | | | |
|---|----|--|-------------------------|--------------------------|
| Interpretation/ scientific implications | 18 | a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results ² . c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research. | Page 10-12/Line 313-378 | Discussion/Paragraph 1-5 |
| Generalisability/ translation | 19 | Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology. | Page 10-12/Line 313-378 | Discussion/Paragraph 1-5 |
| Funding | 20 | List all funding sources (including grant number) and the role of the funder(s) in the study. | Page 12/Line 381-386 | Funding/Paragraph 1 |

From:

Animal Research: Reporting In Vivo Experiments

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References:

1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ 340:c332.

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*As the checklist was provided upon initial submission, the page number/line number reported may be changed due to copyediting and may not be referable in the published version. In this case, the section/paragraph may be used as an alternative reference.