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| ITEM RECOMMENDATION |
| Title | 1 | Provide as accurate and concise a description of the content of the article as possible | Title  |
| Abstract | 2 | Provide an accurate summary of the background, research objectives, includingdetails of the species or strain of animal used, key methods, principal findingsand conclusions of the study. | Abstract |
| INTRODUCTION |
| Background | 3 | a. Include sufficient scientific background (including relevant references toprevious work) to understand the motivation and context for the study, andexplain the experimental approach and rationale.b. Explain how and why the animal species and model being used can addressthe scientific objectives and, where appropriate, the study’s relevance tohuman biology. | paragraph 1-3 |
| Objectives | 4 | Clearly describe the primary and any secondary objectives of the study, orspecific hypotheses being tested. | paragraph 3 line6-7 |
| 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 guidelinesfor the care and use of animals, that cover the research. | Ethics approval and consent to participate |
| 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 allocatinganimals 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 studydesigns were carried out. | Materials and Methods 2.6 |
| Experimentalprocedures | 7 | For each experiment and each experimental group, including controls, provideprecise 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). | Materials and Methods 2.6 |
| Experimentalanimals | 8 | 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).b. Provide further relevant information such as the source of animals,international strain nomenclature, genetic modification status (e.g. knock-outor transgenic), genotype, health/immune status, drug or test naïve, previousprocedures, etc | Materials and Methods 2.6 |
| Housing andhusbandry | 9 | Provide details of:a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage orhousing; bedding material; number of cage companions; tank shape and materialetc. for fish).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, environmentalenrichment).c. Welfare-related assessments and interventions that were carried out prior to,during, or after the experiment. | Materials and Methods 2.6 |
| Sample size | 10 | a. Specify the total number of animals used in each experiment, and the numberof animals in each experimental group.b. Explain how the number of animals was arrived at. Provide details of any samplesize calculation used.c. Indicate the number of independent replications of each experiment, if relevant. | Materials and Methods 2.6 |
| Allocating animalsto experimentalgroups | 11 | a. Give full details of how animals were allocated to experimental groups, includingrandomisation or matching if done.b. Describe the order in which the animals in the different experimental groupswere treated and assessed. | Materials and Methods 2.6 |
| Experimentaloutcomes | 12 | Clearly define the primary and secondary experimental outcomes assessed(e.g. cell death, molecular markers, behavioural changes). | Materials and Methods 2.6 |
| Statistical methods | 13 | a. Provide details of the statistical methods used for each analysis.b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals,single neuron).c. Describe any methods used to assess whether the data met the assumptionsof the statistical approach. | Materials and Methods 2.10 |
| RESULTS |
| Baseline data | 14 | For each experimental group, report relevant characteristics and health status ofanimals (e.g. weight, microbiological status, and drug or test naïve) prior totreatment or testing (this information can often be tabulated). | Results 3.3 Body weight gain |
| Numbers analysed | 15 | a. Report the number of animals in each group included in each analysis. Reportabsolute numbers (e.g. 10/20, not 50% 2 ).b. If any animals or data were not included in the analysis, explain why. | Results 3.3, 3.4,3.5 |
| Outcomes andestimation | 16 | Report the results for each analysis carried out, with a measure of precision(e.g. standard error or confidence interval). | Results 3.3, 3.4,3.5 |
| Adverse events | 17 | a. Give details of all important adverse events in each experimental group.b. Describe any modifications to the experimental protocols made to reduceadverse events. | N/A |
| 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, anylimitations of the animal model, and the imprecision associated with the results 2 .c. Describe any implications of your experimental methods or findings for thereplacement, refinement or reduction (the 3Rs) of the use of animals in research. | Discussion paragraph 1-2 |
| Generalisability/translation | 19 | Comment on whether, and how, the findings of this study are likely to translate toother species or systems, including any relevance to human biology | Discussion paragraph 3 |
| Funding | 20 | List all funding sources (including grant number) and the role of the funder(s)in the study. | Funding |
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as possible.

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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.

Objectives 4 Clearly describe the primary and any secondary objectives of the study, or

specific hypotheses being tested.

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.

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.

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).

Experimental

animals

8 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).

b. Provide further relevant information such as the source of animals,

international strain nomenclature, genetic modification status (e.g. knock-out

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