A Gold Standard Publication Checklist to Improve the Quality of Animal Studies, to Fully Integrate the Three Rs, and to Make Systematic Reviews More Feasible

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Summary — Systematic reviews are generally regarded by professionals in the field of evidence-based medicine as the highest level of medical evidence, and they are already standard practice for clinical studies. However, they are not yet widely used nor undertaken in the field of animal experimentation, even though there is a lot to be gained from the process. Therefore, a gold standard publication checklist (GSPC) for animal studies is presented in this paper. The items on the checklist have been selected on the basis of a literature analysis and the resulting scientific evidence that these factors are decisive in determining the outcome of animal studies. In order to make future systematic reviews and meta-analyses of animal studies possible, to allow others to replicate and build on work previously published, diminish the number of animals needed in animal experimentation (*reduction*), improve animal welfare (*refinement*) and, above all, improve the quality of scientific papers on animal experimentation, this publication checklist needs to be used and followed. We have discussed and optimised this GSPC through feedback from interviews with experts in the field of animal experimentation. From these interviews, it became clear that scientists will adopt this GSPC when journals demand it. The GSPC was compared with the current instructions for authors from nine different journals, selected on the basis that they featured a high number of publications on animal studies. In general, the journals' demands for the description of the animal studies are so limited that it is not possible to repeat the studies, let alone carry out a systematic review. By using the GSPC for animal studies, the quality of scientific papers will be improved. The use of the GSPC and the concomitant improvement in the quality of scientific papers will also contribute to decreased variation and increased standardisation and, as a consequence, a reduction in the numbers of animals used and a more reliable outcome of animal studies. It is of major importance that journal editors become convinced of and adopt these recommendations, because only then will scientists follow these guidelines to the full extent.

Key words: *animal experimentation, meta-analysis, publication checklist, scientific quality, systematic review.*

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Introduction

A systematic review (SR) is a literature review focused on a single question which tries to identify, appraise, select and synthesise all available high-quality research evidence relevant to that question (1). SRs are generally regarded by evidence-based medicine professionals as the highest level of medical evidence, and they are already standard practice in clinical studies. However, SRs are not yet widely used nor undertaken in the animal experimentation field, although there would be a lot to be gained from the process. A systematic approach to incorporate all available relevant literature into the design of an animal experiment is a prerequisite for research which is of high scientific quality. Good science, from a scientific as well as an animal welfare point of view, is the basis of the book, *The Principles of Humane Experimental Technique*, by Russell and Burch (2). In this book, they recommend that the Three Rs principles (*Refinement*, *Reduction* and *Replacement*) should be applied whenever possible in animal studies. Besides producing high-quality research, SRs of animal experiments will result in direct implementation of the Three Rs. SRs may provide the proper argumentation to decide which animal model will give the best answer to the (clinical) research question (3, 4) and to detect whether there are gaps in scientific knowledge that require new animal experiments (*replacement* and *refinement*). This will also aid in preventing unnecessary duplication of animal experiments (*reduction*), and thus discourage unnecessary animal use and time loss. A SR of animal studies will also lead to a better interpretation of the already existing scientific results from animal experiments, through which a better

translation to the clinic (translational research) and more guarantees for patient safety can become reality.

Pound *et al.* (5) showed that proper analysis of animal experiments by executing a SR could improve the decision making in whether or not to start a clinical trial. Macleod *et al.* (6, 7) have focused on the need for SRs in the field of stroke research, and have stressed the urgent need for improving the design, execution and reporting of animal studies.

Within the clinical setting, SRs have become an essential routine. Surprisingly, SRs are not yet standard when undertaking animal studies. This may relate to the fact that all animal studies differ in design, which makes an evaluation in a systematic manner a challenge. Moreover, there are no clear guidelines for writing a SR in the animal experimentation field, and because many papers on animal experiments are incomplete in reporting the necessary details (8) or are of poor scientific quality (4, 9, 10), systematic analysis cannot easily be performed.

The random allocation of experimental units to treatment groups and blind assessment of the treatment effects are standard and obligatory in human clinical trials, but are still not widely applied when performing animal experiments. In publications on animal studies, it is often left unmentioned whether or not randomisation and blinding have been performed (8). We would like to endorse the statement by Professor Ian Roberts: "We are only asking that the same standards as are applied in human research are applied to animal research" (11).

Also, the importance of reporting husbandry conditions and the basic characteristics of animal models is underrated, even though there is much evidence to show the importance of these parameters (Table 1). For instance, mice housed in standard cages without cage enrichment show impaired development, abnormal repetitive behaviour and an anxious behavioural profile (12). Accordingly, when the availability of cage enrichment and the way of housing is not reported, interpretation of the results may be biased, because anxiety could be a result of the treatment and/or a result of the way of housing.

In order to make performing a SR possible in the future, to allow others to replicate and build on work previously published, and to improve the quality of scientific papers about animal experimentation, we suggest the use of the gold standard publication checklist (GSPC), which is presented in this paper. This list can be, and indeed should always be, used when designing experiments and reporting data, and subsequently, it will improve animal welfare (*refinement*) and reduce the number of animals needed in an experiment (*reduction*). Over the last decade, many publications have appeared on proper experimental design, statistics, reporting, etc. (7, 9, 10, 13–21). However, because this information is not always easy to find, e.g. in the case of text books or less well-known databases, we have assembled this information in an easy-to-use GSPC. This GSPC was discussed and optimised through semi-structured interviews with expert scientists from the animal science field. From this discussion, it became clear that scientists will adopt the demands of journals. Therefore, we have also investigated to what extent the current guides for authors from nine journals, selected because they publish many papers on animal studies, comply with our GSPC, and on which specific items improvement is necessary.

Table 1: Explanatory literature highlighting the importance of certain items mentioned in the publication checklist

Items from publication checklist	References
Experimental design	9, 15
Temperature	29, 30
Ventilation	29, 31–33
Humidity	34–37
Lighting	36, 38
Bedding	29, 39–41
Cage size/cage space/group size	29, 42–44
Cage enrichment	29, 44–47
Individual housing	29, 48–51
Cage change	33, 52, 53
Handling/transport	52, 54–56
Nutrition	57, 58
Water	59–61
Blinding	8, 62
Randomisation	15, 8, 62

Methods

In order to develop a GSPC, we made extensive use of the literature (14, 15, 20, 22–24), the *Guide for Authors* from the *Laboratory Animals* journal, and experts in animal science within the Radboud University Nijmegen Medical Centre, The Netherlands.

Our group of experts consisted of 15 scientists who perform animal studies, of whom three are medical doctors specialising in anaesthesiology, neonatology and pharmacology, respectively, three are professors in nuclear medicine, laboratory animal science and orthopaedics, respectively, two are animal welfare officers, six are post-doctoral researchers in the fields of either tumour immunology, rheumatic diseases, pharmacology/

toxicology, nuclear medicine or psychology, and one is a PhD student in neurology. This panel was approached for feedback, in order to optimise the GSPC. They were individually interviewed for 1.5 hours, in a semi-structured manner, in which feedback on the checklist was requested. These interviews were also audiotaped. Their comments were used in the optimisation of the GSPC and are partly reported in the current paper.

After optimising the GSPC, we selected nine journals which publish papers on animal experiments. It was the aim to find journals in this category which varied in both their impact factors, and the types of biomedical research they included. For the selection, a search was performed within PubMed in the English language, with 'mice' as a MeSH Major Topic and the following additional limits: published in the last 10 years and being a (Journal Article *or* Research Support NIH Extramural *or* Research Support NIH Intramural *or* Research Support *or* Non-US Government, Research Support *or* US Government Non-PHS). This resulted in 5060 hits, and from this we selected the 20 most frequently-occurring journals (which we termed 'List 1'). Subsequently, these journals were ranked according to their impact factors (termed 'List 2'). From these two lists, seven journals, which scored highest on both lists, were chosen. The journals *Nature* and *Science* were also selected, because of their scientific prestige.

The guide for authors from the following journals: *Proceedings of the National Academy of Sciences of the USA, Journal of Immunology, Journal of Comparative Neurology, Journal of Nutrition, American Journal of Pathology, Laboratory Animals, Experimental Animals, Nature* and *Science,* were then compared to the GSPC. We counted how many items in the methods and results section of our GSPC were mentioned in the guide for authors in each case. The maximum score for each journal was 74 points.

Results

In order to improve the quality of scientific publications on animal experimentation, and to make performing SRs in the animal science field more feasible, Figure 1 lists the items which ought to be included in every paper about animal experimentation. From the semi-structured interviews, it was concluded that most scientists could see the advantage of using this checklist (10 of the 15 scientists). However, a few of them were of the opinion that the GSPC describes too many details irrelevant to the outcome of the studies. All panel members indicated that they would be willing to include all of these checklist items, if journals demanded it. However, it was felt that this information should be very concise and should not distract from the main message of the paper. Making the GSPC information available on the World Wide Web, in a journal supplement form, was encouraged by all 15 panel members. Even though there is scientific evidence from the published literature that the mentioned items are relevant (see also Table 1) and can interfere with and bias the results of animal studies, this is not common knowledge. Only four out of the 15 panel members directly recognised the value of all the items mentioned. One panel member suggested that animal facilities should provide protocols with their standard housing conditions and other standards according to the checklist, which would facilitate the collection of, and reference to, the information.

In addition, we compared the guides for authors from nine different journals (*Proceedings of the National Academy of Sciences of the USA, Journal of Immunology, Journal of Comparative Neurology, Journal of Nutrition, American Journal of Pathology, Laboratory Animals, Experimental Animals, Nature and Science)* with the GSPC (see Table 2). Strikingly, none of the nine journals asked for a description of the experimental unit and experimental design used. None of the journals asked for a description of the way randomisation was executed or whether authors were blinded to the treatment modality, whereas randomisation and blinding are basic principles requested in clinical research nowadays. Also, none of the author guidelines asked for a description of the reasons why (and how many) animals had been excluded, even though this might result in a different interpretation of statistical outcomes.

Many other parameters, such as housing/husbandry conditions, are only mentioned in the guidelines for authors from one journal, and details on nutrition in only two of the selected journals. Seven out of the nine journals demanded that their authors give a description of compliance with national regulatory principles, and an ethical and qualitative assessment. Remarkably, for four of these journals, these two items covered 50% of their total demands (Table 2). Five out of the nine journals gave only an overall statement about the *Materials and Methods* section: that the documentation of the methods and materials used should be sufficient to permit replication of the research.

Figure 2 shows a scatterplot of the evaluation of the guides for authors from the nine journals. The percentages of items scored on the GSPC are indicated on the y-axis, and the impact factors of the journals are presented on the x-axis. *Laboratory Animals* had the highest score, as 54% of the items mentioned in our GSPC are requested in their *Guide for Authors.* Overall, this graph strongly suggests that journals with high impact factors have low demands concerning the level of detail of

Figure 1: The Gold Standard Publication Checklist (GSPC)

Introduction:

Background information

— Description of the literature concerning the topic of the paper, including a short (global) description about how the results have been achieved/obtained

— Description of the gaps in the current knowledge concerning the topic

— The aim or objective of the current study

The research question or hypothesis

— Specific and focused

— Use the **PICO(T)** mnemonic, if possible:

- **P**atient Group or Animal species
- **I**ntervention (or exposure)
- **C**omparison/**C**ontrol Group
- **O**utcome measure

If applicable:

- **T**ime (duration of intervention)

The clinical relevance or other relevance of research

— Reasons why a specific animal model has been chosen; and

— The specific characteristics of the animal model

Methods:

Experimental design (if possible)

— For example:

- Completely randomised design
- Block design
- Factorial design
- Repeated measures design
- Sequential design

Experimental groups and controls

— Quarantine and acclimatisation period after transportation to animal facility

— Species

— Designation of strain (exact genetic code)

— Origin and source of animals

— Genetic background (outbred, inbred, F1 hybrid, mutant, transgenic, congenic, consomic, etc.) and generation

— Definition of the experimental unit (individual animal/animals in one cage)

— Number of animals per group (and possibly power and sample size calculations)

— Sex

— Age (at the beginning and the end of the experiment)

— Weight (at the start of the experiment)

— Microbiological status

- Conventional/specified pathogen-free (SPF)/gnotobiotic, germ-free
- Measures to protect microbiological status (for example, open-system, closed-system (SPF), individually ventilated cage racks, isolation unit)

Figure 1: (continued)

Experimental groups and controls (continued)

— Housing: Animal room
- Temperature ± range (regulated or not)
- Relative humidity ± range (regulated or not)
- Ventilation
  - Over-pressure or under-pressure
  - Air changes per hour
- Lighting
  - Natural or artificial
  - Number of hours light per 24 hours
  - Light intensity
  - Time when light is switched on
  - Transitional decrease in light intensity
- Noise (music, etc.)

— Housing: Cages
- Type and size
- Number of animals per cage (and if individually housed, why?)
- Bedding (reference; if not, type). Is batch analysis certificate available? Pre-treatment?
- Presence and type of cage-enrichment
- Frequency of cage change
- Frequency of handling

— Nutrition
- Type (natural-ingredient diets, chemically-defined diets or purified diets)
- Composition or batch number (if possible, use a reference)
- Pre-treatment
- Feeding regimes (*ad libitum*, meal feeding, restricted, etc.). If not *ad libitum*:
  - Amount of food given
  - Frequency and time of feeding

— Water
- Type (analysis certificate available?)
- Pre-treatment (concentration of acidification or chlorination)
- Water schedule
  - Quantity (*ad libitum*?)
  - Frequency of water supply (in case of restriction)
- Frequency of change
- Bottles or automatic watering system

— Method of allocation to treatment group: i.e. randomly assigning animals to a specific group

— Description of how the disease or intervention is defined in the animal

— Description of the reasons to exclude animals from the experiment

— Description of the control groups in the experiment, and an explanation of why these specific control groups are important for answering the research question

Regulations and ethics

— Description of compliance to national regulatory principles

— Description of the ethical and qualitative assessment by an independent organisation within the institute (e.g. Institutional Ethics Committee)

Figure 1: (continued)

The intervention

— Time schedule
  - Day and time of intervention within experiment
  - Time between intervention and sampling or processing

— Type of intervention
— Description of operation techniques or other techniques and materials used
— Dose and/or frequency of intervention (when applicable)
— Administration route (enteral [oral or via the anus]/parenteral/trans-dermal)
— Drugs and dose tested (product name, manufacturer, concentration)
— Other products used (product name, manufacturer, concentration)
— Method and time of sampling (blood, urine, etc.)
— Anaesthesia (duration, type of drug and method)
— Analgesia (type of drug and method)
— Euthanasia (type of drug and method)
— Description of general wellbeing of the animal during and at the end of the intervention and — in the case of compromised wellbeing — what relieving measures have been taken

Outcome

— Description of parameters of interest, and the method of determination
  - Inclusion also of important physiological parameters and reference values to define wellbeing of the animal

— Description whether, or how, the staff was blinded to the treatment modality
— Description of the statistics used

Results:

— Description of the main results
— Numbers and reasons of premature deaths during the experiments (short description of autopsy findings)
— Excluded animals (numbers and reasons why they were excluded)
— Total numbers of animals included in the statistical analyses
— Short description/explanation of included animals with peculiarities
— Power analysis after adjustment for diseased and excluded animals (to determine the reliability of the study)
— Description of the most important relevant physiological parameters during intervention (like temperature, body weight, heart rate, etc.)

Discussion:

— Discussion of principal findings
— Discussion of the (indirect) clinical and overall scientific relevance of the outcome
— Definition of whether or not follow up studies are necessary

the description of animal experiments featured in the papers they publish.

Discussion

In this paper, we have presented a GSPC which is intended for general use by all scientists who perform animal experiments. The checklist has been developed primarily because the scientific quality of animal experiments urgently needs improvement (6, 9, 10, 17), but also because it will facilitate future SRs and meta-analyses on animal studies, and it will allow others to replicate and build on previously-published work. Moreover, the GSPC will aid the implementation of the Three Rs principles of Russell and Burch (2) in many different ways. The use of the GSPC will improve animal welfare (*refinement*) and reduce the number of animals needed in an experiment (*reduction*). Moreover, it is expected that, despite the use of a reduced number of animals, a scientifically more valid answer can be obtained. This is supported by the 1970–2000 publication analysis by Carlsson *et al.* (19), which indicated that, over time, the number of animals used for one research article had

fallen by about 50%, whereas the reported details on the animals, materials and methods used had doubled. Although well-designed and well-executed animal experiments are a condition for translational research, many papers involving animal experimentation are still incomplete in their reporting (6, 7, 9, 15). Because of that, most experiments cannot be repeated reliably by others, even though reproducibility of experiments is one of the main principles of experimental science. In addition, incomplete reporting causes difficulties in the interpretation of the results of studies, and makes the execution of systematic reviews impossible.

It has been known for a long time that controlling the variation within an experiment improves the quality of the research, and diminishes the numbers of animals needed in an experiment without losing scientific information (15). However, many scientists and journal editorial boards still appear to underestimate the importance of controlling and reporting these details. The excuse for reporting in an incomplete manner that was most frequently mentioned by our panel members, is the fact that many journals have a space limitation on the submitted papers. However, most journals are now electronically available, and have the facility to publish extra information in the form of an electronic supplement, without using space in the hard copy. This facilitates reporting according to the GSPC. Moreover, it is essential that journal editors underscore the need for all the details of an animal experiment to be published, not only as essential ingredients for a paper of good scientific quality, but also in order to permit the experiment to be repeated by others. Only if journals start to require the (electronic) publication of these details, will scientists be willing to make the effort. Other drawbacks raised by our panel members are that reporting all of the details mentioned in the GSPC is very time consuming, and that the importance of several of these items is not supported by evidence. With regard to the first drawback, we suggest that animal facilities provide a helping hand by writing standard operating procedures (SOPs) according to the items on the checklist, for each of the different animal species in the facility, and make these available to the customers, e.g. by publishing them on the Internet. Scientists will then be able to refer to most of the items in the GSPC by referring to an Internet site, or they can add these texts in electronic supplements to the article. In this way, space limits can be adhered to, and the replication of experiments and scientific quality will improve. Because they consider some items on the GSPC to be irrelevant to the outcome of animal experiments, scientists do not feel the obligation to report sufficient corresponding details. In Table 1, an overview is given of the literature which proves the scientific relevance and significance of these items, and thus underlines the need for their inclusion.

It is quite clear from our analysis of the guides for authors from the nine journals, that the majority of them do not require detailed information about the animal experiments featured in the articles they publish. Almost all the journals agreed on the necessity of mentioning one particular item: seven out of nine guides for authors required a statement about compliance with national regulatory principles and a description of the ethical and qualitative assessment of the experiment. This was also previously found by others (18). However, compliance with regulatory and ethical principles does not automatically imply that basic elements for good science have been met. Moreover, it does not provide a basis for the ability to repeat a study reliably.

Certain basic considerations, which are necessary for good experimental science, were not mentioned at all in any of the guides for authors: none of the nine journals asked for a description of the experimental design used, which is quite surprising, since only a well-designed experiment will give valid answers and should be considered ethically acceptable (15). In addition, none of the journals asked for a description of the method of randomisation or whether authors were blinded to the treatment modality, whereas these concepts are now widely-accepted basic principles to prevent bias in clinical research.

None of the guidelines requested a description of the experimental unit or reasons why, and how many, animals were excluded from the experiment and/or the analysis. This might result in an erroneous interpretation of statistical outcomes, and could subsequently increase the potential hazards involved in translating positive experimental outcomes to possible clinical benefits.

Earlier reports have proposed the use of quality criteria for animal experiments (10, 17, 18, 25), since standardisation of the design and the outcome parameters of animal experiments may facilitate the comparison of different studies and thus the gaining of better insight into the questions under consideration (17). Smith *et al.* (10) reported on the description of animal use in scientific papers, focusing on a selected number of items involving animal use and housing. Festing and Altman (14) published a checklist with a focus on husbandry conditions only, and, in 2005, Alfaro published a list with recommendations for reporting (18). This list by Alfaro did not describe all the items of importance, as a description of the experimental design and the method of allocation to a treatment group, and whether or not the scientist was blinded for the outcome measure, were not mentioned. In addition, no description of the general wellbeing of the animal was requested.

Our current GSPC is a complete checklist that will hopefully be fully adopted by scientists and

Table 2: Scores of the guides for authors from the nine different journals, on items included from the publication checklist

No.	Methods:			PNAS	J. Im.	J.C. Neur.	J. Nut.	A.J. Path.	Lab. An.	Exp. An.	Nat.	Sci.
1	Experimental design (if possible) *For example:* completely randomised design; block design; factorial design; repeated measures design; sequential design											
2	Experimental groups and controls:	Quarantine and acclimatisation period after transportation to animal facility							*			
3		Species		*	*	*	*		*	*		
4		Designation of strain (exact genetic code)			*	*	*		*	*	*	
5		Origin and source of animals				*	*		*	*		
6		Genetic background and generation				*			*			
7		Definition of the experimental unit										
8		Number of animals per group (and possibly power and sample size calculations)				*			*		*	
9		Sex				*	*		*			
10		Age (at the beginning and the end of the experiment)				*	*		*			
11		Weight (at the start of the experiment)					*		*			
12		Microbiological status							*			
13			Conventional/specified pathogen-free (SPF)/ gnotobiotic, germ-free						*			
14			Measures to protect microbiological status						*			
15		Housing: animal room	Temperature ± range (regulated or not)						*			

Table 2: continued

No.	Methods (continued):			PNAS	J. Im.	J.C. Neur.	J. Nut.	A.J. Path.	Lab. An.	Exp. An.	Nat.	Sci.
16		Relative humidity ± range (regulated or not)							*			
17		Ventilation							*			
18			Over/under pressure						*			
19			Air changes per hour									
20		Lighting										
21			Natural or artificial									
22			Number of hours light per 24 hours						*			
23			Light intensity									
24			Time when light is switched on						*			
25			Transitional decrease in light intensity									
26		Noise (music, etc.)										
27	Housing: cages	Type and size										
28		Number of animals per cage (and if individually housed, then why?)							*			
29		Bedding (reference; if not, type). Is batch analysis certificate available? Pre-treatment?							*			
30		Presence and type of cage-enrichment							*			
31		Frequency of cage change										
32		Frequency of handling										

This table shows which items from the publication checklist are also requested in the Guides for Authors *of the* Proceedings of the National Academy of Sciences of the USA *(PNAS),* Journal of Immunology *(J. Im.),* Journal of Comparative Neurology *(J. C. Neur.),* Journal of Nutrition *(J. Nut.),* American Journal of Pathology *(A. J. Path.),* Laboratory Animals *(Lab. An.),* Experimental Animals *(Exp. An.),* Nature *and* Science. *M = materials; D = drugs.* * = *information requested in guide for authors.*

Table 2: continued

No.	Methods (continued):			PNAS	J. Im.	J.C. Neur.	J. Nut.	A.J. Path.	Lab. An.	Exp. An.	Nat.	Sci.
33	Nutrition						*		*			
34		Type (natural-ingredient diets, chemically-defined diets or purified diets)					*		*			
35		Composition or batch no. (if possible, use a reference)					*		*			
36		Pre-treatment							*			
37		Feeding regimes (*ad libitum*, meal feeding, restricted, etc.). If not *ad libitum*, amount of food given; frequency and time of feeding							*			
38	Water											
39		Type (analysis certificate available?)							*			
40		Pre-treatment (conc. of acidification/chlorination)							*			
41		Water schedule							*			
42			Quantity (*ad libitum*?)									
43			Frequency of water supply (in case of restriction)									
44		Frequency of change										
45		Bottles or automatic watering system										
46	Method of allocation to treatment group: i.e. randomly assigning animals to a specific group											
47	Description of how the disease or intervention is defined in the animal											

Table 2: continued

No.	Methods (continued):			PNAS	J. Im.	J.C. Neur.	J. Nut.	A.J. Path.	Lab. An.	Exp. An.	Nat.	Sci.
48		Description of the reasons to exclude animals from the experiment										
49		Description of the control groups in the experiment										
50	Description of compliance to national regulatory principles			*	*	*	*	*	*			*
51	Description of the ethical and qualitative assessment by an independent organisation within the institute			*	*	*	*	*	*			*
52	The intervention:	Time schedule							*			
53			Day and time of intervention within experiment						*			
54			Time between intervention and sampling or processing						*			
55		Type of intervention										
56		Description of operation techniques or other techniques and materials used				*	*M					
57		Dose and/or frequency of intervention (when applicable)										
58		Administration route (enteral [oral or via the anus]/ parenteral/transdermal)										
59		Drugs and dose tested (product name, manufacturer, conc.)				*D	*D					
60		Other products used (product name, manufacturer, conc.)				*	*		*			
61		Method and time of sampling (blood, urine, etc.)										

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Table 2: continued

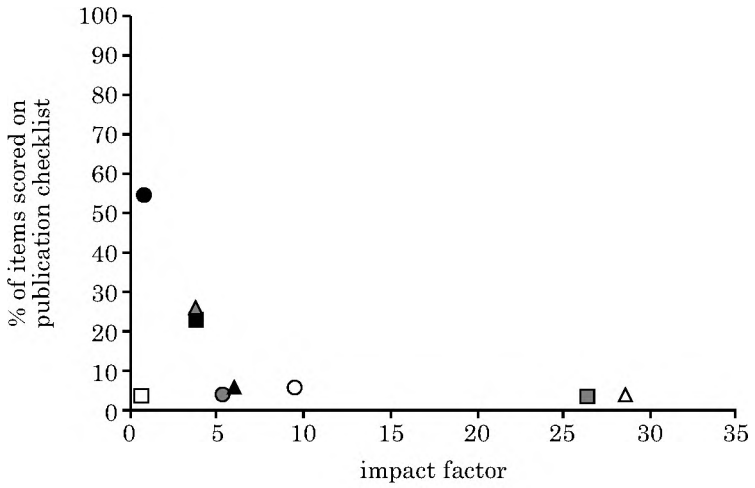
No.	Methods (continued):			PNAS	J. Im.	J.C. Neur.	J. Nut.	A.J. Path.	Lab. An.	Exp. An.	Nat.	Sci.
62		Anaesthesia (duration, type of drug/method)				*D	*D		*D			
63		Analgesia (type of drug/method)				*D	*D		*D			
64		Euthanasia (type of drug/ method)				*D	*		*			
65		Description of general well-being of the animal during and at the end of the intervention										
66	Outcome:	Description of parameters of interest, and the method of determination										
66			Inclusion also of important physiological parameters and reference values to define wellbeing of animal									
67		Description whether, or how, the staff was blinded to the treatment modality										
68		Description of the statistics used				*	*	*	*		*	
No.	**Results:**											
69	Numbers of, and reasons for, premature deaths during the experiments (short description of autopsy findings)											
70	Excluded animals (number and reason why they were excluded)											
71	Total number of animals included in statistical analysis											
72	Short description/explanation of included animals with peculiarities											

Table 2: continued

No.	Results (continued):	PNAS	J. Im.	J.C. Neur.	J. Nut.	A.J. Path.	Lab. An.	Exp. An.	Nat.	Sci.
73	Description of the most important relevant physiological parameters during intervention									
74	**Overall statement about materials and methods**	*		*	*	*				*
	Total:	**4**	**4**	**17**	**19**	**4**	**40**	**3**	**3**	**3**
	% (out of 74)	**5**	**5**	**23**	**26**	**5**	**54**	**4**	**4**	**4**
	Impact factor	9.6	6.1	3.9	3.8	5.5	0.9	0.6	28.8	26.4

This table shows which items from the publication checklist are also requested in the Guides for Authors *of the* Proceedings of the National Academy of Sciences of the USA *(PNAS),* Journal of Immunology *(J. Im.),* Journal of Comparative Neurology *(J. C. Neur.),* Journal of Nutrition *(J. Nut.),* American Journal of Pathology *(A. J. Path.),* Laboratory Animals *(Lab. An.),* Experimental Animals *(Exp. An.),* Nature *and* Science. *M = materials; D = drugs. * = information requested in guide for authors.*

Figure 2: A scatterplot showing the percentage of items scored on the publication checklist and the corresponding impact factors of the nine journals whose guides for authors were analysed in the study



○= Proceedings of the National Academy of Sciences of the USA;▲= Journal of Immunology; ■= Journal of Comparative Neurology; △ = Journal of Nutrition; ● = American Journal of Pathology; ● = Laboratory Animals; □ = Experimental Animals; ■ = Science; △= Nature.

journals. The GSPC checklist includes more-precise details of what a scientist should consider when reporting, e.g. housing conditions, nutrition, drinking water supply and elements of the intervention, as compared to all other recommendations or checklists published previously.

It is advisable to include the determination of important physiological parameters as a reference value for the wellbeing of the animals in all animal studies, since only "happy animals make good science" (26). By publishing these reference values, important background information on animal welfare can accumulate over time. Furthermore, we suggest that scientists should also perform a power analysis after completion of the experiment, to justify the numbers of animals used and to check whether the power of the experiment has been sufficient to draw any conclusions. Executing a power analysis after the experiment is easy, because, at that time, parameters such as sample size and effect size will have been determined.

In conclusion, we present in this paper a GSPC which should be easy to use and, when used by scientists to its full extent, will allow others to replicate and build on previously published work. Ultimately, with better reporting, SRs of high quality will become feasible. The use of the GSPC will also improve the quality of scientific papers on animal experimentation, firstly, by decreasing variation and increasing standardisation, and secondly, by aiding in the optimal planning and design of animal studies. As a consequence, the numbers of animals used in science would also be expected to fall. It is clear that the GSPC can have a major impact on direct and indirect implementation of the Three Rs principles. In addition, an improved experimental design contributes to a better translation to the clinic and increases patient safety (6, 27, 28). Scientists ought to make all the individual animal data available on the World Wide Web, in the form of an electronic supplement to the journal, as this will make the execution of meta-analyses of animal studies possible in the future. When following the GSPC and making all the data available, science on animal studies becomes more transparent, which is also important to meet societal concerns. Therefore, it is of the utmost importance that journal editorial boards and scientists adopt the recommendations mentioned in this paper.

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