Considering and reporting sex as an experimental variable II: an update on progress in the British Journal of Pharmacology

CONSIDERING AND REPORTING SEX AS AN EXPERIMENTAL VARIABLE II: AN UPDATE ON PROGRESS IN THE BRITISH JOURNAL OF PHARMACOLOGY

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1 Background

The escalating concern about the use of only males in biomedical research (e.g., Heidari et al., 2016), prompted the Senior Editorial Board (SEB), under the leadership of the Editor-in-Chief (EiC) of the *British Journal of Pharmacology* (*BJP*), to publish an Editorial that addressed this issue (Docherty et al. 2019). The article highlighted some sex differences in vulnerability to, and expression of, diverse medical disorders, as well as the efficacy and risk of harmful side-effects of treatments in women. Even that handful of examples provided compelling evidence to justify the decision that, in future, manuscripts submitted for publication in the *BJP* should be based on studies that had included both sexes in the experimental design unless there was a clear justification for using only one sex.

An exemption from this policy would be when the subject of the research is confined to one sex, such as, for example, investigations focused on the uterus or prostate. However, we recognized that there would be a time lag between the publication of our Editorial with this new guidance and the subsequent publication of manuscripts that adhered to advice detailed in that article. For that reason, we continued to consider reports of studies that had been carried out before publication of our guidance, even if they had used only one sex (either male or female). However, in such instances, authors were expected to include a plausible scientific justification for not using both sexes. Also, when the use of a single sex was accepted by the Editors, we asked that the manuscript should include a statement in the Discussion to acknowledge that the research findings might not generalise to the other sex and to consider what the findings might mean for the other sex.

Our intervention was timely. Since 2019 there has been an explosion of interest in addressing diversity issues within the biomedical sector. This has included high profile calls to action in the academic literature across the specialities (e.g., the cardiovascular and neurological fields (Vogel et al., 2021; Waters et al., 2021)) as well as the formal recognition by national governments of the issues and a commitment to develop policy and strategies to improve the balance in research studies (e.g., in the UK https://www.gov.uk/government/consultations/womens-health-strategy-call-for-evidence/womens-health-strategy-call-for-evidence). Three years later we now wish to determine whether our interventions have had any impact.

This Editorial reports findings from an audit of recent articles published in the *BJP*, which was carried out to determine whether the introduction of the guidance, coupled with a number of initiatives to raise the profile of the issues, has led to any improvement in this matter. The primary objective was to ascertain whether or not the proportion of publications reporting studies that used both sexes has increased since 2019 and, for those that used only one sex, whether (and if so, how) authors justified that decision.

2 Audit: Time-range, sampling procedure and scope

Time-range

From the perspective of the *BJP*, two key milestones contributed to the effort to persuade researchers to study both sexes in their experiments. The first was in 2016, when both the SAGER guidelines (Heidari et al., 2016) and a policy statement from the National Institutes of Health in the USA (NIH 2016) were published. The latter explained why, in the future, the research funded by NIH should include both males and females in the experimental design.

The results of the audit, which are presented and discussed in this Editorial, start at 2014 to have some information on the 'baseline situation' before NIH activated that policy, in addition to the impact of our own interventions. We realised that there would be a lag between initiation of a policy and percolation into the literature, but some increase in the use of both sexes might be expected, if only because of heightened awareness of the problem.

The next milestone was the publication of the *BJP* Editorial in November 2019. With the anticipation of a gradual change in scientific mindset, we expected the number of publications between 2020 and 2022 that reported the use of both sexes to show an upward trajectory. This is not least because referees and editors of the *BJP* would examine authors' compliance with the journal's policy on the incorporation of both sexes into the experimental design as a factor to consider when deciding whether to recommend a manuscript for publication.

Sampling procedure

The audit sampled publications from all volumes of the *BJP*, published between January 2014 and December 2022. Two issues were sampled for each volume (calendar year); these were issue 4 (i.e. February) and issue 20 (i.e., October), as a matter of routine. Because several Themed Issues comprised mainly reviews, with few original research reports, it was sometimes necessary to include additional issues of each volume (3 and 11 and, subsequently, consecutive issues), to ensure that the audit included at least 20 papers per volume.

Studies using fish, embryos and haploid cells (oocytes) were excluded because identifying their sex is not straightforward. For the same reason, immortal cell lines were also not included because, with few exceptions, the sex of the donor is not documented. Apart from those exclusions, all original research papers that qualified for the audit in each issue were included in the process so as to avoid any risk of sampling bias. These articles reported experiments regardless of whether they had used animals of any species (including humans), tissue samples or primary cultures, or whether the experiments were conducted *in vivo*, *ex vivo* or *in vitro*. Consequently, the number of papers audited in different issues varied slightly and ranged from 20-28 per volume (year), giving a total of 168 in all.

Publications that reported the use of the same sex(es) for all experiments in the series reported in the manuscript were given a single score in the audit. However, some studies involved the use of different sexes in different experiments in a series. In such cases, the use of males and/or females and/or both was scored for each individual experiment within each publication.

Scope

The main point of interest was to determine whether there was any increase in the proportion of studies that had used both sexes. However, that question gave rise to several others that are equally important. All the points that were included in the audit are listed in full in **Table 1.**

3 Key findings

Studies using only one sex, or both, and their justification

As is evident from the bar chart in Figure 1, there has been little change in the exclusive use of males (>60%) since 2014. A promising decline between 2018 - 2019 was reversed in the following two years and so the proportion of studies in 2022 (21%) that used both sexes is similar to that in 2014 (19%). Despite increasing awareness of the need to study both sexes, only two papers (one each in 2020 and 2021) discussed the limitations of using only one sex, or implied that the findings might not generalize from one to the other. This was despite some authors including text in the Discussion that identified other limitations of their research. A surprisingly large proportion of studies (22%) did not specify the sex used in the experiments, even though authors are required to sign a Declarations of compliance with the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020) and the *BJP* Instructions for Authors, both of which require this information. This omission even applied to studies of humans and other large mammals (e.g., pigs).

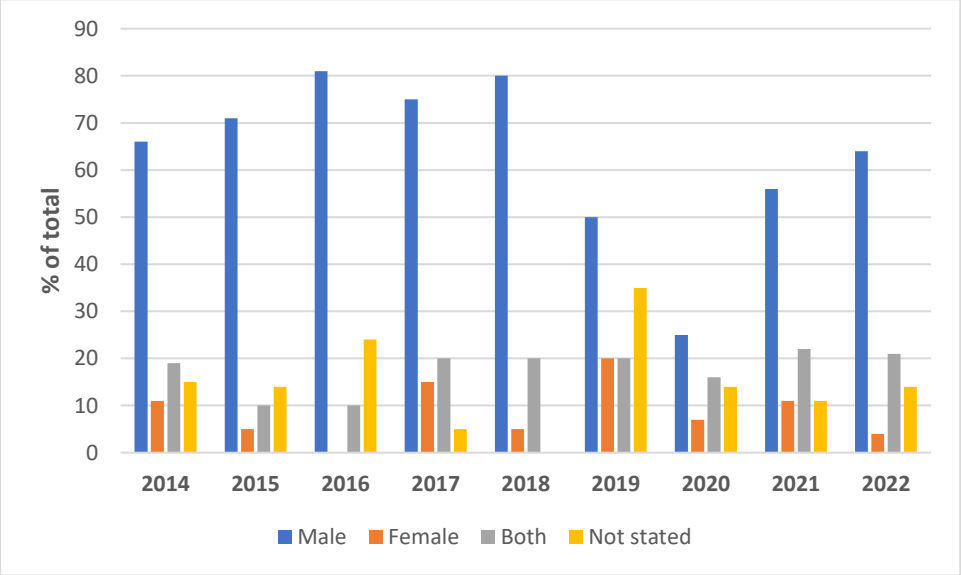

Figure 1. The percentage of studies published in the British Journal of Pharmacology that used either only males, only females, both males and females, or where the sex of the experimental unit was not stated in the manuscript. [The first editorial to discuss the use of both sexes in experiments published in the *BJP* was published in November 2019 (Docherty et al., 2019)]

Across all years, a large majority of studies used only one sex without providing any justification for that decision (Figure 2). However, there is an encouraging increase in the proportion of studies that now justify the use of only one sex: from 0% (2014 – 2016) to nearly 30% in 2022. The nature of the justifications differed considerably. Some authors opted to use the sex that expresses the higher incidence of the disorder in humans: e.g., eating disorders (females), resistance to metabolic syndrome with high fat diet (females), aggression (males), lower pain threshold (females) or where a gene linkage was traced to the Y chromosome.

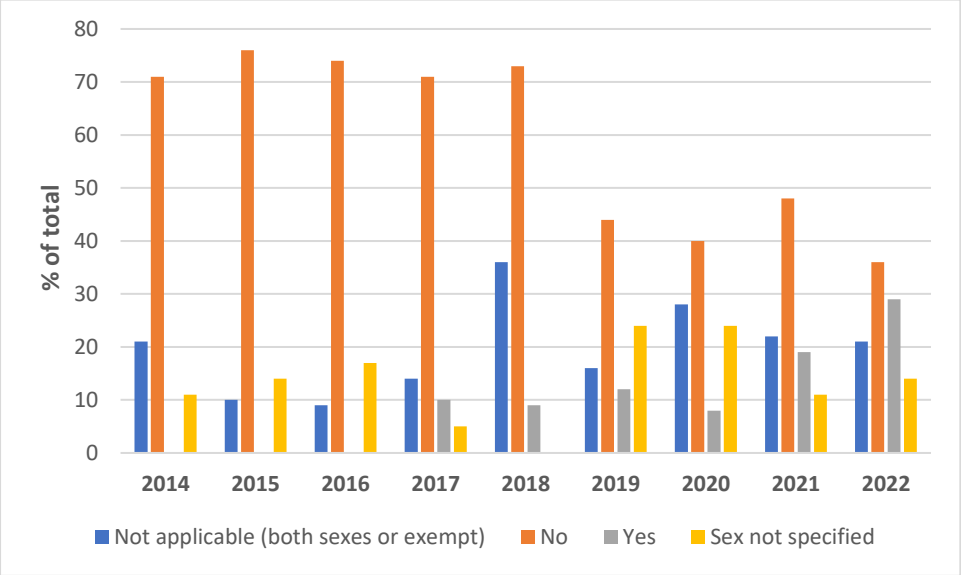

Figure 2. The percentage of studies published in the *BJP* that included a justification for using experimental units of only one sex in the experiments. [The first editorial to discuss the use of both sexes in experiments published in the *BJP* was published in November 2019 (Docherty et al., 2019)]

The most common justification for using only one sex (males) was the higher variability of female response. Given the well-documented evidence for progesterone metabolites as allosteric modulators of the $GABA_A$ receptor, for example (Bäckström et al., 2022) , it would be surprising if there were not slow cyclical fluctuations in the physiology and behaviour of females. However, a growing number of studies challenge this suggestion,

with several assessments/studies providing evidence that increased variability of females is not a justifiable reason for studying exclusively males: a view supported by recent meta-analyses (Becker et al., 2016; Kaluve et al., 2022). Whether or not the variability of male and female rodents is similar across all fields of preclinical research remains to be seen, but it is worth bearing in mind that studies of such questions, even in humans, are neither straightforward nor conclusive (e.g., Davies et al., 1999)

Experimental design and data analysis

Regarding studies that used both sexes, it was striking that this was rarely considered as a relevant factor in the experimental design. Only 7 publications specified that 'Sex' was taken into account for randomization of the experimental units to different treatments (Figure 3). By contrast, 14 studies did not randomize (or counterbalance) sex across the different treatments and 18 did not mention this point at all. In some studies (even of humans), the numbers of the two sexes were not balanced at the start of the experiment. For example, in one study the ratio of males to females was 4:1. In such cases, neither randomization of the samples from both sexes to different experimental treatments nor meaningful interrogation of the data for sex differences would have been feasible. One study used both sexes, but the experiments were carried out independently (to avoid "confounds"). Yet, a sex difference in the response of interest was reported, despite the lack of randomization (or counterbalancing) of the study.

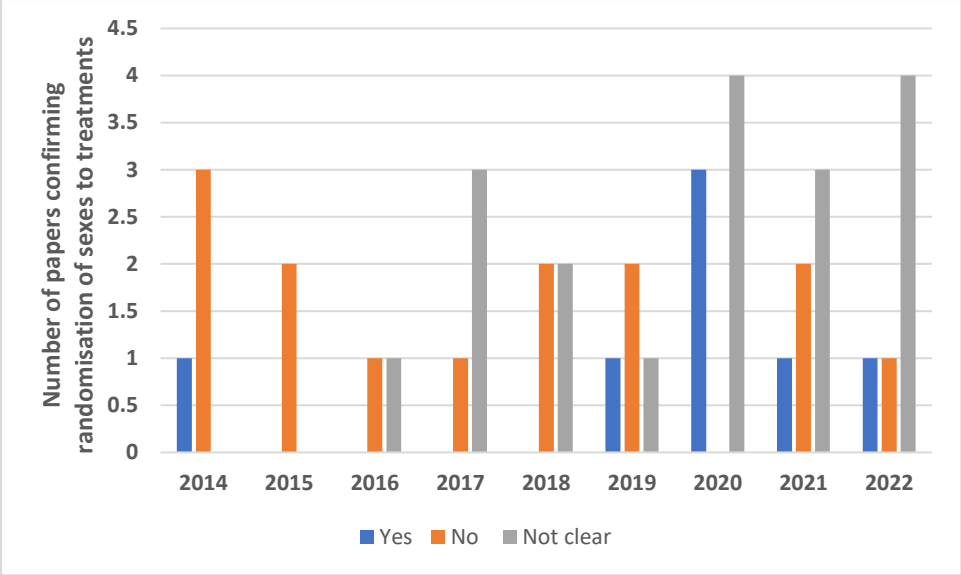

Figure 3. The number of publications (by year) in which randomization of sex to different experimental treatments was confirmed, or not, or whether it is not clear from the description of the experimental design

In respect of statistical analysis of the data, only 9 of the 40 studies that had used both sexes included 'Sex' as a factor in the statistical analysis of which 5 reported a sex difference as a finding. However, a common feature of studies of the remainder was to pool the data from both sexes without first validating that step: i.e., there was no primary factorial analysis to test whether there was an interaction between the factor, Sex, and others in the design. Yet, only if it is confirmed that the effect of the experimental intervention does not differ in males and females would such pooling of the data produce a safe conclusion.

Finally, only 4 papers specified the sex of the subject in the title of the publication. Many more papers (36) specified the sex of the subjects in the abstract, but this information did not increase during the period covered by the audit (Figure 4). Although some authors included text in the discussion dealing with limitations of the study, only 2 papers included the use of only one sex or acknowledged that the findings might not generalize to the other sex (both published after the first BJP Editorial on this topic).

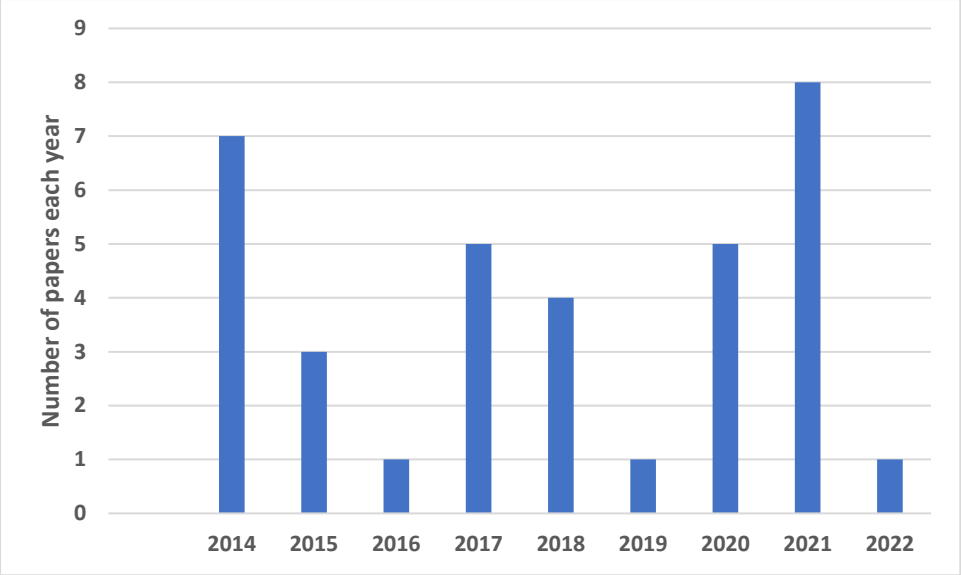

Figure 4. The number of papers each year that specified the biological sex of the experimental unit in the abstract

4 Conclusions

Overall, our audit indicates that progress in persuading researchers to study both sexes has been disappointing. Moreover, it is evident that manuscripts are being accepted for publication in the *BJP* that do not comply with our recommendation that both sexes should be integral to the study design, unless there is a clear justification for not doing so. It would not be desirable to impose a draconian rule that papers will not be accepted for publication unless they are compliant with our guidelines on this point. This is not least because the animals have been used for research already and it would be unethical do deny dissemination of the findings. Also, the audit makes it clear that, unless there is a profound change in research practice, then only a small handful of papers each year would qualify for publication in the *BJP.*

What is needed is for the *BJP*, and other journals, to find a way to convince researchers that the study of both sexes is fundamentally important for scientific discovery and the equitable development of effective therapies: women's health simply cannot be marginalised. But, as our audit shows, despite the increased awareness of sex bias in biomedical research, more needs to be done to convince researchers to act on this awareness. Perhaps the reluctance to adopt practices that are essential for delivering inclusive research relates to a perception of economic pressures. However, as highlighted in the previous Editorial (Docherty et al., 2019) it is essential to correct the misconception that using both sexes inevitably increases the total number of animals needed for the experiments. As was explained in that article, an appropriately powered study that includes sex as an experimental factor can use the same number of animals, regardless of whether only one, or both, sexes are used.

The announcement, in September 2022, that the United Kingdom Research Institute (UKRI) now requires the research they fund to study both sexes, aligning their policy with that of the National Institute for Health in the USA, will surely be helpful (UKRI 2022). It will be interesting to repeat the audit in 4 years' time, to confirm whether this policy has any effect on manuscripts submitted to the *BJP*, or whether researchers will continue to assert that their experiments qualify for an exemption.

Meanwhile, referees and Editors are well placed to maximise compliance, within the constraints of the experiments that were carried out. For instance, if only one sex were used, they can ensure that: 1) the justification has scientific merit; 2) the title and abstract specify the sex of the subjects that were used; and 3) the limitations of using only one sex are discussed fully.

5 Summary of key points for authors, editors and referees

- The *BJP* requires sex to be considered as an experimental variable for all experimental reporting. This will affect the details of the experimental design that are documented or, in the absence of a design incorporating both sexes, a reasonable justification for that approach.

- We strongly suggest that all experiments (in vivo, ex vivo, and in vitro, if relevant) should include both sexes, unless there is a specific justification or exemption, such as e.g. when using immortalised cell lines or tissue derived from a sex organ.

- If only one sex has been used, this should be specified in the title and/or abstract of the manuscript

- Multifactorial designs should be used to study the overall effects of Sex, Treatment (i.e., the experimental intervention), and their interaction.

- Authors should consider the implications for the study findings when testing males and females in proximity, and describe these implications in the discussion of the manuscript.

Audit questions
• Field (keyword)
• Species used
• Type of study: in vivo / tissue samples in vitro / ex vivo / primary culture
• What sex(es) were used
• If only one sex was used, was a justification provided
• Was there an undisputable justification for using only one sex (e.g., studies of the vas deferens or prostate)
• Did the justification rely on the use of a single sex being 'standard practice' in the research field
• If only one sex was used, was the sex specified in the title
• If only one sex was used was the sex noted in the abstract
• Did the experiment use a randomised factorial design, with Sex treated as a categorical factor, and was this explicit in Methods and Stats
• If the study used both sexes, did the statistical analysis of the results consider Sex as a factor in the analysis, or were the data from both sexes pooled without any interrogation of a possible sex difference
• Did the findings reveal a sex difference in the measure of interest and, if so, was this mentioned in the discussion
• If using only one sex, did the Discussion acknowledge that the findings might not apply to the other sex

Table 1. The list of questions addressed in the audit