Sex Balance in Preclinical Animal Models—Have We Made Progress?

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ABSTRACT

The NIH announced new policies in 2014 aimed at requiring NIH-funded researchers to address sex balance in preclinical cell and rodent models. Although exclusion of women in clinical research was identified as problematic and addressed decades ago, the preclinical research world has not followed suit. The NIH identified this continued reliance of animal studies on a single sex, often male, as inappropriate: "consideration of sex is a critical component of rigorous experimental design".[1] These changes took effect in Fiscal Year 2016 grant applications for projects funded in Fiscal Year 2017. Sales data from commercial animal vendors is a unique source of information to judge whether the new NIH policies are affecting animal usage. We analyzed Taconic Biosciences' mouse and rat sales data for non-profit institutions in the United States. For the most common research models, there was little to no movement towards equal usage of males and females. These common strains and stocks all display bias towards a particular sex which has remained unchanged from 2013 through 2018. For transgenic models, most of which have more narrowly defined uses compared to standard inbred strains and outbred stocks, the results are similar. Each model maintains a similar sex bias over time, with some fluctuation seen by year. From an animal welfare perspective, a move towards sex parity in rodent research would reduce the numbers of animals produced but not put to research use. The nature of breeding is that generally equal numbers of males and females are produced for each line, but sex bias in usage means a large percentage of one sex may not be used experimentally. No strong evidence for movement towards parity of rodent usage by sex is evident in Taconic's sales data. Research thus continues to potentially overlook influence of sex in many experiments, and vendors continue to produce animals of the non-preferred sex which may not be used experimentally.

METHODS

Shipment records from Taconic Biosciences for commercially available mice and rats were reviewed for the period Jan 1, 2013, through Aug 31, 2018. The dataset was filtered to include only shipments made to non-profit institutions in the United States, including both academic and governmental organizations. The data was further filtered for specific mouse and rat strains/stocks of interest and then for sales by sex for each animal model. Sales of retired breeders and pregnant animals were excluded from the data. Sales at all commercial health standards were included in the data. Time periods are reported as calendar year, not US government fiscal year.

Specific models included in the evaluation were:

Common name	Nomenclature	Taconic model #	Model type
B6	C57BL/6NTac	B6	Inbred mouse
BALB/c	BALB/cAnNTac	BALB	Inbred mouse
129S6	129S6/SvEvTac	129SVE	Inbred mouse
NOD	NOD/MrkTac	NOD	Inbred mouse
Swiss Webster	Tac:SW	SW	Outbred mouse
Sprague Dawley®	NTac:SD	SD	Outbred rat
NCr nude	CrTac:NCr-*Foxn1*^{nu}	NCRNU	Immunodeficient mutant mouse
C.B-17 scid	C.B-*Igh-1*^{b}/IcrTac-*Prkdc*^{scid}	CB17SC	Immunodeficient mutant mouse
APOE4	B6.129P2-*Apoe*$^{tm3(APOE*4)Mae}$ N8	1549	Transgenic mouse
Tau	STOCK Tg(Prnp-MAPT*P301L)JNPL3Hlmc	2508	Transgenic mouse
Stat1 Knockout	129S6/SvEv-Stat1^{tm1Rds}	2045	Transgenic mouse

RESULTS

Figures 1–11 show the breakdown by sex (male or female) of units shipped to US non-profit and government customers for the specified model, by year for the period 2013–2018. 2018 data represents the period Jan 1, 2018 through Sep 3, 2018.

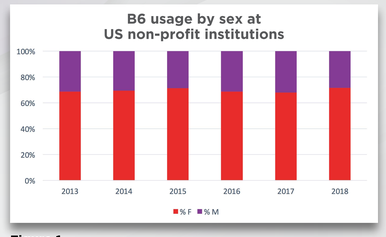


Figure 1

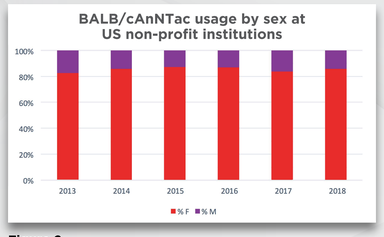
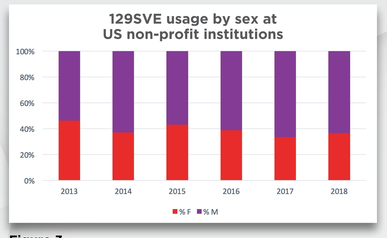


Figure 2



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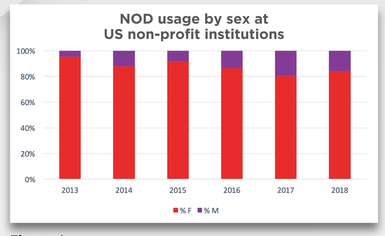
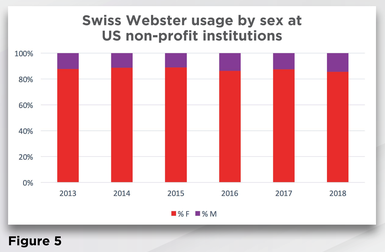


Figure 4



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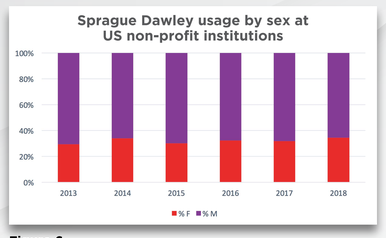
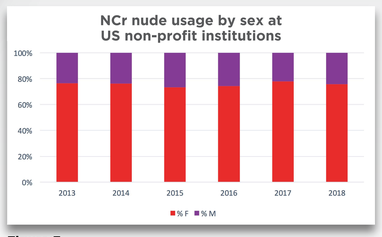


Figure 6



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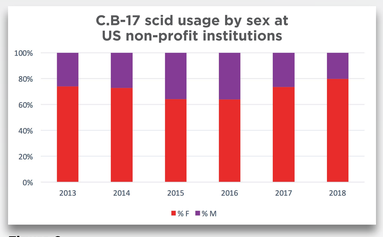

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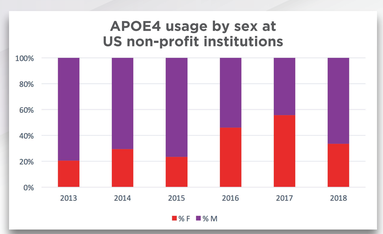

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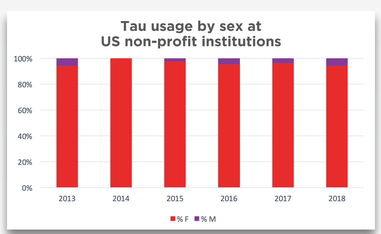

Figure 10

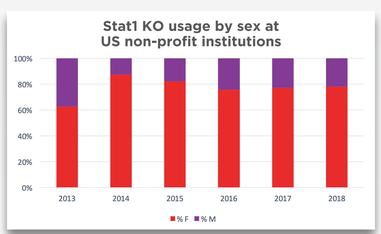

Figure 11

DISCUSSION AND CONCLUSIONS

General purpose inbred and outbred mouse and rats are standard models with a wide range of research applications. For these common research models, there was little to no movement towards equal usage of males and females as seen in unit sales by Taconic to US non-profit and government researchers. As shown in Figures 1–3 and 5–6, the inbred strains B6, BALB/c and 129S6, and outbred strains Swiss Webster and Sprague Dawley® all display biases towards a particular sex; these biases have remained unchanged from 2013 through 2018.

Standard strains and stocks are used for a very wide variety of research applications and experiment types, which may make more detailed analysis difficult. Yet if we focus on special purpose strains, including the NOD mouse and various immunodeficients and transgenics, most of which have more narrowly defined uses compared to standard inbred strains and outbred stocks, the results are similar. Each model maintains a similar sex bias over time, with some fluctuation seen by year. No strong evidence for movement towards parity of rodent usage by sex is evident in Taconic's shipment data.

The NOD inbred strain is a special purpose model of Type 1 diabetes. As shown in Figure 4, ≥80% of all NOD mice shipped to US non-profit and government customers were females during the time period studied, despite the fact that incidence of Type 1 diabetes in the US is higher in males than females.[2] The immunodeficient NCr nude and C.B-17 scid models both show predominant usage of females, which is consistent with the bias towards females as tumor xenograft hosts. The males sold were likely for use in male-specific tumor studies such as prostate cancer or for adoptive transfer inflammatory bowel disease studies which have commonly used male C.B-17 scids.[3] Usage of the Tau Alzheimer's disease model revealed nearly all mice shipped to US non-profit researchers were females, with female mice comprising 94–100% of total volume each year from 2013 through 2018. Drilling down to the individual order level for these specialty strains, a few orders clearly source full and equal cohorts of both sexes for a study, but the vast majority of cohort orders remain for a single sex only.

The experimental decisions which drive sex bias in mouse and rat models are likely to be based on a combination of myth and reality as well as history and custom. Sex bias varies by research discipline, with neuroscience, pharmacology, and physiology skewed in favor of males and reproduction and immunology skewed in favor of females, as measured by the ratio of published papers reporting on only male versus only female study subjects.[4]

Sexually mature males may be more aggressive than females and thus require different (more costly) housing, but that does not apply equally across all strains and stocks. A default assumption should not be made that males are inappropriate because of potential housing problems. In neuroscience, the commonly held assumption that females are more variable because of reproductive hormonal fluctuations is used to justify use of males only, but a meta-analysis of neuroscience studies including both male and female rats found "that even when female rats are used in neuroscience experiments without regard to the estrous cycle stage, their data are not more variable than those of male rats."[5] For some strains, phenotypic differences may influence sex bias. For example, the prevalence of diabetes in NOD mice is higher in females compared to males[6] and female Tau mice are reported to have more severe disease pathology compared to males[7], so the choice to preferentially use females for these strains may be a rational one for certain types of studies. However, neglecting males for these studies may be a missed opportunity in terms of identifying factors which influence disease phenotype by sex.

The impact of sex bias in preclinical studies is broad, from impacts on animal welfare through applicability of research findings to the full human population. Because roughly equal numbers of males and females are produced through breeding, predominant usage of a single sex for particular models results in large quantities of non-primary sex animals which are not put to experimental use. From an animal welfare standpoint, a move towards sex parity in preclinical research would ideally not increase the overall numbers of animals used, but rather reduce the overall numbers of mice and rats produced. As study subjects become more evenly distributed across males and females, lower total production is required. Beyond the 3Rs, the drive to increase consideration of sex as a biological variable is to improve the quality of preclinical research results and ensure they apply to both men and women.

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