NIH Request for Information:
Consideration of Sex as a Biological Variable in Biomedical Research

Analysis of Public Comments

May 19, 2015

Executive Summary

In May 2014, National Institutes of Health (NIH) Director Dr. Francis Collins and NIH Associate Director for Research on Women's Health Dr. Janine Clayton published a *Nature Commentary*, Policy: NIH to balance sex in cell and animal studies[1], stating NIH plans to adopt a new policy requiring the consideration of sex as a biological variable (SABV) in preclinical research. While women now account for roughly half of all participants in NIH-funded Phase III clinical trials, basic and preclinical biomedical research has more often focused on male animals and cells. A closely related concern, addressed in a January 2014 *Nature Commentary*[2] by Dr. Collins and NIH Deputy Director Dr. Lawrence Tabak, is that failure to consider SABV at the preclinical stage may undermine the transparency and generalizability of research findings and weaken the value of national investments in biomedical research. Drs. Collins, Tabak, and Clayton emphasized that just like randomization, blinding, and sample-size calculations are basic elements of rigorous experimental design, so too is consideration of SABV. Considering sex in NIH-funded studies strengthens the foundation of biomedical research and maximizes our understanding of male and female biology and health outcomes.

To inform policy development, NIH formed the Trans-NIH SABV Working Group. On September 11, 2014, the Working Group released a Request for Information (RFI): Consideration of Sex as a Biological Variable in Biomedical Research (NOT-OD-14-128) to gather input from the research community and other interested stakeholders. NIH invited community feedback on several topic areas: whether consideration of SABV is an issue affecting reproducibility, rigor, and/or generalizability of research findings; areas of science (e.g., cardiovascular disease, neuroscience) and phases of research (e.g., basic, translational, clinical) with greatest opportunity of need for consideration of SABV; impediments to consideration of SABV in research; and suggestions of ways in which NIH could best facilitate consideration of SABV in research. After 44 days of public comment, 222 responses were received from professional societies, patient advocacy groups, privacy groups, and individual scientists. The comments reflected a variety of scientific perspectives from basic, clinical, and translational areas of inquiry.

Analysis of the RFI revealed areas of collective significance to respondents, while also capturing respondents' specific viewpoints and suggestions. A vast majority of respondents (87 percent) supported the notion that consideration of SABV is an issue affecting the reproducibility, rigor, and/or generalizability of research findings. Respondents commented that certain areas of science and phases of research would benefit in particular from SABV policy. These areas included neuroscience and neurological disorders, pharmacology and immunology, and cardiovascular disease. RFI respondents also identified areas of science and phases of research in which such a policy may have more limited benefit or may be cost prohibitive to implement. Almost all respondents (86 percent) noted at least one concern about implementing SABV policy, with cost and methodological and experimental concerns most frequently mentioned. RFI respondents suggested that NIH raise awareness regarding the importance of SABV in research design and analysis, provide the community with resources and training in this area, consider modifications to application and review processes, and provide guidance to the scientific community regarding NIH's expectations for applicants.

[1] http://www.nature.com/news/policy-nih-to-balance-sex-in-cell-and-animal-studies-1.15195
[2] http://www.nature.com/news/policy-nih-plans-to-enhance-reproducibility-1.14586

Report on the Results of the RFI:
Consideration of Sex as a Biological Variable in Biomedical Research

Design of the Request for Information (RFI): Consideration of Sex as a Biological Variable (SABV) in Biomedical Research (NOT-OD-14-128) focused on gathering input from the research community and other interested stakeholders about the consideration of SABV in research practices. A web-based form was available to the public from September 11, 2014, to October 24, 2014 (NOT-OD-15-012). Comments were also accepted via email. The RFI invited comment on the following six considerations:

- Whether consideration of sex as a biological variable is an issue affecting the reproducibility, rigor, and/or generalizability of research findings.
- Areas of science (e.g., cancer, neuroscience) or phases of research (e.g., basic, translational) conducted with animals that have the greatest opportunity or need for considering sex as a biological variable.
- Areas of science or phases of research conducted with cells and/or tissues that have the greatest opportunity or need for considering sex as a biological variable.
- Main impediments (e.g., scientific, technical, and other) to considering sex as a biological variable in research.
- Ways in which NIH can facilitate the consideration of sex as a biological variable in NIH-supported research.
- Any additional comments you would like to offer to NIH about the development of policies for considering sex as a biological variable in research involving animals, tissues, or cells.

Characteristics of Respondents: 222 responsive submissions were received; 195 (88 percent) were submitted by individuals, and 27 (12 percent) were submitted on behalf of associations or institutions. The latter group included statements from 11 academic institutions (colleges, universities, or university centers), 8 professional associations, 5 research/patient advocacy organizations, and 3 non-profit research institutions and/or hospitals. (See Table A1 in the Appendix for a complete list.) Because very few substantive differences were found in the responses of organizations as compared to individuals, the data in this report reflect their combined response (i.e., “respondents”). All percentages in the report use the total number of respondents (N=222) as a denominator.

Analysis of the Results: Ripple Effect Communications, Inc. provided a content analysis of the RFI responses. (See Table A2 in the appendix for a description of the coding process and all codes and sub-codes incorporated in the analysis.)

Topics of Response

Feedback clustered around 7 primary topics (Table 1). Categories 1 to 5 adhere closely to the format of the RFI questions, while 6 and 7 capture additional themes raised by respondents. Not all respondents provided feedback to all topics (response rates are included in parentheses). While the majority of respondents provided feedback to categories mapping specifically to the RFI (1-5), just over half of respondents provided additional input on the form the policy should take (category 6), and roughly one-third of respondents commented on the relationship of such a policy to the broader scientific enterprise (category 7).

RFI respondents frequently distinguished *science-related* aspects of considering SABV in research (a primary focus of responses to categories 1-3) from *policy* or *policy implementation* of SABV in research (a primary focus of response to categories 4-7). Respondents' interpretation of forthcoming NIH SABV policy framed the nature of their feedback. For example, there was significant speculation (and an underlying assumption) that the forthcoming policy would require all NIH-funded researchers to include both sexes in all NIH-funded research studies.

Table 1. Primary categories of response to the RFI

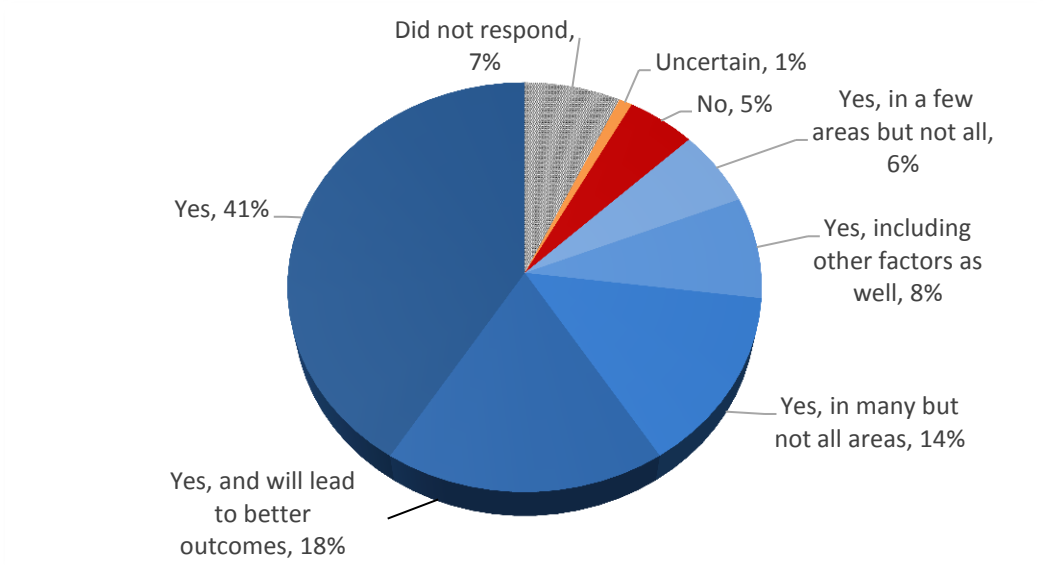
Primary Categories of Response	Focus of Feedback
In response to RFI Questions	
1) Relationship of SABV to rigor, reproducibility, and generalizability (93%)	Science
2) Areas and phases with opportunity for SABV in animal research (85%)	Science
3) Areas and phases with opportunity for SABV in cell/tissue research (64%)	Science
4) Impediments to considering SABV in research (86%)	Policy
5) Ways in which NIH can facilitate consideration of SABV in research (81%)	Policy
Related and/or Supporting Themes	Policy
6) Suggested approaches for policy development and form (54%)	Policy
7) Relationship of the policy influence on the broader scientific enterprise (36%)	Policy

Note: Values reflect the percentage of total RFI respondents (N=222). Categories are not mutually exclusive.

Sex as a Biological Variable in Biomedical Research

Nearly all RFI respondents (93 percent) provided feedback to the first RFI prompt, "Whether consideration of SABV is an issue affecting the reproducibility, rigor, and/or generalizability of research findings." A vast majority of respondents (87 percent) made declarative statements or clearly implied that sex is an important biological variable in research (Figure 1). These responses included approximately 40 percent who indicated "yes" with no additional qualifications or clarifications, and nearly 20 percent who affirmed the statement while also indicating that considering SABV will provide better outcomes for science. Approximately one-third provided an affirmative response but with qualification. These included statements that sex is important in many but not all scientific areas or, conversely, in a few areas but not in all. Other respondents raised the importance of SABV in addition to and in comparison to other factors (examples given included age, species, breed/strain, and hormone levels). These respondents noted that the importance of individual factors might vary with the mechanism or outcome under study. Small subsets of respondents disagreed with the first RFI prompt or were uncertain regarding the effect of sex on rigor, reproducibility, and/or generalizability. Finally, some respondents suggested the existence of fields in which sex would not be expected to have an impact and/or fields that are sex-specific, such as those focusing on prostate or ovarian cancer.

Figure 1. Support for whether consideration of SABV is an issue affecting reproducibility, rigor, and/or transparency in research findings.



Note: Values reflect the percentage of total RFI respondents (N=222). Categories are mutually exclusive.

Areas of Science and Phases of Research Opportune for Considering SABV

Areas of Science

RFI respondents identified many areas of science and phases of research that they believe are opportune for considering SABV in 1) animal and 2) cell/tissue research. A higher percentage of respondents provided feedback regarding animal research than cell/tissue research (74 percent versus 34 percent). The collective feedback included a set of 22 fields and areas. (See Table A3 in the appendix for the complete list with frequency counts.) The following were the top five most frequently mentioned fields:

- Basic neurobiology and neurological disorders
- Behavioral neuroscience, mental disorders, and disorders of addiction
- Pharmacology and immunology
- Cardiovascular disease
- Endocrinology (in cell/tissue research)
- "All Areas"

Neuroscience, neurological and neurobiological areas featured prominently in respondent comments. Examples include neuronal development; brain mapping; sensory processing and perception; stroke; and neurological disorders, such as concussion, migraine, and Parkinson's disease. Cardiovascular disease was also cited frequently among respondents, who mentioned the high impact of cardiovascular disease on the human population and known sexual dimorphism in humans. Pharmacology and immunology were also mentioned frequently because of their overarching impact on an organism and the existence of sexually dimorphic responses to medications.

Some respondents to RFI prompts about animal and cell/tissue research did not specify any particular area of science but stated that sex is an important variable across all areas of preclinical research. Others suggested that no areas of science should be excluded until there is evidence that sex differences in a given

area do not have an effect in humans. Additional fields of particular relevance for SABV studies were suggested in smaller numbers. These include digestion and metabolism, autoimmune disorders, cancer research, reproductive health, aging, and muscular and skeletal system research. Related to cell/tissue research, respondents also suggested stem cell approaches, "-omics" fields, epigenetics, and cell signaling.

Respondents provided diverse explanations for these choices. Many relied on areas of personal expertise, providing examples where sex differences arose in the course of their conducting research; others provided NIH with references to publications illustrating such examples, or to additional areas known in their field that contain sex differences. Many appeared to identify fields based on their relevance to human health and/or known human sex differences.

Phases of Research

The RFI also inquired about phases of research most opportune for the consideration of SABV. Again, there was a higher rate of response regarding animal research versus cell/tissue research (51 percent versus 28 percent). For animal research, translational research was cited most often (28 percent), followed by basic, clinical, and "all phases" in roughly equal proportions (17 percent, 13 percent, and 16 percent, respectively). Many respondents indicated that it was best to focus on the earliest possible phase of research, because these studies inform more costly and/or complicated studies in later phases (e.g., clinical trials).

For cell/tissue research, relatively few RFI respondents (10 percent of respondents or less) mentioned basic, translational, and "all phases." An additional 10 percent of respondents specified "No Phase," suggesting little relevance for the consideration of SABV in cell/tissue research. Respondents provided feedback regarding the challenges faced with incorporating SABV in cell and tissue lines. Many mentioned that sex seems less relevant, difficult to determine, and/or challenging to control for in immortalized cell lines. They pointed out that changes that occur during the processes of immortalization and propagation may be more salient than the sex of the organism from which the cells are derived. Typical cell culture conditions, which include reagents such as animal serum, may be biased by sex *a priori* due to the fact that these reagents are obtained from animals and thus contain sex-specific hormones from the source animal. Others distinguished between the lack of relevance of sex in immortalized cell lines, which may have lost their sex-specific characteristics; and the relevance of sex in primary cell and tissue cultures, which retain sex-specific characteristics.

Impediments to Considering Sex as a Biological Variable in Research

The majority of RFI respondents (86 percent) provided feedback regarding a range of potential impediments associated with the consideration of SABV in research. This feedback clustered into three main themes (not mutually exclusive): cost concerns (65 percent of respondents), methodological and experimental concerns (62 percent of respondents), and limited understanding of SABV in the scientific community (20 percent of respondents). (See Table A4 for complete details on responses to each category.)

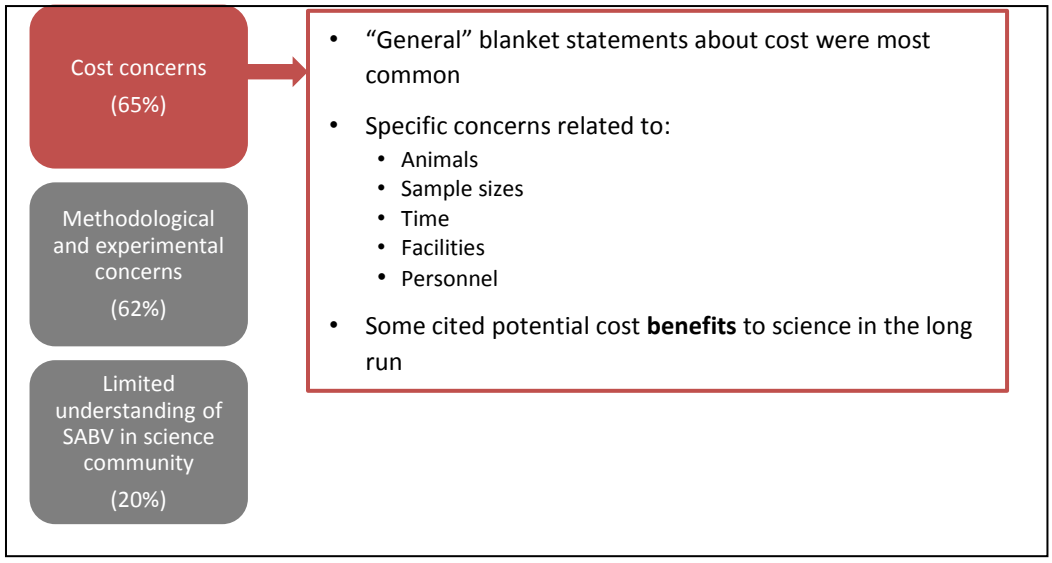
Cost Considerations

A majority of respondents (65 percent) indicated cost as a significant impediment to conducting research that considers sex in design and/or analysis (Figure 2). This feedback ranged from broad statements about

increased costs to particular areas of concern. For example, respondents mentioned increased costs related to the need for additional animals; increased sample size; and the cost of additional time, personnel, and/or facilities needed to conduct this research. Respondents who use mutant mouse lines or non-human primates expressed specific concerns about costs related to breeding, housing, and maintaining their animal colonies. These respondents felt that because of the extra cost of breeding and housing their animals, research using transgenic mice or non-human primates would incur especially significant costs.

Even when in favor of an NIH policy for including SABV in research studies, respondents voiced concern about costs in the context of current grant funding levels. However, a small number of respondents noted that the proposed NIH SABV policy may reduce costs in the long run. They suggested that using both males and females would increase the generalizability of findings, thus hastening successful drug development and lessening costs associated with oversights incurred by not including both sexes.

Figure 2. Feedback regarding **cost concerns**



Note: Values reflect the percentage of total RFI respondents (N=222). Categories are not mutually exclusive.

Methodological and Experimental Concerns

A similar proportion of respondents (62 percent) identified methodological issues and experimental concerns as potential impediments to considering SABV (Figure 3). Although respondents presented great variability in their feedback, three areas captured the bulk of the feedback regarding these concerns: sample availability (26 percent), estrous cycle concerns (23 percent), and lack of sufficient methodological and experimental expertise within the scientific community (22 percent).

Sample Availability. Respondents raised a few repeated challenges regarding sample availability in the consideration of SABV in research. In the case of *in vitro* experiments, for example, male and female counterparts of many common cell lines have not been generated, and direct comparisons across sexes may be difficult to perform. In research involving non-human primates, a potential increased demand for female animals may put strain on breeding colonies and, in turn, have the unintentional consequence of compromising projects that focus on female-specific health areas. Respondents also

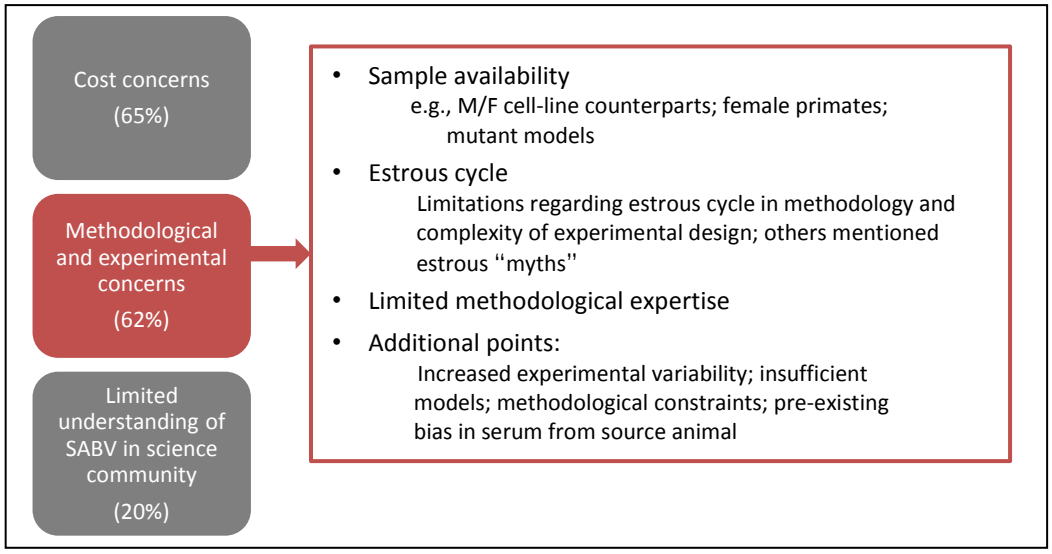
noted that research with mutant mice models may also experience particular challenges, given that the yield of a specific genotype can be low in a given litter.

Estrous Cycle Concerns. Respondents also noted concerns over the added methodological complexity that is required to control for the estrous cycle in female animals. The suggestion was that proper monitoring requires additional levels of consideration, such as evaluating sets of animals at various stages in their cycle. A small percentage of respondents, however, noted misconceptions regarding the estrous cycle and that the inclusion of female animals does not add notable variability to a study.

Limited Expertise. Respondents also noted that many researchers may lack the proper methodological knowledge necessary to execute experiments utilizing both sexes. Including both sexes in the absence of sufficient methodological expertise may result in improper experimental design and statistical analysis (e.g., not adequately taking into account power considerations, adjusting analyses for disaggregated data). Many respondents related this concern to that of the technical aspects of controlling for estrous cycle fluctuations.

Additional Points. Additional points were raised in smaller numbers. These include concerns about added variability introduced in experiments with the inclusion of a second sex, the technical difficulty of determining the sex of cell lines or animals, and the validity of some methods used by researchers to control for sex (for example, ovariectomization over vasecomization). Respondents also raised concerns about the hormonal composition of media or serum used for cell/tissue incubation, if that medium has a hormonal and sex composition different from that of the sample under study.

Figure 3. Feedback regarding **methodological and experimental complexity**



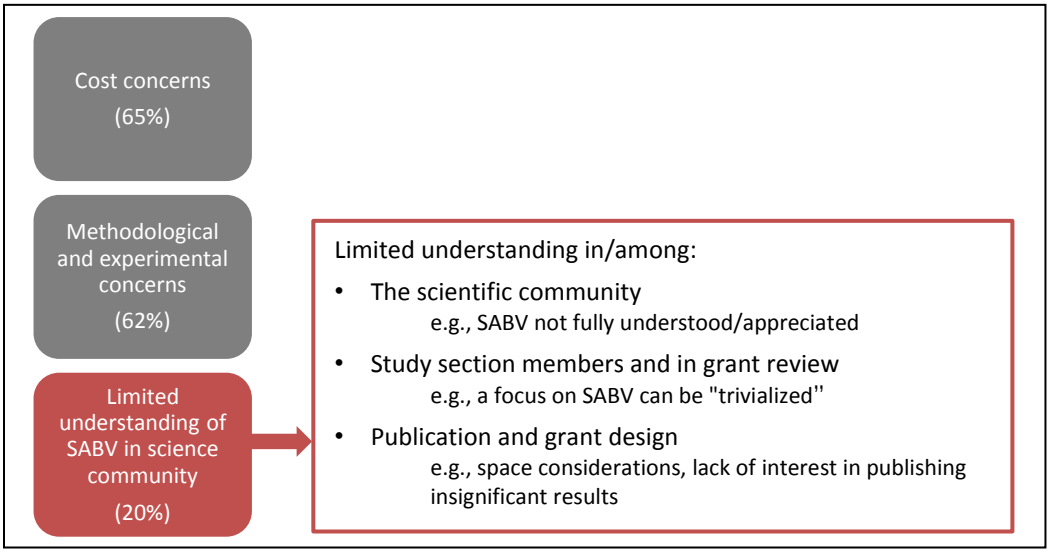
Note: Values reflect the percentage of total RFI respondents (N=222). Categories are not mutually exclusive.

Limited Understanding of SABV in the Scientific Community

Approximately 20 percent of respondents focused on impediments related to limited understanding of the importance of SABV in the scientific community: among individual researchers, study section members, and in grant applications and publications (Figure 4). These respondents presented concerns that the scientific

community does not fully understand or appreciate the importance of work that considers SABV, especially in grant review and publication. Respondents described perceptions that the study of sex differences is frequently "trivialized" by study sections or is often viewed as too descriptive or not mechanistic enough to warrant strong reviewer support. Similarly, respondents raised the point that existing publication standards, especially with regard to interest in publishing null or insignificant findings, are a limitation to the process of considering SABV in research.[3] Respondents also discussed space considerations (in both journals and grant applications) as a practical limitation that can hinder the consideration of SABV.

Figure 4. Feedback regarding **practical roadblocks and limited understanding of evaluators**



Note: Values reflect the percentage of total RFI respondents (N=222). Categories are not mutually exclusive.

How Can NIH Facilitate Consideration of Sex as a Biological Variable?

The majority of RFI respondents (81 percent) provided feedback regarding types of actions and/or resources NIH could provide to help researchers comply successfully with policies regarding the consideration of SABV. Feedback from respondents focused upon four main areas: 1) provision of resources (funding, training, and databases/tools), 2) careful consideration and modifications to the NIH grant application and review process, 3) guidance to the scientific community regarding NIH's expectations for applicants, and 4) raising awareness regarding the importance of SABV in research design and analysis.

Table 2 presents these primary areas of response with examples for each. More than half of RFI respondents (58 percent) suggested that NIH could offer tangible resources to help with implementation of a new policy, with a strong emphasis on funding. Respondents also suggested that NIH provide training

[3] After the RFI was conducted, journal guidelines on this topic were released and can be found here (11/7/2014): *Science* Editorial: "Journals Unite for Reproducibility" (http://www.sciencemag.org/content/346/6210/679.full), *Nature* Commentary: "Journals Unite for Reproducibility" (http://www.nature.com/news/journals-unite-for-reproducibility-1.16259), NIH Website: "Proposed Principles and Guidelines for Reporting Preclinical Research" (http://www.nih.gov/about/reporting-preclinical-research.htm).

resources and scientific tools such as courses, workshops, and databases. Specific suggestions include the development of inexpensive methods for genotyping sex in embryonic mice, services to rapidly measure hormone levels, or public databases that detail the sex of commonly used cell lines or of lines that are useful for studying sex differences.

Respondents also asked that NIH provide standards for implementation and execution of science that considers SABV, and for reporting data by sex. Approximately one-third of RFI respondents (31 percent) suggested that NIH ask applicants to provide a justification for their approach to considering SABV in their research applications. They also requested resources and/or training for reviewers to assess applicants' plans to address SABV in research design and analysis and asked NIH to ensure accountability of grantees.

Table 2. How NIH can facilitate consideration of SABV

Categories of response	Examples
Provide resources (58%)	
• Increase funding (50%)	Issue RFAs; increase R01 budgets; increase caps on modular budgets
• Provide training (18%)	Integrate SABV into training curriculum; hold NIH-sponsored events (e.g., workshops/webinars, conferences)
• Support/develop tools and resources (14%)	Establish databases to assist researchers (e.g., sex of commonly used cell lines, sex-matched cell lines, sex differences in animal traits, or lists of expert researchers); establish specimen repositories; provide literature review tools and resources
Modify the grant application and review process (46%)	
• Require justification (30%)	Require explanations for how SABV will be factored in experimental design and analysis in grant applications
• Train reviewers (27%)	Provide training to reviewers to assess the applicants' SABV considerations
Provide guidance and promote standards (45%)	
• Provide best practices (31%)	Give clear language and practical direction for grant applications and reviewers
• Require transparency (23%)	Require reporting of sex of animals or cells at time of publication and in progress reports.
• Ensure accountability (5%)	Promote grantee compliance with expectations; develop metrics for policy evaluation
Raise awareness (12%)	
• Raise awareness (12%)	Utilize media outlets to raise awareness to general public; partner with organizations to promote consideration of SABV

Note: Values reflect the percentage of total RFI respondents (N=222). Categories are not mutually exclusive.

Suggested Approaches for Policy Development

Approximately half of respondents (54 percent) provided policy suggestions. Among the most common (31 percent of respondents) was a preference for what might be called a "flexible" approach, anchored by the

notion that consideration of SABV be implemented on a "case by case" or "targeted" basis. "Flexibility" garnered different meanings. Some respondents thought it may depend primarily on the research question, while others suggested it may apply to certain fields and not to others. Still others suggested that attention to SABV should be balanced across research projects in a given area/field of science. However conceived, advocates of a flexible approach often offered concerns regarding an assumed forthcoming "one-size-fit-all" blanket policy. Many respondents said that a blanket policy would be particularly inappropriate for studies using acutely scarce resources (e.g., non-human primates), or experiments involving decidedly sex-specific fields, such as prostate cancer or female reproductive health.

Smaller percentages of RFI respondents suggested that NIH provide financial incentives to entice researchers to consider SABV, such as longer grant durations or higher funding caps, or, conversely, that NIH "do nothing." Still others suggested that NIH consider piloting SABV policy in certain research fields, within the NIH intramural program, or in certain extramurally funded research centers.

Potential Policy Influences on the Scientific Enterprise

Finally, about one-third of respondents (36 percent) commented on the potential SABV policy in relationship to the broader context of the U.S. scientific enterprise. In small numbers, points were raised about the impact that an SABV policy may have on scientific progress in the United States, including concerns that it could slow progress during times of intense funding constraints. Others mentioned a perception that SABV policy interferes with the open pursuit of science. Respondents also provided feedback relating policy to ethics in science. For example, many related SABV policy to the ethics of inclusion in research and the development of treatment and clinical interventions applicable to all people; some respondents believed that an SABV policy may promote unnecessary use of animals; others suggested that an SABV focus may amplify erroneous beliefs that women and men are universally or comprehensively different in all areas.

Summary and Conclusions

The majority of RFI respondents agreed with the tenor of the viewpoints expressed in the May and January 2014 *Nature* commentaries[4] that SABV is a factor affecting the reproducibility, rigor, and/or generalizability of research findings in biomedical research. While the sample of RFI respondents represents myriad stakeholders in the scientific community – including the membership base of several sizeable scientific professional associations – the small and non-random sample of responses (N=222) raises challenges for the interpretation of categorical percentages. Nonetheless, these RFI responses provided a range of concerns that NIH will find helpful in developing and implementing SABV policy. Respondents' feedback appeared to be generally informed by the desire to optimize human health outcomes, whether by clarifying known human health disparities, as in the areas of cardiovascular disease and mood disorders, or by studying organismal biology, such as neural and endocrine systems.

[4] http://www.nature.com/news/policy-nih-to-balance-sex-in-cell-and-animal-studies-1.15195; http://www.nature.com/news/policy-nih-plans-to-enhance-reproducibility-1.14586

Respondents noted several challenges that may influence their ability to consider SABV as effectively as possible in their research programs. Most heavily cited were cost concerns related to animals, sample sizes, time, personnel, and facilities. Respondents differed, however, in the extent to which they anticipated that increased costs would impede research. Some remarked that considering SABV in the context of a particular research question outweighs increased cost concerns, whereas others noted that additional required resources may slow overall scientific progress. In nearly equal proportions, respondents raised methodological and experimental considerations, noting that many researchers lack the capacity (e.g., the expertise and tools) to consider SABV in their studies in a meaningful or accurate way. Respondents noted that if a blanket policy is issued, a lack of knowledge with respect to proper experimental design and data analysis may confound research findings and fail to improve scientific outcomes. Some respondents reported specific concerns about practical limitations, such as the existence of sex-matched cell lines or the availability of female animals for research (e.g., non-human primates).

RFI respondents offered a series of suggestions toward a nuanced approach that would allow investigators the discretion to decide if consideration of SABV is appropriate for their proposed project: the most common of which is one promoting consideration of SABV on a case-by-case basis (e.g., per application). Based on the assumption that SABV policy changes are forthcoming, respondents suggested concrete steps to advance the study of both sexes in biomedical research. These include: 1) the provision of resources that include funding, tools, and training; 2) leadership to ensure transparency, accountability, and best practices in the scientific community; 3) modifications to the current application and review process that include justification for including (or not including) SABV; and 4) education for reviewers about the nuances and importance of studying both sexes. NIH appreciates the feedback received through this RFI and will consider the comments and suggestions, as well as other factors, in developing SABV policy.

Acknowledgements

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Ripple Effect Communications, Inc.: Amy Bielski, Erica Husser, Jennifer Pohlhaus, Elizabeth Sillman, and Elyse Sullivan.

Appendix

Table A1. Organizations that provided statements of response

Professional Associations

- American Psychological Association
- Society for Pediatric Research
- American Society of Clinical Oncology
- American Society of Primatologists
- Federation of American Societies for Experimental Biology
- Association of American Medical Colleges
- The Endocrine Society
- Education Committee of Society for Behavioral Neuroendocrinology
- American Physiological Society (submitted independently from the RFI)

Non-profit/Advocacy Organizations

- Society for Women's Health Research (2)
- Sex and Gender Women's Health Collaborative
- Physicians Committee for Responsible Medicine
- Prevent Blindness

Institutes and Centers

- Connors Center for Women's Health and Gender Biology
- Institute for Gender in Medicine
- Texas Heart Institute, Regenerative Medicine Research
- TTUHSC Laura W. Bush Institute for Women's Health
- Tulane Nat'l Primate Research Center (2)
- Southwest National Primate Research Center
- Emory University
- Laboratory of Neuroendocrinology, Brain Research Institute, UCLA
- The University of Texas System
- University of Texas Health Science Center at San Antonio
- Women's Health Research Institute at Northwestern University
- National Primate Research Centers
- Oregon Health and Science University

Table A2. Conceptual framework and codes[5]

	Primary Category	Code Group	ID	Sub-code
1	Sex as a biological variable in preclinical research	Yes	A01	Yes
			A03	Yes-only a few areas
			A04	Yes-many not all areas
			A05	Yes-other factors as well
			A06	Yes-better outcomes
		No	B01	No
			B02	No-only a few areas
		Unknown	C01	Unknown
2	Phases and areas of research: animal research	Phase of research	E00	All
			E01	Basic
			E02	Translational
			E03	Clinical
			E04	No Phase
		Specific scientific area	F00	All
			F21	Known only
			F01	Endocrinology
			F02	Reproductive
			F03	Basic neurobiology and neurological disorders
			F04	Behavioral neuroscience, mental disorders, and disorders of addiction
			F05	Cardiovascular
			F06	Aging
			F07	Autoimmune
			F08	Pharmacology
			F10	Cancer
			F11	Dermatology
			F12	Pulmonology
			F13	Digestion and metabolism
			F14	Immune response
			F15	Muscular and skeletal system
			F16	Developmental
		Animal type/sample type	W00	Animal studies
			W05	Mouse
			W06	Rat

[5] To determine primary areas of feedback across the set of respondents, Ripple Effect Communications developed an iterative coding process using the RFI framework as a guide for the initial set of response categories (*Primary Category*). Coding proceeded inductively with new codes introduced to account for emerging themes. After all of the comments had been coded once, each was reviewed a final time in the context of the complete set of codes. In addition, the process included a nested coding strategy to account for the respondents' general, overarching response categories (*Code Group*), as well as the specific or clarifying statements made in support of these views (*Sub-code*).

	Primary Category	Code Group	ID	Sub-code
			W07	Hamster
			W08	Rabbit
			W09	Nonhuman primate
			W10	Human
3	Phases and areas of research: cell/tissue research	Phase of research	G00	All
			G01	Basic
			G02	Translational
			G03	Clinical
			G04	No Phase
		Specific scientific area	H00	All
			H23	Known only
			H01	Transplantation
			H02	Endocrinology
			H03	Reproductive
			H04	Biopsies
			H05	Cardiovascular
			H06	"-omics"
			H07	Cell signaling
			H08	Stem cell research
			H09	Cancer
			H10	Apoptosis
			H11	Pharmacology and immunology
			H12	Epigenetics
			H13	Basic neurobiology and neurological disorders
			H14	Behavioral neuroscience, mental disorders, and disorders of addiction
			H16	Aging
			H18	Dermatology
			H19	Digestion and metabolism
			H20	Pulmonology
			H21	Developmental
		Animal type/sample type	X00	Animal studies
			X05	Mouse
			X06	Rat
			X07	Hamster
			X08	Rabbit
			X09	Nonhuman primate
			X10	Human
4	Impediments to considering sex as a biological variable	Cost concerns	J00	Cost-general
			J01	Cost-animal

	Primary Category	Code Group	ID	Sub-code
			J02	Sample size
			J03	More time needed
			J04	Personnel increase
			J05	Facility cost increase
			J07	Cost effective
		Concerns about methodology and experimental design	M01	Sample availability
			M02	Methodological constraints
			M03	Estrous limits
			M04	Estrous myth
			M05	Expertise required
			M06	Insufficient models
			M08	Increased variability
			M09	Pre-existing bias in serum
		Practical roadblocks	L01	Limitation of physical space
			L02	Perspectives of study section
			L03	Limitations of grant design
			L04	Challenges with publishing
		Limited understanding	Z00	Understanding
5	Concerns about policy influence on the scientific enterprise	Scientific enterprise	N01	Interferes with pursuit of science
			N03	Further squeeze on funding
			N04	Slow scientific progress
			N02	Unnecessary use of animals
			N05	Ethical issues
6	Suggested approaches for policy development	Alternatives	Y01	Do not do anything
			Y02	Recognition as encouragement
			Y03	Financial incentive
			Y00	Consider proposals individually
			Y04	Pilot test the policy
7	Types of resources suggested for compliance with pending policy	Provide resources	R01	Increase funding
			R02	Create tools
		Effective leadership from NIH	R03	Provide training
			Q01	Require transparency

Primary Category	Code Group	ID	Sub-code
		Q03	Provide best practices
		Q04	Ensure accountability
	Modify the application and review process	P01	Require justification
		P02	Train reviewers
	Raise awareness	S01	Raise awareness

Table A3. Areas of research with greatest opportunity or need for consideration of SABV in research

Scientific Areas	Animal N	Cell/Tissue N
Basic neurobiology and neurological disorders	58	23
"All areas"	47	37
Pharmacology and immunology	43	16
Behavioral neuroscience, mental disorders, and disorders of addiction	39	3
Cardiovascular	35	15
Endocrinology	33	18
Digestion and metabolism	24	5
Autoimmune	17	-
Cancer	16	11
Known Only	16	5
Reproductive	12	2
Aging	10	6
Muscular and skeletal system	8	-
Developmental	6	2
Pulmonology	6	1
Dermatology	1	1
Stem cell research	-	14
"-omics"	-	12
Cell signaling	-	11
Epigenetics	-	8
Transplantation	-	4
Apoptosis	-	3
Total	**164**	**75**

Note: Values reflect the number of mentions by respondents. Categories are not mutually exclusive.

Table A4. Impediments to considering sex as a biological variable in biomedical research

A4a. Feedback regarding cost concerns

Category of Response	Percent of RFI Respondents
Cost Concerns	
• General cost concerns (blanket statements)	40%
• Costs related to:	
○ Animals	24%
○ Sample size	18%
○ Time	12%
○ Facilities	9%
○ Personnel	7%
• Cost benefits	3%

A4b. Feedback regarding methodological and experimental concerns

Category of Response	Percent of RFI Respondents
Methodological and Experimental Concerns	
• Sample availability	26%
• Estrous cycle	
○ Estrous limits	23%
○ Estrous "myth"	6%
• Limited expertise	22%
• Increased experimental variability	11%
• Insufficient models	9%
• Methodological constraints	7%
• Pre-existing bias in serum	5%

A4c. Feedback regarding practical roadblocks and limited understanding of evaluators

Category of Response	Percent of RFI Respondents
Limited understandings of the importance of SABV in:	
○ The scientific community	20%
○ Among study section members and in grant review	10%
○ Publication and grant design	2%

Note: Values reflect the percentage of total RFI respondents (N=222). Categories are not mutually exclusive.