NIH's Duty to Sepsis Research¹

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INTRODUCTION

Sepsis, defined by the Center for Disease Control and Prevention as "the body's extreme response to an infection,"² strikes more than a million U.S. residents every year³ and kills nearly 270,000 of these individuals.⁴ According to a 2019 report from the National Advisory General Medical Sciences Council (NAGMSC) Working Group on Sepsis, "Despite decades of intensive study of the underlying mechanisms of this condition, no new drug or significantly new diagnostic technology has emerged. Dozens of prospective trials of agents or strategies targeting the inflammatory basis of sepsis have failed."5 Similarly, a vast body of peer-reviewed research, discussed herein, details the failings of sepsis experiments on nonhuman animals to yield benefits in the treatment of human sepsis.

In their report, the NAGMSC Working Group on Sepsis recommended that the National Institute of General Medical Sciences (NIGMS, the National Institutes of Health's (NIH's) primary funder of sepsis research) "rebalance" their sepsis research-funding portfolio to "include a more clinical focus."⁶ In a Notice of Information issued by NIGMS following the NAGMSC report, the institute indicated its intention to support more sepsis research that "uses new and emerging approaches, such as clinical informatics, computational analyses, and predictive modeling in patients, and new applications of high-resolution and high-throughput bioanalytical techniques to materials obtained from septic patients" and called the support of "[s]tudies using rodent models of sepsis" a "low priority."7 In other words, NIGMS intends to prioritize funding human-relevant sepsis research

⁵ National Advisory General Medical Sciences Council Working Group on Sepsis. Final Report. https://www.nigms.nih.gov/News/reports/Documents/nagmsc-working-group-on-sepsis-final-report.pdf. Published May 17, 2019. Accessed October 3, 2019. (Ex. 4)

¹ This report contains AMA-style citations for citations to scholarly articles and websites and Bluebook-format legal citations.

² Center for Disease Control and Prevention. What is sepsis? https://www.cdc.gov/sepsis/what-is-sepsis.html. Updated July 20, 2018. Accessed October 19, 2019. (Ex. 1)

³ Hershey TB, Kahn JM. State sepsis mandates—a new era for regulation of hospital quality. N Engl J Med. 2017;376:2311-2313. (Ex. 2)

⁴ National Institute of General Medical Sciences. Sepsis. <u>https://www.nigms.nih.gov/education/pages/factsheet_sepsis.aspx</u>. Updated September 2019. Accessed October 19, 2019. (Ex. 3)

⁶ Ibid.

⁷ National Institute of General Medical Sciences. Notice of Information: NIGMS Priorities for Sepsis Research. https://grants.nih.gov/grants/guide/notice-files/NOT-GM-19-054.html. Released July 29, 2019. Accessed October 3, 2019. (Ex. 5)

over sepsis experiments on animals. This change is significant considering that in fiscal years 2014-2018, approximately 75 percent of funded NIGMS applications involved the use of vertebrate animals.⁸

The NAGMSC Working Group acknowledged, "The utility of rodent models as a path to new therapies or diagnostics faces substantial doubt in the broader scientific community." In fact, at least 15 peer-reviewed publications over the past 16 years have described how sepsis in humans fundamentally differs from sepsis in other animals. Many have specifically discussed how the use of mice, the most commonly used animal in sepsis experimentation (and basic experimentation in general), has contributed to the lack of progress to develop treatments in this area. As the NAGMSC Working Group stated: "Historical emphasis on a small set of animal systems has not captured what is known of the complex background upon which clinical sepsis is typically encountered" and "[t]he relevance of the most frequently studied model systems [mouse experiments] are in question, raising concerns that current 'best practice' may not be a reliable tool in making translatable discoveries."⁹

In the following, we discuss factors limiting the translation of experiments on mice and other animals to clinical interventions for humans and provide examples of human-relevant methods for sepsis research that should replace animal experimentation. Taken together, the included evidence supports only one conclusion: that making sepsis experiments on rodents a "low priority" is not sufficient; federal funds must not be used for sepsis experiments on non-human animals within any agency of NIH.

The Secretary of Health and Human Services "shall" carry out in the Public Health Service—of which NIH is an agency—duties "relating to the causes, diagnosis, treatment, control, and prevention of physical and mental diseases and impairments of man."¹⁰ NIH has described its role as that of "steward of medical and behavioral research for the Nation."¹¹ Such stewardship means that NIH "is responsible to Congress and the U.S. taxpayer for carrying out its mission in a manner that not only facilitates research but does so cost-effectively and in compliance with applicable rules and regulations."¹²

To fulfill its human-health-centered purpose,¹³ the Secretary—through the NIH Director—"shall ensure" that NIH-supported research first undergoes¹⁴ "appropriate technical and scientific peer review."¹⁵ In determining the scientific merit of an application, the "scientific peer review group *shall* assess the overall impact that the project could have on the research field involved."¹⁶ The peer review groups are required to consider, "among other pertinent factors . . . the adequacy of the approach and methodology proposed to carry out the research,"¹⁷ "[t]he innovativeness and originality of the proposed research,"¹⁸ and "[t]he adequacy of the proposed protection for . . . animals . . . to the extent they may be adversely affected by the project proposed in the application."¹⁹

In describing its scoring criteria for assessing a grant proposal's scientific and technical merit, the NIH has elaborated on the requirement that the approach and methodology of a proposal be adequate:

⁸ *Id.* at 4

⁹ *Id*. at 4

¹⁰ 42 U.S.C. § 241(a).

¹¹ NIH Grants Policy Statement I-40, Oct. 2018, *available at* <u>https://grants.nih.gov/grants/policy/nihgps/nihgps.pdf</u>. (Ex. 6) ¹² *Id*. Indeed, there is a longstanding federal directive for agencies to use public funds efficiently. *See*, *e.g.*, 31 U.S.C. § 712; Exec. Order No. 13, 450, 72 Fed. Reg. 64519 (Nov. 13, 2007).

¹³ 42 U.S.C. § 282(b); 42 U.S.C. § 241(a).

¹⁴ *Id.* § 282(b)(9).

¹⁵ *Id.* § 289a(a)(1).

^{16 42} C.F.R. § 52h.8. (emphasis added)

¹⁷ *Id.* § 52h.8(b).

¹⁸ *Id.* § 52h.8(c).

¹⁹ Id. § 52h.8(h).

Are the overall strategy, methodology, and analyses well-reasoned and appropriate to accomplish the specific aims of the project? Have the investigators presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed? Are potential problems, alternative strategies, and benchmarks for success presented? If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed? Have the investigators presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects?²⁰

Likewise, NIH has elaborated on the innovation criterion:

Does the application challenge and seek to shift current research or clinical practice paradigms by utilizing novel theoretical concepts, approaches or methodologies, instrumentation, or interventions? Are the concepts, approaches or methodologies, instrumentation, or interventions novel to one field of research or novel in a broad sense? Is a refinement, improvement, or new application of theoretical concepts, approaches or methodologies, instrumentation, or interventions proposed?

Does the design/research plan include innovative elements, as appropriate, that enhance its sensitivity, potential for information or potential to advance scientific knowledge or clinical practice?²¹

NIH has also explained that in evaluating the use of live vertebrate animals as part of its assessment of scientific and technical merit, the committee will consider the following factors:

1) proposed use of the animals, and species, strains, ages, sex, and numbers to be used; 2) justifications for the use of animals and for the appropriateness of the species and numbers proposed; 3) adequacy of veterinary care; 4) procedures for limiting discomfort, distress, pain and injury to that which is unavoidable in the conduct of scientifically sound research including the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices; and 5) methods of euthanasia and reason for selection if not consistent with the AVMA Guidelines on Euthanasia.²²

Consideration of these factors concerning the use of animals aligns with the statutory directive for NIH to support research that does not use animals and that reduces the number of animals used and the pain and distress produced in animals used in experimentation.²³

The peer review group's assessment is one layer in the two-layer review process for NIH grant applications: "The peer review system used by NIH, often referred to as the 'dual review system,' is based on two sequential levels of review for each application—initial review by an IRG [Integrated Review Group] or SRG [Scientific Review Group], and a second level of review by the IC [Institute & Center] National Advisory Council/Board."²⁴ "The [Advisory] Council reviews applications not only for scientific and technical merit, as judged by the SRG, but also for relevance to the IC's programs and priorities."²⁵ The Advisory Council has three options upon its review: "The Council may concur with the SRG's recommendation, may decide not to recommend an application on the basis of program or policy considerations, or may recommend deferral of an application and refer it back to the

²⁰ NIH, Understanding Funding Opportunity Announcements: PA-18-345 16, *available at* <u>https://grants.nih.gov/grants/Annotated FOA.pdf</u>. (Ex. 7)

 $^{^{21}}$ *Id*.

²² *Id.* at 18.

²³ 42 U.S.C. § 283e(a)(1)(A)-(C).

²⁴ NIH Grants Policy Statement, *supra* note 11, at I-68.

²⁵ *Id.* at I-72.

SRG for re-review."²⁶ In carrying out their duties, Advisory Councils may recommend funding "for projects which show promise of making valuable contributions to human knowledge."²⁷

In addition to a funding application's scientific merit, the Secretary's evaluation of an application "*shall* take into account" both the significance and the feasibility of the project and "the likelihood of its producing meaningful results."²⁸

It is concerning that, in the NAGMSC report, the Working Group concluded, "The review process used by NIGMS via the Center for Scientific Review may not be well positioned for the receipt of translatable proposals. Applicants know 'what works' at study section and are 'writing to the reviewer' rather than writing to the clinical problem. Without translationally experienced reviewers in the room, this may pose a systematic bias against proposals seeking primarily to develop therapies and diagnostics rather than pursue disease mechanism."²⁹ The Working Group found that "[f]ew members of the most commonly employed study sections for the NIGMS sepsis portfolio bring translational expertise."³⁰ If NIGMS is to tip the balance of funded sepsis research towards human-relevant projects and away from experiments on mice and rats, its study sections must also be rebalanced to favor members who have expertise in human-relevant translational research. The same is true for NIH as a whole if the agency is to improve the productivity of NIH-funded sepsis research.

The NIH has a straightforward mandate to fund research only in support of the PHS' statutory purpose of improving human health and only after NIH considers the proposal's scientific merit, including the adequacy of the methodology to carry out the research, the innovativeness of the proposed research, and the adequacy of protections for any animals proposed to be used. Notwithstanding the more than \$1.4 billion³¹ in federal funding since 1985 and decades of experimentation, sepsis experiments on other species do not improve human health. As detailed herein, copious scientific studies, a comprehensive review of sepsis experimentation, and the NIH's own writings show that sepsis experiments on animals inherently pertain to a *fundamentally different condition* from sepsis in humans. The record makes clear that sepsis studies in mice or other nonhuman animals are an inappropriate methodology to research sepsis in humans. Accordingly, for NIH to continue to fund such experiments—in spite of this knowledge—is arbitrary, capricious, an abuse of discretion, not in accordance with law, in excess of statutory jurisdiction and authority, and without observance of procedure required by law. *See* 5 U.S.C. § 706.

²⁶ Id.

²⁷ 42 U.S.C. § 284a(a)(3)(A)(ii).

²⁸ 42 C.F.R. § 52.5(a) (emphasis added).

²⁹ *Id*. at 4

³⁰ *Id*. at 4

³¹ NIH RePORTER. https://projectreporter.nih.gov/reporter.cfm. Accessed April 5, 2019. Queries: (Text Search: (sepsis AND (mice OR mouse OR mus)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (rat OR rattus)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (rat OR rattus)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (rabbit)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (dog OR canine OR canis)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (pig OR porcine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (sheep OR ovine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (cow OR bovine OR bovinae)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (cow OR bovine OR bovinae)) (Advanced); Search in: Projects Limit to:Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (cow OR bovine OR bovinae)) (Advanced); Search in: Projects Limit to:Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (monkey OR macaque OR baboon OR marmoset OR tamarin)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (monkey OR macaque OR baboon OR marmoset OR tamarin)) (Advanced); Search in: Projects Limit

I. Poor Translation of Non-Human Sepsis to Human Sepsis

For at least 16 years, researchers have documented the failure of animal sepsis experiments to result in treatment for human clinical sepsis. The following are excerpts of such acknowledgments from peer-reviewed publications that demonstrate that approval of sepsis studies in mice and other species is arbitrary, capricious, an abuse of discretion, not in accordance with law, in excess of statutory jurisdiction and authority, and without observance of procedure required by law because of the abundant evidence that they do not translate to treatment for humans— making the approach and methodology fundamentally inadequate, failing to innovate for the improvement of human health, lacking adequate protections for animals who are being used in experiments long known to be futile for NIH's legislatively granted funding purpose, and unlikely to produce meaningful results.

In 2007³², Daniel Rittirsch, L. Marco Hoesel, and Peter A. Ward of the University of Michigan Medical School wrote:

[I]dentification of mediators in animals, the blockade of which has been protective, has not translated into clinical efficacy in septic humans.

Numerous therapeutic attempts have targeted proinflammatory mediators and have had promising effects when used in animal models of sepsis, but virtually all have failed to demonstrate clinical efficacy in human clinical trials³³....Although typical symptoms such as hyperthermia (progressing to hypothermia), tachycardia, and tachypnea can be observed in septic animals, other parameters such as levels of pro- and anti-inflammatory cytokines differ between animals and humans with sepsis, providing a possible explanation as to why human clinical trials based on effective treatment strategies in animals have failed. As a result, the initial euphoria and optimism to find a potent therapeutic strategy for septic patients were dampened, and pharmaceutical companies often consider sepsis research to be a "graveyard" rather than a promising investment with payoff....³⁴

In a 2012 *Nature Medicine* article by Kathleen Raven titled, "Rodent models of sepsis found shockingly lacking," Shaw Warren, an infectious disease specialist at the Massachusetts General Hospital in Boston, was quoted as having said: "The mouse models really don't reflect the human condition."³⁵ In this same article, Mitchell Fink, a surgeon at the University of California Los Angeles, was quoted as having said: "Clearly, current animal models seem to be incapable of predicting results in human trials of new agents."³⁶

In their 2018 paper, Steve Timmermans and Claude Libert of the VIB Center for Inflammation Research and Ghent University in Belgium provided a broader context to the issue and echoed previous writings on the topic in this excerpt:

³² In this 2007 article, authors Rittirsch, Hoesel and Ward present strong and irrefutable evidence that animal sepsis experiments have failed to translate into human benefit. Yet, for unknown reasons (but possibly to not appear as a traitor to the many animal experimenters within the research community), they do an about-face in their concluding statements and fail to advocate for the elimination of animal sepsis experiments, even though their own analyses have clearly laid the groundwork for reaching such a conclusion. Here, the authors make statements suggesting animal models are useful, but that we must better understand their limitations. As no further progress has been made in sepsis treatment and we are still lamenting the failure of animal sepsis experimentation 12 years later, clearly understanding the limitations is not enough. Animal sepsis experimentation must be replaced with human-based methods.

³³ There are no treatments or cures for sepsis that have been derived from animal experiments.

³⁴ Rittirsch D, Hoesel LM, Ward PA. The disconnect between animal models of sepsis and human sepsis. *J Leukoc Biol*. 2007;81:137-143. (Ex. 8)

³⁵ Raven K. Rodent models of sepsis found shockingly lacking. *Nat Med.* 2012;18(7): 998. (Ex. 9)

³⁶ Raven

Over the past decades, huge investments have been made both by academic researchers and pharmaceutical companies, but no obvious candidate drugs have survived clinical trials and hit the market. Several companies have had major financial drawbacks due to these failed attempts, and therefore, the fear for investment in sepsis research is substantial. One can wonder why these immense efforts have not led to therapeutic success so far. While one can criticize the setup of many clinical trials...a major issue is the translation of pre-clinical animal work to the real clinical situation....[T]here are substantial basic physiological differences between mice and humans. For example, given the fact that sepsis involves a significant metabolic crisis, mice, which have a much larger surface/volume ratio than men, go into hypothermia, while humans do not. Also, the husbandry of mice in animal houses with specific pathogen-free conditions may lead to very unnatural gut microbiota compositions, leading to immature deviant immune responses compared to the natural outside world....Hence, research breakthroughs yielding new candidate drug targets, by directly analyzing human sepsis patient data, are important but remain rather underexplored.³⁷

Though mice are the most commonly used animals, rats, dogs, cats, pigs, sheep, rabbits, horses, and primates, including baboons and macaques, have also been used in sepsis experimentation. Regrettably and bewilderingly, the NAGMSC Working Group indicated that the use of "larger animal models" would provide benefits over experiments on mice.³⁸ There is no evidence for such a conclusion. "Larger animal models" are currently used in attempts to study human sepsis and, despite their use, there is still no cure for this condition. No non-human species reproduces all physiologic features of human sepsis; therefore, they should not be used for research into the human condition when there is copious evidence, including the following excerpts from peer-reviewed publications, that sepsis experiments on any non-human species have failed to improve treatment for the condition in humans. In 2005, Jon A. Buras of Beth Israel Deaconess Medical Center, Bernard Holzmann of Technische Universitat Munchen, and Michail Sitvosky of Northeastern University also discussed baboon sepsis experiments:

Unfortunately, there are significant differences between each of the current animal sepsis models that prevent any single one from emerging as the perfect vehicle for sepsis drug discovery....The success of anti-TNF therapy and corticosteroids in the baboon model and subsequent failure in clinical trials helped cast a disparaging shadow on the clinical relevance of this sepsis model. Such failures supported the claim that the baboon model represented an intoxication model rather than one of infection, as the bacterial load in these models (1011 and 2.5 x 1010 E. coli per kg) was considered to be within the intoxicating range....On the basis of these data, one review of animal models concluded that "The moral of the TNF story is similar to that of the steroid story, which is that clinical trials based on inappropriate pre-clinical studies have little chance of clinical success."³⁹

In 2004, Howard Hughes investigator Charles T. Esmon of the Oklahoma Medical Research Foundation and the University of Oklahoma Health Sciences Center wrote of the broad failures of endotoxic animal sepsis experiments, with examples from rodent and baboon studies:

Inhibiting coagulation with natural anticoagulants has consistently resulted in inhibition of the inflammatory cytokine response in animal models...whereas these inhibitors have not proven to be effective in inhibiting the inflammatory response in human models of endotoxemia....There are several possible explanations for the differences in the results....First, the animals may simply respond

³⁷ Timmermans S, Libert C. Learning lessons in sepsis from the children. *Molecular Systems Biology*. 2018;14:e8335. (Ex. 10)

³⁸ *Id*. at 4

³⁹ Buras JA, Holzmann B, Sitkovsky M. Animal models of sepsis: Setting the stage. *Nat Rev Drug Discov*. 2005;4:854-865. (Ex. 11)

differently. Endotoxin may not replicate the response to bacterial infusion, particularly because endotoxin probably triggers less complement activation and lacks the bacterial DNA that can activate toll receptors triggering innate immunity. Second, the bacterial challenges in human volunteer studies are much less severe than in the bacterial infusion studies in baboons.

In the baboon model...E. coli is infused to initiate a septic response. Fibrinogen consumption is extensive and inflammatory cytokine levels are very high (about 100 ng/mL for interleukin-6)....In contrast, in human volunteers subjected to experimental endotoxin challenge, interleukin-6 levels only reach about 3– 5 ng/mL....Thrombin–antithrombin complex levels (100 ng/mL) are about 30-fold lower than in the baboon model. Given the potential of the coagulation system to augment the inflammatory system, particularly when coagulation enzymes contact extravascular cells as may be the case in severe sepsis...many of the interactions that probably augment the inflammatory response are missing in this model of human endotoxemia.

...[R]odents and baboons are housed in feces-contaminated environments. Many of the animals used for studies of sepsis are coprophagous, potentially allowing the development of resistance and the use of alternative mechanisms to minimize the shock reactions. This hypothesis is consistent with the observation that baboons, rats, and mice are all much less sensitive to endotoxin than humans. The response to infection varies.⁴⁰

Esmon also provided the following table demonstrating experimental differences between the condition in baboons and. humans:⁴¹

	Baboon Severe Sepsis	Human Endotoxemia
TFPI dose	2000 ng/mL	150 ng/mL
Peak IL-6	50 ng/mL	4.5 ng/mL
TAT	>10,000 ng/mL	90 ng/mL

Table 2. Severe sepsis in baboons vs. human endotoxemia

TFPI, tissue factor pathway inhibitor; IL-6, interleukin-6; TAT, thrombin-anti thrombin.

In 2016, Sudhir Verma of the University of New Dehli wrote, "A number of animal models have been developed to faithfully mimic human sepsis but unfortunately the interventions that have been shown protective in animal sepsis fail in human clinical trials."⁴²

Therefore, it is vital that human sepsis research have its basis in human physiology, not that of other animals. Human-relevant methods for sepsis research are discussed later in this report.

In 2005⁴³, Buras, Holzmann, and Sitvosky wrote:

⁴⁰ Esmon CT. Why do animal models (sometimes) fail to mimic human sepsis. *Crit Care Med.* 2004;32(5):S219-S222. (Ex. 12)

⁴¹ Esmon

⁴² Verma S. Laboratory Animal Models to Mimic Human Sepsis: A Review. *RRJZS*. 2016;4(2):34-39. (Ex. 13)

⁴³ Like Rittirsch, Hoesel, and Ward, authors Buras Holzmann, and Sitvosky present a strong argument for discontinuing the practice of using non-human animals to study human sepsis, but vacillate when the time comes to bring their analysis to its logical conclusion. They posit that it "might still be possible to improve animal models by better staging of sepsis." However, as we now know fourteen years later, no efforts to improve animal sepsis experimentation have resulted in any tangible benefits for human patients, nor are any conjectural modifications of sepsis experiments on other species likely to yield such benefits due to the inherent disparities between species discussed at length in the literature.

Drug development typically begins with preclinical animal model studies and progresses to human clinical trials. This process has been remarkably unsuccessful with respect to the development of human sepsis therapies....This raises the question whether animal studies of sepsis are sufficiently reliable to predict successful human therapy....

The failure of clinical trials has often been blamed on flawed animal models of sepsis....

Animal models of sepsis do not completely recreate the human disease or involve identical care delivered to human sepsis patients.⁴⁴

In 2014, Meredith Bara and Ari R Joffe of the University of Alberta wrote, "Of concern, the literature has shown a poor translation rate of AR [animal research] to human medicine. This is true in critical care, for example, in the fields of sepsis...."⁴⁵ In a recent report, an international working group consisting of 31 experts from 13 countries, which convened to address problems with animal sepsis modeling, wrote: "With the ultimate goal to reduce mortality/morbidity in patients, animal modeling of diseases has been limited by poor translation....This is often fueled by the low fidelity of available model systems..., their inappropriate study designs...and selective use of animal data...."⁴⁶

The inability of sepsis experiments on non-human animals to lead to sepsis treatments for humans has long been evident. As Olivier Huet of Alfred Hospital and Judy B. de Haan of the Baker IDI Heart and Diabetes Institute in Melbourne, Australia wrote in 2014, "After more than 40 years of basic research on septic shock and more than 30 phase II or III clinical trials, septic shock remains a disease without a specific treatment."⁴⁷

Despite more than \$1.4 billion⁴⁸ in NIH funding since 1985, from taxpayer dollars the agency is authorized to spend for the improvement of human health, sepsis experiments on mice and other animals have not resulted in a viable treatment for human sepsis. This failure is due to inherent species differences and, relatedly, inherent problems with sepsis-induction methods and protocol design, as described below. It is imperative that NIH cease funding experimentation it has long known to lack translatability to human health and that is notoriously plagued

⁴⁴ Buras, *et al*.

⁴⁵ Bara M, Joffe AR. The ethical dimension in published animal research in critical care: the public face of science. *Crit Care*. 2014;18:R15-R21. (Ex. 14)

 ⁴⁶ Osuchowski MF, Ayala A, Bahrami S, *et al.* Minimum quality threshold in pre-clinical sepsis studies (Mqtipss): An international expert consensus initiative for improvement of animal modeling in sepsis. *Shock.* 2018;50(4):377-380. (Ex. 15)
 ⁴⁷ Huet O, de Haan JB. The ethical dimension in published animal research in critical care: the dark side of our moon. *Crit Care.* 2014;18:120-121. (Ex. 16)

⁴⁸ NIH RePORTER. <u>https://projectreporter.nih.gov/reporter.cfm</u>. Accessed April 8, 2019. Queries: (Text Search: (sepsis AND (mice OR mouse OR mus)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: All Fiscal Years); (Text Search: (sepsis AND (rat OR rattus)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: All Fiscal Year: All Fiscal Year: All Fiscal Years); (Text Search: (sepsis AND (hamster OR Cricetinae)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Years); (Text Search: (sepsis AND (guinea OR cavia)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Years); (Text Search: (sepsis AND (guinea OR cavia)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Years); (Text Search: (sepsis AND (dog OR canine OR canis)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Years); (Text Search: (sepsis AND (pig OR porcine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Years); (Text Search: (sepsis AND (gig OR porcine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Years); (Text Search: (sepsis AND (pig OR porcine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Years); (Text Search: (sepsis AND (gig OR porcine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Years); (Text Search: (sepsis AND (pig OR porcine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Years); (Text Search: (sepsis AND (cow OR bovine OR ovine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: All Fiscal Years); (Text Search: (sepsis AND (monkey OR macaque OR baboon OR marmos

by poor scientific practices; to do otherwise is arbitrary and capricious, an abuse of discretion, not in accordance with law, in excess of statutory jurisdiction and authority, and without observance of procedure required by law.

a. Species Differences

Mice are not chosen as experimental subjects due to their similarities with humans, but for convenience. As Verma wrote in 2016,⁴⁹

The mice are [*sic*] most popularly used pre-clinical animal model for sepsis research. Being small in size, easy to rear, little or no harm to laboratory personnel and availability of inbred strains and diagnostic/immunological assay kits are some of the key features that mice are preferred over large animals including dogs, cats, horses and non-human primates.⁵⁰

As Timmermans and Libert noted, there are substantial species differences among mice, humans, and other animals that limit the inherent translational ability of non-human animal sepsis experiments to human sepsis treatment. These differences, including the genes and proteins involved in inflammation and immunity, as well as anatomical dissimilarities, make it arbitrary and capricious to fund research on human sepsis that involves the use of other species when NIH has a clear, statutory mandate to fund research that fulfills the PHS' purpose of improving human health. The agency has acknowledged its role as steward of medical research and its corresponding obligation to American taxpayers to support research in a manner that is both cost-effective and compliant with applicable law. Funding sepsis experiments—to the tune of \$125,991,490 in active projects alone as of April 5, 2019⁵¹—on non-human animals, considering the underlying species differences and documented barriers to translatability described herein, is in contravention of the directives that grant proposals justify the appropriateness of using a particular species and that the NIH support research that does not use animals, as well as of the criteria of originality, innovativeness, adequate approach and methodology in the scientific review of grant proposals, significance, and likelihood to produce meaningful results. The well-documented species differences described below foreclose a strategy to "ensure a robust . . . approach" and, certainly, to "address relevant biological variables,"⁵² as well as any significance of the research, likelihood of the research to produce

⁴⁹ Verma, like their colleagues, despite an entire manuscript devoted to addressing the many problems associated with animal sepsis experimentation, ends with a few sentences stating that the use of animals can still provide some insights and that new models should be explored. As has been demonstrated in this report, these are the same arguments made at least 12 years prior, yet nothing has changed in this field. How many times must a model fail before a new path is chosen? After how many years and how many unsuccessful human trials is it justified to abandon the faulty paradigm? ⁵⁰ Verma

⁵¹ NIH RePORTER. <u>https://projectreporter.nih.gov/reporter.cfm</u>. Accessed April 5, 2019. Queries: (Text Search: (sepsis AND (mice OR mouse OR mus)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (rat OR rattus)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (rat OR rattus)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (rabbit)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (dog OR canine OR canis)) (Advanced); Search in: Projects Limit to:Project Search in: Projects Limit to:Project (sepsis AND (pig OR porcine)) (Advanced); Search in: Projects Limit to:Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (pig OR porcine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (sheep OR ovine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (cow OR bovine OR bovinae)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (cow OR bovine OR bovinae)) (Advanced); Search in: Projects Limit to:Project Search: (sepsis AND (monkey OR macaque OR baboon OR marmoset OR tamarin)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (monkey OR macaque OR baboon OR marmoset OR tamarin)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects)

⁵² See Understanding Funding Opportunity Announcements: PA-18-345, *supra* note 20, at 16.

meaningful results, and justification of the appropriateness of using such species to study sepsis for the benefit of human health.⁵³

Species differences that preclude sepsis experiments on animals from having clinical relevance to the condition in humans have been discussed in a number of peer-reviewed publications. The most striking demonstration of species differences between mouse and human sepsis was presented in a landmark study involving the collaboration of 39 researchers from institutions across North America, as well as international experts. The results of this ten-year study, led by Stanford University's Junhee Seok, were published in 2013. Seok and colleagues compared data obtained from hundreds of human clinical patients with results from experiments on mice to demonstrate that, when it comes to serious inflammatory conditions such as sepsis, burns, and trauma, humans and mice are not similar in their genetic responses, as described in the following excerpt from the published paper:

Murine models have been extensively used in recent decades to identify and test drug candidates for subsequent human trials....However, few of these human trials have shown success⁵⁴....The success rate is even worse for those trials in the field of inflammation, a condition present in many human diseases. To date, there have been nearly 150 clinical trials testing candidate agents intended to block the inflammatory response in critically ill patients, and every one of these trials failed....Despite commentaries that question the merit of an overreliance of animal systems to model human immunology...in the absence of systematic evidence, investigators and public regulators assume that results from animal research reflect human disease. To date, there have been no studies to systematically evaluate, on a molecular basis, how well the murine clinical models mimic human inflammatory diseases in patients.

In this article, we report on a systematic comparison of the genomic response between human inflammatory diseases and murine models.

These results show that the genomic responses to different acute inflammatory stresses are highly similar in humans, but these responses are not reproduced in the current mouse models. New approaches need be explored to improve the ways that human diseases are studied.

...[T]here was very low correlation of expression changes between human genes in any of the conditions and their mouse orthologs in the mouse models ($R2 \le 0.09$, ~47–63%). By random chance, 50% of the genes between two uncorrelated conditions are expected to change in the same direction. Furthermore, there was poor correlation of the mouse gene orthologs with each other among the three murine models ($R2 \le 0.13$, ~39%–63%).

...[W]ith comparable sample sizes, the human conditions introduced a more profound genomic response (more than or equal to three times more genes) than the corresponding mouse models.

An inherent assumption in the use of murine models to mimic human systemic inflammation is that the time course of injury and repair between the species is similar....Although in all conditions, the gene response time occurred within the first 6–12 [hours], the recovery time differed dramatically. Genomic disturbances recovered in the murine models within hours to 4 [days] but lasted for 1–6 [months] or more in patients.

...[T]here was great variability between the three murine models...suggesting that mouse responses differed not only from the human conditions but also from one another

⁵³ See id. at 18.

⁵⁴ No human trials using murine models or other non-human animals in the area of sepsis have shown success.

...In every pathway, human endotoxemia had much higher similarity to human injury than seen in the mouse models.

...[I]ndividual gene activation in the human conditions was not necessarily predicted by the ortholog in the corresponding mouse model in either direction or magnitude....

We were surprised by the poor correlation between the genomic responses in the mouse models and those responses in human injuries, especially given the worldwide prevalence of the use of mice to model human inflammation.

...[T]he genomic responses in patients correlated well with each other, whereas this response was mimicked poorly by the mouse models. The same relationship held for the directionality of the gene response. For example, in humans, correlations of 0.91 (trauma), 0.55 (ARDS), 0.54–0.79 (sepsis), and 0.50 (infection) were observed with percentage of the genes changing in the same direction of 97%, 84%, 86–92%, and 83%, respectively. For murine orthologs, there were correlations between 0 and 0.08, with 47–61% of genes changing in the same direction—random chance would predict 50%.

Studying disease in patients is much more complex than studying model systems. In the trauma patients that we studied, patient heterogeneity existed in the most relevant characteristics.... However, despite of [*sic*] these heterogeneities, we observed highly consistent genomic response in patients between trauma and burns in contrast to the lack of correlations in the murine models.

There are multiple considerations to our finding that transcriptional response in mouse models reflects human diseases so poorly, including the evolutional distance between mice and humans, the complexity of the human disease, the inbred nature of the mouse model, and often, the use of single mechanistic models....In addition, differences in cellular composition between mouse and human tissues...can contribute to the differences seen in the molecular response. Additionally, the different temporal spans of recovery from disease between patients and mouse models are an inherent problem in the use of mouse models. Late events related to the clinical care of the patients (such as fluids, drugs, surgery, and life support) likely alter genomic responses that are not captured in murine models.

The evolution of the immune system for any species is, at least in part, a direct consequence of the microbe-exerted selection pressure for that species. Recent articles have highlighted tradeoffs that species make to balance often opposing evolutionary strategies for resistance vs. tolerance or resilience to infection....Relative to the human response, mice are highly resilient to inflammatory challenge....For example, the lethal dose of endotoxin is 5–25 mg/kg for most strains of mice, whereas a dose that is 1,000,000-fold less (30 ng/kg) has been reported to cause shock in humans....The extreme sensitivity of humans relative to mice to massive inflammation may result in genomic responses that reach an upper threshold in each human disease, whereas the resilience of mice may prevent maximum responses and lead to greater heterogeneity.

The prevailing assumption—that molecular results from current mouse models developed to mimic human diseases translate directly to human conditions—is challenged by our study."⁵⁵

Their findings are summarized in this excerpt from the paper's abstract:

Here, we show that, although acute inflammatory stresses from different etiologies result in highly similar genomic responses in humans, the responses in corresponding mouse models correlate poorly with the

⁵⁵ Seok J, Warren HS, Cuenca AG, *et al*. Genomic responses in mouse models poorly mimic human inflammatory diseases. *PNAS*. 2013;110(9):3507-3512. (Ex. 17)

human conditions and also, one another. Among genes changed significantly in humans, the murine orthologs are close to random in matching their human counterparts (e.g., R2 between 0.0 and 0.1). In addition to improvements in the current animal model systems, our study supports higher priority for translational medical research to focus on the more complex human conditions rather than relying on mouse models to study human inflammatory diseases.⁵⁶

NIH Director Francis Collins authored a blog about these results, meaning that since at least 2013, NIH has recognized that mouse sepsis is not a suitable surrogate for studying human sepsis and that the use of mice has contributed to substantial losses in the path of developing a treatment for human sepsis. In Collins' words:

[W]hen it comes to molecules designed to target a sepsis-like condition, 150 drugs that successfully treated this condition in mice later failed in human clinical trials—a heartbreaking loss of decades of research and billions of dollars.

Mice, however, apparently use distinct sets of genes to tackle trauma, burns, and bacterial toxins—when the authors compared the activity of the human sepsis-trauma-burn genes with that of the equivalent mouse genes, there was very little overlap. No wonder drugs designed for the mice failed in humans: they were, in fact, treating different conditions!

...Mice, as the authors note, are more resilient to infection and mount a much more regulated immune response to pathogens than humans. While it takes relatively few bacteria in the bloodstream to make humans critically ill, it takes a million-fold more bacteria to sicken a mouse. Perhaps this is because mice nose around in some filthy places and can't afford to overreact to every microbe?...

[T]his study's implications may well go beyond mice and sepsis. It suggests that we should not assume a mouse's drug response will always accurately predict a human's.

The new study provides more reason to develop better and more sophisticated models of human disease. More than 30% of all drugs successfully tested in animals later prove toxic in human trials. The NIH plans to commit \$70 million over the next five years to develop "tissue chips"—miniature 3-D organs made with living human cells—to help predict drug safety and efficacy....Though this is high-risk research, these chips may ultimately provide better models of human disease and biology than the use of animals."⁵⁷

Dr. Collins hit the nail on the head—more than one billion taxpayer dollars and decades of research have yielded no benefit to human health because treatments developed on mice treat a different condition from human sepsis. While Dr. Collins, perhaps not wanting fully to concede that the agency that is the steward of the nation's medical research repeatedly authorized ineffectual experiments paid for by the American taxpayer, equivocated that research on mice "*might*" someday still "teach" researchers something, it is evident that the only thing that mice have yet taught researchers or likely will in the future—on the taxpayer's dime—is that sepsis experiments on mice are irrelevant to sepsis in humans.

In addition to the landmark study by Seok, *et al.*, other peer-reviewed publications have also detailed the ways in which human sepsis does not resemble the conditions observed in experiments on animals. In 2007, Rittirsch, Hoesel, and Ward wrote:

⁵⁶ Seok, et al.

⁵⁷ Collins F. Of Mice, Men, and Medicine. NIH Director's Blog. <u>https://directorsblog.nih.gov/2013/02/19/of-mice-men-and-medicine/#mo</u>. Published February 19, 2013. Accessed November 30, 2018. (Ex. 18)

[T]here are differences between rodents and humans on the molecular level. For instance, human C-reactive protein (CRP), an acute-phase protein and popular marker for inflammation, is known to be an activator of the complement system in humans, but in contrast, rat CRP does not influence the complement system....The fact that there are differences between TLRs in mice and in humans could also affect interpretation of sepsis studies in the two species....Therefore, the simple transfer of knowledge from animals to humans is highly illusive."⁵⁸

In 2014, Mitchell Fink of the David Geffen School of Medicine at the University of California Los Angeles wrote:

Unfortunately, the animal models that have been used for this purpose have often yielded misleading findings. It is likely that there are multiple reasons for the discrepancies between the results obtained in tests of pharmacological agents in animal models of sepsis and the outcomes of human clinical trials. One of [sic] important reason may be that the changes in gene expression, which are triggered by trauma or infection, are different in mice, a commonly used species for preclinical testing, and humans. Additionally, many species, including mice and baboons, are remarkably resistant to the toxic effects of bacterial lipopolysaccharide, whereas humans are exquisitely sensitive.⁵⁹

Bara and Joffe, in 2014, wrote, "[R]esponses to interventions are different in different species due to in-principle differences in initial conditions of complex systems (the organism) resulting in different genomic (and hence functional) outcomes....⁶⁰

In 2015, scientists from Rutgers University wrote:

Emerging -omics tools that are capable of examining physiologic responses at the systems level are promising, especially for complex conditions, such as sepsis....The major caveat related to these tools is that since the biological processes are analyzed at a higher level, inter-species differences become as relevant to the response as the sought-after question itself. Therefore, utility of the animal models has been questioned recently in the scientific community....⁶¹

This means that new approaches to sepsis research, such as those using tissue chips and –omic technology, must be inherently human-based to avoid the pitfalls of extrapolating results from other species. Fundamentally, non-human animal-based approaches to sepsis research lack external validity, as inter-species differences make it so that these studies cannot be reliably applied to other species or settings.

In the *Journal of Translational Medicine*, a 2018 review by Pandora Pound of the Safer Medicines Trust and Merel Ritskes-Hoitinga of Raboud University Medical Center in the Netherlands discussed species differences as an insurmountable problem of external validity throughout animal experimentation, defined as "the extent to which research findings derived in one setting, population or species can be reliably applied to other settings, populations and species,"⁶² for *all* preclinical animal models. The authors discussed the concerning trend among those involved in animal experimentation to minimize species differences and their effects on external validity:

⁶² Pound P, Ritskes-Hoitinga M. Is it possible to overcome issues of external validity in preclinical animal research? Why most animal models are bound to fail. *J Transl Med.* 2018;16:304. (Ex. 21)

⁵⁸ Rittirsch, et al.

⁵⁹ Fink MP. Animal models of sepsis. *Virulence*. 2014;5(1):143-153. (Ex. 19)

⁶⁰ Bara, *et al*.

⁶¹ Kamisoglu K, Haimovich B, Calvano SE, *et al.* Human metabolic response to systemic inflammation: assessment of the concordance between experimental endotoxemia and clinical cases of sepsis/SIRS. *Crit Care.* 2015;19(71):1-10. (Ex. 20)

We noted that those conducting preclinical animal research appear to downplay the problem of animalhuman species differences but interestingly, other researchers and commentators in the field do similarly. Although they may briefly acknowledge that species differences constitute a problem for external validity, the tendency is to focus on other, potentially modifiable, aspects of external validity....This is perhaps understandable, since acknowledging the issue of species differences entails confronting the possibility that the preclinical animal research paradigm no longer has a great deal to offer. That possibility is alarming, not only to scientists who conduct animal research but also to those attempting to improve it. Yet there is a way forward. Research methods and technologies that are physiologically relevant to humans obviate the need for animals and thus eliminate the problem of animal-human species differences. As a recent industry report...concluded, the time has come to humanise medicine. For the sake of patients and animals, we agree.⁶³

This reluctance to discuss or accept the full consequences of inherent species differences—which should render the use of non-human species to study human conditions obsolete—is also true for those scientists who are writing about the problems associated with non-human animal sepsis experimentation, many of whom have been cited in this report. This is an unfortunate problem and one that could partially explain why sepsis research has not sooner experienced a much-needed shift of resources towards human-based methods. Statements that clearly testify how the use of non-human animals has not resulted in benefits for human sepsis patients and that the reasons for this are due to inherent and insurmountable species differences should be taken at face value, even when, a few sentences later, authors become apologists for experiments on animals, possibly to save face or not appear controversial.

In 2015, an expert working group consisting of veterinarians, animal technologists, and scientists with knowledge relevant to sepsis experimentation wrote:

It is essential to acknowledge that all experimental models have limitations and that an animal model can never fully replicate all of the features of human disease. Where predictive validity is poor, any benefit that may result from an animal study is limited and such work is hard to justify on ethical grounds.⁶⁴

Thus, there is a vast body of evidence that sepsis experimentation on mice cannot surmount the marked physiological differences between mice and humans and yield treatments beneficial to humans; indeed, sepsis in the two species is two different conditions—as acknowledged more than six years ago by NIH.

b. Sepsis Induction Methods

There are a number of ways by which experimenters induce sepsis or a sepsis-like condition in non-human animals. These can include infection of one animal with excrement from another, induction of sepsis via pneumonia or other organ infections, or the insertion of a stent allowing an animal's stool to enter their abdominal cavity. In the following section, we will focus our discussion on the most commonly used methods,⁶⁵ which involve injection of a toxin, usually lipopolysaccharide (LPS), and cecal ligation and puncture (CLP). As demonstrated by the excerpts below, *all* models of experimental sepsis in non-human animals are fundamentally flawed; therefore, funding experiments that rely on these methods will not achieve NIH's mandate to approve studies for the improvement of human health or its peer-review criteria for that purpose. Experimentation that has long been known to be unsuited for its purported aim of improving human health—the only aim in accordance

⁶³ Pound, Ritskes-Hoitinga

⁶⁴ Lilley E, Armstrong R, Clark N, *et al.* Refinement of animal models of sepsis and septic shock. 2015;43(4):304-316. (Ex. 22)

⁶⁵ While this discussion covers the most commonly used sepsis induction methods, *all* methods of inducing sepsis in other animals are fundamentally flawed due to inherent and insurmountable species differences.

with the grant-making authority bestowed by Congress on the agency—cannot be considered to be original and innovative; it does not "challenge and seek to shift current research . . . paradigms" or "include innovative elements . . . that enhance its . . . potential to advance scientific knowledge."⁶⁶ The approach and methodology of such experimentation likewise is not "well-reasoned and appropriate to accomplish the specific aims of the project," is far from "robust," and cannot adequately "address relevant biological variables"⁶⁷ when the most pertinent biological variable is that sepsis in humans is a *different condition* from sepsis in other species. For that reason, there can be no adequate protections for and justification for the use of animals in sepsis experiments that receive federal funding for the benefit of human health. Funding sepsis experiments on non-human animals despite the available evidence is arbitrary and capricious.

i. Endotoxic Models

In endotoxic models, a mouse or other animal is injected with a bacterial toxin, typically LPS. As described by Riedemann, Guo, and Ward, "LPS, the main component of the Gram-negative bacterial cell wall, was known to stimulate release of inflammatory mediators from various cell types and induce acute infectious symptoms when injected into animals."⁶⁸ Endotoxic models have been criticized as a particularly poor model for human sepsis and perhaps not even an accurate model for murine sepsis.

Riedemann and colleagues provided the following figure, "Possible reasons for failure in sepsis trials," to illustrate some pitfalls of this model:

⁶⁶ See Understanding Funding Opportunity Announcements: PA-18-345 16, supra note 20, at 16.

⁶⁷ See id.

⁶⁸ Riedemann NC, Gui RF, Ward PA. The enigma of sepsis. J Clin Invest. 2003;112: 460-467. (Ex. 23)



Authors of other peer-reviewed publications have provided the following commentary on endotoxic models:

In 2007, Rittirsch, Hoesel, and Ward wrote:

[I]t turns out that the LPS experimental model and sepsis in humans differ in several key points, especially in the profile of cytokine release. Cytokine levels (TNF-alpha, IL-6, CXC chemokines) peaked much later and occurred at much lower levels in human patients with sepsis as well as in the model of cecal ligation and puncture (CLP; described in detail below) when compared with effects of LPS infusion....Such findings suggested that the LPS model may not accurately reflect sepsis in the CLP model or in septic humans.⁶⁹

Verma wrote the following in 2016:

⁶⁹ Rittirsch, et al.

[C]omparison of response to endotoxin in mouse and human has demonstrated that mice are relatively more resistant to endotoxin...The endotoxin dose leading to 50% mortality in mice (i.e., LD50) is about 1–25 mg/kg...whereas in humans it is 2-4 ng/kg....

The bolus injection based sepsis model differs from human sepsis in showing a very rapid and transient increase in systemic cytokine levels, which is not the case in human sepsis. Human sepsis is characterized by prolonged elevation of systemic cytokines that are several orders of magnitude lower than endotoxicosis models.

[The bacterial infection model] fails to recapitulate many important clinical features of sepsis [and is] a potential model of intoxication with endotoxins rather than true septic model.⁷⁰

In 2005, Buras, Holzmann, and Sitovsky wrote:

Most laboratory animals, including mice, are relatively resistant to endotoxin and require administration of high LPS doses to induce the shock state....

[I]t seems that prolonged low-dose infusion of LPS does not reproduce important aspects of the immune response to a septic challenge caused by infection.

Inhibition of inflammatory mediators proved beneficial in endotoxicosis models; however, neutralization of inflammatory mediators has failed to demonstrate substantial benefit in clinical trials.

It is important to note that clinical sepsis differs from this response by showing a prolonged elevation of systemic cytokines and by exhibiting serum cytokine concentrations that are several orders of magnitude lower than in endotoxicosis models.

Furthermore, comparison of the response to endotoxin in humans and mice has demonstrated that mice are relatively more resistant to endotoxin, further limiting the extrapolation of studies between species....

Bolus injection of LPS commonly induces a hypodynamic cardiovascular state immediately and unfortunately does not reproduce the haemodynamic changes observed in human sepsis and some infection models....⁷¹

In 2014, Fink wrote: "[T]he temporal kinetics and the magnitude of ... changes from normal physiology are often different from what is observed in acute murine endotoxemia."⁷² In 2015, Lilley and the expert working group discussed how LPS sepsis "models" do not model sepsis at all:

Lipopolysaccharide causes a severe systemic inflammatory response in the absence of an ongoing infection, bypassing opsonization, and does not create a model of sepsis per se.

There are a number of potential confounding factors with [live bacterial infection models], including the choice of bacterial strain, the bacterial load, and the susceptibility of the host animal and the compartment of infection.⁷³

⁷⁰ Verma

⁷¹ Buras, *et al*.

⁷² Fink

⁷³ Lilley, et al.

ii. Cecal Ligation and Puncture

In CLP, experimenters cut open an animal's abdomen, puncture their intestines with a needle so that bacteria will leak out, and then close the perforated intestines back into the abdominal cavity. The mice then endure widespread pain, with the worst symptoms in the abdomen, and eventually become so sick that they are unable to move. They experience fever, chills, diarrhea, difficulty breathing, lethargy, disorientation, shock, multiple organ failure, and, eventually, death.⁷⁴ Despite being considered the gold standard for animal sepsis modeling by animal sepsis experimenters, CLP experiments do not bypass the fundamental problems with genetic and physiological species differences, are confounded by abscess formation, and, importantly, have also failed to translate to improvement of sepsis for humans.

In 2005, Buras, Holzmann, and Sitovsky wrote:

The host response to CLP is an attempt to wall off the infected/ inflamed area through the creation of an abscess....A certain percentage of animals successfully contain the infection, do not progress to septic shock and recover fully....The ability of the host to survive by containing the infection suggests that therapies promoting abscess formation would improve outcome following CLP. It is therefore possible that an agent primarily enhancing abscess formation could be confused with a therapy for septic shock.

Variability within the CLP model itself might be underappreciated. Although the trends in sepsis outcomes might be similar, it is somewhat more difficult to compare experimental results between laboratory groups. A number of factors contribute to the variability within the CLP model, including age, sex and strain....Variability is highlighted by comparison of control mortality rates and time of death between laboratories reporting the same experimental techniques. Such a comparison might reveal different outcomes despite use of the same experimental system. For example, two studies evaluating the role of IL-10 in sepsis used female C57BL/6 mice and double-puncture CLP with a 20- and 22-gauge needle....Survival was significantly greater in the 20-gauge needle puncture group (>90%) compared with the 22-gauge group (~25%). These results would not be predicted given the well-documented positive correlation of needle size with mortality....⁷⁵

Two years later, in 2007, Rittirsch, Hoesel, and Ward noted that variability is still a troubling issue in the CLP model, "[A] concern of the CLP model is consistency."⁷⁶

In 2012, Ward indicated that another five years later, experimenters using the CLP sepsis "model" have still failed to make simple changes to their study design to attempt to address the method's consistency problems:

[T]he CLP model has several complexities that influence outcomes: effects of age and gender; the tremendous heterogeneity of immune and inflammatory responses related to genetic strains of mice; influences of therapeutic interventions; and dramatic differences in outcomes based on technical approaches (number of cecal punctures, needle size, amount of cecum ligated....

In rodents injected with LPS, plasma mediators peak in the first several hours versus in CLP with mediator peaks much more slowly developing, over the first 48 [hours]....To what extent can these two models be compared is questionable as is whether either model mimics events developing in humans with sepsis.⁷⁷

⁷⁴ Rittirsch, *et al*.

⁷⁵ Buras, et al.

⁷⁶ Rittirsch, et al.

⁷⁷ Ward

It is important to remember that while some of these variabilities could be addressed, even perfectly reproducible CLP experiments are still plagued by a lack of external validity caused by inherent species differences that will prevent reliable translation to the human clinical setting.

In 2014, Fink wrote:

[A]t a gene expression level, acute systemic inflammatory responses in mice appear to be quite different from those that occur in humans. ...[S]epsis in humans is, by and large, a disease that occurs at the extremes of age....In contrast, in the vast majority of studies using the murine CLP model, sepsis is induced in young adult animals without any co-morbid conditions....[T]he temporal course of the septic process—from the onset of symptoms to death—in the murine CLP model is compressed into an interval that lasts at most a few days. In contrast, patients with lethal sepsis often survive for weeks before succumbing to their illness.⁷⁸

In the 2019 NAGMSC report, the Working Group acknowledges that "the cecal ligation and puncture model does not share the inciting pathophysiology of most human sepsis and is demonstrably difficult to standardize across operators and laboratories" and that "[c]ecal ligation by its nature casts a surgical, rather than medical, subspecialty perspective on sepsis, casting doubt on its utility in studying the majority of human experience."⁷⁹

The following tables point out the disadvantages of the various sepsis induction methods:

From the 2004 paper, "Animal models of sepsis: Setting the stage"⁸⁰ by Buras, Holzmann, and Sitkovsky:

78 Fink

⁷⁹ *Id.* at 4

⁸⁰ Buras, et al.

Box 2 | Caveats of sepsis animal models

Endotoxaemia

- Single toxin may not mimic responses in human sepsis
- Variable haemodynamic responses with different doses and infusion rates
- Intra- and inter-species variability in response to toxin

Bacterial infection

- Requires growth and quantification of bacteria prior to administration
- Significant inter-laboratory variability
- Large quantity of bacteria used may elicit confounding toxicosis response
- Host response is dependent on infecting bacterial strain
- · Different host responses with infection of different compartments
- Variable host response dependent on bacterial load and infusion time
- Genetic background affects host responses to specific pathogens
- Human therapy potentially withheld could detract from validity of the rapeutic agent

Caecal ligation and puncture

- Multiple bacterial flora
- Inter-laboratory variability
- Human therapy potentially withheld could detract from validity of the rapeutic agent
- Age variability
- Strain variability
- Potential of ascribing sepsis therapy success to enhanced abscess formation mechanism

Colon ascendens stent peritonitis

- Multiple bacterial flora
- Human therapy potentially withheld could detract from validity of therapeutic agent
- Less characterized haemodynamic response
- Less experience to identify possible confounding variables

"Comparison of various commonly used animal models of sepsis"⁸¹ by Sudhir Verma, 2016:

SEPSIS MODEL	TYPE	VARIABILITY FACTORS	ADVANTAGES	DISADVANTAGES
Endotoxemia/ Toxicosis Model	Injectable	 Type of toxin used Dose Route of administration Fluid Resuscitation Host species and strain 	 Simple and reproducible Induced response is acute Highly controlled and standardized model 	 LPS mediated signaling is strict- ly TLR-4 dependent Does not mimic human sepsis in terms of cytokine profile Variability in dose, toxin and route of administration
Bacterial Infection Model	Injectable	 Bacterial load Route of administration Time of infusion Bacterial and host strain Antibiotic/fluid resuscitation 	 Presence of bacterium al- lows insights into mecha- nisms of host response to pathogens 	0.0
Colon Ascendens Stent Peritonitis (CASP)	Surgical	 Stent lumen diameter Load of stool transferred into peritoneum Sex, age and strain 	 Polymicrobial Presence of infection focus 	 Surgically more difficult Length of colon is not defined Less characterized hemody- namic phase
Cecal Ligation and Puncture (CLP)	Surgical	 Needle size and number of puncture Amount of cecum ligated/ necrosis induced Uncontrolled bacterial load Antibiotic/ fluid resuscitation Sex, age and strain 	 Simple procedure Presence of infection focus Polymicrobial Mimics human sepsis most Prolonged and lower elevation of cytokines as in human 	 Abcess Formation Variability in severity due to differences in experimental procedure

c. Protocol Design

Aside from inherent species differences, which nullify the external validity of non-human animal sepsis experiments, and the inconsistencies between experimental animal sepsis modeling and the human clinical situation, fundamental issues with protocol design influence the internal validity of animal sepsis studies. Internal validity can be defined as "the scientific robustness of a study's design, conduct, analysis and reporting."⁸² Poor internal validity means the data being produced is likely not reflecting reality, even for those animals being used, and that the results from these experiments may not be reproducible, a critical aspect of the scientific process that speaks to the potential truthfulness of a finding. Sepsis experiments on non-human animals are not only unsound when applied to other situations and species (lacking external validity), but also when held against themselves (lacking internal validity); therefore, funding such experiments would be arbitrary and capricious. NIH's very raison d'être—its statutory mission—is for the advancement of human health. Congress has authorized the agency to fund research for the fulfillment of that purpose, with clear legislative direction to discern grant proposals' scientific and technical merit, including the originality and innovativeness of the proposed research, the adequacy of the proposed approach and methodology, and the adequacy of protections for animals the researcher proposes to use. NIH has long known that funding sepsis experimentation on mice and other species does not yield advancements in human health but instead treats a "different condition[]." The agency cannot comply with its governing statutes and regulations while continuing to heap taxpayers' dollars upon fundamentally flawed sepsis experimentation.

Issues with animal sepsis protocol design have been repeatedly discussed in the literature. The following are excerpts from relevant peer-reviewed publications.

In 2004, Esmon wrote:

⁸² Pound, Ritskes-Hoitinga

There are major differences between the way septic patients are managed and the way animals are managed in experimental sepsis studies. The vast majority of septic patients are placed on a ventilator, and fluids or vasopressors are given to help maintain blood pressure. In contrast, these procedures are seldom employed in studies of animals. Thus, if the experimental drug worked primarily by improving lung function in the animal, this advantage might be negated in patients because of mechanical intervention.... Because the animal studies lack both optimized ventilator support and blood pressure support, it is even more difficult to extrapolate the outcomes to septic human patients.

Most animal studies are performed acutely in young healthy animals, whereas a significant percentage of the clinical population is elderly with many secondary complications (e.g., diabetes, systemic vascular disease, high blood pressure, immune suppression, cancer). Furthermore, unlike the acute challenge presented in most animal studies, many of the septic patients' clinical histories indicate a relatively slow onset of the disease. In many animal studies, intervention occurs before or during the very early stages of sepsis, when inflammatory cytokine levels are still rising and both organ damage and vascular leakage are minimal. In contrast, current treatment strategies are started when many (probably most) patients are switching from a proinflammatory cytokine response to an anti-inflammatory response and organ damage is already apparent. Patients are also generally receiving some form of supportive therapy (e.g., fluids, vasopressors, ventilators). Because these are seldom used in the animal model, their effect on a particular drug response is difficult to assess. In the animal model, a well-defined bacterial strain, endotoxin challenge, or, in the most complex case, cecal ligation puncture at a defined site is employed to bring about the onset of sepsis. Generally, bacterial proliferation can be controlled in these situations by selecting the appropriate antibiotic (if desired). In human sepsis, the pathogenic bacteria are often not known, mixed infections involving both Gram-negative and Gram-positive bacteria are common, and antibiotic treatment is incomplete or ineffective. Anti-inflammatory strategies that impair bacterial killing may be helpful in cases in which antibiotics were effective and harmful when they were not. Thus, an intervention in human sepsis is attempted at a later stage and under very different conditions than it is during efficacy testing in animal models.... Differences in the nature of the initiating agent causing sepsis and the lack of co-morbidities in the animal models probably contribute to some of the differences in animal studies and clinical trials in sepsis.

Another major difference between the animal studies and the human septic patient treatment groups has been the dosages of natural anticoagulants employed. In animal studies, antithrombin...tissue factor pathway inhibitor...and activated protein C...are all used at much higher concentrations than in the patient studies....This could have been tolerated due to a reduced susceptibility to bleeding in the young animals relative to septic human patients. Alternatively, the natural anticoagulants employed in the animal studies were usually derived from different species (often human), and because some of these anticoagulants have strong species specificity...the anticoagulant responses may be lower in the experimental animals than in humans.

The vast majority of animal studies use healthy, young adult animals as the model system. In most cases, the animals have no co-morbidities (e.g., diabetes, hypertension, cancer) and start with normal leukocyte counts....When bacteria are used to initiate the septic challenge, they are usually a single species with known antibiotic sensitivity. Often, septic human patients have one or more of the above clinical conditions. Cancer patients may have decreased leukocyte counts and, as a result, impaired ability to kill bacteria. In addition, many pathologic bacteria produce toxins in addition to endotoxin, so clearing these bacteria is particularly important. Reports vary, but up to 50% of sepsis patients may have antibiotic-resistant bacteria or may receive an antibiotic that is not completely effective. In cases such as these, inhibiting the innate immune response would increase the probability of bacterial proliferation.

Sepsis is more common in neonates and the elderly than it is in young adults. However, most of the animal studies of experimental sepsis are performed in young adults. In human neonates, concentrations of the coagulation factors, especially the vitamin K factors, are very low. This changes the regulation of blood coagulation considerably, probably making it easier to elicit either hemorrhage or thrombosis in response to inflammation....

Thus, it is not surprising that studies performed with candidate drugs on previously healthy animals at the peak of life might give different results than those seen in elderly humans, especially as reflected by the risk of adverse effects to drugs.

Failure to extrapolate physiologic responses from animals to patients may be due to several factors: differences in infective agents, differences in age, the existence of co-morbidities in the patients, timing of treatment, differences in dosages and different dose-dependent efficacies when the drug is derived from a different species, differences in the use and effectiveness of antibiotics, whether the pathogen is largely in the organs or the bloodstream, and lack of intensive care in the animal studies.⁸³

Esmon succinctly listed some of these concerns in the following table⁸⁴:

Experimental Sepsis	Septic Patients
Usually young adults	Often neonates or elderly
Endotoxin/defined organism	Unknown/often multiple organisms
Usually blood borne	Often in tissue
Antibiotics +/-	Antibiotics often ineffective
Defined intracellular (Th2)/extracellular (Th1)	Mixed/unknown (Th1/Th2)
Treatment usually early	Treatment usually late
Onset usually rapid	Onset usually slow
Co-morbidities—seldom	Co-morbidities—common Hypertension Cancer, low neutrophils Atherosclerosis Diabetes
Auto-antibodies (APL)?	50%
Ventilator used—seldom	Ventilator used—usually
Coprophagy/unclean environment—usually High level of anticoagulant tolerance Tolerance without some hemorrhage	Coprophagy/unclean environment—seldom Low level of anticoagulant tolerance Tolerance with some hemorrhage

Table 1. Animal models versus septic patients

Th, T-helper cell; APL, antiphospholipid.

Also in 2005, Buras, Holzmann, and Sitovsky wrote:

Two drawbacks common to all sepsis models with respect to clinical relevance are the timing of disease development and lack of supportive therapeutic interventions. Animal sepsis models are constructed to develop reproducible and rapid disease as compared with human sepsis. The onset and progression of sepsis to multi-organ failure occurs in hours to days in most animal models, whereas in human patients this progression occurs over days to weeks. Furthermore, human patients are promptly treated with various standard therapies such as source control (identification of infection with physical removal by invasive procedures), oxygen therapy, intubation and mechanical ventilation; fluid, antibiotic and

⁸³ Esmon

⁸⁴ Esmon

vasopressor therapy; and nutritional support). Given the lack of supportive interventions in animal models, caution must be exercised before extrapolating efficacy results from animal to human disease.⁸⁵

Buras and colleagues provided this list of factors that increase variability in different types of animal sepsis experiments:⁸⁶

Box 3 Variability factors
Toxicosis models
• Type of toxin utilized (lipopolysaccharide subtype, use of sensitizing agent [d-Gal])
Lethal or sub-lethal dose
Route of administration
Fluid resuscitation
Host species and strain
Bacterial infection models
Bacterial load
Route of administration
Timing of infusion
Bacterial strain
• Host strain
Antibiotics/fluid resuscitation
Caecal ligation and puncture model
Needle size used for perforation
Number of perforations
Amount of caecum ligated/amount of necrosis induced
 Uncontrolled bacterial load (amount of stool milked into peritoneum)
Antibiotic/fluid resuscitation
• Sex, age and strain
Colon ascendens stent peritonitis model
Stent lumen diameter
Load of stool transferred into peritoneum

In 2007, Rittirsch, Hoesel, and Ward wrote:

Obviously, there are clear differences between laboratory animals and patients. Mice and rats are housed in specific pathogen-free areas, may often be inbred strains, have the same age and weight, and most importantly, do not have comorbidities (such as diabetes, hypertension, and pre-existing immunosuppression among others) seen in septic humans. In light of the fact that most humans with sepsis are 50 years old, and as most mice used in sepsis are 3 months old (with an average lifespan of 24 months), it is possible that there is a "disconnect" between the study of mice and humans with sepsis. Furthermore, the experimental models have a precisely known time period. In contrast, we encounter

⁸⁵ Buras, et al.

⁸⁶ Buras, et al.

patients of different ethnicities, ages, and weight, and most of the time, we are uncertain when the symptoms first emerged.⁸⁷

In 2012, Ward wrote:

Several similar features occur in septic humans, but clinical interventions such as antibiotic therapy and fluid resuscitation make the sequence of events less definitive than those found in animal models of sepsis....

[R]esuscitative measures in humans may alter the pathophysiological manifestations of sepsis. Most CLP or endotoxemic studies employ 25 g C57Bl/6 male mice which are 1–2 months old. This would be roughly equivalent to young humans (approximately 4 years old), while in most clinical trials the mean age of patients is 60 years. Accordingly, this may be one reason why animal data cannot be reliably extrapolated to humans.⁸⁸

In 2012, Raven wrote:

[R]esearchers usually use young, healthy, inbred mice that die fairly quickly after disease onset. "This is very different from the human situation," notes [Kevin Tracy of the Feinstein Institute for Medical Research in interviews conducted by the author]. "Death and other end points [in humans] develop over weeks and months."

The unspoken truth is that when identical insults are given to identically aged groups of animals—even from the same litter—we see a variable individual effect," says Mervin Singer, an intensive-care specialist who studies mouse models of sepsis at University College London [in interviews conducted by the author].⁸⁹

In 2014, describing the CLP model specifically, Fink wrote:

[S]epsis in humans is, by and large, a disease that occurs at the extremes of age.... In contrast, in the vast majority of studies using the murine CLP model, sepsis is induced in young adult animals without any comorbid conditions. It is noteworthy in this regard that CLP-induced sepsis in young adult mice is not associated with the development of acute kidney injury (AKI), a common problem in human sepsis, whereas CLP-induced sepsis in aged animals does lead to development of AKI...septic patients typically receive multiple forms of supportive care, including: mechanical ventilation, if indicated; resuscitation of intravascular volume; infusion of vasopressors and/or inotropic agents; administration of anti-microbial agents; renal replacement therapy, if indicated; surgical source control, if indicated; and enteral (or, less commonly, parenteral) administration of nutritional supplements. Some of these forms of supportive care, such as the administration of antibiotics, are sometimes included in murine studies. However, the more complex interventions, such as renal replacement therapy or prolonged mechanical ventilation, are rarely, if ever, employed....⁹⁰

In 2015, Lilley and colleagues wrote:

⁸⁷ Rittirsch, et al.

⁸⁸ Ward PA. New approaches to the study of sepsis. *EMBO Mol Med.* 2012;4:1234-1243. (Ex. 24)

⁸⁹ Raven

⁹⁰ Fink

In the clinical setting, sepsis mortality is highest in very young and elderly patients who often present with comorbidities, representing [*sic*] highly heterogeneous population. By definition, this means that there is no single animal model that fully recapitulates the clinical sepsis syndrome.⁹¹

In 2016, Verma wrote:

It is also difficult to extrapolate animal model studies results to human because of the following reason: while designing an in vivo experimental setup, pre-requisite taken into consideration is to regulate the underlying confounding variables. To achieve so, healthy, inbred animals of same sex, age and weight are chosen to limit the baseline variability. Also, the experimental insult is kept constant ensuring same stimulus is provided to each animal for the purpose of comparing the results. But it is a well-known clinical reality that the patients are outbred; have variable age, sex and weight; have variable health parameters and different causes of sepsis.⁹²

Verma also provided a similar illustrative table, which echoed earlier concerns:⁹³

Factor	Clinical Condition	Experimental Condition
Genetic pool of sex	Heterogenous of both the sexes	Homogenous of single sex
Age	Variable (More of neonatal and elderly)	Usually young
Species	Single i.e., Human	Variable i.e., Mice, Rat, Pigs, dogs, etc.
Treatment Differences	Active treatment including resuscitation, antibiotics, organ support, etc.	No organ support, No or limited antibiotics and resuscitation
Infection	Natural	Induced
Type of microbes	Usually virulent	Usually avirulent
Degree of insult	Variable	Uniform
Time course	Natural	Imposed

Table 3. Difference between clinical and experimental conditions of Sepsis.

Also in 2018, Laudanski and colleagues of the University of Pennsylvania, Emory University, and the Philadelphia College of Osteopathic Medicine repeated these concerns:

Animals are often inbred and kept in sterile conditions, leading to high homogeneity. While reducing interindividual variability is helpful in pure laboratory research, the applicability of the research may be compromised by the lack of diversity typical of human patients. It is also questionable how well the environment of the animal facility resembles natural conditions. Stable temperature, rigorously controlled diet, multiple barriers to prevent infection, and limitations on animal mobility due to housing constraints are far from typical clinical environments.⁹⁴

The 2019 NAGMSC Working Group found that "[i]mportant comorbidities (age, diabetes, pre-existing organ failure, malignancy) and life-sustaining acute therapies (mechanical ventilation, renal replacement therapy) are difficult to incorporate into existing models of disease."⁹⁵

The long-standing deficits in scientific rigor and reproducibility characteristic of non-human animal sepsis experimentation are clear. While it may appear that some of the problems with variability and consistency could be resolved with improvements in study design, the refusal of experimenters to solve these problems in the more than 15 years since they were brought forth by Esmon demonstrates a concerning laziness and inflexibility prevalent within the community conducting these experiments. The refusal to correct even simple issues, such as

⁹¹ Lilley, *et al*.

⁹² Verma

⁹³ Verma

⁹⁴ Laudanski K, Stentz M, DiMeglio M, Furey W, Steinberg T, Patel A. Potential pitfalls of the humanized mice in modeling sepsis. *Int J Inflamm.* 2018;6563454. (Ex. 25)

 $^{^{95}}$ *Id.* at 4

animal age, represents an enormous waste of resources. Without rigor, it cannot be assured that the results obtained in these experiments have any applicable validity for the species or strains of animals involved, much less for humans. Moreover, addressing problems with study design does not erase the immutable species differences that most undeniably inhibit translation to the clinical setting.

II. Welfare Concerns with Animal Sepsis Experiments

In addition to lacking relevance for sepsis in humans, sepsis experiments on non-human animals involve significant suffering for the animals used, who experience fever, chills, diarrhea, difficulty breathing, disorientation, shock, and, finally, multiple organ failure and death as a result.⁹⁶ To inflict this degree of suffering on animals in experiments that are known to have no relevance to human health is insupportable. By law, NIH must consider the adequacy of the protections for the animals proposed to be used in a grant application and support research that does not use animals, that reduces the use of animals, and that reduces the pain and distress produced in animals in experimentation. When the purported objective of a grant proposal—and the only permissible objective in accordance with NIH's purpose of improving human health—is to develop a treatment for sepsis in humans, it should go without saying that there cannot be adequate protections after *decades* of evidence that such experimentation causes significant pain and distress in animals while failing to yield any treatment beneficial to humans—and indeed while being incapable of yielding such a benefit due to insurmountable differences between the species. Furthermore, as described below, the welfare issues inherent in sepsis experiments on mice and other species impact translation of those experiments—that already lack translatability to sepsis in humans.

According to Lilley and the expert working group of veterinarians, animal technologists, and scientists:

Sepsis research represents an area where many of the models used have the potential to cause high levels of suffering for animals.

The surgical implantation of devices required for biomarker measurements has the potential to cause postsurgical pain, infection, and wound breakdown.

Animals with sepsis are likely to have special husbandry needs. Highly debilitated animals will have limited ability to move around the holding cage and will have difficulty in feeding, drinking, and maintaining body temperature.⁹⁷

Importantly, pain and distress further weaken any potential translational benefit of animal sepsis experiments. Bara and Joffe discussed this in 2014:

[A]nimal pain and distress introduce uncontrolled confounding variables to research, violating the assumption of normal physiology and behavior....Pain and distress can impair brain development, cognition, memory, spatial learning, resistance to stress-induced pathology, immunocompetence, disease progression, and behavioral expression....Description of and attention to anesthesia, analgesia, and husbandry, including veterinary knowledge, are critical to producing scientifically reliable results....

[P]ain and distress may confound the study results, and may thus be a reason for the poor translation of AR [animal research] to humans.⁹⁸

⁹⁶ Rittirsch, et al.

⁹⁷ Lilley

⁹⁸ Bara, Joffe

Thus, sepsis experiments on animals—while yielding no benefit for humans suffering from the condition involve a high degree of pain and distress that affect the validity of the experiments and that cannot pass muster under scientific review for the adequacy of protections for the species and justification for their use.

III. Human-Relevant Sepsis Research Methods

More than twenty-five years ago, Congress required the Director of NIH to develop and implement "a plan...for the [NIH] to conduct or support research into...methods of biomedical research and experimentation that do not require the use of animals;...for establishing the validity and reliability of [these] methods...; ...for encouraging the acceptance by the scientific community of such methods that have been found to be valid and reliable; and...for training scientists in the use of such methods that have been found to be valid and reliable."⁹⁹ Nothing in the law requires that researchers use non-human animals to study and find treatments for sepsis in humans. In 2015, the expert working group of veterinarians, animal technologists, and scientists issued a report on the implementation of the 3Rs in sepsis research.¹⁰⁰ The authors stated: "The decision to use animals in sepsis research must only be taken after all *in vitro* and *in silico* approaches and human clinical studies have been ruled out based on scientific or ethical grounds. Knowledge of the strengths and weaknesses of, and alternatives to, *in vivo* sepsis models is essential to choose the most appropriate model system to use."¹⁰¹

The group noted several methods that could be used instead of animal models:

[*I*]*n vitro* cell culture models may be useful to analyze specific aspects of the biological response and may be used in the initial steps toward understanding mechanisms

In addition to conventional two-dimensional monolayers, there is growing use of organotypic threedimensional (3D) cell culture models to explore infectious disease mechanisms. Three-dimensional cell cultures more closely mimic the morphological and functional features of their *in vivo* parental tissues....These 3D systems have enormous potential for bridging the gap between cell-based research and animal models for studying both host-pathogen interactions and human disease progression, as well as for the development of novel drugs and therapeutics....Infection mechanisms explored using 3D culture models include *Pseudomonas aeruginosa* lung infections....*Salmonella typhimurium* in the small intestine...hepatitis C virus in the liver...and LPS in lung and liver models....*In vitro* models can be used to investigate specific aspects of sepsis pathophysiology, allowing a more focused approach to be used in animal models that might better translate into the human condition.

In addition, *in vitro* reconstitution of disease-related cell types or tissues could be used in the development of synthetic human models that would also improve current disease models....Moreover, new genomic information, such as the availability of personal genomes...or exomes...to capture the disease heterogeneity directly from patients or systematic interpretation of genome-wide signatures in human diseases...will complement or even replace the need for mouse models in disease discovery and drug development.¹⁰²

Lilley and colleagues also noted:

^{99 42} U.S.C. § 283e(a)-(b).

¹⁰⁰ Lilley, et al.

¹⁰¹ Lilley, *et al*.

¹⁰² Lilley, et al.

Considerable advances have been made in detailing the molecular mechanisms underlying the acute inflammatory response, and the complexity of this process makes inflammation a prototypical case study for the application of systems and computational biology.¹⁰³

In the enclosed exhibit¹⁰⁴, we provide a comprehensive list of human-relevant, basic and mechanistic sepsis studies published on or after February 19, 2013 (the publication date of Director Collins' blog admitting that human sepsis and mouse sepsis are "different conditions"¹⁰⁵) and indexed in NIH's PubMed database that do not involve the use of non-human animals. The existence of these 239 publications further demonstrates that funding experiments for the treatment of human sepsis on nonhuman animals when there are human-relevant methods for studying the condition is not in accordance with the laws that govern NIH's funding authority.

In addition, public funds used for sepsis experiments on non-human animals detract from what could be spent improving and enhancing human epidemiological and clinical sepsis research. There may have already been a breakthrough in clinical sepsis research. Physicians have recently had impressive results by treating sepsis patients with an intravenous vitamin C combination.¹⁰⁶ One patient whose chance of dying from sepsis was nearly 100 percent was well enough to leave the intensive care unit within seven days of receiving this treatment.¹⁰⁷ An estimated 10 to 20 percent of intensive care specialists around the world have already started using this therapy, and studies involving 13 hospitals are underway to confirm its efficacy.¹⁰⁸ Importantly, these successes have been achieved using only human patients, not mice or other animals, and many patients were helped tremendously in the process.

IV. Conclusion

Despite the overabundance of evidence, much of which is presented here, some animal experimenters continue to insist on the utility of using mice and other animals to study human disease, including human sepsis. Claims that the use of animals has led to biomedical advancements are stated without any cited references. These claims are assumed and not evidence-based. This is true for many of the manuscripts cited in this report, with a bold, unsubstantial declaration praising the use of animals sometimes sandwiched in between facts about the lack of value of non-human animal sepsis experiments.

It is sometimes stated that studying *why* mice experience sepsis differently from humans is important for developing treatments for this condition.¹⁰⁹ This argument is senseless. One does not study a human patient with Alzheimer's to discover why they do not have muscular dystrophy. One should not study mouse sepsis to learn about human sepsis.

Despite the \$129,443,886 *currently* awarded by the National Institutes of Health for sepsis experiments on non-human animals,¹¹⁰ patients have nothing to show for it. The NIH is authorized by statute to fund research that

¹⁰³ Lilley, et al.

¹⁰⁴ Ex. 26

¹⁰⁵ Collins

¹⁰⁶Marik PE, Khangoora V, Rivera R, Hooper MH, Catravas J. Hydrocortisone, vitamin C, and thiamine for the treatment of severe sepsis and septic shock: A retrospective before-after study. *Chest.* 2017;151(6):1229-1238. (Ex. 27) ¹⁰⁷Harris R. Can a cocktail of vitamins and steroids cure a major killer in hospitals? NPR.

https://www.npr.org/sections/health-shots/2018/05/11/609149556/can-a-cocktail-of-vitamins-and-steroids-cure-a-majorkiller-in-hospitals. Published May 11, 2018. Accessed October 19, 2019. (Ex. 28)

¹⁰⁸*Ibid*. ¹⁰⁹ Collins

¹¹⁰ NIH RePORTER. https://projectreporter.nih.gov/reporter.cfm. Accessed October 11, 2019. Queries: (Text Search: (sepsis AND (mice OR mouse OR mus)) (Advanced); Search in: Projects Limit to:Project Abstracts; Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (rat OR rattus)) (Advanced); Search in: Projects Limit to:Project Abstracts; Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (rat Search: (sepsis AND (ratbit)) (Advanced); Search in: Projects); (Search in: Projects)

improves *human* health. To continue to fund experiments on other species in light of the extensive evidence that there are insurmountable differences between sepsis in humans and sepsis in other animals—that indeed they are two disparate conditions—disregards the funding criteria of innovation, originality, approach and methodology, significance and likelihood of producing meaningful results, and protections for animals and is arbitrary, capricious, an abuse of discretion, not in accordance with law, in excess of statutory jurisdiction and authority, and without observance of procedure required by law.

in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (dog OR canine OR canis)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (pig OR porcine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (pig OR porcine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (sheep OR ovine)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (cow OR bovine OR bovinae)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (cow OR bovine OR bovinae)) (Advanced); Search in: Projects Limit to:Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (cow OR bovine OR bovinae)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects); (Text Search: (sepsis AND (monkey OR macaque OR baboon OR marmoset OR tamarin)) (Advanced); Search in: Projects Limit to:Project Abstracts;Project Title; AdminIC: All; Fiscal Year: Active Projects)