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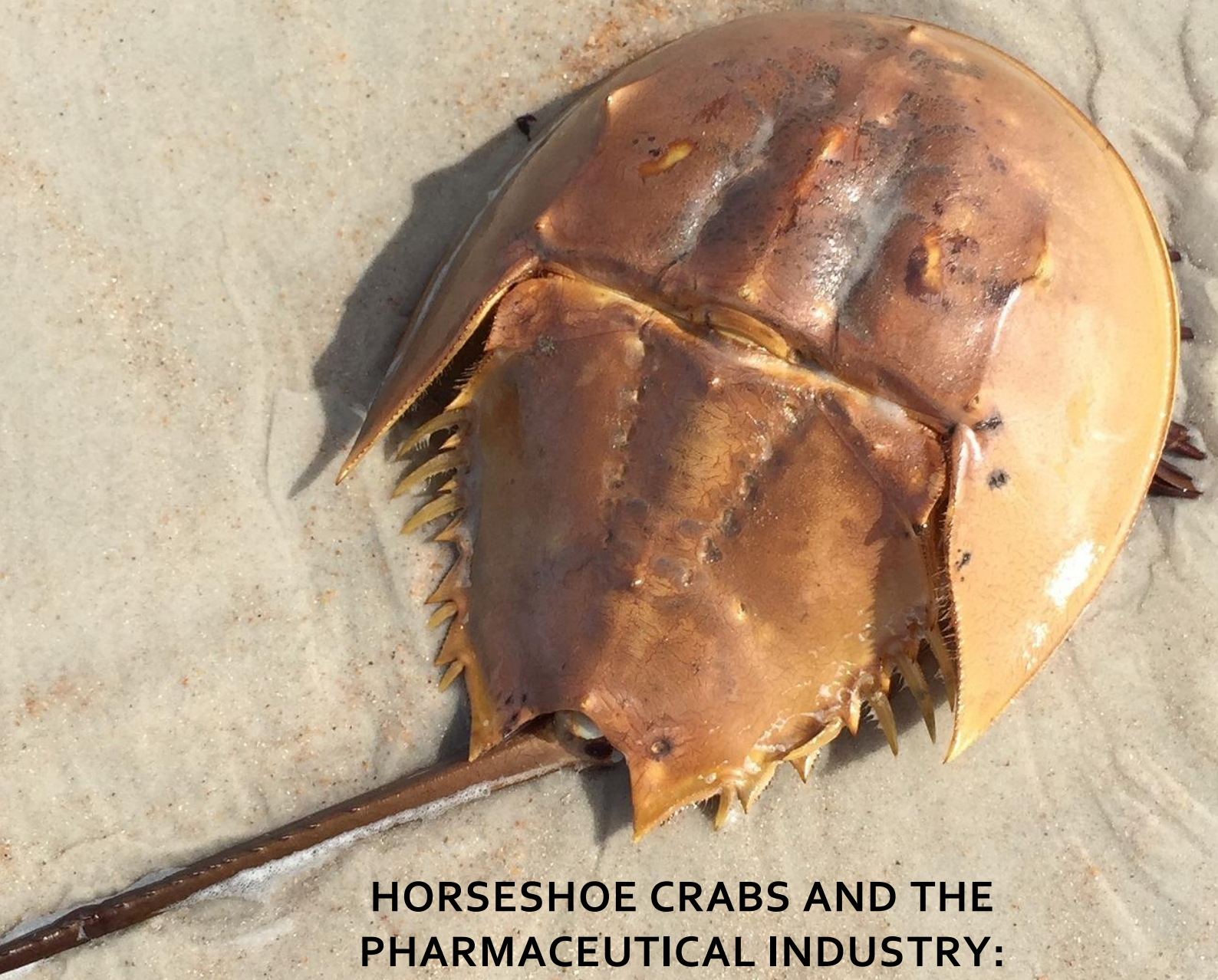

# HORSESHOE CRABS AND THE PHARMACEUTICAL INDUSTRY: CHALLENGES AND ALTERNATIVES

*PROJECT REPORT*

RSPCA

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*Image credit:* Pixabay

# INTRODUCTION

Endotoxins are bacterial components that can cause systemic toxicity if they enter the mammalian blood stream. Testing for the presence of endotoxins is vital for the safe use of vaccines, injectable medicines, and medical devices in human and veterinary medicine. In North America and Europe the primary method for endotoxin testing is the Limulus Amebocyte Lysate (LAL) test. This utilises the coagulative properties of Atlantic horseshoe crab (*Limulus polyphemus*) blood to detect endotoxins, linking this immunologically unique and ancient species to the global supply chains of modern health and medicine. The development of the LAL test served as a replacement to previous methods of endotoxin testing which involved injecting samples into rabbits (the Rabbit Pyrogens Test) – a significant reduction in harm to animals.

However, procuring blood for this (regulatory mandated) testing involves capturing & bleeding over 500,000 crabs from wild populations each year. The continued use – and potential rise in use given increasing global demand for pharmaceuticals – of horseshoe crabs is prompting growing questions around welfare and sustainability. There are growing debates around Limulus' sentience and capacity to suffer, with animal protection groups posing welfare and ethical questions. Whilst the crabs are returned, alive, to the sea following the collection of their blood, and some view the bleeding process as harmless, there are increasing discussions about the impact that capture and bleeding can have on crab health and mortality.

This report explores what a social science perspective might add to understanding the debates surrounding the use of horseshoe crabs in endotoxin testing. It draws on qualitative research with stakeholders, alongside documentary and policy analysis to examine the various perspectives, positions, and sides of debates about horseshoe crabs. This report attempts to represent the wide diversity of stakeholder perspectives about the biomedical use of horseshoe crabs in a balanced manner.

The two major themes responsible for the current contestation – the impact that the biomedical industry has, or does not have, on horseshoe crabs, and the current replaceability of horseshoe crabs within contemporary pharmaceutical processes – are discussed. Building on this, the report then discusses the role that ideas about animal welfare have traditionally played in shaping conversations about horseshoe crabs, examining why the biomedical use of *Limulus* has remained outside of, and resistant to, an engagement with the welfare concerns that underpin the social acceptability of the biomedical usage of other animals. Bringing conversations about horseshoe crabs into connection with wider discussions about animal welfare more broadly offers many opportunities for restructuring debates in helpful ways. Particularly, the 3Rs framework – the ambition to, where possible, replace, reduce, and refine the biomedical use of animals – emerges as a useful and appealing concept to a wide variety of the stakeholders involved in discussions about horseshoe crabs. As conversations about the biomedical use of horseshoe crabs are starting to change there is also a need for more openness and transparency. This is increasingly a staple of best practice within the broader biomedical industry, and greater openness here would afford an important opportunity to reassure publics and other stakeholders about the level of care involved in contemporary biomedical use of horseshoe crabs. The pharmaceutical reliance on horseshoe crabs is growing as a topic of public interest, without assurances and openness about humane care, it may be that public opinion shifts from an understanding of the (currently perceived) need to utilise horseshoe crabs in a care-full manner, to one that rejects this as a tolerable practice. With welfare potentially rising on the agenda, there are opportunities – and demands – for animal welfare organisations to become involved in, and help shape, future discussions about horseshoe crabs.

This report is intended to be an overview of the topic. It is aimed at the multiple stakeholder and public audiences interested in the matter of horseshoe crabs and animal welfare. Additional scientific articles are in development in peer-reviewed academic journals and books, which will expand on the themes identified here.

## CONTENTS



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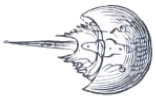
Additionally, support from the RSPCA's Science and Policy Group, and the Animal Research Nexus project has been crucial in the production of this research and report.



## 1. WHAT CAN A SOCIAL SCIENCE PERSPECTIVE ADD?

The use of horseshoe crabs in endotoxin testing engages a complex range of stakeholder perspectives and involves multiple 'epistemic communities' each with their own shared beliefs, working practices, and criteria for assessing validity that lead them to form different understandings. The serious public health concerns posed by endotoxins means debate over the use of horseshoe crabs is growing. However, it involves considerable uncertainties and diverse views. Discussions about the biomedical use of horseshoe crabs are becoming increasingly polarised. Krisfalusi-Gannon et al. (2018: 10) suggest that the drivers for horseshoe crab protection 'are both environmental and economic'. This may be true, but they are also social and cultural. As Davies et al. (2016) argue, social science research can make a significant difference to laboratory policy and practice, opening up understandings of the social, economic and cultural processes that influence practices of laboratory animal science, and the wider social contracts that enable public acceptance of the scientific use of animals.

The use of horseshoe crabs in endotoxin testing is thus a complex scientific **and** societal issue, situated at the interface of human, animal, and environmental health. Social science can help understand the 'shape of the conversation'. This involves considering who is included in a conversation about the use of horseshoe crabs and how it is framed, focussing on understanding the various perspectives, positions, and sides of the debate in order to try and move discussions forward in productive ways (Cassidy, 2019). This is less about definitively identifying what is factually or morally 'right' and instead exploring what different stakeholders believe, and why they believe the things that they do, to try and answer the question, why has this become an issue?

**Understanding the attitudes underpinning current and future horseshoe crab use across a complex range of stakeholder perspectives can be aided by social science.**

### WHAT DO SOCIAL SCIENTISTS DO?

Social scientists use qualitative methodologies to map how different discourses are forming, intersecting, and impacting practices. Here, this has involved documentary and policy analysis alongside empirical interviews with stakeholders.

Interviewing is one of the most commonly used research methods in the social sciences. Interviews enable researchers to learn from interviewees' perspectives, their situated and contextual experiences, and their attitudes and feelings towards – in this case – horseshoe crabs and endotoxin testing. Thirteen interviewees were selected from across the broad spectrum of groups with a stake in the biomedical use of horseshoe crabs; manufacturers, biotechnology companies, regulators,

### WHAT IS SOCIAL SCIENCE?

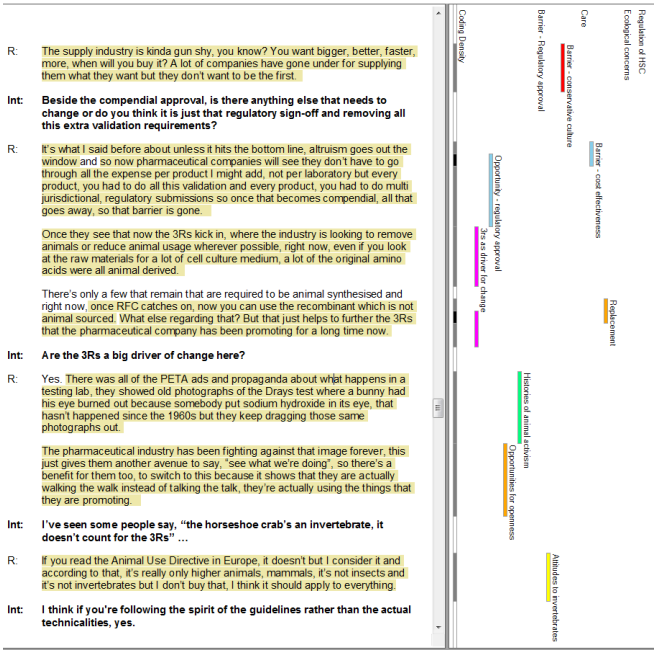
Social science is, broadly, the study of human society and social relationships. Social scientists explore how people interact with one another, how shared cultures develop, and how people influence the world. Social science can help explain how society works, what different groups expect, and inform governance and policy.

## WHY ARE SOCIAL SCIENTISTS INTERESTED IN HORSESHOE CRABS?

Social scientists working within the broad field of science and technology studies seek to understand how scientific research and technological innovation are affected by, and affect in turn, society, politics, and culture. This involves exploring the different ways that scientific knowledge is produced and contested, and how different 'claims' to evidence, truth, and ethics are made and invoked in ways that influence practice.

More recently, social scientists have become interested in how the use of animals in biomedicine is impacted by social, cultural, and political relations, and particularly, the contemporary challenges emerging as scientific practices and social expectations change. This involves examining how different interests are spoken for, and decisions made, around biomedical use of animals.

pharmaceutical scientists, conservationists, animal-welfare groups, academic researchers. These individuals were located across the UK, Europe, and North America. This approach enabled a narrow but deep focus. Conversations with these stakeholders explored their perspectives and concerns relating to the current and future roles of horseshoe crabs within practices of endotoxin testing. These interviews provided an opportunity to understand the priorities of stakeholders. They highlight key connections, themes, and questions, and help identify and address the challenges and opportunities that exist for change regarding the biomedical use of horseshoe crabs. All of these conversations were recorded, with interviewee's consent, and transcribed to allow analysis. One additional stakeholder opted to submit written responses to the questions that structured the interviews, to allow them to collect the information required. It is common practice within social scientific research to anonymise participants for reasons of confidentiality and ethical research practice. This is particularly the case here, given the sensitive nature of animals' involvement in testing, along with the need for sensitivity around commercial interests. As such, all interviewees have been assigned attributions based on their broad sector of work. The research was assessed and approved by the University of Exeter's Ethics Committee. Analysis involved a type of thematic analysis – a widely used method within qualitative research – that allows the comparison of themes across different perspectives, understanding what is important to different people, and identifying where there are differences and similarities between different groups. Following analysis, a cross-section of respondents were invited to review a draft of this report and provide feedback on the initial themes discussed. Through examining different perspectives about evidence, validity, risk, we can begin to understand why the biomedical use of horseshoe crabs has become a contested issue, and try to suggest what frameworks might help to enable better conversations in the future.




*Example of part of a transcript 'coded' using NVIVO, a qualitative analysis software package.*


*Wordcloud generated from qualitative interviews*

## 2. HOW DID HORSESHOE CRABS END UP INTERTWINED WITH THE GLOBAL SUPPLY CHAINS OF MODERN HEALTH AND MEDICINE?

The biomedical use of horseshoe crabs is simply the latest set of human-animal relations in which horseshoe crabs have become entangled as a commodity. Indeed, the harvest and use of Limulus is not a recent phenomenon at all, with masses crabs having been utilised as fertiliser since the 18th century. Kreamer and Michel's (2009) historical research notes that in 1880 over 4 million crabs were harvested from Delaware Bay alone – not accounting for elsewhere along the Atlantic coast.

Biomedical interest in horseshoe crabs began growing towards the end of the nineteenth century, when the animal was recognised as an important comparative experimental laboratory animal. Horseshoe crabs were large, readily available, and 'easy to maintain in laboratory aquaria' (Leibovitz and Lewbart, 2003: 246).

Between 1950-51, Frederik Bang injected various bacteria into the circulatory system of horseshoe crabs. Bang had speculated that species of ancient origin might reveal primitive immunological functions (Levin et al., 2003). He found that gram-negative bacteria produced intravascular clotting – but gram-positive bacteria did not (Bang, 1953). A series of later collaborations with Jack Levin enabled the discovery that endotoxin was the key factor in the clotting of Limulus blood. By 1968, Levin and Bang had recognised that the sensitivity of the system would make a highly applicable method for assaying bacterial endotoxin, with the reaction being able to be adapted to a convenient *in vitro* test.

At the time the official test for endotoxins was the Rabbit Pyrogen Test (RPT), which involved injecting a sample of a product into the ear vein of three rabbits, and regularly monitoring febrile-response. Here, parallels with the current situation emerge – in that critical to the acceptance of any new bioassay is the demonstration that it correlates with existing and established assays. For LAL to replace RPT, scientific, pharmaceutical, and regulatory communities needed to be convinced that LAL was more sensitive and specific, and reassured that LAL did not fail to detect any endotoxins of importance to quality testing (Levin et al., 2003).

### Why is history important?

*Understanding the use of horseshoe crabs in endotoxin testing as a complex scientific and societal issue requires a historical perspective to see how debates, policy, and practice have been shaped over time. Particularly as many of the discourses and narratives produced to enable and affirm the continued collection of horseshoe crab blood rely on historicised comparisons and justifications. Relations with horseshoe crabs are always contrasted with prior valuations of the 'usefulness' of the crab (Gisler and Michael, 2011). The current controversy over alternatives to crab-blood derived reagents links to previous disputes, scepticisms, and uncertainties involved in ensuring the safety of parenteral medicines and devices from contamination by endotoxin.*

The timely opportunity provided by the advent of short-lived radiopharmaceuticals created a demand for pyrogen testing that could not be met by the rabbit assay (because of the rapid decay, the large volume required for testing, the risk of radiation exposure, and the problems of waste disposal) (Swan, 2002). Thus in 1969, James Cooper, working under Levin and Henry Wagner began investigating the applicability of the LAL test to these radioactive drugs and comparing the RPT with the LAL test. Their work found a strong correlation between the rabbit's febrile response and the LAL reactivity, indicating that the LAL test

was an indicator of biological response and thus an effective means of *in vivo* pyrogen testing (Cooper et al., 1971). Cooper would go on to conduct further studies establishing the feasibility of LAL as an alternative to the RPT for the detection of endotoxin in pharmaceutical drugs (Cooper et al., 1972).

> "When you look at it, it was probably a good 15 or more years before the LAL test was really accepted as a substitute for the rabbit pyrogen test." – Interviewee, regulatory sector.

However, as Levin (2019) reflects, despite the demonstration of a correlation with established assays for endotoxin, some scepticism remained (again, a situation with great parallels to the present disputes around alternatives). This challenge emerged because the greater sensitivity of LAL 'made it impossible to directly compare the results of the LAL test with other assays at the lowest concentrations of endotoxin which LAL could detect' (Levin, 2019: 6). Large studies were needed to document efficacy of LAL.

In 1973 the FDA announced their decision to regulate the sale and use of LAL, stating that LAL was not suitable as a replacement, and that the RPT remained the required final pyrogen test, but recognised that LAL had value as an in-process test (National Archives And Records Administration, 1973). This would create a mechanism for the generation of mass amounts of data about LAL as well as build the capacity for people to gain experience using this new technology.

In 1977, the FDA announced that LAL could be used as a final test alternative to the RPT in biological products and medical devices (but not yet human drugs) – providing approval was first obtained from the appropriate bureau of the FDA – noting that since the '73 decision, production techniques had been standardized, LAL sensitivity had been improved, and questions concerning specificity had been answered (National Archives And Records Administration, 1977). Despite this, Levin et al. (2003) recall that industry acceptance and adoption was slow, with many companies having years of experience with the RPT. The RPT, though expensive, was familiar, and viewed as more straightforward. There was also fear that LAL, through its greater sensitivity, would result in a greater rejection of products, or introduce a greater threshold of regulation (Levin et al., 2003).

The start of the 1980s saw the FDA announce draft guidelines for use of the LAL test as an end-product testing method for endotoxins in human and veterinary injectable drug products, and invited pharmaceutical manufacturers to comment and share data. Under these draft guidelines, manufactures would need to 'validate' their use of the LAL test, for each individual product, demonstrating the sensitivity of their LAL, the lack of product inhibition or activation of the test for each drug product formulation, and the presence or absence of any nonendotoxin pyrogens in the drug product formulation (National Archives And Records Administration, 1980a: 3669). The same year saw the equally important publication of 'General Chapter <85> Bacterial Endotoxins Test' by the United States Pharmacopeial Convention (USP), making the LAL test a Compendial method (Levin, 2019). LAL was increasingly gaining acceptance, and more importantly, being positioned in the regulatory frameworks that would enable its use. By 1985 it was noted in the Federal Register that 'the Limulus test is widely used' in demonstrating the presence of endotoxin (National Archives And Records Administration, 1985: 3101). Finally, in December of 1987, the FDA published a 'Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices' (Food and Drug Administration, 1987). This document was the culmination and finalisation of the 1980 draft guidelines, and set forth the official conditions for the use of LAL in lieu of the rabbit pyrogen test.

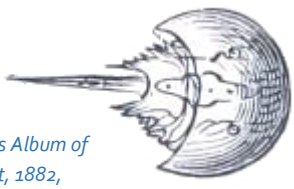


*Image credit: Hill's Album of Biography and Art, 1882, Thomas E. Hill.*

### Beginning to care for crabs

An early example of an attempt to embed a regulatory expectation of a level of welfare for horseshoe crabs can be seen in 1978 with the formal introduction of what became known as the 'return to the sea' policy.

This followed proposals by Cooper, Hochstein, and Seligmann some years earlier. According to one account, Cooper had heard that a company in Ann Arbor, Michigan were taking horseshoe crabs, bleeding them to make LAL, and then destroying the crabs in an autoclave. Cooper did not feel this particularly prudent, having seen from his own research that horseshoe crabs could survive the process (Atlantic States Marine Fisheries Commission, 2009). The policy stated that 'to guarantee that the manufacture of LAL will not have an adverse impact on existing crab populations, the horseshoe crabs shall be returned alive to their natural environment after a single collection of their blood'.

Additionally, the regulatory note stressed the importance that the horseshoe crabs 'from which blood is collected for production of the lysate, shall be handled in a manner so as to minimize injury to each crab' (National Archives And Records Administration, 1978: 35732–35733). This policy was written into the Federal Register from 1978 until 1996, acting as part of the licensing requirements to be a manufacturer of LAL, until it was rescinded as part of the Clinton administration's 'Reinventing Government' reforms – though remains honoured and considered best practice by many biomedical companies today (Atlantic States Marine Fisheries Commission, 2009: 6).

Whilst simultaneously framed at ensuring the sustainability of supply-chains, the introduction of this policy was a significant move, embedding an initial regulatory expectation of a culture of care and level of welfare for horseshoe crabs. For some however, this was not enough, and in a 1980 copy of the Federal Register the FDA noted they had received a letter requesting 'a restriction be imposed on the collection and bleeding of the crabs during their spawning seasons' – an early indicator of concern for crabs and the impact of this new technology on Limulus populations. The FDA's response was a blunt rejection of this idea, despite acknowledging that 'although at present little is known about the effects of bleeding on crabs returned to the wild' (National Archives And Records Administration, 1980b: 32297) (a situation many conservationists might argue remains to this day).

## 3. GLOBAL CONSIDERATIONS

**This report focusses specifically on the Atlantic horseshoe crab (*Limulus polyphemus*) and the Limulus Amebocyte Lysate (LAL) test.**

However, global endotoxin detection is also dependent upon the Tachypleus Amebocyte Lysate (TAL) test, produced in China, and utilized for analogous testing by Asian and Pacific-based pharmaceutical manufacturers (Krisfalusi-Gannon et al., 2018). Although, this involves a complex geography, as many multi-national pharmaceutical companies that manufacture products in China ultimately test their end products with LAL as they are selling worldwide (Gauvry, 2015). This involves the utilisation of *T. tridentatus* (commonly known as the Chinese horseshoe crab, Japanese horseshoe crab, or tri-spine horseshoe crab), a species which is listed as endangered (Laurie et al., 2018).

With the population of *T. tridentatus* declining drastically there is speculation that this could lead to a compensatory spike in the global demand for LAL (Krisfalusi-Gannon et al., 2018). There is concern that 'regulations to protect the harvest of crabs for biomedicine are not as successful in Asia as in the United States', and that bleeding practices often involve 'draining to death', rather than the catch and release fisheries practiced in America (Laurie et al., 2018; Moore, 2017: 120).

> "They use TAL because TAL is cheaper than LAL, and LAL has import tariffs associated for bringing it in country, so they use TAL and TAL is responsible for an awful lot of problems that we're having with the Asian horseshoe crab, not all of them but it is one of them. [Conservationists] have been trying to get the pharmaceutical companies to not use TAL, to use LAL or rFC where appropriate. What [they're] working towards is the total stoppage of TAL as an endotoxin test, period. Use LAL, or if you're able to, use rFC, because *Limulus* is going to have to absorb TAL. *Limulus* comes from the East Coast and unlike in Asia, our population is, it wasn't always this way, it's very well regulated, we are regulated for sustainability."
> – Interviewee, conservation sector.

The different values and ethics invoked in the biomedical utilisation of horseshoe crabs are shaped by localised cultures. Indeed, there is concern that local social norms are the predominant factor in determining harvest practices, rather than a wider ethical framework. For example, in the IUCN Red List account of *T. tridentatus* Laurie et al. (2018: 20) note that 'Bokang Marine Biological (Zhanjiang A&C Biological), a subsidiary of Charles River Laboratories of the USA' are one of the eight manufacturers of the TAL reagent based in China. In the US, Charles River Laboratories claim they are 'proud to play a role in alleviating pressures on horseshoe crab populations through tireless conservation efforts, active animal welfare campaigns' (Charles River Laboratories, 2020b), a position which would appear to conflict with the practices of over-exploitation and unrestricted capture impacting *T. tridentatus*. It is an example of why claims to be involved in conservation of horseshoe crabs are often contested and viewed with a cynical lens.

> "They are trying to convince everybody that they are the gold standard of horseshoe crab conservation, where the exact same company in Asia is harvesting horseshoe crabs, bleeding them to death, and they're contributing to the decline of the population. How do you square that?"
> – Interviewee, conservation sector.

## 4. CURRENT CONTROVERSY

Current controversy about the biomedical use of horseshoe crabs is located around two separate – though interrelated – themes: the **impact** that the biomedical industry has – or does not have – on horseshoe crabs, and the current **replaceability** of horseshoe crabs within contemporary pharmaceutical processes.

*Image credit: Blood is extracted from horseshoe crabs at a biotech facility. Lonza / Reuters.*

### What can social science tell us about controversies?

*Current debate about horseshoe crabs can be understood as something of a 'knowledge controversy'. That is, 'an academic and/or policy and/or public debate centred upon questions of scientific knowledge, expertise and evidence' (Cassidy, 2019: 13).*

*Often in situations of knowledge controversies, the scientific knowledge is still in the 'process of being built' and hence the contestation is operating in an arena in which much is still highly uncertain and speculative, adding to the complexity (Cassidy, 2019: 14).Indeed, Turnhout et al. (2019) observe that the focus of contention is often on the processes and methods by which facts are produced.*

*Of course, whilst at their heart, these controversies are framed as being about who is factually 'right' in an interpretation or presentation of 'knowledge', this is entwined with contestations over the potential economic, political, and societal implications of that knowledge, and therefore simultaneously about values and interests, as much as about 'facts' (Turnhout et al., 2019). The social context in which knowledge controversies play out influences which facts are readily accepted and which are contested (Turnhout et al., 2019).*

## BIOMEDICAL IMPACT?

The number of crabs delivered to biomedical facilities has increased from 335,501 crabs in 2004 to 575,760 crabs in 2017 (peaking with 622,098 in 2012). The Atlantic States Marine Fishery Commission (ASMFC) – the regulatory body around the horseshoe crab suggests that biomedical use is now 'fairly stable'(2019: ii). However, there is concern that growing global demand (and emerging markets) for vaccines, pharmaceuticals and medical devices will require an increasing supply of LAL (Krisfalusi-Gannon et al., 2018). Whilst additionally, the rise of personalized medicine could necessitate individualised product testing on a per use basis, adding further pressure (Porzio, 2018).

Concern for horseshoe crabs most frequently circulates at the population level (rather than the impact on individual crabs), and their role within wider ecosystems. Overt worries about the survival of migratory shorebirds that rely on the consumption of horseshoe crabs eggs – such as Red Knots – are often more visible social markers of concern, rather than anxieties about the crabs themselves. Despite the desire of some media outlets to frame the topic as one of great importance to animal rights activists, i.e. 'crab blood used to make covid-19 vaccine outrages animal rights activists' (de Ferrer, 2020), discussions and contestation about horseshoe crab use have mostly emerged from conservation groups. It is only more recently that animal welfare groups have begun to think about the impacts of biomedical bleeding on horseshoe crabs, and the issue remains a low priority for most animal rights groups (see page 21 for the difference between animal welfare and animal rights, and why this matters for discussions of horseshoe crabs).

The Atlantic horseshoe crab has been classed as 'vulnerable' by the IUCN, with populations trending to 'decreasing' (Smith et al., 2016). However, the extent to which the biomedical use of crabs has an impact here is contested.

> "If you look at the peer reviewed stock assessment survey, it said that the LAL industry actually has negligible impact on the horseshoe crab population status, negligible impact, so that's good."
> – Interviewee, biotechnology sector.

> "So let's not talk about the biomedical industry, let's talk about erosion, let's talk about development, let's talk about all of these things in terms of protecting the horseshoe crab population. I'm willing to accept that the biomedical industry does have some horse in that race but I'm also convinced that they're not the culprit here."
> – Interviewee, regulatory sector.

The numbers of crabs collected for biomedical purposes are frequently contrasted against the earlier fertilizer industry, as well as the contemporary bait fishery, which utilises horseshoe crabs as bait to catch eel and conch (whelk) – a process that involves a 100% mortality rate. Harvests here peaked in 1999 at 2.6 million horseshoe crabs, with 994,491 harvested in 2017) (Atlantic States Marine Fisheries Commission, 2019). Similarly, the ASMFC (2019: 13) suggests that bycatch and 'discard mortality may be comparable to or greater than mortality from other sources (bait landings plus biomedical collection)'. An appendix in the ASMFC's 2019 Horseshoe Crab Benchmark Stock Assessment and Peer Review Report estimates that the number of horseshoe crabs that die as a result of the bleeding process 'is about 7% of the horseshoe crabs that die as bait for eel and conch, and a miniscule fraction, 0.2%, of the estimated number of horseshoe crabs in Delaware Bay alone' (ASMFC, 2019: 270).

> "And the bait industry doesn't get any media attention, that takes a million crabs and chops them up every year. It's like can the bait industry reduce their reliance first?"
> – Interviewee, pharmaceutical sector.

However, as Bolden (2019: 504) summarises, it is 'unproductive to pit the bait fishery take against the biomedical take'. Indeed, as Davies (2019) has explored in the context of laboratory animals more broadly, drawing comparisons to the high numbers of animals used in other sectors does not tend to reassure publics nor create a strong basis from which to argue an ethical point. Some manufacturers were also uncomfortable with efforts to shift the 'blame' onto the bait industry, and the construction of an oppositional dualism between bait and biomedical. All of the actors around the horseshoe crab are important partners in working to achieve conservation, sustainability, and animal welfare goals.

The extreme focus on population statistics, and attempts to attribute the vulnerability of the horseshoe crab to the biomedical industry, is unhelpful. However, though the LAL industry may have a negligible impact on the population status of horseshoe crabs, this does not negate the need for a focus on the welfare experiences of the horseshoe crabs that are bled, ensuring that a high level of care is practiced and implemented throughout the process.

There is an oft-cited idea within literature discussing the bleeding of horseshoe crabs that 'bleeding the crabs does not appear to harm them in any way' (Loveland, 2002: 99). As Moore (2017) argues, there is a concerted effort by industry to position the bleeding of crabs as easy, harmless, and quick. Moore (2017: 112) suggests that the bleeding process involves collecting 'between 25% and 40% of a crab's blood. To put this into context, in the UK, under the 'Animals (Scientific Procedures) Act' (ASPA), the 'withdrawal of blood samples (>10 % of circulating volume) in a conscious animal within a few days without volume replacement' would be classified as a 'moderate procedure'. That is, a procedure 'where animals are likely to experience short-term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress' and 'are likely to cause moderate impairment of the animal's wellbeing or general condition' (Home Office, 2014: 118–120).

One of the most contentious aspects of the debate about horseshoe crab use is around the mortality rates associated with bleeding the crabs. Mortality comprises two parts, 'observed mortality' from collection to release, and 'post-bleeding mortality'. For observed mortality, the ASMFC report that 'all facilities observed mortalities less than 10% while crabs were in their possession, with all currently operating facilities observing mortalities less than 4%' (2019: 41). However, Krisfalusi-Gannon et al. (2018: 5) note that the percentage of horseshoe crabs that died prior to being bled more than doubled between 2008 to 2012, and suggest that a culture of deleterious harvest and transportation practices exists.

Post-bleeding mortality rates are uncertain and complicated to model. The ASMFC assumes a 15% mortality rate for bled and released crabs. However, this 15% rate is highly contested and widely ranging in published literature – Hurton and Berkson's (2003) experiments resulted in a post-bleeding mortality rate of 8.3%, whilst Leschen and Correia's (2010) study concluded a post-bleeding mortality rate as high as 29.8%. Whilst debates over methodologies for modelling post-bleeding mortality rates will continue to rage, it is the subsequent action over this mortality – whatever the rate – that might be expected by publics. The 1998 Fisheries Management Plan for horseshoe crabs established a biomedical mortality threshold of 57,500 crabs. If exceeded, this threshold 'triggers the Management Board to consider action'. However, in the words of the Atlantic States Marine Fisheries Commission (2019: 36), 'the threshold has been exceeded every year since 2007 with the exception of 2016, although no management action has occurred'.

Along with mortality rates, there is growing interest in considering the 'sub-lethal' effects of biomedical bleeding. Anderson et al. (2013) found that bled horseshoe crabs, post-release, suffered from significant changes in activity levels and behavioural rhythms, reductions in hemocyanin levels (potentially affecting

immune function), and decreased reproductive fitness (prompting concerns about longer-term population impacts). However, again, there is dispute about whether these studies represent industry standard practices. For example, whilst Owings et al. (2019) found that bled animals approached mating beaches less than control animals during the first week after release, some commentators have questioned the representability of the methodology which involved simulating the time crabs might spend on the deck of a boat or a dock prior to transport to biomedical facilities by placing the animals next to a space heater in a greenhouse.

> "The fact is that the vast majority of them are returned back, maybe more research needs to be done on are those horseshoe crabs okay once we put them back in the ocean. Are they okay? Because of course, that's an issue as well, if you give blood, you want to be okay when you walk out."
> – Interviewee, regulatory sector.

As a wild-caught animal, horseshoe crabs are also at risk of harm during the collection process, Leibovitz and Lewbart (2003) report that traumatic injuries are common in harvested crabs; from fractures in exoskeletons if the animals are dropped onto ship's deck, to injuries acquired when crabs are dragged over the rough surface of the ocean bottom in the nets of collecting vessels, or even wounds induced by the crabs puncturing each other from being compressed together in storage and transport. The ASMFC introduced 'best management practices' in 2011 to remedy this, introducing a focus on the proper care and handling of horseshoe crabs (Atlantic States Marine Fisheries Commission, 2019), though concern of these additional stressors remains.

> "I go out with our fishermen and I audit their practices. In our contract with them, we have it specified as per the best practices document and so it's actually in our contracts with our fishermen on how they are to handle the horseshoe crabs. They're treated very gently and they're brought back to the same spot where they were taken, within 24 hours, the shells are marked so they're not re-bled in the same year."
> – Interviewee, biotechnology sector.

*Image credit: "Horseshoe Crab" by Smithsonian Institution – licensed under CCo 1.0*

## REPLACEABILITY?

A synthetic substitute to horseshoe crab blood was introduced in 2001 - laboratory-synthesized genetically engineered recombinant Factor C (rFC) (Ding and Ho, 2001), becoming commercially available in 2003 (Bolden, 2019). However, uptake of this replacement was extremely limited due to the availability and market-dominance of the LAL test, combined with concerns about a single-source and supply of the synthetic, cautions over the validation of the alternative, and a lack of regulatory requirements to consider alternatives to testing in non-vertebrates.

> "The rFC reagent has been commercially available for about 15 years but it has found little acceptance in the market place. First generation recombinant products had issues with reagent availability, cost, special equipment requirements and product performance in conjunction with the regulatory situation. "
> – Interviewee, biotechnology sector.

More recently, there has been a renewed attention on replacements to the LAL test, emerging as a result of concerns relating to horseshoe crab populations and as recombinant reagents have become commercially available from multiple manufacturers (Bolden and Smith, 2017). One review of the performance of rFC as an endotoxin detection method suggested it is equivalent to, or better than, LAL in terms of the ability to detect and quantifiably measure bacterial endotoxin (Maloney et al., 2018). However, others have been less positive about the potential to move to this alternative on a routine or commercial basis, citing concerns about the ability of the alternative to achieve adequate specificity (Dubczak, 2018).

Reich et al. (2014: 2) compared the ability of commercially available endotoxin tests to quantify naturally occurring endotoxins in 20 different water samples, finding that 'all endotoxin methods correlated well with each other (>94.4%), demonstrating that recombinant Factor C (rFC) assays are suitable alternatives to Limulus Amebocyte Lysate (LAL) assays for testing naturally occurring endotoxins in water'. However, the 'missing' the 5.6% here has been seized on by Dubzcak (2018) (and others) as representing 'an unacceptable patient safety risk for many'. Dubczak goes on to argue that 'results have never shown it to be superior to the compendial method' and provides examples 'where rFC under-predicted endotoxin contamination'. Similarly, Wassenaar and Zimmermann (2018) note instances where rFC 'turned out to be insensitive' and 'performed rather poorly'. However, more recently there have been further studies that have demonstrated 'recovery rates closer to 100%' (Piehler et al., 2020: 10). Over a period of six years Piehler et al. (2020: 10) compared classical LAL and next generation rFC-based test methods, concluding that 'rFC represents a very reliable model, equivalent or even superior to LAL and suitable for routine bacterial endotoxin testing'. However, concerns still remain. Recently, Tsuchiya (2020: 36751) has suggested that the specificity of LAL to endotoxin is achieved by both Factor C and Factor B, and that such a finding represents 'the risk of the use of recombinant Factor C (rFC) reagents'.

The topic of alternatives here has generated much discussion, with debates becoming increasingly polarised. Indeed, Guest (2019) (a scientist working on drug safety) describes inquiring about the technologies available for endotoxin testing, and receiving a 'completely polarised message'. Guest goes on to explain, "in all honesty, with such a conflicting view, it was hard to trust the information".

There is concern here from some stakeholders that *'commercial interests of alternate methodologies or technologies will and do capitalize on the spread of mistruths and incorrect information and in some cases are the source.'* (interviewee, biotechnology sector). Whether this is true or not is difficult to ascertain – and indeed, there is also suspicion from those involved in alternative approaches that 'traditional' organisations have been involved in 'marketing against' the replacement. It highlights the growing polarisation, contestation, and mistrust that increasingly characterises, shapes, and ultimately limits conversations about alternatives in this

space. As another interviewee from the biotechnology sector summarised, there's *'just a massive amount of politics behind it'*. Thus representative of why, in some ways, a social science approach to this 'controversy' might be helpful.

It is not the place of this report to debate the science of current technologies and whether they are fit for purpose, however there is no question that their existence has changed the very shape of discourse around alternatives here:

> "Before you couldn't say that, now you can say it, particularly since there is an alternative, so it's just changed the whole conversation. So now if the alternative is no good, that's a different conversation, let's talk about the efficacy of the alternative, but there **is** an alternative."
> – Interviewee, conservation sector.

However, regulatory requirements for standardised procedures frequently complicate ambitions for the replacement of animals. In the case of rFC, regulatory approval has been slow to emerge. The FDA issued guidance allowing for the use of recombinant factor C instead of LAL-based assays in 2012, though this was still considered an 'alternative test' and subject to validation requirements, rather than considered a fully equable replacement. This was followed by revisions to the European Pharmacopoeia in 2016, which included Recombinant Factor C (rFC) as an alternative method, again subject to validation requirements. However, this amendment specifically attested that 'the use of alternative reagents such as recombinant factor C as a replacement to the amebocyte lysate eliminates the use of a reagent extracted from live animals'.

Whilst scientific (and perhaps, corporate) consensus over rFC is still playing out, at a session of the European Pharmacopoeia (Ph.Eur) Commission in 2019, the 'test for bacterial endotoxins using recombinant factor C (rFC)' was designated as a new general, standalone, test as of July 2020 This will, at least within Europe, put the synthetic test on an equal footing with crab-blood tests. However, pharmaceutical manufacturers operate in a globalised market, and without harmonisation across the various Pharmacopoeias, there is still a long road for the alternative to gain industry confidence and uptake. The lack of alignment between global regulators is a particular challenge:

> "So that specifically will help, if you're a small European based manufacturer and you only sold in Europe. You could immediately switch to that and that would be great. Directionally, it's great, it's awesome and we're very supportive. However, it's tough for us because we operate globally."
> – Interviewee, pharmaceutical sector.

The United States Pharmacopoeia (USP) had similarly proposed the inclusion of recombinant factors for endotoxin testing in chapter <85> Bacterial Endotoxins, which would have likewise given rFC equal status. However, in May 2020 the USP announced that they had 'decided to cancel this proposal and start the development of a separate chapter that expands on the use, validation, and comparability of endotoxin tests based on recombinantly derived reagents' (USP-NF, 2020a). Whilst this decision has sparked an outpouring of attention and criticism in popular media – with the USP's decision framed as an outright 'rejection' of the replacement (Arnold, 2020; Reuters, 2020), there is more hope to be found than these articles would imply. The USP claim to have a 'commitment to the introduction of recombinant Factor C' and suggest that their plan to introduce rFC guidelines within a standalone chapter, is aimed at helping to facilitate broader adoption by manufacturers and a response to the existing regulatory landscape in the US (USP-NF, 2020b). The directionality of change is positive, but the speed of transition is disappointing.

There is a concern that a turn to synthetic alternatives might actually result in more harm to horseshoe crab populations; rather than being a high-value 'catch and release' asset within the biomedical economy, the rise of alternatives may shift the crab's status as a commodity back to that of fishing bait. For example, Charles River Laboratories, a manufacturer of LAL, suggest on their website that:

> 'Many companies think that moving away from LAL to reduce the use of the HSC will help them comply with 3R principles. This idea should be carefully evaluated, as moving to rFC could produce counterproductive effects by endangering the conservation efforts [...] Without the need for LAL in biomedical use, the legal protection of the horseshoe crab is not guaranteed in the future, and they would again fall prey to overfishing and use as bait.' – Charlies Rivers Laboratories (2020)

Conservation is positioned here as a way of practicing care, performing stewardship, and offsetting harms to some crabs through providing affordances to the species at large. However, the idea that horseshoe crabs are only afforded protection and conservation by an ongoing exploitation of the species is one that did not appeal to everyone, and adds another level of complexity and contestation around the replicability of horseshoe crabs. The likelihood of a rise in the bait fishery as a result of biomedical reduction or replacement is debated, given that there are already strict quotas on the bait industry.

The potential replacement of the biomedical use of horseshoe crabs also draws concerns about the potential economic impact of this on local communities.

> "So you're using a threatened and endangered species but these fishermen, their livelihood is at risk and so is it human welfare, is it animal welfare? And all that tension exists."
> – Interviewee, pharmaceutical sector.

*Image credit: "Horseshoe Crabs" by U. S. Fish and Wildlife Service - Northeast Region – licensed under CC PDM 1.0*

## 5. WHAT ROLE FOR WELFARE?

"It's like welfare people have completely ignored horseshoe crabs."
– Interviewee, conservation sector.

Whilst some would argue that from the outset 'LAL manufacturers were concerned about the welfare of the horseshoe crab' (Novitsky, 2002: 79), this often appears conflated with ideas of conservation. In such that the focus is themed around preservation, survival, rather than welfare as a concept focussed on the quality of life of animals (Fraser et al., 1997). The ethics produced here are concerned with a species and population level vulnerability and sustainability, rather than focussed on the experiences of, and potential harms to, the individual crabs.

"There is no evidence that this practice impacts the population."
– Interviewee, biotechnology sector.

Taking an animal welfare perspective, that the practice does not cause impacts at a population level is, perhaps, not enough. Regulation too is aimed at implementing quotas on horseshoe crab use as a mechanism for ensuring conservation (notably, of endangered shorebird species, not crabs themselves), rather than out of concerns for the minimizing harm or suffering to crabs. The idea that we should only care about horseshoe crabs in as much as they impact birds, is somewhat problematic. Wider conceptualisations of 'animal welfare' are rarely invoked. It's perhaps a crude measure, but the phrase 'welfare' does not appear once in The Atlantic States Marine Fishery Commission's 271 page '2019 Horseshoe Crab Benchmark Stock Assessment and Peer Review Report'.

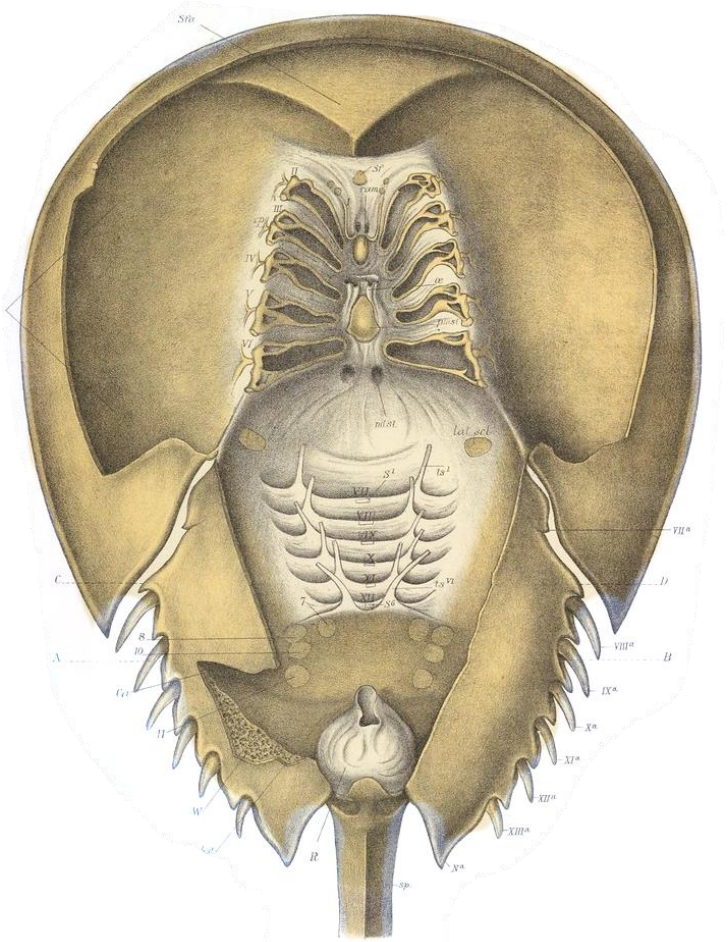


*Image credit: 'Anatomy of Limulus' - Biodiversity Heritage Library – CC PDM 1.0*

## WHY IS CRAB WELFARE HARD TO THINK ABOUT?

Thinking about horseshoe crabs as a welfare issue and priority is challenging for multiple compounding reasons. Horseshoe crabs are a wild invertebrate, managed as a fishery, and bled through a process widely imagined as both non-invasive and a force for conservation, in order to produce a reagent that is positioned as an *in vivo* alternative.

The use of horseshoe crabs in endotoxin testing is a complex scientific and societal issue, situated at the interface of human, animal, and environmental health. It is a valuable case study to think through how people approach and engage with issues of animal welfare and ethics, as **it doesn't easily fit into existing ethical frameworks.**

At present, horseshoe crabs are outside of the scope of most formal legislation regulating animal use; not considered a 'protected' animal.

### A REAGENT, NOT AN EXPERIMENT?

Whether horseshoe crabs are 'used' for biological testing is a matter of interpretation; certainly they are not used *in vivo*, but acquiring their blood is vital in underpinning endotoxin testing.

> "The LAL industry does not use crabs for testing. Only a small proportion of blood is removed from the animal which is then later refined and processed into a final product that supports in-vitro testing."
> – Interviewee, biotechnology sector.

The EU's Directive 2010/63/EU asserts that 'wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure'. There is obvious room for confusion and contestation here, about whether LAL 'entails the use of live animals'. Definitional angst abounds about what counts as 'science', a 'procedure', and even 'an animal'. One interviewee from the biotechnology sector (involved in the manufacture of LAL) described the challenge of trying to get users of LAL *"to at least understand that this is not mana from heaven, this is derived from a living animal".*

The invisibility of the horseshoe crab – as opposed to the embodied presence of other animals enrolled within the modern biomedical industry – has resulted in relatively little engagement with the ethical frameworks and social contracts that mediate the use of animals in science.

### A WILD ANIMAL?

Fundamentally, horseshoe crabs are wild animals. The welfare of wild animals is comparably neglected when contrasted with the focus applied to ensuring the welfare of captive animals, particularly laboratory animals (Palmer and Greenhough, In Preparation). Concern for wildlife is often expressed as a focus on preventing death rather than attempts at improving welfare directly (Wolfensohn and Honess, 2007). As Davies (2019) notes, people's ways of thinking about the ethics of animal care differ dependent on spatial contexts. That is, what publics expect, and think of as acceptable practices, varies according to the imagined spaces in which we place animals.

> "I was speaking to a colleague, and one of her comments was that she was okay with lab rabbits but she was more uncomfortable with the wild animal aspect."
> – Interviewee, pharmaceutical sector.

Crabs positioning as 'wild' within human classifications and imaginations confuses the ethics that are invoked in our utilisation of them.

### AN INVERTEBRATE?

Invertebrates are generally exempt from formal animal welfare legislation (Herrmann and Jayne, 2019). For many, there is contestation about

whether, as an invertebrate, the process of collecting blood from the crabs causes 'pain'.

> "Since those horseshoe crabs are invertebrates and arthropods, I guess the whole concept of pain and so on is not applied to them that much. I guess just because they're not as similar to us as other vertebrates."
> – Interviewee, biotechnology sector.

However, ideas – and public expectations – around invertebrates are changing, and whether the continued exclusion of horseshoe crabs from welfare considerations continues, remains to be seen.

## A FISHERY, NOT A LABORATORY?

Despite their pharmaceutical use, as a wild-caught species, horseshoe crabs are most often imagined through the regulatory lens as a fishery, rather than a laboratory species, and thus managed with a similar set of ethics to other fisheries. Being conceptualised as a fishery is important, as the spatial imaginations invoked can alter both the practice and representation of science, and the ensuing social expectations and public accountabilities (Palmer et al., In Preparation).

Fisheries management plans have their own vocabularies, concepts, and priorities. Thus, horseshoe crab use is discussed in terms of 'maximum sustainable yields' and 'mortality rates' rather than the language of 'replacing, reducing, and refining' or 'harm-benefit analysis' that underpins (and indeed, enables) the laboratory use of many other species, demonstrating how different regulatory regimes act to configure ways of practicing and performing care and welfare.

## AN *IN VITRO* ALTERNATIVE?

LAL is commonly understood and positioned as an *in vitro* test, serving as a 'replacement' in itself as an alternative to the *in vivo* Rabbit Pyrogen Test (RPT), the previous regulatory standard for pyrogen testing.

> "We knew obviously that the lysate test was a move away from the rabbit pyrogen test, that had been the requirement beforehand, so it was seen as less of an animal test than perhaps the rabbit pyrogen test."
> – Interviewee, contract research sector.

The discursive framing affirming LAL as *in vitro* alternative renders invisible the ongoing role that live animals play in its production. Of course, this depends on what is taken to constitute an 'animal' here. At present, horseshoe crabs are outside of the scope of most formal legislation regulating animal use, not considered a 'protected animal'.

Language, history and narratives shape what regulations are, what they do, and how they change. As discussed in the earlier section on history, the move to LAL for bacterial endotoxin testing certainly enabled the phasing out of large colonies of rabbits, producing a significant reduction in harm to animals (and to the technicians responsible for injecting the rabbits with samples). However, as Zhang (2018) notes, 'the LAL test still required the use of animals, but the grisly process of sticking needles into animals became hidden and outsourced to a different part of the supply chain'. The tendency to refer to this transition as a 'replacement' – though commonplace – is challenging, and would be better understood as a 'partial replacement', in that the test still involves the use of cells taken from animals.

*Image credit: Hill's Album of Biography and Art, 1882, Thomas E. Hill. Public domain.*

## What is animal welfare?

Animal welfare is a multifaceted, variable, evolving, and highly debated concept. Mellor and Reid (1994; 12) describe 'good welfare/well-being is the state of being manifest in an animal when its nutritional, environmental, health, behavioural and mental needs are met'. They go on to explain:

> *"When applied to animals, including humans, the term 'welfare' (or 'well-being') usually denotes an absence of 'suffering' or an absence of what might be argued are major components of suffering - i.e. anxiety, fear, pain and distress. That word usage seems to be especially prevalent in the research context where our objective is to minimise the cost to other animals of their use in experiments. Suffering of almost any sort is taken to represent an unpleasant, undesired state of being which is the outcome of the impact on the animal of noxious stimuli, whatever their origin or type. A wide variety of circumstances can lead to suffering and suffering takes many forms. Suffering can be acute or chronic, it or its components can have a range of intensities, it can manifest predominantly as anxiety or fear or pain or distress or different combinations of these phenomena, and the body responses can be mainly physical, largely mental or both physical and mental." – Mellor and Reid (1994; 5)*

Increasingly, rather than just an avoidance of the imposition of negative experiences, animal welfare involves enabling positive experiences too. Proponents of animal welfare seek to improve the treatment and well-being of animals. One particular route for thinking this through is the 'Five Domains' model:

> *"The five domains of the model are nutrition, environment, health, behaviour and mental state. Factors considered in the first four domains give rise to affects which are assessed in the fifth (mental) domain. The model is not a definition of animal welfare, nor is it a representation of body structure and function. Rather its primary purpose is to facilitate systematic, thorough and coherent animal welfare assessments, with a focus on both welfare compromise and enhancement." – Mellor and Burns (2020; 151)*

Such an approach attempts to avoid the potential for confusion between understanding animal welfare as simply animal health (or even, not dying). As Duncan (1993) states, "neither health nor lack of stress nor fitness is necessary and/or sufficient to conclude that an animal has good welfare. Welfare is dependent on what animals feel.".

Whilst often conflated with the phrase 'animal rights', animal welfare is a separate approach, which does not necessarily advocate the abolition of the use of animals that an animal rights perspective would bring. Importantly, different groups have different interpretations of what animal welfare is. Publics tend to understand welfare as meaning that animals can experience positive emotions, while scientists interpret animal welfare as primarily the absence of suffering (Miele et al. 2011). Importantly though, the issue of public perception should not be confused with welfare (Barnett et al. 2001). Whilst many stakeholders will not know what 'good welfare' entails specifically for horseshoe crabs, they will expect a humane level of care to be employed.

## WHY IS ANIMAL WELFARE VALUABLE FOR THINKING ABOUT HORSESHOE CRABS?

Horseshoe crab use within contemporary biomedicine is rarely connected to an animal welfare framework. However, animal welfare science, and many of the concepts associated with it, can offer a valuable approach for approaching – and importantly, communicating – the biomedical use of horseshoe crabs.

> "As we are going to continue to bleed animals … we should do it as humanely as possible."
> – Interviewee, conservation sector.

The use of animals for biomedical purposes continues to be an area of public and scientific debate. What is socially acceptable in terms of the biomedical utilisation of animals has changed over time in response to changes within science and across society (Davies et al., 2016). It is also shaped by regional cultures, values, and politics – as can be seen by the different regulatory status given to alternatives to crab-blood derived testing between Europe and the US. Public support in utilising animals is conditional, based on the extent to which there are alternatives, the processes through which harms to animals are minimized and the benefits to human (or animal) health maximised (Davies et al., 2016).

> "It is imperative that we strive for the highest standards of animal welfare in the research environment, not only because of our ethical responsibility to the animals we use, but also because of our responsibility to the community in which we work. We are accountable to that community and we must be seen by that community to behave in a caring and disciplined manner. If we are to continue in our researches, we need to retain its respect." – Mellor and Reid (1994: 12)

There is a growing interest amongst animal welfare and protection groups and broader publics in campaigning for the humane treatment of crabs, lobsters and other decapod crustaceans (Fiorito et al., 2014). Though technically horseshoe crabs are not 'true crabs' (they are instead classed as belonging to the subphylum Xiphosura, an order of arthropods related to arachnids), these campaigns highlight how ideas – and public expectations – around invertebrates are changing. To draw on Davies (2019) again, people's ways of thinking about the ethics of animal care differ dependent on spatial contexts. That is, what publics expect, and think of as acceptable practices, varies according to the imagined spaces in which we place animals. Thus, whilst horseshoe crabs may be sourced via a fishery, their later movement into a laboratory space – for the purposes of producing human health – means that they are associated with an expectation of care and welfare in line with what is extended to other laboratory animals.

A focus on welfare is not about the abolition of collecting blood from horseshoe crabs. Whilst media articles may often structure people concerned about animal welfare as diametrically opposed to LAL (Reuters, 2020), this is an over simplification and shallow understanding. As the quote introducing this section highlights, a focus on welfare is about ensuring that the entire experience from the crab's point of view – from initial capture, transport, cleaning, restraint, bleeding, transport, release, recovery – are done with care, to a humane standard. This whole-process approach is important, as discussions of animal welfare often overly focus on the one technical act when imagining laboratory animals' lives.

> "I want to be very clear that the people who are doing the harvesting also care for these crabs, they want to see them alive, they don't want them to be dying in their hands or anything, nobody wants that."
> – Interviewee, communications sector.

The LAL industry may have a negligible impact on the population status of horseshoe crabs, however, this does not negate the need for a focus on the welfare experiences of the horseshoe crabs that are bled, ensuring that a high level of care is practiced and implemented throughout the process.

> "On welfare, I've seen research about the behaviour changes post bleeding and things like that, so that's where you can be kind of callous about it and say there's no impact, but as a good scientist, you can't say, being really objective, you can't say that there's not a probability that there's not an impact on welfare and behaviour"
> – Interviewee, pharmaceutical sector.

Of course, it is easy to place all of the burden of welfare on the manufactures of LAL who bleed the crabs, however, everyone within the pharmaceutical supply chain who uses LAL is implicated in minimising the impact on the crabs and recognising and addressing ethical issues. This may involve recognising that LAL is derived from a living animal, taking steps to ensure efficient and non-wasteful use, exploring opportunities to replace, reduce, or refine use, and questioning and holding suppliers to account about how welfare considerations are implemented in their manufacture of LAL.

*Image credit: Eric A. Lazo-Wasem, CCo 1.0*

## 6. MOVING FORWARD WITH THE 3RS

The 3Rs – the ambition to where possible, **replace**, **reduce**, and **refine** the use of animals – are established and accepted worldwide as the best framework for governing animal-dependent science, playing an integral role in ensuring high standards of ethical consideration whilst also maximizing the potential for high-quality science (Kirk, 2017). The 3Rs, first formulated by Russell and Burch (1959) in their book 'The Principles of Humane Experimental Technique' have become central to how the use of animals in scientific procedures is socially understood, politically imagined, and (inter)nationally regulated (Davies et al., 2018). They exist as a means of weaving together 'good science, good care, and socially acceptable practices' (Davies et al., 2018), providing a pathway to aligning both 'moral and scientific values within a pragmatic ethical framework' (Kirk, 2017).

Despite the global reliance on this immunologically unique and ancient species and its centrality and indispensability amongst the supply chains of modern health and medicine, the pharmaceutical utilisation of horseshoe crabs to produce the LAL test is rarely viewed through a 3Rs framework. However, there is growing interest amongst stakeholders in utilising a 3Rs framework as a route to engage in animal welfare.

> "Any responsible organization will go through a process of reducing, refining and replacing as part of its operational and strategic growth plans. The LAL industry is no different and in fact is expected to be constantly getting better at doing this, hence waste is reduced, consumption is reduced and we can offer alternatives to customers."
> – Interviewee, biotechnology sector.

This is an important point. Much of the oppositional discourse that has built up around the biomedical use of horseshoe crabs acts to remove the agency of manufacturers as being responsible for, and indeed leading on, cultivating change. As an interviewee from the biotechnology sector explained, alongside debates and questions initiated by conservationists and animal protection groups, '*an increased philosophical approach to corporate sustainability has also contributed to more recent questions around the use of horseshoe crabs.*'.

Different stakeholders see different value and possibilities in each of the individual 'Rs', to the point of substantial friction between those who advocate focus on 'replacement' above 'reduction'. Ideas about replacement have tended to dominate discussions, a focus that has detracted from equally important efforts towards developing alternative approaches involving reduction and refinement.

> "The focus of the pharmaceutical industry is typically a well-balanced approach to sustainability efforts spanning all 3Rs. When it comes to the LAL industry, which is one of the pipelines feeding into pharma, R for replacement became the sole focus over the past decade."
> – Interviewee, biotechnology sector.

Moving forward, as the quote below describes, applications of the 3Rs in the context of horseshoe crabs should be viewed as the 3Rs in concert.

> "I like your 3Rs because I'm involved in all of them. They're all important and the thing is that everybody has to recognise that all of them are important and they all interact."
> – Interviewee, biotechnology sector.

## PERSPECTIVES ON OPPORTUNITIES FOR REPLACEMENT

Replacement refers to the replacing of animals with non-animal methods wherever feasible. Bolden and Smith (2017: 405), note that 'replacing the animal-derived LAL with rFC for endotoxin testing is consistent with the 3 R principles (replacement, reduction, refinement) for more ethical and sustainable use of animals for testing'. They go on to explicitly link their interest in using recombinant factor C to replace LAL for endotoxin testing to their company's 'commitment to animal welfare and conservation'. However, as discussed earlier, the topic of replacements has generated much discussion and speculation, and the replaceability of horseshoe crabs is highly contested, with views ranging from a belief that:

> "Conversion to rFC would result in a 90% reduction in the demand for LAL, which means that mortality resulting from bleeding would decrease by an estimated 100,000 horseshoe crabs annually."
> – Interviewee, conservation sector.

To others who were adamant that:

> "Recombinant factor C is not going to "save" the horseshoe crab."
> – Interviewee, biotechnology sector.

A particular challenge here, as the final quote astutely identifies, is that the shape of the conversation about replacement is focussed on the idea of 'saving' the horseshoe crab:

> "Much of the discussion regarding replacement of the LAL test is occupied by a narrative that suggests: a) the population is in peril , b) replacing the LAL test with something else will save the horseshoe crab."
> – Interviewee, biotechnology sector.

As such, discussions are mired in an unconstructive rhetoric that leads to defensive comparisons with other aspects affecting crab population vulnerability. Whilst these are valid concerns, and the horseshoe crab is indeed threatened by multiple compounding factors, this obscures arguments for replacement that are about reducing suffering to individual animals. Discussions about replacements for horseshoe crab blood would be better to frame themselves in terms of how they are restructuring the harm-benefit equations involved in the biomedical use of these animals.

However, despite the existence of a replacement many stakeholders felt that opportunities were highly constrained by regulation, and that '*unless it was included in a pharmacopeial method'* (interviewee, contract research sector), they were unlikely to consider replacing their LAL use. Presently, with the alternative being considered as an 'alternative test' (in the US at least), it was seen as an unattractive option. The additional validation involves a considerable amount of additional time and expense, and as one interviewee from the contract research sector explained, '*the likelihood of any company doing a validated alternative is not great because of the amount of validation that is required'*. Others argued that whilst yes, the validation was something of a hurdle, the extent of this had been greatly inflated and could be negated over time through experience and, ultimately, through a corporate commitment to animal welfare, above what was easy.

Despite the approval of rFC by European regulators (and the potential acceptance by US regulators in the future) there remains little actual incentive to switch to the replacement. Conventional LAL testing is still allowed, and is very much embedded in the licenses of existing products, the skillsets of technicians, and the cultures and practices of endotoxin testing. Thus, whilst the European Pharmacopoeia decision has been much celebrated, the regulatory approval is only the first step. What needs to follow is large scale cultural change within the sector that foregrounds the ethical benefit of adopting animal-free alternatives.

Some stakeholders question whether this turn to welfare and the 3Rs is an attempt to capture market-share through a cynical branding move. Indeed, some questioned whether efforts at 'replacement' and the invocation of synthetic biology may be better targeted at other sectors of horseshoe crab use:

> "There's so much money being put on the publicity of rFC, if they put half of that onto a legitimate effort for bait replacement, it would be a good day."
> – Interviewee, biotechnology sector.

This, in particular, was seen as a potential way of alleviating pressure on horseshoe crabs (and the associated mortality of using them as bait) whilst mitigating the impact on local economies and communities that relied on fishing horseshoe crabs.

Concerns over patient safety were for many the bottom line. In a conservative, risk-averse sector, whilst many were encouraged by the promise of replacement, there was a desire for more data to emerge before people would feel confident to make this transition.

> "I can't emphasise enough how much that patient centric approach is personally for me, it's critical, I don't want to have to question myself that I got it wrong. I'm sure it's fine! But I'd like to see more data on it and I think there will be some more data coming out."
> – Interviewee, pharmaceutical sector.

Questions remain as to what level of evidence is required to achieve this confidence, and how to achieve industry acceptance. Some argued that much of the desired evidence is already available, and thus, the focus may need to be on education and improving access to the existing evidence.

> "That's where the effort needs to go and we think there's an overwhelming amount of data that supports it, it's just overcoming some of the political realities I think now, and just get in there [...] There's actually a good deal of data out there and so we're just trying to hope to direct people to that body of work, to show them there has been a lot of data out there and published."
> – Interviewee, pharmaceutical sector.

With many amongst life sciences organisations increasingly embracing sustainability and corporate social responsibility, moving towards a level of replacement was viewed as something that was only going to grow in priority and significance in the future.

> "Given growing global sustainability efforts by many life science based companies, recombinant reagents may have a fairly significant role to play in the future given that the reagents are non-animal based."
> – Interviewee, biotechnology sector.

Ultimately, replacing LAL will take time, confidence, and data, and will involve a plethora of approaches, rather than being 'one-size fits all'. Total replacement may never be achieved. For that reason, there are huge opportunities for reduction and refinement within the process, as one interviewee from the conservation sector concluded, '*as we are going to continue to bleed animals, as we make this migration from LAL to rFC, we should do it as humanely as possible'*.

## PERSPECTIVES ON OPPORTUNITIES FOR REDUCTION

In biomedical research, reduction usually refers to ensuring that the minimum number of animals is used to answer the scientific question, using effective experimental design and statistical analysis to optimise numbers and avoid wasting animals. In the context of this use of the horseshoe crab, reduction involves minimising the number of animals used in a given method or process.

Reduction is increasingly framed as a process in contrast to replacement, as Krisfalusi-Gannon et al. (2018: 9) argue, 'revising the current system to improve efficiencies in horseshoe crab use may be more viable in the near term'. Technological fixes are regularly viewed as the way forward in terms of reduction. For example, one manufacturer has developed new technologies that allow the use of less raw material than traditional endotoxin testing methods. Charles River Laboratories argue that *'if all tests were performed using cartridge technology, today's entire worldwide LAL demand could be met with less blood than from Charles River's current annual quota'* (Charles River Laboratories, 2020a).

> "If you can replace 95% of your tests with a method that uses 99% less LAL, your impact is – I won't go into the maths – but it felt that it wasn't unreasonable that a significant reduction could lead to a massive impact for the better."
> – Interviewee, pharmaceutical sector.

Interviewees felt that engaging with the specific numbers of crabs used biomedically offered a route to creating a localised culture of care and interest in the 3Rs within endotoxin testing:

> "When you translate it back to crabs – very approximately because there's so much variability – but when you can convert number of test vials and lysate for the crab, people are keen to hear that [...] we had challenges, and I said "just remember the drivers for change here, this is your forecasted burden reduction on the crabs and I understand this is difficult but if we can do this quicker, the impact is there" and that worked."
> – Interviewee, pharmaceutical sector.

Creating this engagement is important as the number of crabs bled is ultimately linked to demand and use, and thus the largest opportunities for reduction occur further down the supply-chain.

There is huge scope – though presently, little awareness – for end-users in laboratories around the globe to effect reduction and significantly decrease the amount of crab blood used. AstraZeneca's 2018 Sustainability report describes how as part of their 'Culture of Care' and focus on high standards of animal welfare they were keen to reduce the use of horseshoe crabs in testing, and have invested in new technology 'which will see a reduction in annual lysate (crab blood reagent) consumption from approximately 7.5 litres to just a few hundred millilitres' (AstraZeneca, 2018: 48).

Additionally, Guest (2019) advocates for the automation of endotoxin testing, suggesting it would result in a significant reduction in waste and in invalid tests that need repeating, along with the streamlining of testing plans to increase the number of tests per run, thus reducing total lysate used. Marketing for automation argues that *'the most expensive LAL test is the one that must be repeated because of invalidity'* (Charles River Laboratories, 2020a) – and this is also true for the burden placed on crabs by testing errors.

However, another interviewee from the pharmaceutical sector also reported that ultimately *"some people don't believe that reduction's enough, they want replacement, the ethical quandary of fishing these creatures doesn't sit well with some people"*.

## PERSPECTIVES ON OPPORTUNITIES FOR REFINEMENT

Refinement involves reducing suffering and improving welfare throughout animals' lives, including during and after procedures, and within transport, housing, handling, husbandry and care. Because of the multiple stages involved in procuring horseshoe crab blood – collection, transportation, storage, bleeding, re-release – there is considerable scope for refinement of processes. Avoiding direct sunlight, extreme temperatures and excessive time out of the water are extremely important for the survival of horseshoe crabs (ASMFC, 2019).

Much of the work refining processes of the biomedical use of horseshoe crabs has gone quietly unnoticed, thanks to the industry's tendency towards secrecy. However, the ASMFC's 'best management practices' introduced in 2011 represent a significant step-change in how the welfare of individual crabs was considered at each stage within the collection, bleeding, and release of crabs collected for biomedical purposes.

> "The BMP's take into consideration the different harvest methods, geography, and state regulations form areas where biomedical collection takes place. Because there are variabilities in methods and regulation from state to state, not all the BMPs apply for all collectors. For instance the recommendation for collecting at night is practiced in the Delaware Bay region. In contrast, fishing at night with a dragger is prohibited in Massachusetts. Each manufacturer works with their suppliers to maintain the best possible conditions for the crabs capture, and release which is typically within 24 hours. It is well known that keeping the animals cool and wet with a swift return to the natural environment benefits the crab. The manufacturers have been practicing this for decades before there were any regulations."
> – Interviewee, biotechnology sector.

Whilst some were reassured by the stated efforts towards refinement, and the acts of care they involved, this was not shared by everyone. For example, one interviewee from the conservation sector described how *'you can find the guidelines and stuff, the recommendations, the best practices but that's not to say that they're actually following those'*. Several manufacturers commented that they are routinely audited and inspected by regulators, with strict mandates and conditions of operation imposed at State levels. However, at a broader (public) level, opportunities for witnessing refinement are limited, and with little openness in the sector, much has to be taken on good faith that moves towards more refined, less harmful, methods are taking place. Some manufacturers reported that they were increasingly inviting customers to view or 'audit' their practices too – again creating mechanisms for pharmaceutical end-users of LAL to play a role in driving change here.

Krisfalusi-Gannon et al. (2018: 4) suggest that 'improved harvest practices have the potential to reduce mortality rates during biomedical harvest by more than half'. They question a range of possibilities from removing a smaller volume of blood per drawing, to the scope of using indwelling catheters, and even the potential to develop processes of plasmapheresis and reinfusing crabs. However, it is still early days for refinement in this area. Krisfalusi-Gannon et al. go on to argue that 'to achieve the lowest horseshoe crab mortality and highest blood quality during biomedical bleeding, a more systematic understanding of the nuances of the optimal horseshoe crab environment, feeding and care would be required' (2018: 9). However, there is a hesitancy to explore refinement in case this implied an admittance or acceptance that current standards and practices were not adequate at safeguarding animal welfare.

> "That's a hard thing to get them to swallow, to change their operational position and that would further have to make them kind of suggest that

> their processes, to some extent, are deleterious to the species. And can they say that?"
> – Interviewee, biotechnology sector.

There are relatively few published studies that discuss husbandry conditions for horseshoe crabs, and many researchers consider 'captive rearing to be difficult, time consuming and impractical' (Carmichael and Brush, 2012: 39). Despite these challenges, there are aspirations that creating what might be understood as a laboratory 'strain' of horseshoe crab, as opposed to the current wild usage, might offer opportunities for greater care and welfare.

> "We started to aquaculture horseshoe crabs, provide them an optimised management and optimised feed, with the hopes of going a low impact resource harvesting and we have some interesting ideas, that fall in probably the first or second R, it's not necessarily in that Replacement R.[…] That's the luxury we have here because after [bleeding], we then have the opportunity to be three steps from them and care for them and then feed them, whereas opposed to just saying 'you're on your own now'."
> – Interviewee, biotechnology sector.

Aquaculturing might offer opportunities to remove the stresses induced by capture and transport of wild stock. Similarly, Tinker-Kullberg et al. (2020) suggest that aquaculture would allow for taking smaller amounts of blood, more frequently, rather than the current system that can involve collecting 'between 25% and 40% of a crab's blood (Moore, 2017: 112). As discussed earlier, the effect that the amount of blood extracted has on mortality rates, and what the upper limits of this should be for 'best practice', remains a contested area (Atlantic States Marine Fisheries Commission, 2019). Whilst taking less more frequently, may be appealing at first, there are also ethical concerns about the cumulative effect on potential animal suffering, something which must be considered in any harm-benefit analysis of 'refinements' (Davies, 2018). Despite this, Tinker-Kullberg et al.'s (2020: 9) experiments found that aquaculture approaches seemed to indicate 'healthier and less stressed animals', resulting from an optimized environment. Rather than a brief and extractive relationship, aquaculturing crabs might allow for more caring relationships with crabs. Of course, other ethical arguments might question what freedoms are being lost through an enforced captivity. Some expressed a concern too that large-scale efforts at refinement, like aquaculture, detracted from smaller, quieter, efforts that might improve horseshoe crab welfare, efforts such as training or auditing, that might contribute more to the establishment of a culture of care for crabs.

*Image credit: A still from the PBS Nature documentary Crash*

## 7. CAN WE BE MORE OPEN ABOUT CRABS?

Conversations around the biomedical use of animals are starting to change, becoming more common, rather than shrouded in secrecy. Openness and transparency about the use of animals within the biomedical industry is increasingly a staple of best practice, however this has not permeated into discussions about horseshoe crab use.

> "Perhaps the improvements or the changes haven't been well publicised, they're perhaps their own worst enemy, I don't know."
> – Interviewee, contract research sector.

The lack of transparency here exacerbates efforts to cultivate crab welfare, with few opportunities to tell the stories about care that are crucial to maintaining the social contracts that underpin the biomedical use of animals.

> "We don't talk to the press and it's like…'No, that's backwards because if you do that, you are allowing your detractors to make … they're controlling the conversation and if you don't stand your line and keep backing up, your detractors aren't just going to stay there, they're going to keep following you, you need to stand up and say, this is what we do, this is why we do it and we are very proud of what we do because here is the benefit to you', and just lay it out, say 'here's the mortality associated, there is some, we feel bad about it but here is the benefit to the human population'."
> – Interviewee, biotechnology sector.

The reticence of manufacturers to speak to the media only serves to further entrench established positions and heighten controversy here. Some efforts have been made to speak out about the conservation work that is facilitated by the biomedical usage of horseshoe crabs, however, as discussed earlier, people's ways of thinking about the ethics of animal care differs dependent on spatial contexts (Davies, 2019). The assurances being sought here are around how horseshoe crabs are treated within the laboratory specifically, not the wider species context and

### TO WHAT EXTENT IS THIS A 'PUBLIC' ISSUE

***"If you've received a vaccine, used insulin injections, or had an IV in the hospital, you are a consumer of horseshoe crab blood. If you've ever vaccinated a pet, or had a pace maker, stent, or joint replacement implanted, you were also dependent on horseshoe crab blood. In one way or another, we are all consumers of horseshoe crab blood and all of us can play a role in conservation."*** – ERDG, 2013

The quote above from the ERDG (a non-profit conservation organization focussed on horseshoe crabs) demonstrates how the biomedical use of horseshoe crabs benefits huge swathes of the public everyday. Moore (2017: 104) suggests that 'probably every human since the 1970s has directly or indirectly benefited from horseshoe crab blood'. This might be the case, but to what level are people aware, and to what extent do they care?

There is little research about public attitudes to horseshoe crabs and their biomedical use, specifically. However, a number of articles recognise that there has been a growing public awareness and interest in these animals (Eagle, 2002; Shuster et al., 2003). Though wild animals, their movement into a laboratory space places them firmly within public imaginaries as 'laboratory animals' and thus attached to the concerns, expectations, and trusts that publics have about laboratory animals more broadly.

Stories about horseshoe crabs in the press and media generate huge interest, attention, and discussion. The topic is certainly of public interest, and growing.

conservation work that is enabled by the animals use – but exactly how welfare is ensured within their use itself.

Without talking to publics about reasons for bleeding crabs, and the steps taken to improve the process and ensure welfare, it is hard to build public assurance and trust. Given histories of animal rights activism, it is easy to understand nervousness and apprehension about opening up about the utilisation of horseshoe crabs. However, greater openness would go a considerable way to reducing the polarisation over the use of crabs that is occurring, allowing room for more nuance, understanding, and comfortable conversations.

For example, one social media response to a recent Reuters article about the US Pharmacopeia's decision to not yet remove the validation requirements for rFC, posed the simple, but eloquent question: "How about better quality care then?". It highlights again how whilst there may be a broad social acceptability for the laboratory usage of animals, this is conditional that steps are taken to minimize any harms to animals (Davies et al., 2016). Without these assurances, and openness, ideas about socially acceptable scientific practices involving animals are liable to change, and it may be that public opinion shifts from an understanding of the (currently perceived) need to utilise horseshoe crabs through caring methods, to one which rejects this as a tolerable practice.


## WHAT CAN PUBLICS DO?

Despite the widespread public health benefits that are produced through utilising the blood of horseshoe crabs, and the growing public interest in how the animals are used, there is presently little opportunity for publics to shape debates.

***"People who are following a vegan lifestyle, they're obviously doing all they can from a personal level to cut out animal products, but then this is an area that you just can't cut out."***
***– Interviewee, communications sector.***

It is impossible to 'opt out' of being a consumer of horseshoe crab blood. It's perhaps this impotence that fuels the often outraged comments increasingly appearing on social media or in response to news stories about horseshoe crab use.

Processes that aim to create space for public voices in other aspects of the biomedical use of animals – such as the lay (or community) members on Animal Welfare and Ethical Review Bodies in the UK, or Institutional Animal Care and Use Committees in the US – generally do not cover horseshoe crab or LAL use.

Whilst coastal communities in the eastern US can get involved in local conservation efforts as a way to express relations of care, this option is not available to all those who benefit from LAL.

As such, public action around biomedical use of horseshoe crabs is limited, often to sharing the story of crabs use – hence why greater openness here could improve the quality of debates.

A particular challenge here is also disentangling concern for horseshoe crabs from perceptions that are shaped by wider discourses and sentiments – about the scientific use of animals and perceptions of 'big pharma' more broadly. As one interviewee from the biotechnology theorised, *'the vastly uneven attention suggests that the biomedical industry is an easy target as part of the pharma supply chain and false statements on lysate value and mortality inflate the perception of corporate greed.".* Casting manufacturers of LAL as 'the bad guys' as one interviewee put it, is unhelpful. Though equally, framing concern about horseshoe crabs as deriving from anti-corporate narratives also limits opportunities for valuable and valid discussions about the care involved in collecting horseshoe crab blood, and the efforts that are being made to replace, reduce, and refine usage. As another interviewee from the biotechnology sector eloquently summarised: *'Good lord, tone down the rhetoric. The elevated rhetoric does not help anybody [...] Our detractors aren't in their hearts, bad people, they believe what they believe and they are doing their best to make sure that what they believe is carried out, just like our fishermen aren't bad people.'*.

As scholars working on public engagement with science (or, public understanding of science) have noted, it is unhelpful to assume that people need more information to understand science – what has been termed the 'deficit model'. The deficit model assumes that there is a knowledge 'deficit' that can be 'fixed' by giving the public more information (Brown, 2009; Kearnes et al., 2006). However, simply giving more information to people does not necessarily change their views. Instead, the focus should be on better communication, public engagement, and genuine dialogue with publics (Stilgoe et al., 2014).

Dawson and Hoffmeister (2019) lament that 'a number of recently published articles and reports contained information that is inaccurate regarding the horseshoe crab population and its use in the biomedical industry'. However, when information is limited, and possibilities for openness and dialogue curtailed, it is inevitable that this type of discourse develops. There is much concern that some of the studies around mortality rates and sub-lethal effects caused by bleeding do not reflect the actual practices utilised by industry. However, again, with little openness about actual practices, and a culture that encourages an adversarial and oppositional mistrust amongst different stakeholders, this is not productive.

*Image credit: "Horseshoe Crabs" by U. S. Fish and Wildlife Service - Northeast Region – licensed under CC PDM 1.0*

In the UK, the Concordat on Openness on Animal Research launched in 2014, as a set of commitments for UK based life science organisations to enhance their communications about the scientific use of animals. Introducing a similar set of commitments around the biomedical usage of horseshoe crabs could be a highly impactful move for a sector that is still steeped in secrecy and a hesitancy to talk.

**Commitment 1:** We will be clear about when, how and why we use animals in research

**Commitment 2:** We will enhance our communications with the media and the public about our research using animals

**Commitment 3:** We will be proactive in providing opportunities for the public to find out about research using animals

**Commitment 4:** We will report on progress annually and share our experiences

*The Concordat on Openness – Understanding Animal Research (2017)*

All of the manufacturers of LAL make claims around having high quality care and a strong set of best management practices that shape their use of crabs – from collection, transport, bleeding, through to re-release – being open, transparent, and showcasing these instances of care would go a long way to creating a space for better conversations about the biomedical use of horseshoe crabs.

> "If they came together and said, "This is what we do to look after the crabs", that could be quite powerful."
> – Interviewee, pharmaceutical sector.

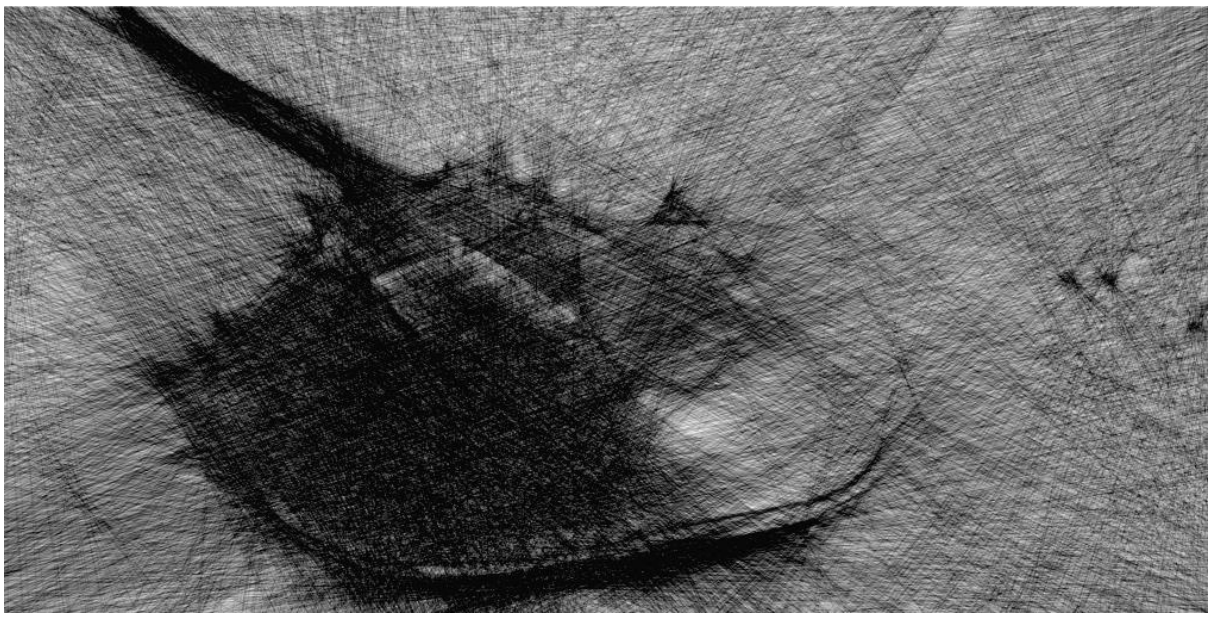


*Image credit: The National Wildlife Federation*

## 8. HOW CAN ANIMAL WELFARE ORGANISATIONS MAKE A DIFFERENCE HERE?

With welfare potentially rising on the agenda, there are opportunities – and demands – for animal welfare organisations to become involved in, and help shape, future discussions about horseshoe crabs. The RSPCA (Royal Society for the Prevention of Cruelty to Animals) is a constructive, science-led animal welfare organisation, committed to promoting the replacement of animals in research and testing wherever possible as a primary goal. The RSPCA has an interest in understanding the processes by which humane alternatives are developed, validated and accepted, so that they can be aware of, and work to resolve, obstacles to implementation that may be due to factors such as perceptions of risk and resistance to change.

Initially, it might seem strange that a UK based organisation would be interested in a species that is found exclusively on the shores of the north-eastern USA. However, the products made from the blood of horseshoe crabs are utilised around the world, particularly in Europe, and the UK. Part of moving the debate about horseshoe crabs forward involves not placing the sole responsibility for the animal welfare impacts on the US-based manufactures, but recognising that all of those involved in the supply chains of endotoxin testing have a role and accountability regarding welfare.

Stakeholders were asked to reflect on what role a constructive, science-led animal welfare organisation, like the RSPCA, could play here. Interviewees saw value in a respected organisation like the RSPCA becoming involved in the conversation, and saw opportunities for the RSPCA to steer discussions in ways that embraced complexity. Four specific themes were identified where it was felt the RSPCA could add value in conversations about horseshoe crabs.

Firstly, that the RSPCA, with its expertise in the regulation, care and use of laboratory animals could advance efforts to integrate a 3Rs focus into the biomedical use of horseshoe crabs:

> "And with the 3Rs … that's something that the RSPCA could be looking at is trying to advance that conversation."
> – Interviewee, conservation sector.

Secondly, the RSPCA was viewed as an actor that could work across perspectives, rather than advocating totalising positions that disenfranchised other stakeholders. Through existing networks and relationships with industry partners, it was felt that the RSPCA could play an important role in educating and encouraging the pharmaceutical sector to more actively think about their use of LAL. Again, this isn't about simply advocating a wholehearted replacement, but working with key decision makers to think about what opportunities they have to replace, reduce, or refine use, whilst questioning and holding their suppliers to account about how welfare considerations are implemented in the manufacture of LAL.

> "The whole moral and ethical obligation or responsibility that a company, the role that a company could play if they chose to play, mainly through educating their supply chain and implementing supply chain changes, an organisation like the RSPCA could make a big difference, particularly in Europe."
> – Interviewee, conservation sector.

Thirdly, given that existing messaging around alternatives is polarised, conflicted, and hard-to-trust (Guest, 2019), the RSPCA was seen as a respected organisation that scientists (in the UK) might approach for more information on alternatives to horseshoe crab derived reagents.

"From the RSPCA's point of view, there are alternatives coming and it's perhaps more of a, 'We need to make sure that there's plenty of alternatives and identify what the pros and cons of each of them are'. Until somebody takes that step and produces some evidence, then people aren't really going to move."
– Interviewee, contract research sector.

Finally, it was felt that the RSPCA's platform offered opportunities to raise the profile of horseshoe crabs, illustrating to different lay-stakeholders why and how horseshoe crabs are used within contemporary pharmaceutical systems. Importantly, there was often a sense here that the RSPCA's voice here would produce a more nuanced explanation, rather than reifying the more 'excitable' approaches regularly found in media.

"I think just communication, [the] RSPCA could illustrate the issue for the general public, try and get people fired up about the issue."
– Interviewee, pharmaceutical sector.

Raising awareness of the use of horseshoe crabs is clearly an important task. However, this needs to be done with nuance, particularly because, as mentioned earlier, the opportunity for different publics to take action is limited h

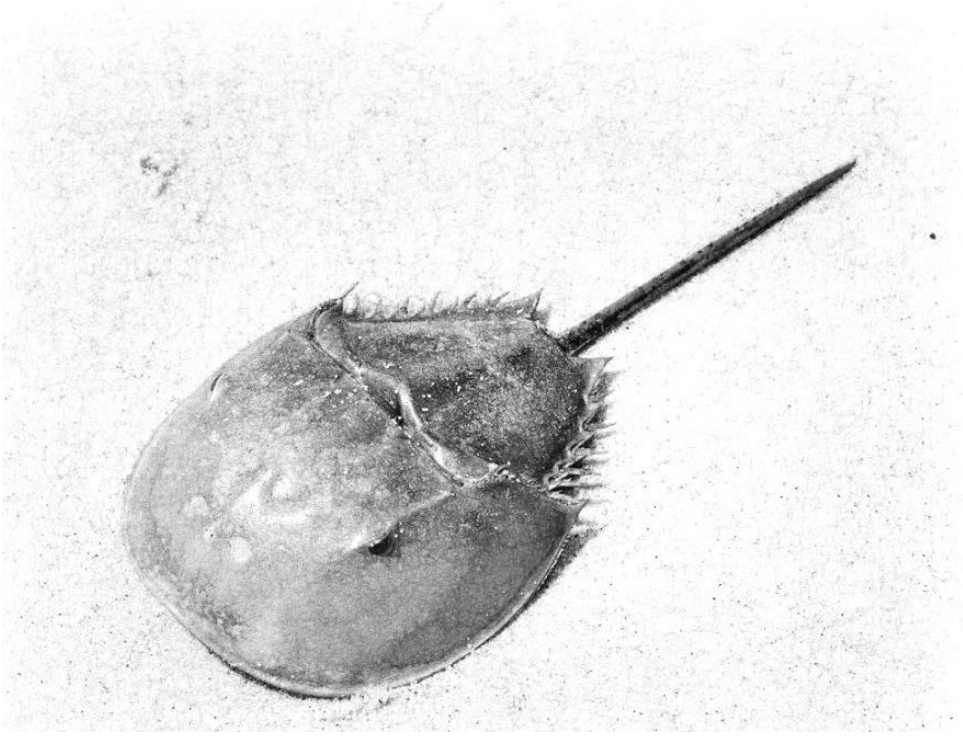


*Image credit: Plant Image Library/C.C. 2.0*

## 9. CONCLUSION

Creating more ethical and sustainable futures for humans and horseshoe crabs alike will require changing the shape of existing conversations about horseshoe crabs. The use of existing ethical, regulatory, and conceptual frameworks like the 3Rs offers huge potential to reframe discussions and find ways to talk about the biomedical use of horseshoe crabs that avoid the growing polarisation, whilst introducing a means of extending – and conveying – the welfare considerations that are increasingly expected across science and society.

Similarly greater openness and transparency would afford important opportunities to further reassure publics and other stakeholders about the level of care involved in the contemporary biomedical use of horseshoe crabs. The pharmaceutical reliance on horseshoe crabs is growing as a topic of public interest, without assurances and openness about humane care, it may be that public opinion shifts from an understanding of the currently perceived need to utilise horseshoe crabs (provided this is done with care), to one which rejects this as a tolerable practice.

Thinking about horseshoe crabs as a welfare or 3Rs priority remains challenging, due to their status as a wild invertebrate that is managed as a fishery and bled through a process widely imagined as both non-invasive and a force for conservation, in order to produce a reagent that is positioned as an *in vivo* alternative. Despite these multiple compounding factors, there are exciting efforts emerging to replace, reduce, and refine contemporary biomedical reliance on horseshoe crabs. The extent to which a turn to welfare and the 3Rs exists as an attempt to capture market-share may be debated. However, it remains the case that efforts to innovate here do offer the potential for improving the level of humane care deployed in the biomedical utilisation of horseshoe crabs – something that is increasingly expected by publics. These expectations of 'good' care will remain on the agenda as the knowledge of the use of these ancient animals grows within public understandings of, and engagements with, science.

Of course, transforming the 3Rs from principles into practice however, remains a challenge, particularly in highly regulated areas, like the pharmaceutical industry (Törnqvist et al., 2014). Within the arena of horseshoe crab use to produce LAL, it is also heavily influenced by economic interests, with existing manufacturers keen to innovate technological fixes that move towards some semblance of reduction, but ultimately, maintain the status quo of crab-bleeding, whilst the replacement products serve to create a pathway for new players to enter a market reportedly valued at $462.38 million in 2014 (Moore, 2017: 119). Many stakeholders were downcast about the possibilities of innovations and refinements in care being taken up within industry at large.

Whilst scientific consensus over whether current replacement technologies are fit for purpose is still playing out, as discussed earlier in this report, there is no question that their existence has changed the very shape of discourse around alternatives here:

> "Before you couldn't say that, now you can say it, particularly since there is an alternative, so it's just changed the whole conversation. So now if the alternative is no good, that's a different conversation, let's talk about the efficacy of the alternative, but there is an alternative."
> – Interviewee, conservation sector.

> "I think what's been really good for the industry over the past few years is that the discussion is there on the table, which it wasn't prior to 2016, everyone was just sort of taking it for granted. There was a bit but it was just people went merrily on their way so I think it's good that we've got the discussion on the table."
> – Interviewee, pharmaceutical sector.

It highlights how conversations about care, welfare, and replacing, reducing, and refining the current use of horseshoe crabs are here to stay. Requests for more data about the efficacy of recombinant Factor C, along with a desire to await the development of more complex alternatives that involve recombinant formulations of the other factors involved in the clotting cascade within 'natural' horseshoe crab blood (such as Factor B and pro-clotting enzymes) will no doubt continue to shape discussions. However, this demonstrates the direction that the industry is moving – ultimately, towards replacement. Questions are increasingly less about could, or should, horseshoe crab blood be replaced, but more about when, and what the threshold of confidence, data, and trust, might be to do this. This discursive move is a significant achievement for all of those concerned about the animal welfare and environmental sustainability impacts of current LAL testing.

There is still a long road for alternatives and replacements to gain industry confidence and uptake, but being a 'compendial test' in Europe represents a significant milestone in the use of non-animal methods. The European decision is a positive result for a species afforded little protection or welfare considerations, despite – as social media reactions to articles about horseshoe crab use regularly demonstrate – a public desire to see more care expressed in the biomedical use of animals. Importantly, this social expectation of care is not just for those animals we find deeply familiar or appealing, but also for enigmatic invertebrates like horseshoe crabs. Cephalopod use is now regulated by law, and evidence is mounting for sentience in decapod crustaceans, with pressure to include them in the UK Animal Welfare Act (Drinkwater et al., 2019).

This research has demonstrated the value that a social science perspective can bring to understanding the current and future roles of horseshoe crabs – and alternatives to crab-derived products – within practices of endotoxin testing. This research was funded for 6 months, and as such, there are certainly limitations to what this type of analysis can achieve (particularly as the research was running concurrently during the COVID-19 pandemic!). However, it hopefully sets the stage for further social scientific work.

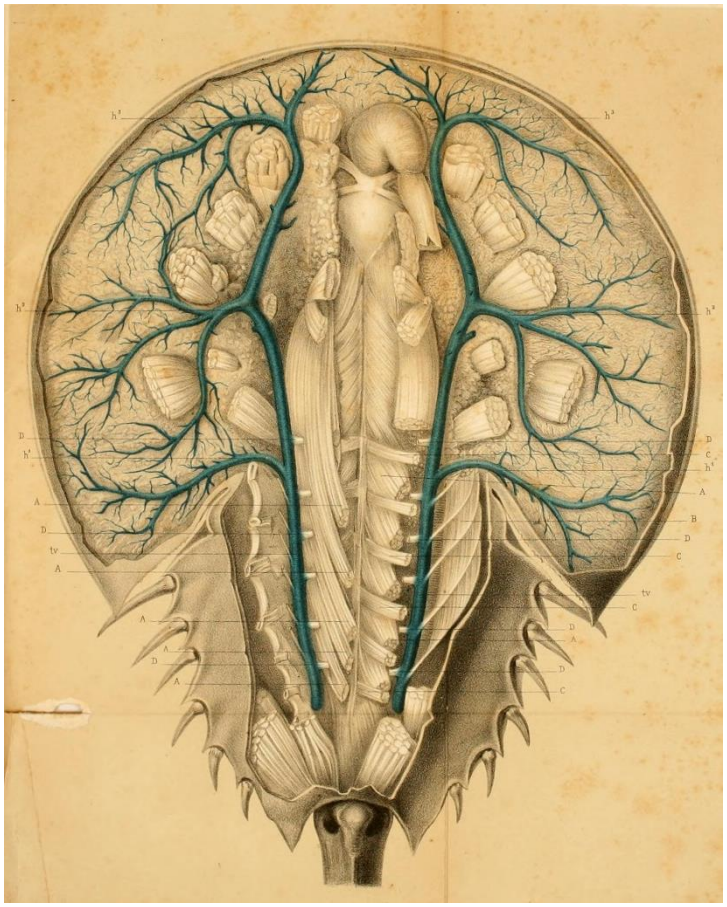


*Image credit: 'Recherches sur l'anatomie des Limules, Paris,1873' – Biodiversity Heritage Library - CC PDM 1.0*

There remains a contested landscape regarding obstacles and opportunities for changing current use of horseshoe crabs within practices and cultures of endotoxin testing. Whilst this research has taken a narrow and deep focused approach, working with key stakeholders, to understand the 'anatomy of arguments' around horseshoe crab use, there is great potential for future social scientific, and wider qualitative, work to map and poll attitudes more widely. Further work to include a focus more inclusive of the TAL sector too would help. Better understanding how different publics perceive, and make value judgements about, horseshoe crabs – as strange, distant, invertebrates – would be helpful. Additionally, with the regulatory changes beginning to emerge within Europe, a significant barrier is removed, and further qualitative research could explore what additional resources or knowledge might be required to change perceptions of risk and resistance.

## FUTURE ROUTES FOR THE BIOMEDICAL USE OF HORSESHOE CRABS

- **Encourage ongoing collection and dissemination of data on recombinant products efficacy, with uptake of these replacements where appropriate.**
- **Encourage open dialogue and discussion about development of alternatives (including current technologies and more complex replacements).**
- **Encourage openness and transparency; find opportunities to showcase practices of care, welfare, and refinements.**
- **Refine animal treatment throughout – from initial capture, transport, cleaning, restraint, bleeding, transport, release, recovery.**
- **Continue to explore potentials of aquaculture for low-impact blood collection.**
- **Reduce and minimise waste through encouragement of automation and cartridge-based technologies.**
- **Recognise a multiplicity of approaches and strategies – there are no 'one size fits all' solutions here. A combination of all 3Rs are needed.**

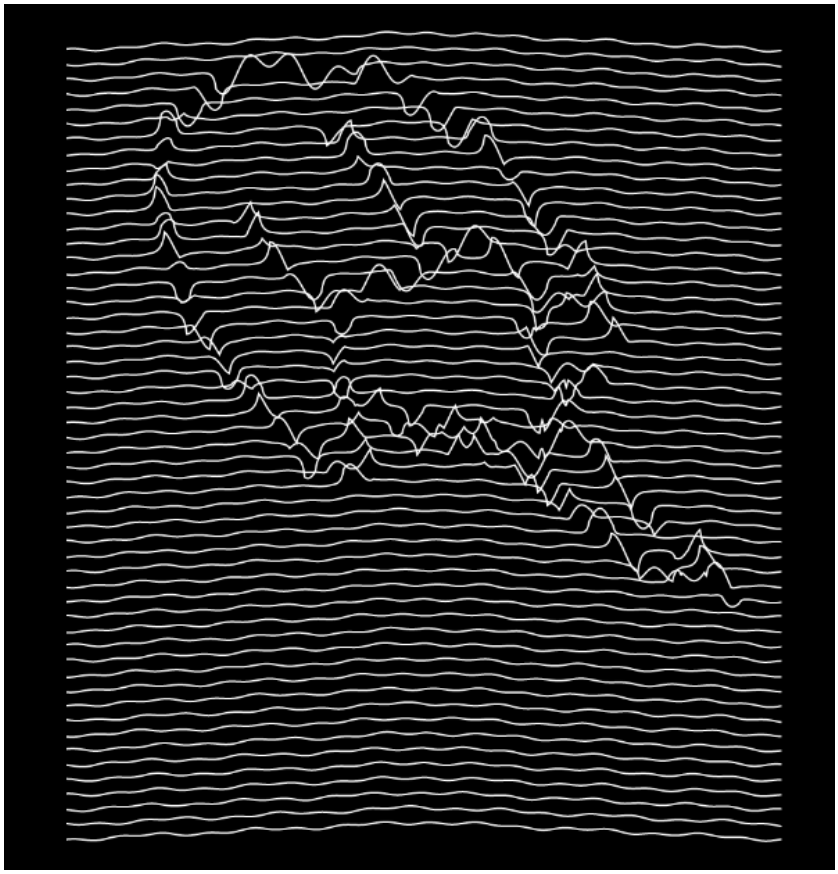


*Image credit: Author, made with* https://garrettdreyfus.github.io/unknownpleasures/

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