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Trends in Biochemical Sciences

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REVIEWS

Review

Friends or Foes? Emerging Impacts of Biological Toxins

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Toxins are substances produced from biological sources (e.g., animal, plants, microorganisms) that have deleterious effects on a living organism. Despite the obvious health concerns of being exposed to toxins, they are having substantial positive impacts in a number of industrial sectors. Several toxin-derived products are approved for clinical, veterinary, or agrochemical uses. This review sets out the case for toxins as 'friends' that are providing the basis of novel medicines, insecticides, and even nucleic acid sequencing technologies. We also discuss emerging toxins ('foes') that are becoming increasingly prevalent in a range of contexts through climate change and the globalisation of food supply chains and that ultimately pose a risk to health.

Highlights

Toxins with nanomolar binding affinities and exquisite target specificities can be powerful tools for fundamental cellular science and medicine. Improved omic techniques can identify toxins from within genomes or venoms, aiding the identification of new naturally occurring molecules, which are more effective drugs/tools in the future.

Drug discovery projects have identified toxins with unique properties and, as exemplified by botulinum toxin and clinical/cosmetic use, can have a billion dollar market potential.

Innovative 'eco-friendly' insecticides derived from venoms are being used in the agricultural sector, exemplifying the diverse potential applications of well-characterised toxins.

Climate change is significantly altering the global distribution of biological toxins, potentially increasing both their discovery and also the likelihood of them affecting humans and animals.

Toxins and Toxinology

In the natural world, toxins are employed for diverse purposes, from the prey-incapacitating molecules found in snake, spider, and cone snail venom, to the defensive compounds harboured by numerous poisonous plant and animal species. While the consequences of human intoxication can be severe (Box 1), the functional diversity of biological toxins has led to their frequent use as experimental tools for studying physiological and pharmacological mechanisms (e.g., synaptic transmission, ion channel subtypes) (Figure 1).

Toxinology involves the identification, characterisation, production, and engineering of biological toxins along with their application or repurposing as research tools and clinical products. As such, toxinology encompasses the study of the evolution, chemistry, biology, and clinical effects of toxins and includes potential biotechnological and/or therapeutic applications.

Experimental applications of toxins as tools for physiologists go back a long time, including Claude Bernard's experiments in the 1800s with curare to demonstrate the existence of chemical signalling between nerves and muscles [2], and Henry Dale's use of muscarine and nicotine to show different subtypes of receptors for acetylcholine [3]. Snake toxins were critical for the first isolation of a receptor for a neurotransmitter [α-bungarotoxin and nicotinic acetylcholine receptors (nAChRs)] [4]. Recently, peptide toxins have been very useful in teasing out the functional importance of different subtypes of ion channels that are critical for neuronal function and cellular signalling [5–7].

An increasing number of toxins are now transitioning from the laboratory to the clinic and the balance of research in this aspect of toxinology is shifting from the classical development of anti-toxins and anti-venoms towards drug discovery [8,9]. The attraction of toxins for drug discovery lies in their often unique selectivity of their biological effects coupled with high potency. Toxic plant alkaloids were the first source of toxin-derived therapeutics, notably

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Box 1. Examples of Venom and Toxin Effects in Humans

Toxins are produced by many types of organisms and upon intoxication can affect cellular functioning in many different ways (Figure 1). When ingested (i.e., for bacterial, fungal, and plant toxins) or injected (i.e., by snakes, spiders, or other animal venoms), toxins can cause serious illness and death. Exposure to toxins of bacterial origin can cause many potentially fatal diseases, including cholera, tetanus, and botulism, and they also contribute to the severe pathologies associated with sepsis and necrotising fasciitis. Tetrodotoxin is generally thought to be produced by bacteria in puffer fish; if ingested, it can block Na^+ channels in nerves and cause muscular paralysis that can be fatal. Fungal toxins include the aflatoxins that can contaminate cereals; they are associated with chronic liver disease and liver cancer. All venoms are diverse mixtures of different toxins, so the effects in humans can be complex and variable [1]. Scorpion venoms contain many toxins that affect ion channels in nerves to cause overstimulation of neuronal signalling that can lead to paralysis. The symptoms of snakebite vary depending on the type of snake. Broadly, elapid snakes (such as cobras and mambas) mainly have neurotoxins that cause neuromuscular paralysis and, potentially, respiratory failure. Viperid snakes (vipers, adders, rattlesnakes) have some components that affect blood clotting and other toxins that cause severe tissue damage. The effects can be fatal or, in the case of local tissue damage, result in life-long disabilities.

digoxin in atrial fibrillation and heart failure, and tubocurarine as a selective muscle relaxant for use as an adjunct to general anaesthetics in major surgery [8]. Snake venoms have also provided leads that have been transformed into successful therapeutics: captopril, the first orally active inhibitor of angiotensin-converting enzyme (ACE), was derived from studies on small peptides known as bradykinin-potentiating peptides, isolated from the venom of the snake *Bothrops jararaca* [10]. Captopril led to major advances in the treatment of patients with high blood pressure and heart failure. Several other venom-derived peptides have led to approved medicines (Table 1), and toxin-related peptides are also being extensively studied for many neurological indications [16]. Perhaps the most surprising toxin that has found medical uses is botulinum toxin, probably the most potent toxin known, but with applications in various movement disorders and migraine, as well as its familiar cosmetic use [17,18] (Box 2).

Collectively, by providing worked examples and case studies, this review article highlights how research in the field of toxinology is now booming and firstly covers the drivers (e.g., technological, climatic) that are aiding and/or stimulating developments in this area. We here demonstrate the considerable impact that toxins are having in a diverse range of sectors (i.e., biosciences, animal/human healthcare, food/water safety) and every year new, emerging toxins are identified or are in development for an increasingly diverse range of applications. This trend will only increase in the future and we have set out a balanced case for why society should now consider toxins as both 'friends and foes'.

Technological Enablers for Toxinology

Technological developments, particularly in the field of 'omics', have greatly facilitated our knowledge of how organisms (e.g., cone snails, snakes, spiders) produce toxins and how these components have evolved [22–24]. **Transcriptomics and proteomics** (see Glossary) have also enabled the sensitive detection and identification of toxins, including those that exhibit unique/specific biological properties. These high-throughput omic approaches have been successfully used as mining tools to identify around 2000 nucleotide and 8000 protein unique sequences from within cone snail venoms alone [24]; the vast majority are yet to be fully characterised for their structural or physiological properties. Current enabling technologies span the molecular, proteomic, chemical, biochemical, and structural fields. For example, toxin identification has been greatly facilitated by high-throughput genomic and transcriptomic sequencing, highly sensitive and specific mass spectrometric chemical detection, and comprehensive proteomic analyses [9,25,26].

One of the enduring challenges that remains for omic approaches is the availability of *in silico* systems that either predict the presence of a toxin with a completely new mechanism of action or can identify the potential for off-target effects that might limit the molecule's usefulness as a

research tool or putative drug therapy. For example, the current limitations of cutting edge tools such as predictive algorithms (e.g., Conosorter or Conoserver) were illustrated when, in an attempt to identify toxins from the venom ducts of cone snails using genetic or transcriptomic data, it was found that that only a proportion of the putative molecules were ultimately found within the proteome [24].

Analytical at-line nanofractionation techniques, which couple high-throughput functional screening with compound identification, have proven to be useful tools for identifying bioactives from complex mixtures [27,28]. Improvements in NMR and ambient mass spectrometry provide the potential for the detection and identification of toxins in foodstuff (see Food and Water Biosafety/Security Sectors). In the field of structural biology, cryo-electron microscopy (cryo-EM) has advanced our understanding of how toxins interact at the molecular level with their targets such as physiological receptors [29,30]. Further, high-speed atomic force spectroscopy (HS-AFM) now allows the interaction of toxins with phospholipids to be studied in real time, providing insights into the functioning and dynamic nature of cell surfaces (see Biosciences Sector).

Perhaps most importantly, many of these approaches have dramatically reduced the amount of starting material required for analysis. Many previously uncharacterised toxin-containing samples (e.g., venoms of small invertebrate species, aquatic toxins produced by marine and freshwater algae or bacteria) are now amenable for compositional and functional characterisation [31–33]. Consequently, several companies now provide libraries of diverse toxins for pharmaceutical screening purposes, and thus toxins remain interesting targets for therapeutic and diagnostic translation [8].

The widespread use of toxins as experimental tools has been further facilitated by developments in synthetic chemistry and **recombinant expression systems** (e.g., bacteria, yeast) making production of research scale quantities of toxins easier. **Solid phase synthesis** of peptide toxins using orthogonal protection strategies now provides a robust and efficient method to produce toxins with the specific disulfide folding networks that are essential for bioactivity [34,35]. Recombinant expression systems have also enabled recent advances, as exemplified by successful production in *Escherichia coli* of complex disulfide bond-rich toxins that require post-translational modification [36]. Whilst the isolation from natural sources or production of synthetic or modified versions of toxins is certainly becoming less challenging for research-scale quantities of material, issues still remain about how to produce the quantities of toxin required for commercialisation of a product.

Toxins as Friends: Advancing Basic Science and Leading to Commercial Developments

Enabling technologies have opened up the potential for identifying, characterising, and exploiting a cornucopia of new and emerging toxins. The following sections highlight the positive impacts these diverse molecules can have in a range of sectors.

Biosciences Sector

Toxins have played an important role in understanding many fundamental physiological processes. In particular, they have helped to elucidate the structure and function of membranes, membrane proteins, and transmembrane signalling.

Ion Channels and the Nervous System

The highly selective binding of peptide toxins to sodium (Na_V), potassium (K_V), or calcium (Ca_V) ion channels has led to their use as exquisitely sensitive tools for studying neural function

Glossary

Antimicrobial peptides (AMPs): are used by multicellular organisms to prevent the development of infection, either directly by disrupting the microbial membranes (e.g., bacteria, virus) or indirectly by stimulating an immune response. They are typically short (<50 amino acids), amphipathic alpha-helical peptides, incorporating positively charged amino acids on one side and hydrophobic residues on the other.

Biosecurity: a field involved in the analysis and management of the risks of the release of plant pests, animal pests and diseases, zoonoses, and genetically modified organisms, and the introduction and management of invasive alien species and genotypes. Adapted from the Food and Agriculture Organization of the United Nations. (http://www.fao.org/biosecurity/).

Biostability and bioavailability: refers to specific characteristics of a drug with regard to what proportion of the original dose reaches the circulatory system (bioavailability) and how long that therapy remains active in the blood (biostability).

Eutrophication: refers to the process by which there is an increase in the nutrient and mineral content of a body of water, triggering the growth of plants and algae.

HAB: harmful algal blooms that predominantly occur due to overgrowth of cyanobacteria, leading to severe oxygen depletion of water and the release of toxins that pose a threat to health of humans and aquatic ecosystems.

Lipid rafts: microdomains within cellular plasma membranes that contain a concentration of protein receptors and/or glycosphingolipids and are thought to be important for key processes (e.g., membrane fluidity, cell signalling, receptor trafficking).

One Health: as defined by the Centres for Disease Control, One Health recognises that the health of people is connected to the health of animals and the environment.

Parenteral: administration of a treatment via a needle.

Peptidomimetic: molecules that mimic the structure and/or function of a peptide. Typically, these are

(Figure 2) [5–7,9,16]. In addition, several reports and filed patents highlighted the potential to exploit the bioactivity of various marine toxins, including saxitoxins, tetrodotoxins (TTX), yessotoxins, palytoxins, brevetoxins, and cyclic imines, for this purpose [37]. In particular, TTX has routine use in the laboratory for the study of Na_V ion channels. The success of these products has led to synthetic toxins being developed as tools for chemical biology that possess higher affinity and specificity than the natural molecule. To aid the identification and development of these novel toxins, high-throughput and/or logical design strategies are used. One such high-throughput method is **phage display**. The production of peptide libraries (in the millions of compounds) with variable sequence and based on known toxin scaffolds is an effective strategy to identify potent and selective ligands. This approach successfully identified a potent and selective toxin probe using a molecular scaffold based on sea anemone toxin ShK specific for the orphan KcsA potassium channel, dysfunctions of which have been linked to autoimmunity [38,39]. A further example is based on a conotoxin from the venom of one of the hundreds of species of marine cone snails; Clark and coworkers designed an α-conotoxin cVc1.1 analogue with >8000-fold selectivity for calcium channels over α9α10 nAChRs. This molecule was used in a mouse model of chronic visceral hypersensitivity to demonstrate that the analgesic effects of the parent toxin were due to a calcium channel block rather than block of acetylcholine receptors [40].

Toxin peptides can be easily modified (e.g., radioactive, fluorescent) to provide analogues to facilitate the characterisation of cell surface receptors and ion channels. Chlorotoxin from the venom of the scorpion *Leiurus quinquestriatus*, has been chemically conjugated to a dye and used as 'tumour paint' because the toxin serendipitously binds selectively to neuronal cancer cells by providing a clear demarcation between cancerous and normal tissue [41]. Peptide toxins can also be structurally modified to provide **peptidomimetics** incorporating functionality (e.g., conformational constraints, non-native amino acids) to overcome some of their physicochemical limitations, including plasma instability and cell permeability, therefore increasing their overall range of applications within the laboratory [42].

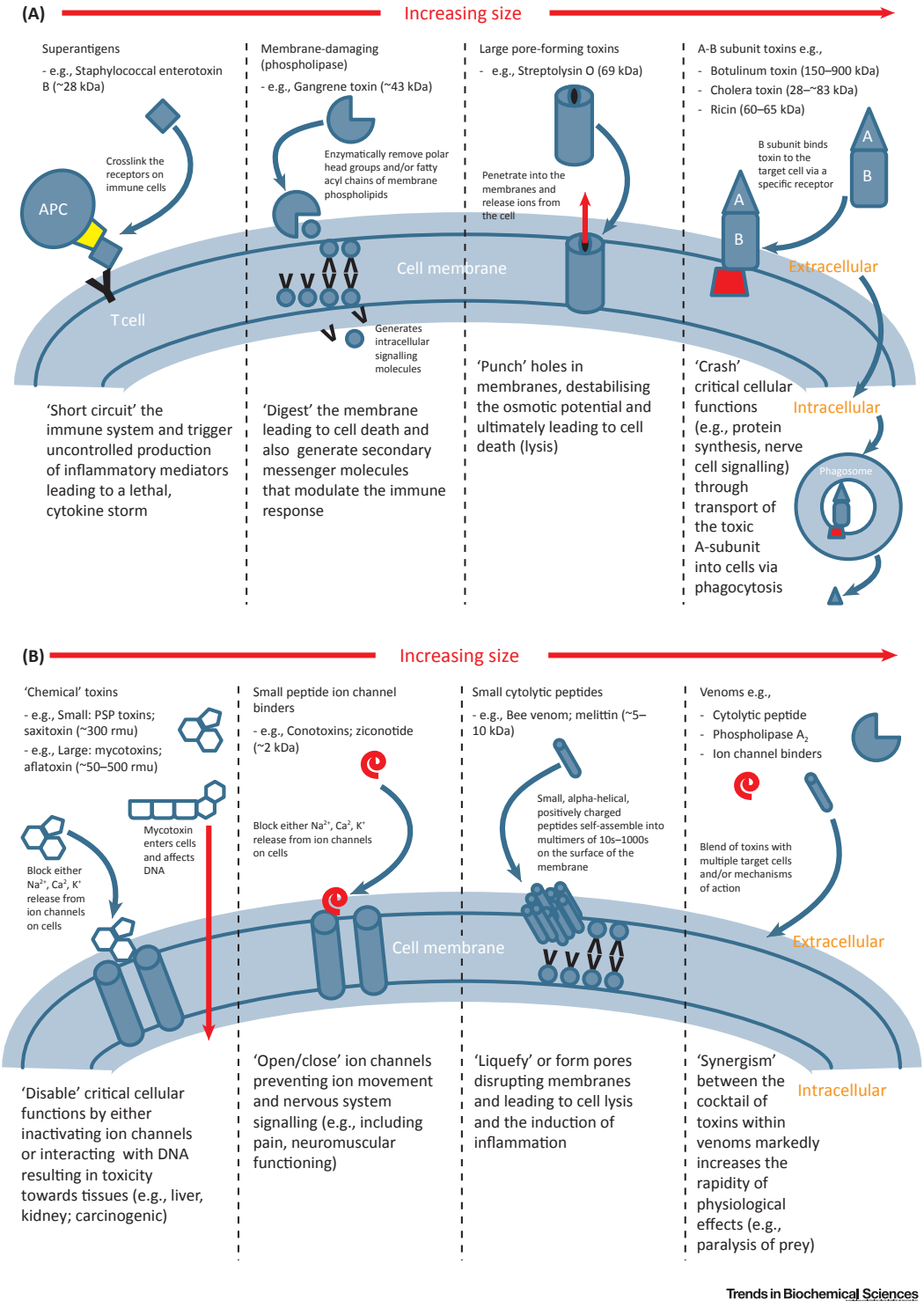
Membrane Structure and Function

Many pathogenic bacteria owe their clinical effects to pore-forming toxins (PFTs) (Figure 1). Toxins that form membrane pores have therefore been studied to determine the mechanism of this membrane disruption. PFTs are also found in diverse eukaryotes ranging from the bee venom cytotoxin mellitin from *Apis mellifera* to actinoporins from sea anemones. More recently, **antimicrobial peptides (AMPs)** from snake and scorpion venoms have been recognised as pore formers. The temporal resolution of mechanisms of action of PFTs (e.g., helping us to understand differences between how peptides insert into membranes) have been improved using the patch clamp technique, initially using artificial lipid membranes and more recently under physiological conditions using mammalian cells [43,44].

The understanding of toxin–membrane and toxin–membrane protein interactions at a molecular level is undergoing a transformative shift, with the development of cryo-EM allowing for high-resolution structural determination without the need for growing crystals. Lysenin, a toxin from the body fluid of the earthworm *Eisenia fetida*, belongs to the aerolysin family of small β-pore-forming toxins [45]. The water-soluble monomeric protein oligomerises in contact with sphingomyelin and forms transmembrane pores [46]. Using cryo-EM, an atomic model of the pore formed in bilayer membranes by the oligomeric protein has been determined [47].

As stated earlier, HS-AFM is a technique that allows structural resolution of molecules to be determined at a nanoscale level in real-time. HS-AFM, in conjunction with quartz crystal

bioinspired small molecules or biopolymers that are derived from peptides.
Phage display: an approach where a gene of interest is cloned into the gene of a phage that encodes a coat protein. Upon expression of the protein from the gene, it is displayed on the surface of the phage, allowing rapid, high-throughput screening ('panning') for proteins that bind to the target of interest (i.e., DNA/protein receptor).
PSP: paralytic shellfish poisoning syndrome experienced following consumption of food products naturally contaminated with saxitoxin produced by algal blooms.
Recombinant expression systems: represents the cloning of a gene encoding a toxin into a plasmid and subsequently into commercial bacterial/yeast strains (e.g., *Escherichia coli*, *Pichia pastoris*) or mammalian cells optimised for protein production.
SNARE proteins: a complex of proteins that form vesicles and that are essential for neurotransmitter release at the presynaptic cleft and nerve cell functioning.
Solid phase synthesis: the stepwise chemical synthesis of a peptide from amino acid building blocks on a solid support, most commonly polystyrene beads.
Strabismus: a medical condition that can be associated with a specific muscle dysfunction, resulting in both eyes being unable to align with each other on an object of interest.
Transcriptomics and proteomics: often referred to as 'omics' and involve high-throughput techniques to characterise the composition of these separate molecules of RNA or protein that make up the sample.
Zoonotic: an infectious disease that can be transmitted to humans from animals.



(See figure legend on the bottom of the next page.)

Table 1. FDA Approved Venom-Derived Therapies for Treating Human Diseases

Drug	Indication	Lead source	Molecular function	Chemical structure	Refs
Captopril	Hypertension and congestive heart failure	*Bothrops jararaca*	Angiotensin-converting enzyme (ACE) inhibitor	Small molecule	[11]
Eptifibatide	Acute coronary syndrome	*Sistrurus miliarius barbouri*	αIIbβ3 receptor	Cyclic disulfide-constrained peptide	[12]
Tirofiban	Acute coronary syndrome	*Echis carinatus*	αIIbβ3 receptor	Small molecule	[13]
Ziconotide	Severe chronic pain	*Conus magus*	N-type Ca_V	Cyclic disulfide-constrained peptide	[14]
Exenatide	Type 2 diabetes	Gila monster (*Heloderma suspectum*)	Glucagon-like peptide-1 receptor	39-residue linear peptide	[15]

Box 2. Case Study: Botulinum Toxin from Biothreat to Wonder Drug

The infamy of botulinum toxin (Latin-derived meaning: sausage poisoning) arose from food poisoning cases in Germany as early as the 1900s. Recognised as the most poisonous natural substance known to man, picogram quantities are lethal in murine models [19]. Interest from a number of regimes led to the toxin becoming a molecule of concern from a defence perspective [20]. Nowadays, botulinum toxin is most commonly associated with cosmetic applications (e.g., antiwrinkling) and is responsible for astonishing global sales of around $3 billion per annum for one product (Botox®) alone.

Botulinum toxin is a 150 kDa A-B subunit toxin (Figure 1). Binding by the B-subunit to the presynaptic membrane of neuromuscular junctions and, by using the zinc metalloprotease catalytic activity of the A-subunit, the toxin cleaves **SNARE proteins** involved in the release of the neurotransmitter acetylcholine. The result is flaccid paralysis of muscle, initially affecting the peripheral nervous system, with death associated with respiratory failure.

Despite being overshadowed by its use in the cosmetics industry, the clinical applications of botulinum toxin have been game changing for the treatment of various neuromuscular disorders. Following FDA approval of the first product in 1978 for the treatment of **strabismus**, a range of other molecules have been developed for use in humans (Table 2). Each has a subtly different composition with either toxin serotypes A and B (of the seven botulinum toxins in total; serotypes A–G) being the main active component, alongside various ancillary proteins that support either the stability or mode of action of the toxin [21]. Their use has revolutionised the treatment of movement disorders ('dystonia') in both adults and children, in most cases reducing/removing the need for corrective surgery. Further recent development has highlighted the potential for the toxin to treat a wider range of neurological conditions, as evidenced by its approval for use in the EU and North America for treating chronic migraines in adults.

microbalance dissipation, has visualised the formation of highly branched membrane defects when AMPs from scorpion venom interact with planar lipid membranes, allowing the separation of fast (within 200 s) and slow (up to 25 min) toxin–membrane interactions [48]. Labelling functionalized AFM tips with toxins such as lysenin or the D4 domain of Θ-toxin (cholesterol-specific) has allowed the identification of specific domains of sphingomyelin and cholesterol in bilayer membranes without the need for fixation or labelling steps, both supporting the concept of **lipid rafts** and offering the possibility of directly observing lipid assemblies in native cells [49].

Nucleic Acid Squence Analysis

Nanopores, created by pore-forming membrane proteins in electrically insulated artificial membranes, can be engineered to detect single molecules passing through the channel they

Figure 1. The Many Sizes, Shapes, and Mechanisms of Action of Toxins. (A) Large toxins and (B) small molecule toxins. Typically, single celled prokaryotes (e.g., bacteria) produce larger toxins as a consequence of having simple protein-folding machinery; however, often small amounts can be very toxic (e.g., botulinum toxin). In contrast, eukaryotes (e.g., spiders, cone snails) can produce incredibly small peptides with highly complicated structures held together with multiple disulfide bonds, creating molecules with high specificity and avidity for their target cell/substrate. APC, Antigen presenting cell; PSP, paralytic shellfish poisoning; rmu, relative mass unit.

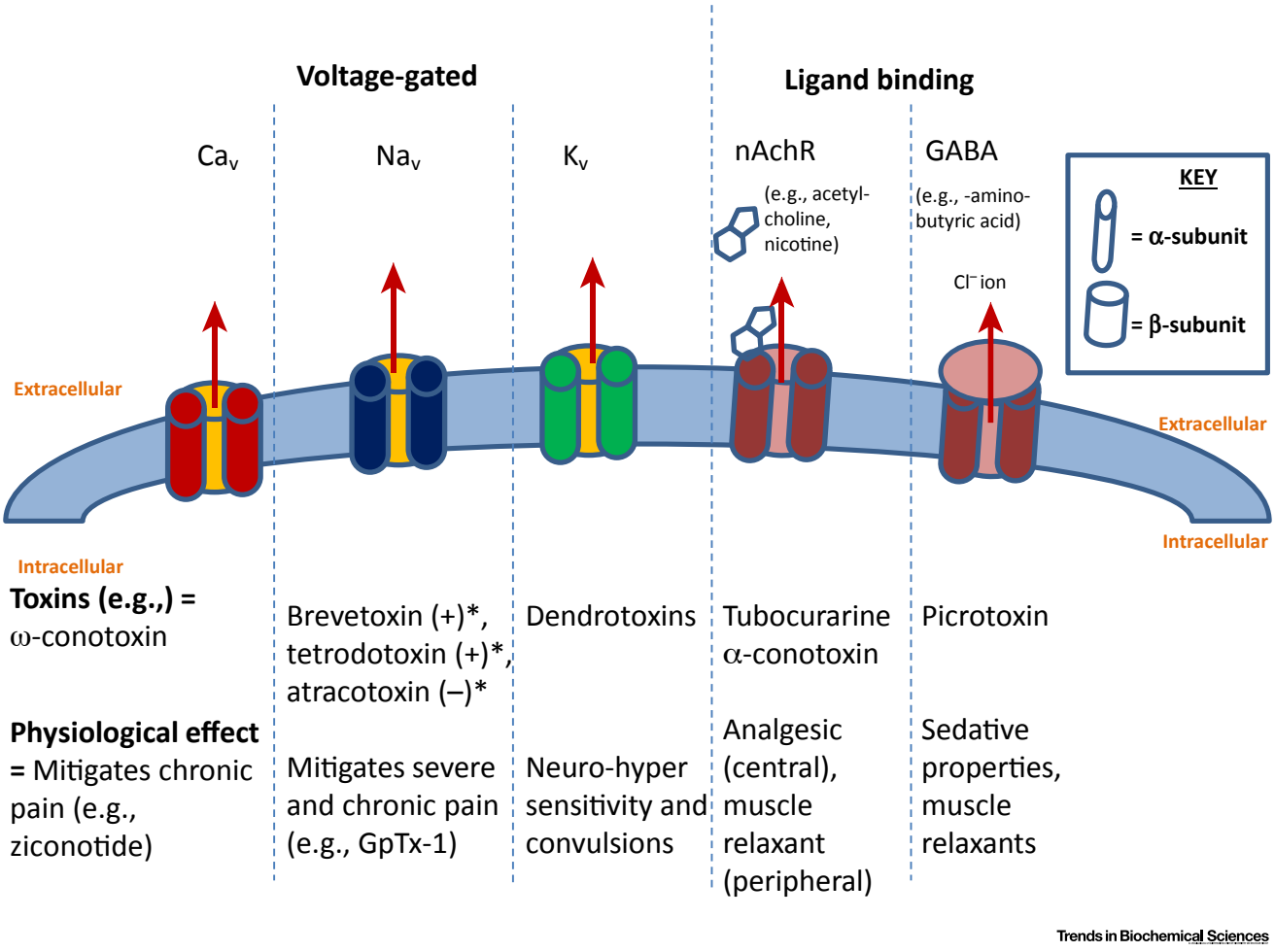


Figure 2. Examples of Voltage-Gated or Ligand-Binding Ion Channels Targeted by Toxins. Only a small subset of ion channel types that are pertinent to the review are highlighted here. Differences in the composition and amino acid sequences of α- and β-subunits of ion channels dictate the nomenclature (e.g., $Na_V1.1$–1.9). Many more exist that provide a diversity of physiological processes. For example, there are toxins with selective actions on calcium-activated potassium channels, inward rectifier potassium channels, chloride channels, acid sensing ion channels, transient receptor potential channels, and ionotropic glutamate receptors. *(+) Open channels and (–) closed. nAChR, nicotinic acetylcholine receptor.

form; more specifically by monitoring voltage-dependent changes in ion currents. The differential detection of individual DNA bases passing through engineered α-haemolysin toxin nanopores has led to the development of revolutionary rapid DNA single molecule sequencers termed the MinION [50,51].

Collectively, the development of new technologies, in parallel with efficient chemistries to produce toxin peptides and peptidomimetics, has led to an increase in the applications in bioscience research that shows no signs of slowing.

Animal and Human Healthcare Sectors

As a result of their diverse and often highly selective functional activities on important physiological processes, toxins have also received attention as potential therapeutics. Indeed, toxinology has already demonstrated impact within a clinical setting for the treatment of

cardiovascular disease (see Toxins and Toxinology). Some emerging areas of interest are in the treatment of pain, as immunotherapies, and for countering antimicrobial resistance (AMR). In particular, venom extracts from species of cone snail, spider, snake, and lizard represent a goldmine of hundreds to thousands of bioactive compounds with peptides as the main components. Typically, the cocktails of peptides employed by venomous species have multiple targets in the body and are designed to immobilise their prey by subverting or inactivating critical physiological pathways/processes. Consequently, research to determine the targets and assess potential applications of these peptide toxins has seen rapid growth in the past few decades given their pharmacological potential [16]. Five peptide toxin-derived drugs from the viper family of snakes (i.e., captopril, eptifibatide, tirofiban), a cone snail (i.e., ziconotide), and a lizard (i.e., exenatide) are currently approved by the FDA for clinical use for a diverse range of medical conditions, including blood pressure, pain, and diabetes (Table 1; Box 3). Many more are in preclinical development, including one from a sea anemone for use in autoimmune diseases (Box 3). There is also a plethora of predominantly enzymatic, snake venom-derived proteins that target natural processes in the circulatory system (i.e., clotting, coagulation) that are in use or being developed as drugs to treat various indications, from heart disease to cancer [52,53].

Pain Therapeutics and Congenital Neurological Conditions

Peptide toxins have potential applications for the treatment of pain with the advantage that they lack the addiction profiles observed with opiate drugs such as morphine. Ziconotide, a synthetic version of a peptide found in the venom of the marine cone snail *Conus magus*, is licensed for use in the treatment of severe and chronic pain (Table 2). The mechanism of action is as a selective N-type voltage-gated calcium channel blocker (Figure 2). However, ziconotide is administered intrathecally (i.e., directly into the spinal fluid) due to severe side effects on oral or intravenous administration. This has prevented widespread therapeutic use of ziconotide and driven current research to focus on developing peptide toxin-derived therapeutics with improved bioavailability and better selectivity [60]. There is clearly potential for the discovery of highly selective and potent peptide toxins that target neuronal ion channels or receptors as analgesics.

Spider venoms predominantly target the nervous system and offer significant potential for the identification of new treatments for pain or neurological disorders. Some research in this area

Box 3. Toxins from Land and Sea to the Clinic

The Gila monster (*Heloderma suspectum*) is a venomous lizard found in south-west USA and north-east Mexico. The venom of this reptile contains many bioactive peptides and enzymes, including exendins, homologues of the mammalian glucagon-like peptides (GLP). In humans, GLP-1 is released from the gut after eating, triggering increases in insulin secretion, decreases in glucagon release, slowing gastric emptying, and decreasing appetite: actions that could contribute to an antidiabetic effect. However, GLP-1 is very rapidly inactivated by dipeptidyl peptidase IV (DPP-IV). Exendin-4 from Gila monster venom is very resistant to the action of DPP-IV [54] and is therefore much longer acting in lowering blood glucose than GLP-1. Consequently, synthetic exendin-4 (exenatide; Byetta) was developed by Amylin Pharmaceuticals for treatment of patients with type 2 diabetes and approved by the FDA in 2005 [55].

The venoms of sea anemones are particularly rich in small peptides that affect sodium and potassium ion channels and thereby disrupt the normal signalling in the nervous system [56]. A component from venom of the Caribbean sea anemone *Stichodactyla helianthus* was first noted for its ability to increase neurotransmitter release by blocking voltage-dependent potassium ion channels [57]. Small structural modifications gave a derivative ShK-186 that had very high potency and specificity for one subtype of potassium channel, K_V 1.3. This was significant because activation of that channel in memory T cells on lymphocytes drives some autoimmune diseases. Studies in animal models of multiple sclerosis and rheumatoid arthritis were positive [58] and ShK-186 was entered into clinical trials by Kineta. Under the trade name Dalazatide, the compound has successfully completed a clinical trial in patients with psoriasis [59].

Table 2. Clinically Approved Products and Applications of Botulinum Toxin

Trade name	Molecule name (active serotype in bold)	Company	Approved applications
BOTOX®	Onabotulinumtoxin**A**	Allergan, USA	Cervical dystonia (neck muscle), severe primary axillary hyperhidrosis (excessive underarm sweating), strabismus (eye alignment), and blepharospasm (eye lid spasm) associated in patients over 12 years old, overactive bladder, muscle stiffness around joints, and chronic migraine
Dysport	Abobotulinumtoxin**A**	Ipsen, France	Cervical dystonia, facial wrinkles, and upper limb spasticity
Myobloc/Neurobloc	Rimabotulinumtoxin**B**	US WorldMeds	Cervical dystonia
Xeomin/Bocouture	Incobotulinumtoxin**A**	Merz, Germany	Cervical dystonia, blepharospasm, facial wrinkles, upper limb spasticity, excessive salivation

focused on targeting $Na_V1.7$ channels on neurons (Figure 2). Amgen tested the GpTx-1 peptide, discovered by high-throughput screening of fractionated venom from the tarantula *Grammostola porteri* [61]. Subsequent structure–activity relationship studies led to the development of potent analogues with excellent selectivity for human $Na_V1.7$ over $Na_V1.4$, which controls excitability of skeletal muscle, and $Na_V1.5$, which is found predominantly in cardiac muscle. This demonstrates the power of logical, structure-based molecular design to improve upon a natural toxin scaffold. In addition, a peptide from the venom of the spider *Heteroscodra maculata*, termed Hm1a, has been found to specifically target $Na_V1.1$ channels and was initially investigated for treating mechanical pain [6]. More recent preclinical research has highlighted the potential of this molecule for treating congenital neurological conditions such as epilepsy: intracerebroventricular administration of Hm1a prevented seizures and premature death in mice [62]. Peptides called mambalgins from the venom of the black mamba snake *Dendroaspis polylepis* act specifically on acid-sensing ion channels and have also shown promise in reducing pain in preclinical studies [5]. Ultimately, to be successful drugs in a clinical setting, these molecules will have to be suitable for oral or intravenous administration as well as having better therapeutic windows, and thus safety profiles, than currently available drugs.

Novel Antimicrobials

AMR amongst pathogenic bacteria, fungi, and parasites is an increasing issue that spans both animal and human healthcare and has led to the creation of the **'One Health'** Initiatives (http://www.onehealthinitiative.com/mission.php and https://www.cdc.gov/onehealth/basics/index.html) [63]. AMR may be triggered by inappropriate or excessive antibiotic prescription in the clinic, or through aggressive use in an agricultural setting (i.e., antibiotic growth promoters in bovine or poultry feeds) contributing to the development of **zoonotic** diseases from pathogenic AMR strains. There is an urgent need for new therapeutic molecules and/or approaches to treat infectious diseases that exhibit resistance to conventional treatments [64,65]. Many AMPs have been isolated from the venom of diverse organisms, including ants, bees, wasps, centipedes, scorpions, spiders, snakes, and cone snails [66]. All have demonstrated efficacy in the test tube towards both gram-negative and -positive bacteria and as topical applications. There is also emerging evidence of their efficacy *in vivo*. One recent example demonstrated the recovery of mice from a lethal bacteraemia caused by clinical methicillin-resistant *Staphylococcus aureus* through the administration of an AMP derived from scorpion venom [67]. However, challenges also remain with respect to the **biostability and bioavailability** of these AMPs such that some form of physicochemical optimisation (i.e., PEGylation, peptide cyclisation) are often required, particularly when considering using routes of administration other than **parenteral** for peptides [68,69]. Aside from AMPs, other venom components have been demonstrated to possess a

range of novel antimicrobial properties, including phospholipase A_2 (PLA_2; Figure 1), metalloproteases, and L-amino acid oxidases (LAAO), and esterases which may lead to antibiotics with novel mechanisms of action [70]. Antimicrobial activity has also been reported for some aquatic toxins [37]. Collectively, this work highlights AMR as an emerging theme within toxinology.

Immune Modulation

The immunomodulatory properties of biological toxins are also being investigated for the treatment of inflammatory disorders, including Crohn's disease and asthma (i.e., cholera toxin) [71]. Promising Phase Ib trial results have been obtained for Dalazatide®, an analogue of the potassium channel blocking toxin ShK from the sea anemone *Stichodactyla helianthus*, for the skin condition psoriasis [59] (Box 3). Finally, subunits of enterotoxins from *Vibrio*, *Escherichia*, and *Pseudomonas* sp. are being used as vaccine adjuvants and have been the subject of a recent review and patent [72,73]

Agricultural Sector

New discoveries are fuelling the interest in using toxins within the agricultural sector as safe, environmentally friendly insecticides. Sero-X, licensed as a bee-friendly insecticide in Australia, contains a cocktail of cyclic peptide toxins isolated from the butterfly pea plant and attacks the gut lining of caterpillars [Australian Government Pesticides and Veterinary Medicines Authority, 2016 (https://apvma.gov.au/sites/default/files/publication/21021-public-release-summary-sero-x-insectidice-containing-clitoria-ternatea-extract.pdf)]. Highly specific insecticides have also been isolated from fungi and spiders. For example, an environmentally friendly insecticidal peptide related to a calcium channel blocking toxin (ω-hexatoxin-Hv1a) from the Australian funnel web spider (*Atrax robustus*) venom has been brought to the market as 'Spear®-T' by Vestaron; molecules from other species of spider are also being investigated as potential novel pesticides [74].

Toxins as Foes: Emerging Impacts of Climate Change, Biosafety, and Biosecurity Implications

Aside from their apparent value as physiological and therapeutic tools for the benefit of mankind, biological toxins are also highly relevant in the wider context of our environment. The risks to human health as a result of new or emerging toxins appearing naturally are clear. Equally, as the prevalence of toxins increases in the environment, so does their accessibility and therefore their potential concern from a **biosecurity** perspective (e.g., contamination of foods, water supplies). Whether naturally occurring or manmade, the prediction of potential risk to humans, rapid identification, and/or timely diagnosis of exposure of toxins represents a significant challenge in a range of contexts.

Food and Water Biosafety/Security Sectors

Global climate change means the niches that can be occupied by toxin-producing species of single cellular organisms, plants, and animals are expanding; particularly those that require warmer climes. In addition, this, coupled with the encroachment of human habitation into new areas of the world, collectively increases the potential/incidence of intoxicating events through exposure (e.g., inhalation, ingestion, injection) to either toxins or toxigenic species in the environment.

Aquatic Food and Water Safety

Aquatic food security is significantly impacted by environmental change [75]. Marine toxins responsible for paralytic, amnesic, and diarrhetic shellfish poisoning (**PSP**, ASP, and DSP) are regulated globally due to the serious health risks to shellfish consumers. However, other marine

toxin groups with potentially serious health consequences remain largely unregulated. The majority of toxins are produced naturally by species of marine harmful algal blooms (mHABs), with others associated with bacteria [76]. Cyanobacterial harmful algal blooms (CHABs) are distributed widely, most notably in freshwater [77], posing a substantial threat to both drinking and recreational water. The natural function of aquatic toxins is unclear and research has primarily focused on the impacts these compounds have on humans. The mechanisms of marine and freshwater toxicity are extremely diverse, and multiple variants of each toxin group produce significant challenges in monitoring to ensure human safety.

Recent years have shown an increase in the frequency of marine toxin-producing blooms and bacteria in many areas of the world, together with increasing reports of new or emerging nonprotein toxins, including polycyclic polyether brevetoxins in New Zealand, ciguatoxins in Spanish fish, and polyhydroxylated natural product palytoxin and ovatoxins in Mediterranean mussels, DSP toxins in the USA, and cyclic imines throughout Europe [78]. There are also prominent examples of the emergence of unexpected bacterial toxins in UK waters (Box 4). CHABs are also occurring with increased regularity in both freshwater and marine ecosystems globally [82].

Expansion of marine toxin-producing mHABs over recent decades is well recognised and has been linked to ballast water transportation, **eutrophication**, and climate change [76]. Climatic change in particular is recognised as a key factor concerning the frequency and severity of natural aquatic toxin occurrence [79,83]. Changes in both mean and extreme values of temperature, light, ocean acidification, wind, precipitation, currents, and nutrients may all contribute to changes in blooms, including germination and growth rates, geographical expansion, as well as cell toxicities, shellfish toxicity [83,84], and incidents of human intoxications [76]. Similarly, expansion of CHABs is influenced by many factors, including iron availability, light, hydrological changes, storm events, drought, precipitation, and industrial pollution [85]. However, the impacts of eutrophication dominate the literature as the main causative factors, particularly in conjunction with higher temperatures [86], and almost every climate change scenario predicts that many freshwater bodies will experience increased temperatures and longer, more intensive periods of thermal stratification, resulting in increased CHABs occurrence and toxicity [87,88].

Box 4. Tetrodotoxin and *Vibrio*

Tetrodotoxins (TTXs) are responsible for the highest mortality rates in all marine intoxications, being potent low molecular weight sodium channel-blocking neurotoxins. They accumulate most commonly in tropical and subtropical regions, particularly in fish from the Tetraodontidae family such as pufferfish. Unlike other marine toxins, these are produced by bacteria, notably *Vibrio* species such as *Vibrio parahaemolyticus*. *Vibrio* growth is strongly dependent on high temperature and low salinity, with increased toxin production anticipated following changes in both parameters, thereby accelerating the spread of pathogenic bacteria and TTXs in northern latitudes [79]. Following the climate change related expansion of *Vibrio* sp. into the marine waters of the southern UK, TTXs were unexpectedly detected in shellfish such as mussels, oysters, and clams, for the first time anywhere in Europe, in some shellfish harvesting sites in southern England [80]. Subsequently, TTXs have been reported in bivalve molluscs from elsewhere in Europe, including Greece, the Netherlands, Spain, and Italy, thereby revealing the potential for TTX shellfish poisoning for consumers of shellfish throughout Europe. The ongoing screening of UK molluscs since the first discovery in the UK revealed the continued presence of TTXs over the following 3 years, but mostly in the warmer summer months and associated with shallow water, estuarine shellfish beds, with low salinity and warmer waters (temperatures >15 °C). As such, there is strong evidence for an enhanced risk of TTX production during times of higher temperatures and precipitation-triggered low salinities, both of which are expected as a result of ongoing climatic change in the UK [81]. This case study represents a unique instance of a new, unexpected, emerging natural aquatic toxin threat, traditionally associated with warm tropical waters and tropical fish species, to consumers of shellfish in temperate regions of northern Europe. It therefore illustrates the potential for dramatic changes to natural toxin populations in the environment, inferring that many other warmer-water HAB and bacterial toxins should not be excluded from future risk assessment and risk management programmes.

Regulations (e.g., food safety, environmental exposure) are absent for the majority of emerging aquatic toxins and, given the limited understanding regarding toxin production and occurrence under climatic change conditions [78], environmental modelling approaches are needed to forecast spatial and temporal aquatic toxicity and related human health hazards [81]. To do this we need to link our developing understanding of environmental impacts on toxin production with the ability to combine improved detection and forecasting systems, including *in situ* ocean observation systems, highly specific and sensitive chemical detection tools, satellite data, and other biological observations into effective operational modelling regimes [76,82].

Increasing Global Prevalence of Mycotoxin-Producing Species

Mycotoxins are a highly diverse range of toxins produced by toxigenic moulds and are found to infect a wide variety of agricultural crops, resulting in significant risk to crop consumers globally. In particular, aflatoxin- and fumonisin-contaminated foodstuffs represent massive health issues, especially in the developing world and for the young. High temperatures, elevated CO_2, and humidity/water availability are known in particular to encourage toxic mould formation and thus climatic change is also having a significant impact on mycotoxin presence, resulting in the spread of mycotoxicity further north within the EU [89], as well as altering the amount of toxin being produced by toxigenic strains [90]. Cutting edge techniques (e.g., ambient mass spectrometry and molecular spectroscopy) will be increasingly important for identification of contaminated feeds and foods. More accurate modelling and prediction methods for the impacts of climate change on mycotoxin production are needed to help address food security concerns [91].

Concluding Remarks and Future Perspectives

Technological developments in omics-based approaches are driving the discovery of new toxins from an increasing array of organisms (i.e., from bacteria to animals). Challenges remain in the availability of suitable (*in vitro*, *ex vivo*, or *in vivo*) models for characterising the biological properties of these novel toxins and enabling their subsequent development within the biosciences or healthcare sectors. There is also an absence of *in silico* tools that aid the prediction and identification of completely new classes of toxins with unique or distinct mechanisms of action. Similarly, new ways to assess the potential for 'off-target' effects on the body would be of significant value when developing toxins for use in veterinary or clinical applications. Although there are a number of exceptions, the mass production of natural bioactives (with potential therapeutic benefits) remains largely challenging given the difficulties of producing industrial scale yields of toxins through culture and/or chemical synthesis (e.g., due to complex disulfide bond formations in many peptides). Once produced, certain peptides may also require chemical modification or formulation in order to improve their pharmacological properties (i.e., biostability, bioavailability), as well as helping to improve potency and target selectivity, thereby reducing the quantities of material required to achieve an identical pharmacological effect. Further work is also required in order to improve how peptide therapeutics will be administered (e.g., formulation towards achieving oral bioavailability instead of injection; delivery to the central and/or peripheral nervous system).

Within the water and food security sector, there is a fundamental need to develop a firm understanding of the relationships that drive climate-induced changes in **HAB** growth, distribution, and toxicity. In conjunction with a better understanding of the biology, developing new satellite imaging and analysis techniques will aid in the prediction of algal bloom formations.

Despite the challenges and outstanding research requirements outlined above (also see Outstanding Questions), for the field of toxinology the positive impact that toxins have made to modern society cannot be denied and that trend looks set to only continue, fuelled by a number of external factors (e.g., technology developments, commercial opportunities). The

Outstanding Questions

What tools can be developed that would allow toxins with completely new mechanisms of action to be identified? As new technological approaches fuel potential drug discovery, the next 'bottleneck' lies within availability of suitable models for evaluating the biological properties of the toxin.

How can we easily mass produce emerging toxins with unique properties but highly complex structures? For a molecule to be of value for drug development it needs to be manufactured on an industrial scale, which represents a significant challenge and a potential barrier to development.

Can peptide-based toxins be adapted or formulated in order to increase their longevity of action and targets within the body? Some peptides will be rapidly broken down and cleared from the body, or are required to be administered by injection, which collectively limits the clinical utility as a treatment.

How and why do algal blooms develop within the environment? The triggers for aquatic toxin production are yet to be elucidated. This makes predicting the emergence of HAB, and therefore the forecasting of toxin production, very difficult.

Key Figure

Current and Future Drivers in the Field of Toxinology

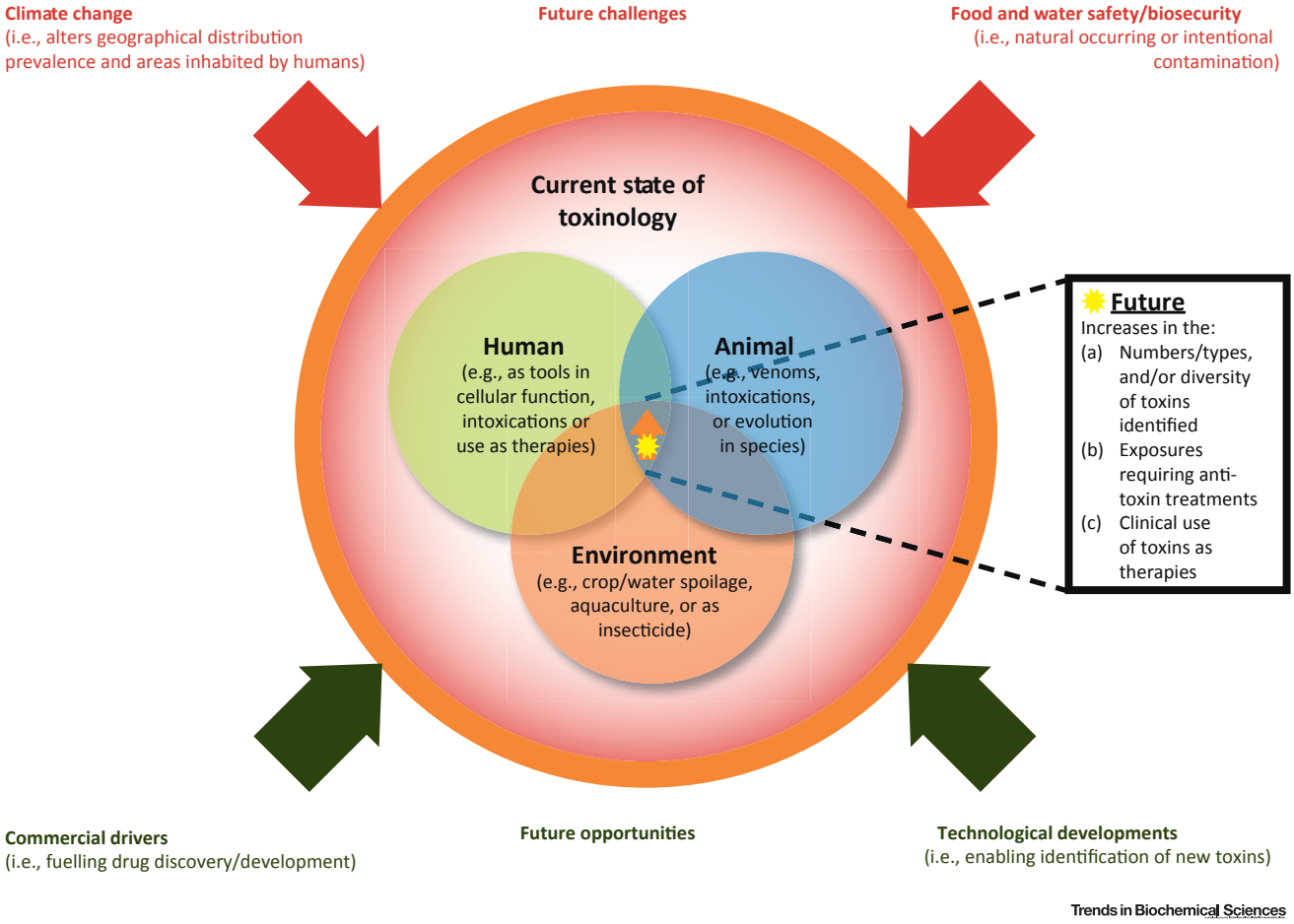


Figure 3. The figure highlights the current state of toxinology where the primary focus of research is on biological toxins that directly affect humans, animals, or the environment. These toxins map to and have a direct impact upon a number of industrial sectors, namely, agriculture, biosciences, food and water biosafety/security as well as human and animal healthcare. Research on specific toxins intensifies when they converge to affect multiple sectors. The key, emerging factors are highlighted that will lead to an increase in the convergence and overall impact of toxins in the future (i.e., positively in green and negatively in red).

rapid assimilation of new technical approaches into toxinology and the increasing connections between research into animal, human, and environmental aspects will be the best approach to meeting the challenges posed by toxins as both 'friends and foes' (Figure 3, Key Figure).

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