OXFORD Toxicology Research

Toxicology Research, 2020, **9**, 676–682

doi: 10.1093/toxres/tfaa063
Advance Access Publication Date: 8 October 2020
Viewpoint

VIEWPOINT

A decade of toxicological trends: what the papers say

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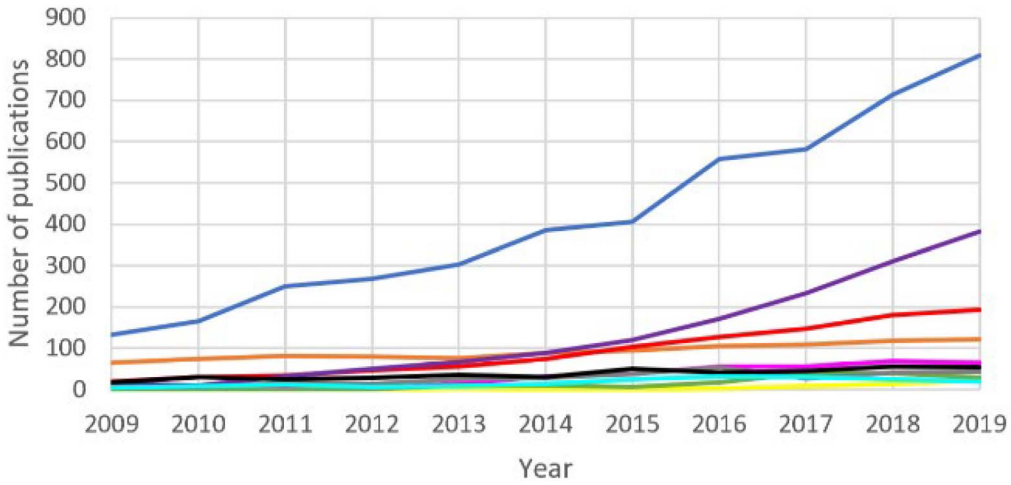
Abstract

Here we look at popular trends and concepts in toxicology over the decade 2009–2019. The top 10 concepts included methodological approaches such as zebrafish and genomics as well as broader concepts such as personalized medicine and adverse outcome pathways. The total number and rank order for each of the top 10 were tracked year by year via PubMed with >9500 papers contributing to the analysis. The data revealed a slow upward trend in the number of papers across all the concepts from 260 in 2009 to >1700 in 2019. Zebrafish, genomics and personalized medicine remained in the top four slots since 2009 with zebrafish dominating the rankings over the entire decade. Genomics was a strong second until 2013 when it was displaced first by the microbiome in 2014 and secondly by personalized medicine in 2015. Other notable trends were the ascendancy of the microbiome and adverse outcome pathways and the descendancy of hormesis and the 3Rs (replacement, reduction and refinement of animals in testing). The observation that the top four slots have been static over the past 4 years suggests that new ideas are introduced and increase in popularity until they find their place in scientific culture. This may suggest that relatively new concepts such as artificial intelligence and microphysiological systems have yet to find their steady state in the rankings. Similarly, as a relatively new player in toxicology, the full impact of the human microbiome on drug efficacy and safety remains to be seen.

Graphical Abstract



Tracking toxicology publications 2009–2019 reveals surprising trends and provides insight into possible uptake of new areas such as CRISPR and AI.FX1

Received: 30 April 2020; **Revised:** 20 July 2020; **Accepted:** 21 July 2020

Key words: toxicology trends, genomics, zebrafish, personalized medicine

Introduction

As with many other disciplines, toxicology has scientific trends that come and go with time. Some of these trending concepts are fleeting, whereas others appear to take centre stage and persist over years or even decades. What trends have we seen over the past decade and what topics are emerging right now? What can this tell us about where the field has been and where it is going? Currently, such topics as artificial intelligence (AI) and the microbiome are increasingly prevalent in the literature, whereas other areas that were 'hot' a few years ago appear less so now. Here we look at popular trends and concepts in toxicology, how they have evolved over the last decade and how they may help inform future directions for the discipline. The study is not supposed to be scientifically rigorous but rather to be a light-hearted read that will provoke discussion within the discipline.

The Concepts

In order to track trends in concepts, several experienced toxicologists were asked to nominate their favourite 'trends' over the decade 2009–2019 where trend was defined as a concept that has a life cycle of emergence, peaking and ebbing. Areas such as hepatotoxicity, cardiotoxicity, *in vitro*, environmental toxicology or renal toxicology were not included in the study since these are arguable core disciplines rather than trends. The top 10 concepts proposed included methodological approaches such as 'zebrafish' and 'genomics' as well as much broader concepts such as personalized medicine and adverse outcome pathways (AOPs). Search terms were determined in order to find the total number of publications in each concept (Table 1). A PubMed search was performed for each year using the search term in conjunction with 'AND toxicology' to limit the output to only the toxicologically relevant results. The frequency of appearance of each of the top 10 concepts in toxicology was then tracked year by year over the past decade to reveal trends and patterns (Fig. 1 and Table 2).

Table 1: Search terms used to analyse trends in toxicologically relevant publications

Concept	Search term(s)
AI	'Artificial intelligence'
Gx	Genomics
Zf	Zebrafish or '*Danio rerio*'
PM	'Personalized medicine' or 'Personalized medicine' or 'Precision medicine'
Mb	Microbiome or microbiota
AOP	'Adverse outcome pathway'
MPS	'Microphysiological systems' or 'Microphysiologic system' or 'Organ on a chip'
RA	Read-across
Hor	Hormesis
3Rs	3Rs

Gx: genomics, Zf: zebrafish, Hor: hormesis, PM: personalized medicine, Mb: microbiome, RA: read-across.

The Trends: Big Picture

One of the most notable findings is that certain concepts have dominated the literature over the last decade: a rank ordering of 'popularity' (Fig. 1) demonstrates that zebrafish, genomics and personalized medicine have remained in the top four slots since 2009. Indeed, zebrafish dominated the rankings over the entire decade. This lack of movement in the 'big three' contrasts sharply with ascendant (the microbiome) and descendant (hormesis) trends elsewhere. Interestingly, genomics was a strong second until 2013 when it was displaced first by the microbiome and secondly by personalized medicine. AOPs shows a general upward trend, whereas 3Rs (replacement, reduction and refinement of animal use in research) [1] and read-across are generally static.

A linear plot of the same data shows a spread in annual hits from <50 to >800 and a slow upward trend in the total numbers of papers from 260 in 2009 to >1700 in 2019 (Table 2 and Fig. 2a). Focusing in on those less represented concepts (<70 hits), there appears to be a lot more movement in trends perhaps suggesting more volatility in these less dominant concepts (Fig. 2b). However, this could just be 'noise' due to smaller numbers. Next, we look at each trending concept and consider its role in toxicology and possible reasons for its position in the rankings.

Zebrafish

Zebrafish were pioneered as a model organism in biomedical research as early as the 1960s due the lower cost, ease to maintain, higher fecundity and less restrictions by law than more complex species [2,3]. The first zebrafish paper was published in 1974 and concerns the toxicity of zinc and cygon. Indeed, early zebrafish publications focused on understanding the impact of environmental toxicants and chemicals on non-mammalian species. It was only later when comparative genomic investigations revealed genome synteny and individual gene conservation between mammals and zebrafish that it was proposed that zebrafish could be relevant for human health [2,4]. The success of this approach and acceptance of zebrafish as a suitable model organism in toxicology over the last 10 years are evidenced in our study where zebrafish is the top concept in the decade we studied (Fig. 1). To understand how long this has been the case, we extrapolated back as far as 1973 and noted a dramatic upturn after 2000 followed by a steady year-by-year increase through the following decade (Fig. 3).

Zebrafish have been used extensively in developmental and environmental toxicology as they have a higher throughput *in vivo* screening capability than other experimental organisms, making them an excellent early indicator of toxicity [5,6]. Zebrafish development has been well characterized, and their pigmentation can be prevented so they remain transparent during development, which allows the adverse effects of chemical exposure on embryogenesis to be assessed quantitatively. Therefore, zebrafish are a valuable vertebrate model used to explore how chemicals influence embryonic development and organ formation [3].

Genomics

Genomics is the analysis of the entirety of an organisms DNA, recognizing the crucial regulatory role of non-coding DNA and

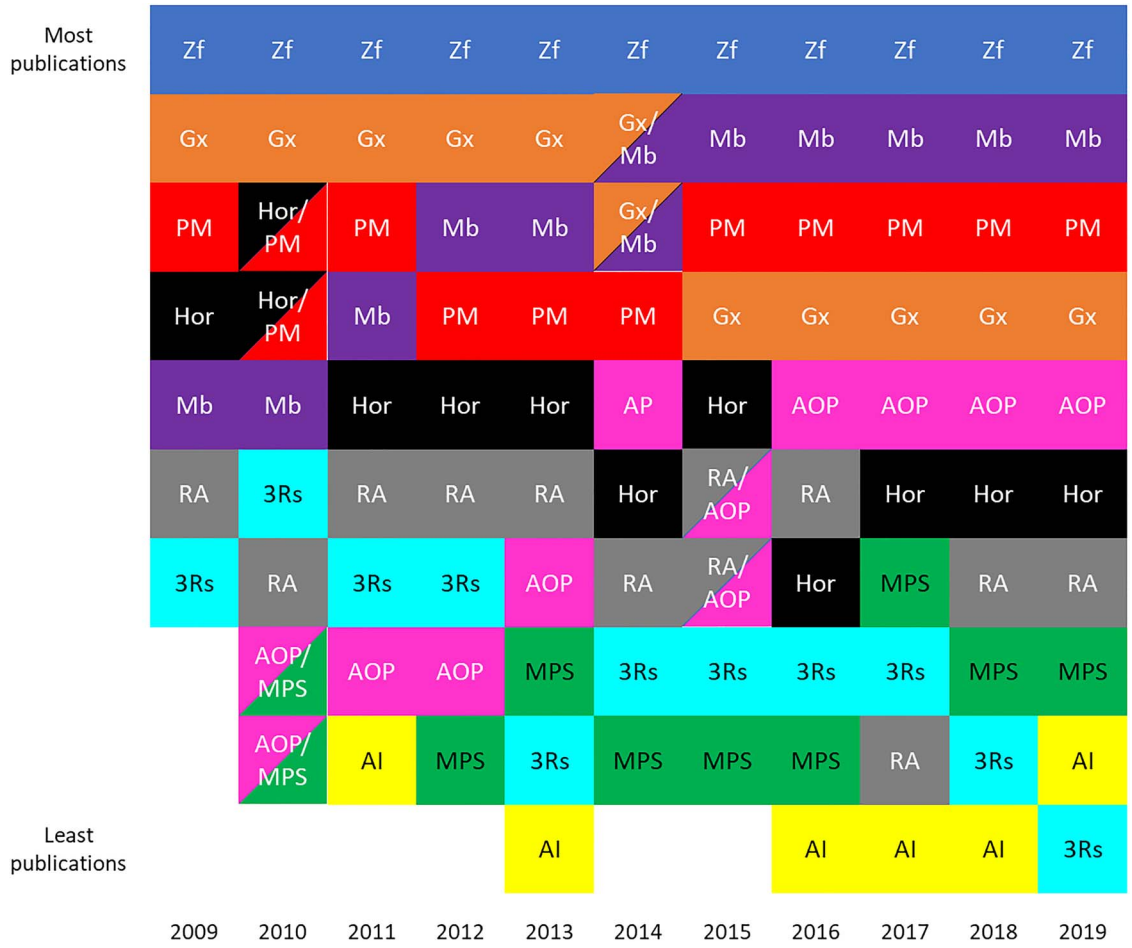


Figure 1: Trends in toxicology publications from 2009 to 2019 where each of the top 10 concepts is ranked based on the number of publications from most to least per year to track changes over time.

Table 2: Total number of toxicologically relevant publications for each concept per year

Concept	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
AI	0	0	1	0	3	0	0	3	8	14	23
Gx	66	75	81	80	76	89	95	106	109	119	122
Zf	132	166	250	269	303	386	406	557	581	714	809
PM	20	31	34	47	56	75	104	128	147	181	194
Mb	14	10	32	51	67	89	120	172	234	311	382
AOP	0	1	2	2	13	33	38	56	56	68	65
MPS	0	1	0	1	10	10	6	18	39	39	28
RA	6	7	16	14	25	28	38	55	26	42	43
Hor	17	31	25	28	36	31	51	41	45	56	55
3Rs	5	9	12	6	8	13	24	33	30	24	20
Total	**260**	**331**	**453**	**498**	**597**	**754**	**882**	**1169**	**1275**	**1568**	**1741**

the complex interactions between multiple genes and the environment [7]. From a toxicological perspective, toxicogenomics is the application of genetics and molecular biology to describe the response to a compound. Compounds with similar toxicity mechanisms should perturb the transcriptome in a similar manner; these transcriptome changes can be used as a predictive markers for toxicity outcome [8]. The use of toxicogenomics has been used in risk assessment to provide additional data to increase the understanding of mechanisms of action [9]. Genomic publications have consistently been in the top four over the last 10 years settling at position 4 in our study, indicating genomics is fully integrated into toxicology and plays a significant role in advancing the scientific basis of toxicity risk assessment. The application of genomic information to toxicology has driven improvements in toxicity testing, cross-species extrapolation, understanding mechanism of action and susceptibility [10]. More recently, genomics has been evaluated for application risk assessment within the context of reducing animal

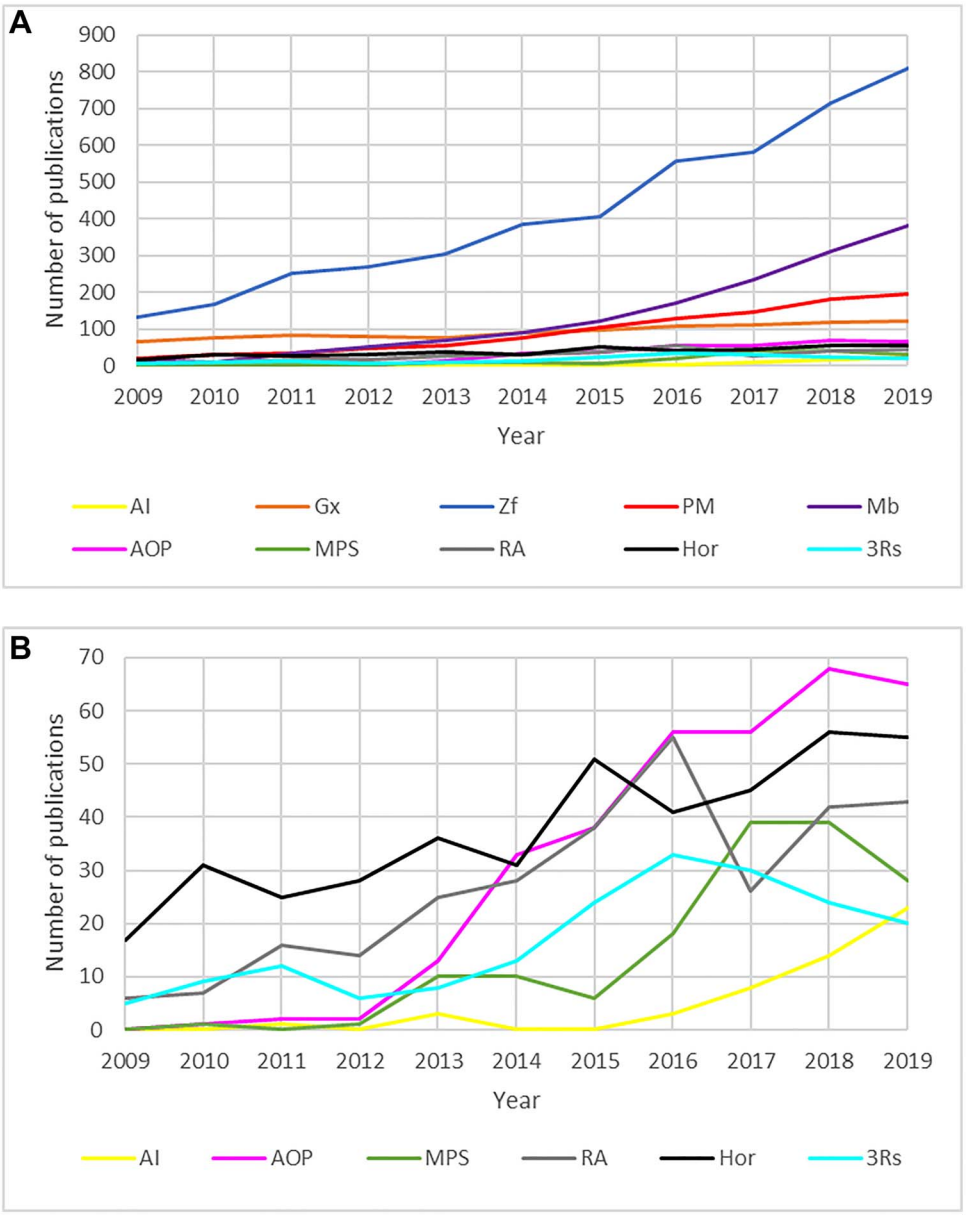


Figure 2: Trends in publications related to toxicology from 2009 to 2019 (**a**) Total number of publications in all concepts per year; (**b**) concepts with <70 publications in 2019.

testing [11]. It will be interesting to see if this potential new application raises genomics up the rankings from its stable slot at position 4.

Microbiota

The microbiota refers to the specific microorganisms found in an environment usually in the context of the skin or gut [12]. The microbiome describes the collection of genomes from all the microorganisms that are found in these environments [13]. The importance of the microbiome in toxicology is mirrored by the substantial increase in publications in the field over the last decade. Drug metabolism by gut bacteria can affect efficacy and safety profile of drugs [14]. The considerable variation in the microbiota from individual to individual may also account for the variation in drug metabolism [15]. A notable discovery is the role of commensal bacteria in modulating antitumor efficacy of some immunotherapies and chemotherapies that has been shown in several animal models [16–19]. These studies have highlighted that bacteria-mediated interactions with patient's immune systems are essential for optimal drug efficacy. It has been shown that specific gut bacterial species can influence the outcomes of therapies in cancer patients [20–22]. These findings demonstrate that a patient's microbiota should be considered when assessing therapeutic intervention because their drug response may depend on their microbiota composition. Over the last 10 years, there has been an explosion in microbiome-related publications in the toxicology field, from only 14 in 2009 to 382 in 2019 (Table 2), although surprisingly the ranking of the microbiome at position 2 has remained constant since 2014 (Fig. 1). As a relatively new player in the toxicology field, the impact of what

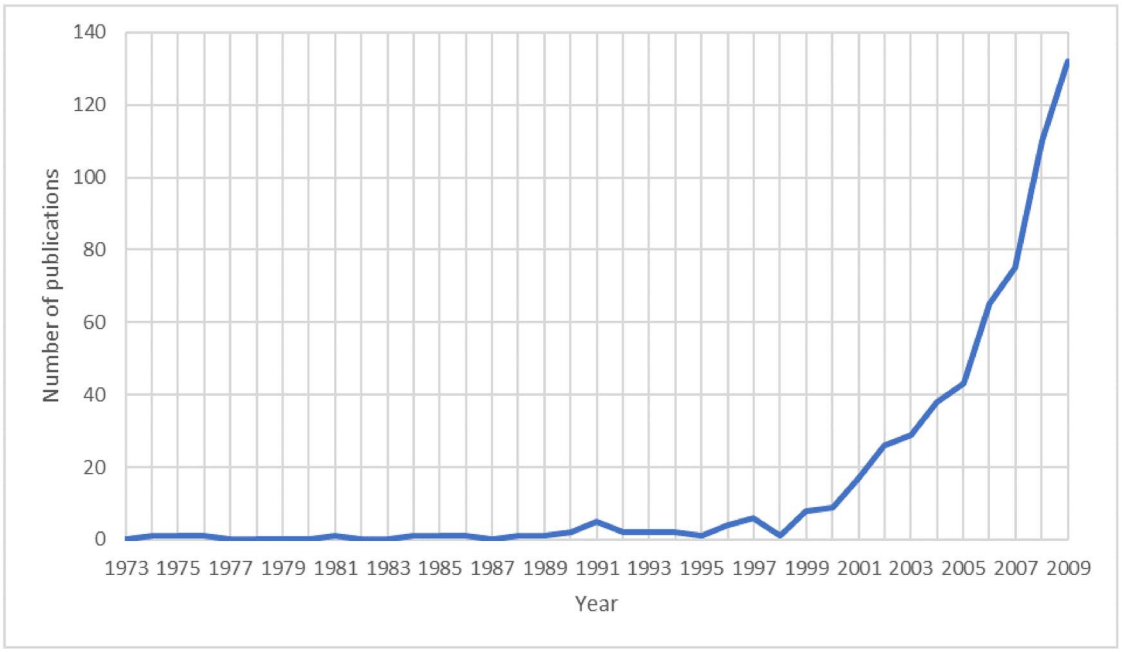


Figure 3: Number of Zebrafish publications 1973–2009.

the human microbiome may have on drug efficacy and safety during development is yet to be seen.

Personalized medicine

Personalized or precision medicine is a treatment methodology that uses new approaches to manage patient's health and tailor therapies based on the individual patient based in their predicted response or risk of a disease, making the treatment as individualized as possible [23]. Toxicologically relevant personalized medicine publications have undergone a substantial growth since 2009 with the increased availability of clinical, genetic, genomic and environmental information from patients. In toxicology, these data help to determine the underlying mechanisms of disease so that the right drug and dose can be selected [24,25]. An individual's response to drugs depends not only on the pharmacodynamics but also pharmacokinetics, which are specific to the individual [24]. An application of personalized medicine in toxicology is CYP 450 genotyping. This test can determine the rate at which certain types of medication will be metabolized by an individual, which is dependent on their genetics. This information can be used to create specific dosing regimens to reduce adverse effects and improve drug efficacy [25].

Personalized medicine has moved around the top four slots over the last decade, stabilizing in third place for the last 5 years, behind the microbiome and zebrafish (Fig. 1). This perhaps suggests that interest in personalized medicine within toxicology has reached steady state.

Hormesis

Hormesis is a term used in toxicology to describe a biphasic dose response with a low dose causing stimulation or a beneficial response and a high dose being inhibitory or toxic [26]. Hormesis has a long history in the work of Arndt and Schulz in the 1880s but more recently there is evidence showing that some anti-anxiety and anti-seizure drugs have hormetic biphasic dose–response relationship [27]. These findings suggest that hormesis should play a significant role when assessing toxicology risk of drugs. However the notion that hormesis is a significant enough issue to be used in all toxicological risk assessment ignores other well-established factors relating to exposure and human susceptibility [28]. The number of hormesis publications per year over the last decade has seen a slow decline. This suggests that hormesis is becoming less important in toxicological research, and that toxicologists have deemed that there is not enough evidence to support the concept being an important part of chemical risk assessment.

Read-across

Read-across is a predictive technique based on the principle that substances with similar chemical structures will have similar properties and thereby have similar toxicokinetic and toxicodynamic properties. Therefore, experimentally derived toxicological data from one substance, referred to as the source chemical, can be read-across to fill the data gap for a second substance, the target chemical, if it has a similar chemical structure reducing the need for an additional toxicology study [29]. Read-across can save time, money and avoids additional animal testing making it a desirable tool in toxicological assessment. The number of read-across publications have increased gradually from 2009 until 2016, many of them describing and assessing the capabilities of read-across as a tool in risk assessment. This growth in publications may be as a result of the establishment of the REACH regulation that provided specific information requirements and guidelines for applying read-across methods [30]. The post-2016 drop in publications may be indicative of less activity in the research area as it has become a well-established methodology in toxicology.

Adverse outcome pathway

AOPs are tools used in toxicology to illustrate the mechanistic basis of toxicological effects in human risk assessment. AOPs

begin with an initiating event followed by a series of intermediate key event steps resulting in an adverse outcome [31]. They are predictive tools that use existing knowledge regarding the linkage between a specific molecular event and an adverse outcome [32]. From the first AOP publications in 2012, there has been a steady increase, as the demand has grown for higher throughput assessment of chemicals with greater accuracy whilst minimizing animal use [33]. AOPs are useful in understanding the mode of action of organ toxicity; there are established AOPs for chemical-induced skin sensitization, cholestasis, liver fibrosis and liver steatosis that have been proven effective in developing novel *in vitro* toxicity screening tests [31]. A battery of assays based on the AOP has been developed to detect the intermediate key events that would lead to the skin sensitization adverse outcome, providing a non-animal route for skin sensitization testing [34]. Although there are concerns that AOPs oversimplify the complexity of biological systems and cannot model multiple stressors, as even a single compound may involve multiple AOPs [33].

3Rs

The concept of the 3Rs were introduced by Russel and Burch in 1959 though publication of 'The Principles of Humane Experimental Technique'. The ideology of replacement is the implementation of methods that avoid or replace the use of animals in research wherever possible. The use of animals was understood to be essential in drug development so reduction is the idea to reduce the number of animal used either by using methods that enable researchers to obtain comparable levels of information from fewer animals or to obtain more information from the same number of animals. Refinement of methods of animal use include minimizing potential suffering and enhancing the animal welfare standards [35]. Animals are used in the safety and efficacy testing of all new drugs in order to meet regulatory requirements and to safeguard human health [36]. Prior *et al.* [37] amongst others have hypothesized whether data from a single species with supplementary *in vitro* data could be justified for general toxicology studies without compromising human safety. However, until there is substantial evidence to support this notion and regulatory authorities have accepted alternative toxicology testing methods it is unlikely that the growth in *in vitro* toxicology will impact on the number of animals used [36]. The toxicologically relevant publications on the 3Rs showed an upturn in 2013 (Fig. 2b), increasing until 2016 but then followed by a decline. This may seem surprising since interest in and adherence to the 3Rs remains highly topical. However, the trends we noted could suggest that the 3Rs as a concept is now embedded in research approaches and that it is not in itself a topic of research.

Microphysiological systems

Microphysiological systems (MPS) are advanced *in vitro* models including systems such as interacting organs-on-chips and 3D organ constructs bioengineered using human cells [38]. The interconnection of organs-on-chips can be used as *in vitro* models to support physiologically based pharmacokinetics and drug discovery and screening [39]. The first publication that described MPS in a toxicology-relevant context was in 2010; since then there has been a gradual increase in publications as MPS technologies advance, making them more relevant for toxicology. The availability of predictive human-based *in vitro* models would reduce preclinical animal testing and help reduce attrition due to lack of inter-species extrapolation [40]. MPS can be used throughout drug development in predictive screens, assessing relevance of *in vivo* findings and helping to define mechanisms of action [41]. However, the complexity of organ toxicity that involves many factors and interplay of different cell types over a long period of time have resulted in the issue being very difficult to address *in vitro*. This has resulted in a slow transition of MPS from the bench into toxicologically relevant assays predictive enough to reduce the need for preclinical studies [40]. This may explain the limited growth and recent decline in the number of MPS publications in the field of toxicology, as MPS have not had a major impact yet. However, the use of MPS in predictive toxicology risk assessment is expected to grow to provide more human-relevant and predicative assays to indicate safety profiles earlier with more certainty.

AI

Machine learning is an area of AI that uses complex algorithms to learn from and make predictions based on already available data [42]. Advanced AI tools can mimic cognitive functions to make predictions using Big Data with high accuracy making it a valuable means of chemical toxicity evaluation [43]. Automated systems can use extensive volumes of molecular profiling data to assess toxicity trends, predict associations and explain adverse outcomes efficiently and accurately [44]. AI-driven *in silico* prediction minimizes risk, reduces cost and allows higher throughput than preclinical and clinical studies. As the data continue to expand and the accuracy continues to improve and the reliance on preclinical and clinical studies may decrease [44]. There have been significant developments in the predictive ability of the AI tools available; however, due to the complexity of toxicology there is some way to go. For example, predictive carcinogenicity models are not precise or reliable enough to completely replace *in vitro* or *in vivo* studies [43]. Within toxicology, discussions on AI seem to have been quite recent (2016 onwards); however, the data show that AI has been around, dropping in and out of the top 10 since 2011.

Future Directions

Our data confirm that toxicology, like other fields, has scientific trends that come and go with time. However, what is surprising is the dominance of certain concepts such as zebrafish over the entire period and that concepts like AI are not as new as one might think. Perception also suggests that 'tool/technology' trends such as genomics, zebrafish and read-across may dominate but that does not appear to be the case with concepts such as the microbiome and personalized medicine taking high-ranking positions. One notable trend is that the top four slots have been static over the past 4 years, suggesting that new ideas are introduced and increase in popularity until they find their place in scientific culture. This may suggest that relatively new entries such as AI have yet to find their steady state in the rankings.

It is interesting to speculate on what new trends will emerge over the coming decade. Tools/techniques such as CRISPR–Cas9, image analysis and next-generation sequencing (NGS) may feature heavily in the general published literature but do not yet feature highly in toxicology publications. For example, CRISPR-Cas9 has been highly prevalent in the literature since around 2017, after the seminal paper from Hendel *et al.* in 2015 [45]. However, there remain very few papers on CRISPR in toxicology. Similarly,

NGS is highly represented in other fields such as human disease research but is less so in toxicology. This suggests that it takes time for these trends to be picked up and used in our field.

Regarding research priorities, it may seem inappropriate to put too much resource into developing tools such as genomics or CRISPR at the expense of applied and/or hypothesis-driven research aimed at decision-making. Overall, our data show an approximate balance between tools and concepts, at least in terms of top-ranking publications. Interestingly, investment and interest in tools such as AI and MPS may be relied on to drive the field of toxicology to its next discoveries since as stated by noble prize winner Sidney Brenner, 'Progress in science depends on new tools, new discoveries and new ideas, probably in that order' [46].

For the future, it may be useful to conduct a second study that evaluates more trends (beyond the 10 explored in this study) and possibly also looks at fluctuations in disciplines within toxicology such as hepatotoxicity, cardiotoxicity and environmental toxicology. These trends, topics and areas could be derived from an open questionnaire to give a more accurate, evidence-based idea of opinions in the field. Such further insights into trends in toxicology could be of great interest generally but of particularly significance for early career scientists as they make important life choices.

Conflict of interest statement. None declared.

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