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Quantifying the pharmaceutical industry's contribution to published 3Rs research 2002–2012

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This project explored the impact of the pharmaceutical industry's contribution to published papers relevant to the 3Rs (reduction, refinement and replacement of the use of animals in research) nearly half a century after the publication of the Principles of Humane Experimental Technique (Russell and Birch 1959). Specifically, the PubMed database was used to search for all papers with an explicit 3Rs objective that were published during the years 2002, 2007 and 2012. Overall, 433 papers with a 3Rs objective were identified in the 3 time periods analysed; there was little change in the total number of published papers in the first two time periods tested (2002, 2007) but this was followed by a substantial (55%) rise in the latter time period (2012). Within this total of 433 papers, the number of published 3Rs papers with industry involvement increased from 20 (2002) through 30 (2007) to 39 (2012). Additionally, the proportion of 3Rs papers involving academia and industry collaboration increased from 40% in 2002 to 61.5% in 2012; the number of multiple affiliation papers also rose during the time period. Other notable trends were the increase in contract research organisation (CRO) involvement in 3Rs research and a slight increase (10%) in the latter time period in those papers describing and presenting original data rather than review/discussion papers. In summary, the reduction, refinement and replacement of animal testing in pharmaceutical drug development depends upon continued and increased collaboration; the data reported herein clearly demonstrate an increased contribution by the pharmaceutical industry to the 3Rs objective along with increasing collaborative efforts between industry and academic institutions.

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Introduction

The Three Rs (3Rs) are a set of principles relating to the ethical use of animals in scientific research.[1] They are the Replacement (the use of non-animal methods over animal methods where possible), Reduction (the use of the minimum number of animals required to achieve the scientific objective) and Refinement (minimising the amount of pain, distress or suffering experienced by the animal or enhancing its welfare) of the use of animals in research.

In the UK, the use of animals in scientific research is regulated under the Animals (Scientific Procedures) Act (ASPA) 1986[2] which permits studies to be conducted using animals only if certain criteria are met. ASPA has recently been revised to transpose European Directive 2010/63/EU[3] on the protection of animals used for scientific purposes and the revised legislation came into force on 1 January 2013. The 3Rs principles are embedded in ASPA – scientists are legally obliged to use non-animal methods where possible, to use the minimum number of animals and to use research protocols that minimise pain, suffering or distress to animals. In this context the EMA's revised paper on replacement of animal studies by *in vitro* models[4] provides information on the conditions and strategy for regulatory acceptance of 3R alternative methods.

In the UK and elsewhere, there are two major scientific sectors within the scope of the 3Rs, academia and industry both with different drivers, aims and needs. Within academia animal usage is almost exclusively for experimentation aimed at generating new knowledge whereas within the pharmaceutical industry an additional component of usage is driven by the need to meet regulatory requirements for safety testing.[5,6] Since assessing efficacy and safety in animals is key to the development of new medicines, the pharmaceutical industry is a key stakeholder in replacement and reduction of animal testing. But how much is this sector contributing to the aims of the 3Rs?

Here we present an analysis of 3Rs publications nearly half a century after the publication of the Principles of Humane Experimental Technique[1] in order to understand the impact of

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the pharmaceutical industry's contribution. We also analyse the collaborative nature of this work and trends in contract research organisation (CRO) involvement.

Methods

The PubMed database[7] was used to search for all papers with a specific 3Rs objective focused on the development of pharmaceutical products. Those solely associated with chemical or cosmetic products and those regarding pharmaceutical products not intended for human use (such as livestock) were excluded. Three time periods (2002, 2007 and 2012) were selected on the a priori assessment of developments in the field of applied life sciences and increasing awareness of the 3Rs principles nearly half a century after the publication of the Principles of Humane Experimental Technique.[1]

In order to pinpoint papers relevant to the 3Rs subject, the PubMed advanced search filters "Title/Abstract" were used in conjunction with appropriate 3Rs terms as described previously[8] with some modifications (summarised in Table 1).

Table 1 A list of search terms used in the analysis. Asterisks depict truncation and were used to capture multiple derivatives of the term

Search Terms: *3Rs terms*
"3Rs"
"Three Rs"
"replacement" + "reduction" + "refinement"
"Animal testing" + "alternative*"
"Animal*" + "testing*" + "reduction"
"Animal*" + "testing*" + "refinement"
"Animal*" + "testing*" + "replacement"
"In vitro" + "animal*" + "alternative*"
"toxicity" + "animal*" + "alternative*"
"animal*" + "husbandry"
"animal*" + "in silico" + "alternative*"
"alternatives to animal testing"
"animal*" + "welfare*" + "laboratory*"
"animal*" + "experimentation*" + "alternative*"
"Pharmaceuticals*" + "animal*"
"Pharmaceutical*" + "animal*" + "alternative*"
"animal*" + "laboratory" + "well being"
"Russell" + "Burch"
"housing" + "animal*" + "laboratory*"
"housing" + "animal*" + "well-being"
environmental enrichment + "animal*"
"in vivo" + "animal*" + "alternative*"
"acute toxicity" + "animal*"
"acute systemic toxicity"
"whole embryo culture"
"*animal**" + "*humane*"
"*reduce*" + "*refine*" + "*replace*"
Extra journals searched
"*ATLA journal*"
"*ILAR Journal*"
"*Developments in biologicals*" *journal*
"*Contemporary topics in laboratory animal science*" *journal*
"*Journal of applied animal welfare science*"
"*ALTEX*"
MESH terms
Mesh term - "*animal testing alternatives*"
Mesh term - "*animal use alternatives*"
Mesh term - "*animal welfare*"

The PubMed advanced search filter "MeSH Terms" was also used where MeSH terms refers to Medical Subject Headings (specific words or phrases tagged/attached to papers by the authors).

Targeted searches were also conducted in journals focusing on 3Rs such as ATLA, ILAR, Developments in Biologicals, Contemporary Topics in Laboratory Animal Science Journal, Journal of Applied Animal Welfare Science and ALTEX.

Once papers were identified and compiled, the PubMed advanced search filter: "Date-publication" was used to quantify papers published in 2002, 2007 and 2012. Author affiliations listed on the PubMed site were used and verified using journal websites together with Company websites to classify whether an organisation was 'industry', 'non-industry' or 'contract research organisation' (CRO). If this was not clear this field was marked as 'unknown'. For the analysis of industry *versus* non-industry, 'CRO' was categorised as a subset of 'industry'.

Neither live animals nor human subjects were used in the study reported in this paper.

Results

Overall number of papers

In total, 433 published papers relevant to the pharmaceutical industry and with a specific 3Rs objective were identified from all sectors across the 3 time periods tested; there was little change in the total number of published papers at the first two time periods selected (2002 and 2007), but this was followed by a substantial (55%) rise during the third period in 2012 (Fig. 1A).

Of the total of 433 3Rs papers identified, 89 (21%) had industry involvement. Over the three time periods analysed, the number of 3Rs papers with industry involvement increased from 20 (2002) through 30 (2007) to 39 (2012) (Fig. 1B), although when expressed as a percentage of all papers there was no clear trend over the 10 year period (2002: 16%; 2007: 26%; 2012: 20%). Those publications where industry involvement could not be defined remained low (0–1.7%) over each of the time periods analysed (Fig. 1C).

Trends in pharmaceutical industry collaboration and CRO involvement

Of the 89 3Rs papers with industrial involvement, there were 39 published by industry alone (defined as no non-industrial authors). However, the percentage went down from 12/20 (2002) through 12/30 (2007) to 15/39 (2012). Thus, there was an increasing trend by industry to work collaboratively with non-industrial partners from 40% of papers in 2002 to 61.5% in 2012 (Fig. 2A).

CRO involvement equally increased from only 2 papers of the 20 (10%) in 2002 to more than 18 of the 39 identified with industrial involvement (46%) by 2012 (Fig. 2B).

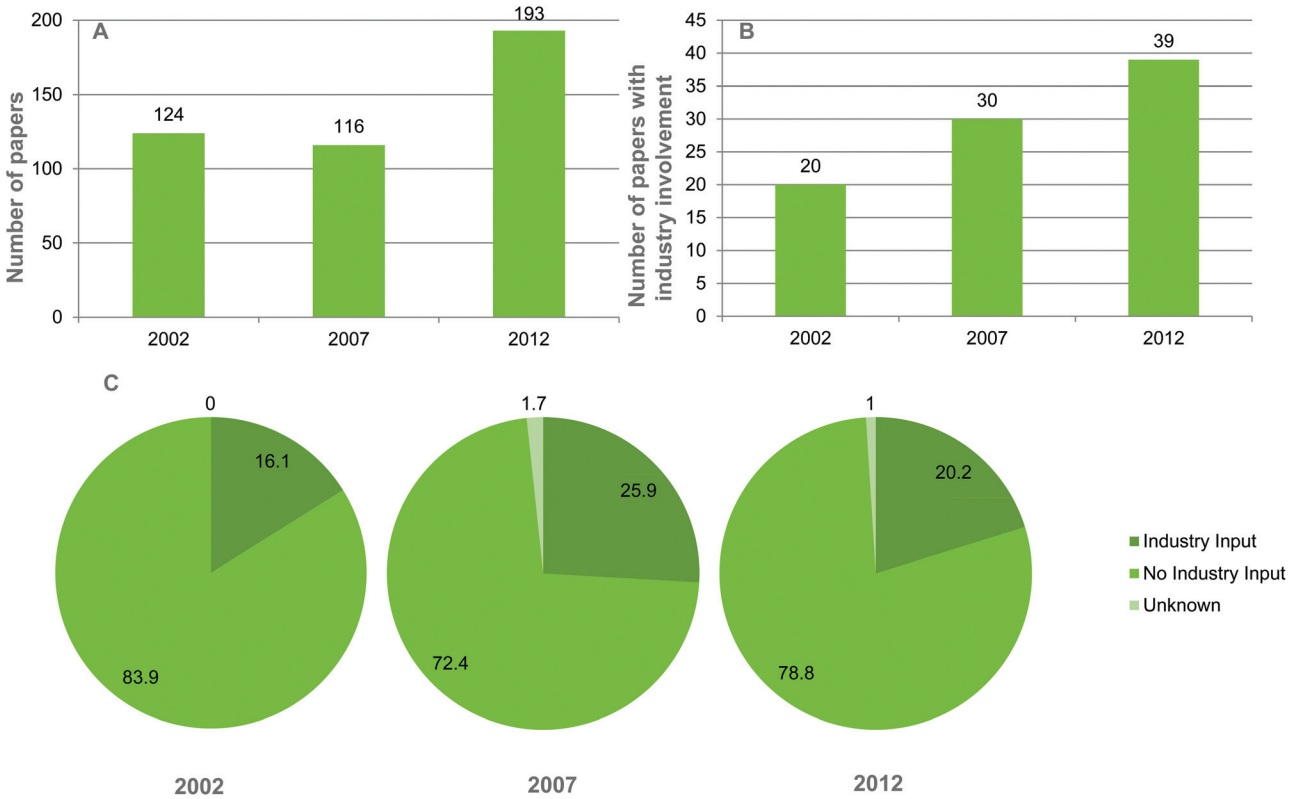

Fig. 1 Papers relevant to pharmaceutical drug development and the 3Rs show as total (A) and those with pharmaceutical industry involvement (B) over 3 time periods (2002, 2007 and 2012). Pie charts depict the percentage of 3Rs papers in 3002, 2007 and 2012 with industry involvement, with no industry involvement or where it could not be defined (C).

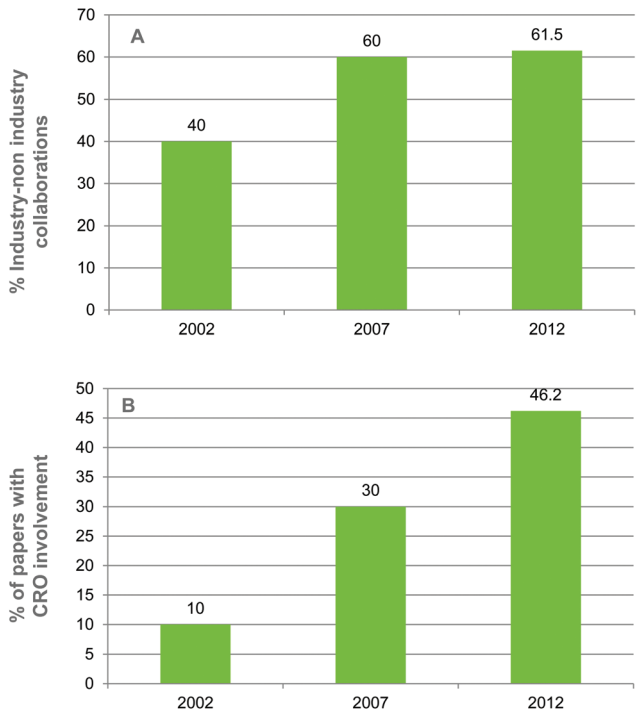

Fig. 2 Trends in papers relevant to pharmaceutical drug development shown as a percentage of papers with pharmaceutical industrial collaboration (A) and CRO involvement (B) over three time periods (2002, 2007 and 2012).

Publication of original research *versus* review articles

Of the 89 published 3Rs papers with pharmaceutical industry input, there was a 10% increase between 2007 and 2012 in the proportion of papers describing original scientific results compared with review papers (Fig. 3A). This was paralleled by a decrease in the number of review papers.

Multiple affiliation papers

An analysis of the affiliations of authors on the 89 papers with pharmaceutical industry involvement also showed a steady increase in multiple affiliations (Fig. 3B). Papers with >2 affiliations increased from 1/20 (5%) in 2002 through to 17/39 (43%) in 2012. Similarly, papers with >4 affiliations increased from 0/20 (0%) in 2002 through to 12/39 (30%) in 2012.

Discussion

The 3Rs form a very important framework to challenge and guide the use of animals in biomedical research. Together with the legislative framework provided by ASPA (1986),[2] the 3Rs ensure that all experimentation is justified by its likely benefit and that animal use is minimised and managed accordingly. There are two major sectors in the scope of the 3Rs, academia and industry, both with different drivers, aims and needs. Within the pharmaceutical industry, one

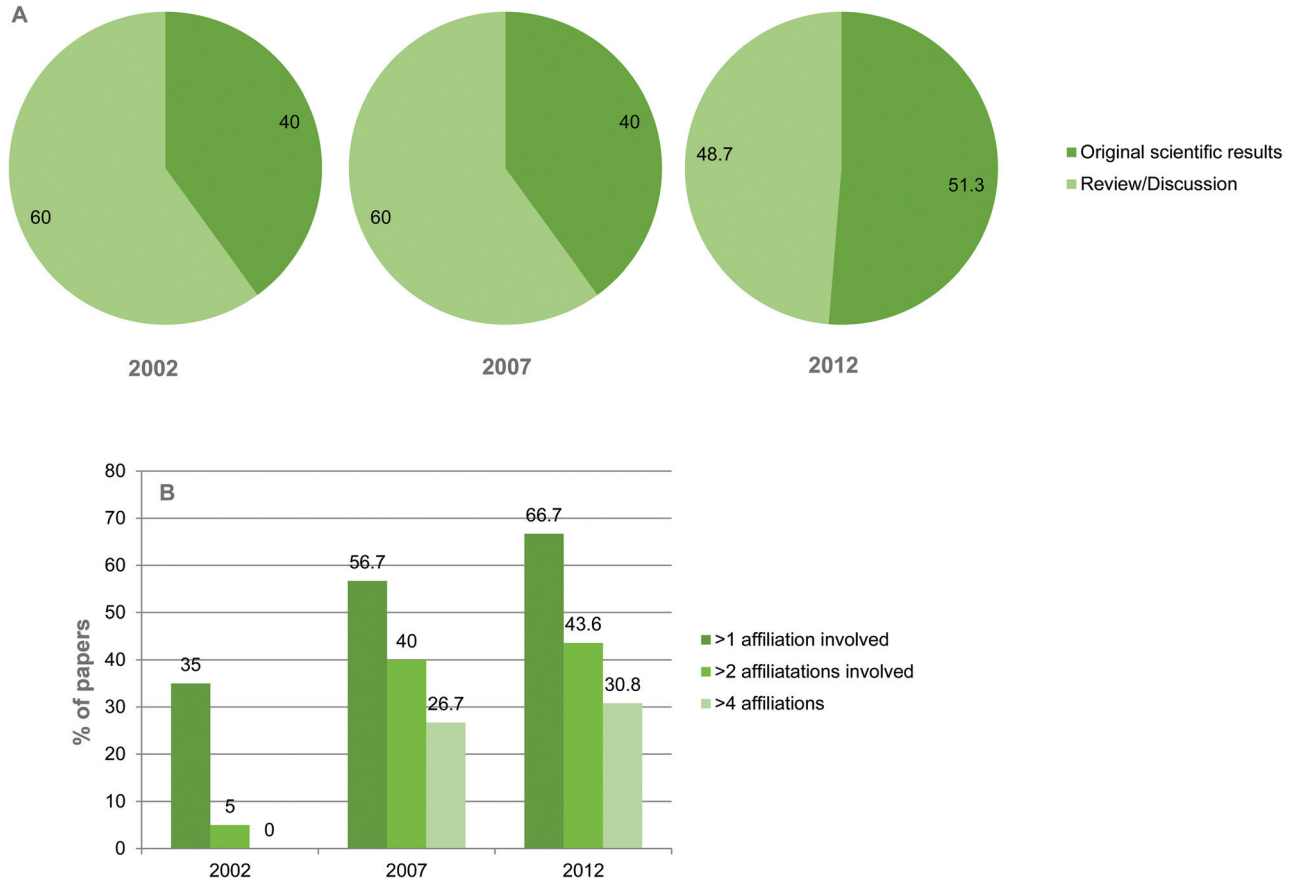

Fig. 3 Trends in original scientific results *versus* review/discussion papers (A) and trends in multiple affiliations (B) for those papers relevant to pharmaceutical drug development and with pharmaceutical industry input over three time points (2002, 2007 and 2012).

component of usage is driven by the need to meet regulatory requirements for safety testing.[5,6] Since assessing efficacy and safety in animals is required in the development of new medicines, the pharmaceutical industry is a key stakeholder in replacement and reduction of animal testing. But how much is this sector contributing to the aims of the 3Rs?

Industry presentations at meetings together with published abstracts and papers would suggest a substantial effort in the in-life experimental work aimed at replacing or reducing numbers of animals. Here, we have analysed publications relevant to the pharmaceutical industry with a explicit 3Rs objective between 2002 and 2012 as a metric for the impact of the pharmaceutical industry's contribution towards the 3Rs initiative.

PubMed was selected as the search engine for this project for its ease of use, advanced filtering functions and broad coverage. Preliminary research (data not shown) showed that the use of additional public databases such as Science Direct and TOXNET did not yield substantial additional data to that found on PubMed. Regarding search terms, Hooijmans and colleagues[8] have described methods for searching for 3Rs; this provided an initial starting point to identify papers, but search terms were added and modified through preliminary research to ascertain which words and phrases were best at delivering papers with an explicit 3Rs objective within the scope of this project.

Inclusion criteria covered those papers with a clear 3Rs focus on the development of pharmaceutical products but not those solely associated with chemical or cosmetic products nor those regarding pharmaceutical products not intended for human use (such as livestock). This exclusion ensured that the results were clearly focused on the question of the pharmaceutical industry's engagement with the application of 3Rs objective. All categories of author affiliation were included except those authored solely by consultants since the sponsor or affiliation was unclear. Papers focused on regulatory changes were included providing the impact was EU wide such as Directive 2010/63/EU.[3] Papers addressing regulatory changes for non-EU countries were not included.

Overall, the data show that involvement by all organisations in 3Rs has been steadily increasing over the last decade. Although pharmaceutical industry involvement as a proportion of all papers published has remained steady, industry's involvement has increased during the last decade following the overall trend of an increased number of papers in 3Rs. Importantly, there has been an increase in the level of industry/non industry collaboration over the decade showing increased partnering with academia in research. Indeed, the results show an

increase in collaboration between all kinds of organisations. Notably, there has been a substantial increase in multiple affiliations, revealing that organisations are increasingly working together towards implementing the 3Rs – sharing ideas, expertise, funding and findings.

Another trend observed in the analysis was the increase over the decade in the number of papers whose authors involved CROs. This is likely driven by two key factors; an increasing tendency of the pharmaceutical industry to outsource its preclinical research to CROs but also an independent engagement of the CROs in driving best practice and new methodologies in animal research. This could also be a reflection of the increase in incubator and start-ups with limited in-house knowledge of the R&D process required for the research and development of medicines.

A number of challenges were encountered in reliably assessing pharmaceutical industry contribution to the 3Rs. Although affiliations were identified for all authors, it was not always possible to verify these independently without payment. In some cases there was no English language website. In these few cases the details made available on PubMed were accepted as correct. Occasionally it was difficult to determine with certainty if a contributing affiliation could be categorised as a CRO; in these cases best judgement was used based on the information available on websites. Another question is whether basing the search on free-to-use and public databases could have limited the search results compared with using pay-for-databases. Nevertheless, we assessed only those papers with open-access, since this is increasingly a source though which authors are publishing papers. Overall, the most important challenge was the risk of underestimating the contribution to the 3Rs; despite the approaches taken to expand and refine the 3Rs search terms used, we have inevitably overlooked relevant papers since authors have used scientific terms rather than recognisable 3Rs terms.

As well as the numerical assessment of the increasing contribution of the pharmaceutical industry to the 3Rs presented here, some specific examples of contributing towards the 3Rs should be highlighted. For example, the *in vitro* micronucleus test for the detection of clastogenic and aneugenic chemicals has progressed from origination[9,10] through to implementation *via* the OECD guideline[11] largely due to a long standing and ongoing academia-industry collaboration as documented by a number of key publications. Notably, the pivotal paper[12] has some 12 authors originating from the UK, the EU, the USA and Japan with around a 50 : 50 split of affiliation from academia and industry. As well as allowing for early *in vitro* detection and screening out of potentially clastogenic and aneugenic compounds, the micronucleus test can provide information on the mechanisms of chromosome damage and micronucleus formation, informing and guiding drug discovery programmes.

In another more recent example, the initiative led by AstraZeneca and the NC3Rs and involving more than 15 Pharmaceutical Companies has challenged the regulatory requirement for acute toxicity testing in pharmaceutical drug development,[13] leading to an eventual change in ICH and EMA guidelines. More recently, academia and the pharmaceutical industry is collaborating to consider the value and need for recovery animals in pre-clinical toxicology testing[14,15] and also the value of the second species in predicting human safety.[14–18]

A driver to note is that many of the on-going *in vitro* initiatives to formally validate alternative and *in vitro* tests are focused on pharmaceuticals as illustrated by Chapman *et al.*[19]– thus the increase in 3Rs publications from the pharmaceutical industry is explained by an increased push in this area. These authors also highlight that sharing cross-company experiences may more readily identify the predictive assays that have been accepted, those that have failed those that merit further validation. Such a cross company data sharing is the focus of an ongoing analysis recently conducted by the ABPI (Roberts *et al.*, manuscript in preparation).

Although there is a shared ambition to use alternatives to animals, replacement depends upon reliable and relevant models. There have been notable successes with *in vitro* test methods validated and accepted in key areas such as genetic toxicology, skin absorption and reproductive toxicology.[19] But there is still much to achieve in this area; many of the *in vitro* assays that have been developed in areas such as genetic toxicology and electrophysiology together with endpoints such as neutral red can give a high level of false positive results which makes extrapolation to the human situation difficult. Additionally, the reliability of *in silico* testing to predict safety signals has been called into question in a recent paper from Cook *et al.* (2014).[20]

The trend towards increased collaboration we have described is of course not just restricted to the 3Rs; it has been widely recognised that success in bringing new medicines to patients will depend upon identifying new sources of knowledge and expertise *via* collaboration.[21] The pharmaceutical industry has a pressing need to improve success rates and also to make failure less costly perhaps *via* the development and validation of higher throughput *in silico* and *in vitro* laboratory tests that could address the main reasons for failure: unexpected toxicity and/or lack of efficacy. This is a key driver for academically-led research aimed at the identification and application of *in vitro*-based tools for use by industry.

The pharmaceutical industry now front-loads toxicity testing, using *in silico*, *in vitro*, and less demanding animal tests at earlier stages of product development to identify and anticipate undesirable toxicological effects; recently an emerging discipline called Green Toxicology[22] has drawn on the experience of the Pharmaceutical Industry to suggests a framework to design safer chemicals by applying some structure/activity rules (SAR) and *in silico* methods to consider possible toxicities before actual synthesis. Overall, current and future emphasis across all industries is in using 21st century toxicology tools hand-in-hand with the 3Rs principles as a preventative strategy to design out undesired human health and environmental effects, increasing the probability of launching a successful, sustainable product.

In summary, animal research is a small but necessary component of the overall R&D process that brings new medicines to patients and the physicians who treat their patients. Moreover, the regulatory environment identifies animal safety testing as a requirement to ensure the safety of patients and volunteers. The data presented demonstrate an increased contribution to the 3Rs and an increased collaboration between the pharmaceutical industry and other institutions. The analysis we have described highlights the reduction, refinement and replacement of animal testing in pharmaceutical drug development as an increasingly important component of the Discovery and Development process that requires continued and increased collaborative working towards a common goal of better science for better medicines.

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