Regulation needs to catch up with innovation.
Henry Paulson
(American public servant, 1946-)

Not all chemicals are bad. Without chemicals such as hydrogen and oxygen, for example, there would be no way to make water, a vital ingredient in beer.
David "Dave" McAlister Barry
(American author, 1947-)

Food for Thought ...

Animal Testing and its Alternatives – the Most Important Omics is Economics

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Abstract

For a long time, the discussion about animal testing *vs* its alternatives centered on animal welfare. This was a static warfare, or at least a gridlock, where life scientists had to take a position and make their value choices and hardly anyone changed sides. Technical advances have changed the frontline somewhat, with *in vitro* and *in silico* methods gaining more ground. Only more recently has the economic view begun to have an impact: Many animal tests are simply too costly, take too long, and give misleading results.

As an extension and update to previous articles in this series written a decade ago, we reanalyze the economic landscape of especially regulatory use of animal testing and this time also consider respective alternative tests. Despite some ambiguity and data gaps, which we have filled with crude estimates, a picture emerges of globally regulated industries that are subject to stark geographic and sectorial differences in regulation, which determine their corresponding animal use. Both animal testing and its alternatives are industries in their own right, offering remarkable business opportunities for biotech and IT companies as well as contract research organizations. In light of recent revelations as to the reproducibility and relevance issues of many animal tests, the economic consequences of incorrect results and the reasons for still maintaining often outdated animal test approaches are discussed.

1 Introduction

About 10 years ago, work by Annamaria ("Antonella") Bottini in the context of a thesis at the London School of Economics, Milan Campus, co-supervised by one of the authors (T.H.), led to a series of publications that addressed economic aspects, globalization, and the regulatory acceptance process of animal testing and its alternatives (Bottini et al., 2007, 2008; Bottini and Hartung, 2009, 2010). Astonishingly, there was hardly any study reported before that addressed this aspect of the debate and the articles in this series, especially Bottini and Hartung (2009), continue to attract many readers: As one indicator, about 10% of the 70,000 reads of articles by T.H. on ResearchGate are owed to this article. Economic aspects actually constitute an important driver for change (Hartung, 2017c), which should prompt the strategic development of safety sciences (Busquet and Hartung, 2017).

Now, it seemed timely to update these figures. In many instances these are simple updates, e.g., the animal use figures in Europe from 2005 were adapted to the most recent available values, i.e., 2011. The costs of animal tests had relied on Fleischer (2007) and were updated now with data from our own survey. Very often, websites served as sources, such as those of trade associations or simply Wikipedia. We cannot quality-control these sources and where discrepant data are found, they are simply presented as such. Some discrepancies arise as different reporting years are found in the sources.

In general, data from the last decade were considered adequate. The restriction to sources in English and German leads

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to an unavoidable trans-Atlantic focus and often only rough estimates of the situation in South-American or Asian countries and elsewhere is possible. All numbers are given in US$ or € and, where necessary, a conversion rate of 1.3 was used, which was roughly the exchange rate earlier in this decade when most reports used here originated. The report is structured by product sectors and this time also includes medical devices and tobacco products, the latter mainly because of the emerging testing needs for lower risk tobacco products.

We focus again on regulatory testing and product development, i.e., the industrial uses of animal testing and its alternatives. Regulatory testing is still a necessary evil. Dee Hock, the former CEO of the Visa credit card association said, "*Heaven is purpose, principle, and people. Purgatory is paper and procedure. Hell is rules and regulations*." With notable differences in approaches on both sides of the Atlantic, Europe has become a pacemaker of regulation with international consequences, while the US, which was rather light on regulations in many areas already, as will be discussed in more detail, goes even further in this direction under the current administration: To quote the current president, "*Excessive regulation is costing America as much as $2 trillion a year. And I will end it very, very quickly*" (Donald Trump). As a consequence, companies in the US have to worry mainly about product liabilities in court ("Tort Law") (Silbergeld et al., 2015), while in Europe, after more extensive testing requirements, companies are largely protected from such liability claims.

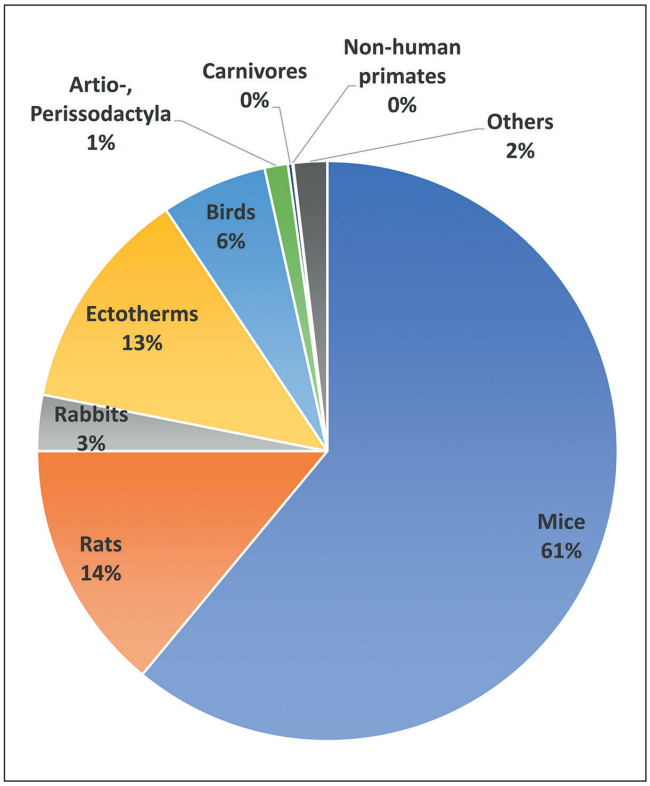

Fig. 1: Number of animals in percentages used in the EU for scientific purposes
Adapted from[1]

2 Animal testing by numbers

Taylor et al. (2008) wrote "*Relatively few countries collate and publish animal use statistics, yet this is a first and essential step toward public accountability and an informed debate, as well as being important for effective policy-making and regulation*". They found estimates of worldwide annual laboratory animal use for 2005 ranging from 28-100 million. Their own estimation, the best we are aware of, collated data for 37 countries that publish national statistics, standardized these against the European definitions and developed a statistical model, based on publication rates, for a further 142 countries. This yielded their most conservative estimate of global animal use of 58.3 million animals in 179 countries as of 2005. By extrapolation, the animals killed for the provision of tissues, animals used to maintain genetically-modified strains, and animals bred for laboratory use but killed as surplus to requirements were added, resulting in 115.3 million animals.

In the EU, the total number of animals used for experimental and other scientific purposes (Daneshian et al., 2015) from the data collected in 2011[1] in accordance with the provision of the Directive for this report is just under 11.5 million (with data from France for 2010). This is a reduction of over half a million animals used in the EU from the number reported in 2008. Notably, the three-year-reporting cycle was interrupted by the introduction of the new Directive 2010/63/EU[2] for the protection of laboratory animals (summarized in Hartung, 2010a); the next statistics will be published only in November 2019 and will likely not be comparable because of the altered reporting standards.

For 2011, as found in previous reports, rodents and rabbits accounted for 80% of the total number of animals used in the EU. Mice were the most commonly used species with 61% of the total use, followed by rats with 14%. The second most commonly used group of animals was, as in previous years, the ectotherm animals (reptiles, amphibians, fish), which represented almost 12.4%. The third largest group of animals used was birds with 5,9% of the total use. The *Artiodactyla* and *Perissodactyla* groups including horses, donkeys and cross-bred animals, pigs, goats, sheep and cattle make up 1.3% of the total number of animals used for scientific purposes in the EU. Carnivores (which include dogs and cats) represent 0.25% and non-human primates represent 0.05% of the total number of animals used in 2011 (Fig. 1).

All websites were accessed in June/July 2018.

[1] http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:52013DC0859&from=EN

[2] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32010L0063

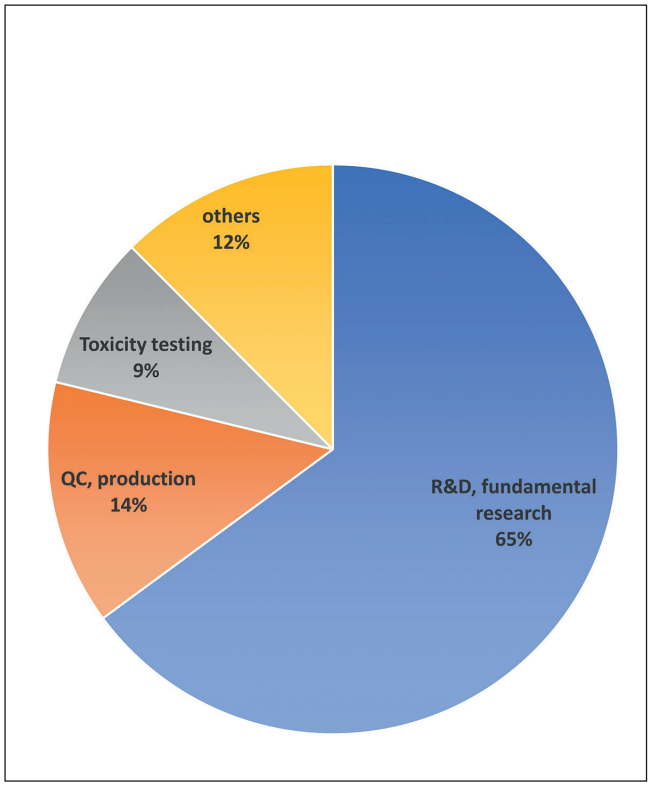

Fig. 2: Animal use for scientific purposes by purpose of the experiments
Adapted from[1]

Tab. 1: Number of animals used for scientific purposes in the USA in 2016[10]

Species	Number of animals	% of total	% change from 2015
Guinea pigs	183,237	22.3%	6%
Rabbits	139,391	17.0%	1%
Hamsters	102,633	12.5%	4%
Non-human primates	71,188	8.8%	15%
Dogs	60,979	7.4%	0%
Pigs	50,226	6.1%	8%
Cats	18,898	2.3%	-5%
Sheep	12,196	1.5%	14%
Other farm animals	40,597	2.5%	0%
Other covered species	161,467	19.7%	24%
Total	**820,812**	**100.0%**	**6.9%**

[10] https://speakingofresearch.com/facts/animal-research-statistics/

Around 65% of animals were used for research and development in the fields of human medicine, veterinary medicine, dentistry, and in biological studies of a fundamental nature. Production and quality control of products and devices in human medicine, veterinary medicine, and dentistry required 14% and other safety evaluation represented 8.75% of the total number of animals used for experimental purposes (Fig. 2).

The data from 2011 still seem to be quite representative as, for example, numbers released every year by the UK Home Office on animal research in Great Britain[3] have remained stably around 4 million and in Germany around 3 million[4] in the years since then.

In the US[5], overall, the number of animals used for scientific purposes covered by the Animal Welfare Act rose by 6.9% from 767,622 (2015) to 820,812 (2016) (Tab. 1). This includes both public and private institutions. These statistics do not include all animals as mice, rats, birds and fish are not covered by the US Animal Welfare Act – though they are still covered by other regulations that protect animal welfare. They also do not include the 137,444 animals that were kept in research facilities in 2016 but were not involved in any research studies. Direct comparison with the EU statistics is impossible as there is no clear relationship. For example, the EU uses proportionally many more rabbits and fewer hamsters than the US and therefore basing a guess of the number of rats and mice used in the US on EU percentages of species would lead to an unacceptably large error. It is possible that the US is actually using many more rodents as such experiments require a minor level of authorization compared to the EU. The pro-animal research advocacy group Speaking of Research stated[5], "*In the UK, where mice, rats, fish and birds are counted in the annual statistics, over 97% of research is on rodents, birds and fish. Across the EU, which measures animal use slightly differently, 93% of research is on species not counted under the Animal Welfare Act (AWA). If similar proportions were applied the US, the total number of vertebrates used in research in the US would be between 12 and 27 million, however, there are no published statistics to confirm this.*"

In Canada[6], in 2016, 4.3 million animals were used in research, teaching, and testing as reported to the CCAC (Canadian Council on Animal Care). The three animal types most often used in

[3] https://speakingofresearch.com/facts/uk-statistics/
[4] https://speakingofresearch.com/facts/animal-research-statistics/german-animal-research-statistics/
[5] https://speakingofresearch.com/2017/06/19/usda-publishes-2016-animal-research-statistics-7-rise-in-animal-use/
[6] https://www.ccac.ca/Documents/AUD/2016-Animal-Data-Report.pdf

2016 were fish (37.2%), mice (34.8%), and cattle (12.2%). The majority of animals (57.3%) were used in studies of a fundamental nature/basic research, representing 2.6 million animals[7]. 14.1% were used for studies for the development of products or appliances for human or veterinary medicine; 12.8% were for studies for medical purposes, including veterinary medicine, that relate to human or animal diseases or disorders; 9.7% were for teaching and training to communicate scientific concepts and develop practical skills and expertise in specific techniques; and 6.1% served for regulatory testing of products for the protection of humans, animals, or the environment.

The total number of animals used in Australia[8] in 2015 was over 9.9 million. South Korea reported 3 million animals in 2017[9] (up 7.2% from the previous year), Norway 11.6 million (notably, two large projects on salmon vaccination alone represented 10.5 million), New Zealand 200,000 in 2015, Israel 1.2 million in 2017 and Switzerland 600,000 animals in 2016[10].

In summary, world-wide animal use appears relatively stable in recent years and the earlier studies (Taylor et al., 2008; Bottini and Hartung, 2009; Daneshian et al., 2015) are not challenged in this respect. An overall market size of about €3 billion for regulatory animal testing as calculated in 2008 is probably still realistic, but we will attempt to refine this below. Quite reassuring, *MarketsandMarkets* released a report on February 15, 2018[11] stating, "*The* in vivo *toxicology market is expected to reach USD 6.14 Billion by 2022 from an estimated USD 4.40 Billion in 2017, at a CAGR of 6.9% ... In 2017, the US is estimated to account for the largest share of the* in vivo *toxicology market, followed by Germany and the UK. However, China is expected to grow at the highest CAGR during the forecast period*". The compound annual growth rate (CAGR) is the mean annual growth rate of an investment over a specified period of time longer than one year.

3 Alternative methods

There are about 50 validated and, in part, accepted alternative methods[12,13,14]. These approved, mainly *in vitro* methods, have not changed gross animal use. Reasons are multiple:

- Most work on alternatives addresses only toxicological tests (7-10% of animal use) (Daneshian et al., 2015).
- The replaced tests were mostly acute and topical tests with relatively small animal numbers.
- The reductions in animals are (over-)compensated by rising numbers in basic research, especially genetically modified mice (Daneshian et al., 2015).
- New regulatory programs such as REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals; EC, 2006) have created additional testing demands.
- The use of alternatives is hindered by non-acceptance in some regions of the world so that global industries have to do the animal test anyway.
- The implementation of alternatives lags behind (see, for example, pyrogen testing, Hartung, 2015)

Overall animal use reduction is probably the wrong metric of success (Leist et al., 2008): Many uses of alternatives are not recorded as such. Outside regulatory toxicology, *in vitro* and *in silico* technologies are indeed being used to a very large extent, and their everyday implementation by far exceeds public perceptions. The performance of the new animal-free approaches is often so high that they have made animal experimentation obsolete or reduced it by 80% and more. In addition to the substitution of *in vivo* testing, the *in vitro* and *in silico* approaches are used as enabling technologies in many fields, sometimes complementing data obtained from animals, sometimes providing for entirely new information. Important use areas comprise among others:

- *Drug discovery:* Since the peak times of animal-based screening in the 1970s and 80s, the use of animals in this area has been reduced by > 80%; there has been, for example, a steady increase in the use of *in vitro* tests by the pharmaceutical industry between 1980 and 2013 (Goh et al., 2015).
- *Non-regulatory toxicology and internal prioritization of drugs:* Non-animal approaches, such as testing for hERG channels, mutagenicity, cytotoxicity, etc. are being used from the earliest stages of chemical profiling; hundreds of methods have been evaluated for specific use within companies, without ever undergoing an official evaluation by EURL-ECVAM, the OECD, or other (inter)national validation bodies.
- *Basic biological research and biomedical research:* Here, the number of animals used per publication has been continuously decreasing over the past 10-20 years, as these approaches have been (i) either completely substituted, e.g., by use of human stem cells, or (ii) as *in vitro* and *in silico* approaches are being used to complement animal data and thus reduce the need for large animal numbers. Put simply, the number of researchers is continuously increasing but animal numbers are largely stable: For example, the number of researchers increased by almost a third (32.2%) in the EU from 2006 to 2016, from 1.42 to 1.88 million[15].
- *Vaccines:* Enormous progress has been made in the areas of safety, quality and efficacy testing of vaccines, where alternatives replace animal-based tests[16,17].

7 https://www.ccac.ca/Documents/Publications/CCAC-Facts-and-Figures.pdf

8 http://www.humaneresearch.org.au/statistics/statistics_2015

9 https://speakingofresearch.com/2018/04/12/rise-in-animal-research-in-south-korea-in-2017/

10 https://speakingofresearch.com/facts/animal-research-statistics/

11 https://www.marketsandmarkets.com/PressReleases/in-vivo-toxicology-testing.asp

12 http://alttox.org/mapp/table-of-validated-and-accepted-alternative-methods/

13 https://ntp.niehs.nih.gov/pubhealth/evalatm/accept-methods/index.html

14 https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance

15 http://ec.europa.eu/eurostat/statistics-explained/index.php/R_%26_D_personnel

16 https://eurl-ecvam.jrc.ec.europa.eu/alt-animal-testing-safety-assessment-chemicals/alt_test_biological_vaccines

17 https://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/biologics-and-vaccines/vaccine-testing/index.html

Tab. 2: Market sizes and estimated growth by *MarketsandMarkets*™
A compilation of study summaries with compound annual growth rates (CAGR) from advertisements of the company is given.

Market	Current worth in billion US$ (year)	Projected worth in billion US$ (in year)	CAGR (%)
High Throughput Screening	13.73 (2013)	19.63 (2018)	7.4
Global Pyrogen Testing	0.46 (2014)	0.82 (2019)	12.2
Global Mycoplasma Testing	0.34 (2014)	0.61 (2019)	14.6
Global Metabolomics	0.57 (2014)	2.10 (2019)	30
Live Cell Imaging	3.57 (2014)	5.45 (2019)	8.8
Global Biologics Safety Testing	1.76 (2014)	3.13 (2018)	12.2
Cell Analysis Market	19.0 (2015)	26.0 (2020)	6.6
Global Cell Culture	11.31 (2015)	18.63 (2020)	10.5
Biochips	7.63 (2015)	17.75 (2020)	14.6
Global Cell-based Assays	10.80 (2015)	18.33 (2020)	11.6
Mouse Model	1.05 (2015)	1.49 (2020)	7.1
Cellular Analysis	28.66 (2016)	41.34 (2021)	7.6
Global Metabolomics	1.03 (2016)	2.39 (2021)	14.6
Biomarkers	27.95 (2016)	53.34 (2021)	13.8
Global Vaccines	32.24 (2016)	48.03 (2021)	8.3
3D Cell Culture	0.47 (2016)	1.35 (2021)	23.6
Global Pyrogen Testing	0.61 (2016)	1.09 (2021)	12.2
Blood Collection	7.65 (2016)	9.90 (2021)	5.3
In Vitro Toxicology Testing	14.15 (2016)	27.36 (2021)	14.1
Global Stem Cell Banking	1.58 (2016)	3.96 (2021)	20.2
Global Regenerative Medicines	17.06 (2016)	49.41 (2021)	23.7
Research Antibodies	9.33 (2017)	12.60 (2022)	6.2
In Vivo Toxicology	4.40 (2017)	6.14 (2022)	6.9
Organs-on-Chips	0.001 (2017)	0.005 (2022)	36.6
In Vitro Toxicology Testing	6.34 (2017)	8.74 (2022)	6.6
3D Cell Culture	0.68 (2017)	1.72 (2022)	20.3
Biological Safety Testing	2.75 (2017)	4.90 (2022)	12.2
Cell Based Assays	13.34 (2017)	19.92 (2022)	8.4
Next Generation Sequencing	5.02 (2017)	12.45 (2022)	20.5
Protein Assays	1.42 (2017)	2.41 (2022)	11.1
Human Liver Models	1.35 (2017)	2.56 (2022)	3.6
Stem Cell Banking	6.28 (2018)	9.30 (2023)	8.2
In Vitro Lung Model	0.19 (2018)	0.42 (2023)	17.5
Stem Cell Assay	0.79 (2018)	1.98 (2023)	20.1
Neuroscience Antibodies and Assays	2.57 (2018)	4.18 (2023)	10.2

- *Biologics:* Recombinant proteins used as drugs (e.g., calcitonin or insulin) pose large problems for animal-based testing and, accordingly, many alternative tests have been developed in this particular field.
- *Clostridial neurotoxins:* Large research programs have led to multiple non-animal test methods in this field. For instance, several proprietary assays are available for the quantification of Botulinum neurotoxin A bioactivity. This is of high importance for products such as "BOTOX" that required hundreds of thousands of animals for potency testing[18].

Some economical metrics of success of new approaches could be market sizes for these technologies (Tab. 2): Estimates from *MarketsandMarkets*, who see *in vivo* toxicology in 2017 at $4.4 billion, see the *in vitro* toxicology testing market at an estimated $6.3 billion in 2017 (press release December 21, 2017), with a CAGR of 6.6%. They see the global cell culture market at $11.3 billion in 2015 (press release November 20, 2017), with a CAGR of 10.5%. In another report (press release July 10, 2017), they estimate the cell-based assay market at $13.3 billion in 2017, at a CAGR of 8.4%. The prohibitive pricing makes the reports themselves inaccessible for academia, but assuming a similar approach in the various reports and taking the summary results at face-value, the press releases indicate that an economy of alternative approaches has developed that is outperforming traditional animal testing. This is in line with our observations that increasing numbers of biotech companies are exhibiting at the pertinent conferences and that alternative methods are now offered in the portfolio of contract research organizations (CRO).

In conclusion, new tests and services are flourishing as part of the biotech revolution. It is extremely difficult to distinguish what tests are in fact replacing directly animal testing and what tests are reducing demands for *in vivo* tests through technological progress. Often the new *in vitro* and *in silico* approaches are enabling technologies, which can do much more than just replace an animal test. Outside of regulatory testing, where we have a formal acceptance step and can monitor animal numbers, it is much more difficult to quantify market shares *versus* animal reduction. The estimates from market research organizations are the best proxy here. A number of market research companies monitor technologies and industries relevant to compound regulation. These reports are in general too costly for academia. However, the public teasers allow extraction of market sizes and projections, as at least *MarketsandMarkets* typically includes these parameters.

Table 2 shows these numbers over the last five years. Notably, source data and methods cannot be scrutinized. It is our personal impression that numbers tend to be inflated, but at least a similar approach in the various reports can be assumed. Although these figures have to be taken with a grain of salt, they do provide a basis for interesting comparisons. The compound annual growth rates (CAGR) are quite remarkable in these biotech sectors.

4 Animal testing and the contract research industry

Most animal testing and alternative methods are currently outsourced to contract research organizations (CRO). Only a few large companies keep experimental toxicologists on staff. Most, however, have only regulatory toxicologists who commission testing. We have argued earlier that the pressure to move from animals to new approaches is actually a business opportunity (Goldberg and Hartung, 2008).

MarketsandMarkets states, "*Some key factors driving market growth are the increasing pharmaceutical R&D activities, mandatory government regulations for animal testing, innovations in animal models, and the development of exclusive* in vivo *toxicology tests. Factors such as increasing research in oncology and personalized medicine and the rising demand for humanized animal models are expected to offer lucrative opportunities for players in the global market*." The following views were made publicly available:

- In 2017, the consumables segment is expected to account for the largest share of the global market. The large share of this segment is attributed to the increasing R&D funding for the development of new transgenic animal models, advancements in the development of genetically modified animals, and increasing pharmaceutical research for developing new drug molecules.
- In 2017, the chronic toxicity testing segment is expected to account for the largest share of the global market. Increasing research focused on drugs used for long-duration therapies such as anti-cancer, anti-convulsive, anti-arthritis, and anti-hypertensives is driving the growth of the chronic toxicity testing market. However, the sub-acute toxicity testing segment is expected to register the highest CAGR during the forecast period.
- The immunotoxicity segment is expected to account for the largest share of the global market in 2017, followed by the systemic toxicity segment. The rising demand for the development of biologics and biosimilars is driving the growth of the immunotoxicity segment.
- Based on the testing facility, the global *in vivo* toxicology market is segmented into outsourced testing facilities and in-house testing facilities. In 2017, the outsourced testing facilities segment is expected to account for the largest share of the global market. The large share of this segment is attributed to the increasing R&D investments and cost-saving strategies of pharmaceutical, biopharmaceutical, and medical device companies, which in turn results in increasing outsourcing of services to CROs.
- Geographically, North America is estimated to dominate the market in 2017 with the highest market share in the *in vivo* toxicology market. The largest share of this regional segment is attributed to the increasing investments in R&D activities, scientific developments in biotechnology, and the presence

[18] Addressed in an ICCVAM/NICEATM/ECVAM Scientific Workshop on Alternative Methods to Refine, Reduce, and Replace the Mouse LD_{50} Assay For Botulinum Toxin Testing (November 13-14, 2006, Silver Spring, MD; http://iccvam.niehs.nih.gov/docs/biologics-docs/BoNTwkshprept.pdf)

of major pharmaceutical and preclinical testing companies in the region. However, Latin America (LATAM) is expected to grow at the highest CAGR during the forecast period. Factors such as the flourishing pharmaceutical and biopharmaceutical industry in Brazil are in turn propelling the drug development activities and increases in the number of CROs that offer cost-effective *in vivo* toxicology services are driving the growth of the global market in Latin America.

- While the overall *in vivo* toxicology market is expected to grow at a high rate, growing pressures to develop and conduct alternatives to animal testing and disadvantages associated with the latter are likely to restrain the growth of this market to a certain extent.

Wikipedia[19] states that as of 2013, there were over 1,100 CROs in the world, despite continued trends toward consolidation. The industry is fragmented, with the top ten companies controlling 55% of the market in 2009. The market was seen for 2018 by *Statista*[20] at $79 billion, with $7.4 billion for discovery, $11.2 billion for preclinical development and safety, $58.5 billion for clinical development, and $2.3 billion for central laboratory.

The major players in the global market include ThermoFisher (US), Danaher (US), Charles River (US), Covance (LabCorp), Eurofins (Luxembourg), Envigo (US), DSI (US), and The Jackson Laboratory (US). IgeaHub[21] considered the top CROs by revenue to be:

1. Laboratory Corporation of America Holdings (Covance) ($10.44 billion revenues in 2017)
2. IQVIA ($9.74 billion revenues in 2017)
3. Syneos Health ($2.67 billion revenues in 2017)
4. Paraxel International Corporation ($2.44 billion revenues in 2017)
5. PRA Health Sciences ($2.26 billion revenues in 2017)
6. Pharmaceutical Product Development (PPD) ($1.90 billion revenues in 2017)
7. Charles River Laboratories International Inc. (CRL) ($1.86 billion revenues in 2017)
8. ICON Public Limited Corporation ($1.76 billion revenues in 2017)
9. Wuxi Apptec ($1.01 billion revenues in 2017)
10. Medpace Holdings, Inc. ($0.44 billion revenues in 2017)

Two examples of those more prominent in toxicology: Charles River Laboratories, for example, achieved a revenue of $1.13 billion in 2012 with 7,500 employees[22]. Covance reached a revenue of $2.6 billion in 2013 with a net income of $179.2 million with more than 12,500 employees[23].

Large parts of commercial animal testing are executed by CROs as companies tend to outsource routine testing. Fleischer (2007) assessed prices and capacities in 2007. During the past years of activity in the preparation of REACH registration dossiers, some quotations were collected (by C.R.) from different CROs that were offering the service. For confidentiality reasons, no indication of the name of the CRO and no reference to the substance are given. Table 3 reports the summary of this survey, separating the price for each individual OECD TG that is generally requested to fulfil the correspondent REACH endpoint. Even though the data set is insufficient to allow a good statistical assessment, these numbers give a realistic picture of the situation as per experience. The costs of good GLP facilities located in the EU are generally comparable and only minor cases of large differences were recorded. There are also many examples of quotations from the same lab made in different years, typically from 2010 to 2018. In general, over this time there was an increase in the cost of *in vivo* studies and a decrease in the cost of the new *in vitro* methods (skin/eye irritation, skin sensitization), considering also that the first proposals were offered when the corresponding OECD guidelines were not yet available.

19 https://en.wikipedia.org/wiki/Contract_research_organization

20 https://www.statista.com/statistics/814232/total-addressable-cro-market-estimates-worldwide-by-function/

21 Luca Dezzani (2018-03-15). "Top 10 Global CROs 2018". IgeaHub Pharmaceutical Club. Retrieved 2018-06-25. (cited from Wikipedia[19])

22 https://en.wikipedia.org/wiki/Charles_River_Laboratories#Animal_rights_issues

23 https://en.wikipedia.org/wiki/Covance

Tab. 3: Price ranges for OECD test guideline studies by various contract research organizations
The costs in column "Full test" are the result of the average of all costs collected in the CRO proposals, indicating in brackets the lowest and highest cost. The column "Full test (extensions)" includes extra costs that should be added.

Endpoint	OECD TG	Average cost (x €1,000) (Fleischer et al., 2007)	Pre-test	Full test average cost (lowest and highest cost)	Full test (extension)	Analytical determination of the dose
Skin Irritation (*in vivo*)	439	1.1		1.2		not required
Skin Corrosion (*in vitro*)	431	not yet available		3.9		not required
Skin Corrosion (*in vitro*)	435	not yet available		2.4		not required
Skin Irritation (*in vitro*)	439	not yet available		2.5 (1.9-4.5)		not required
Eye Irritation (*in vivo*)	405	1.1		1.2		not required
Eye Irritation (*in vitro*) BCOP	437	not yet available		1.8 (1.6-2.0)		not required

Endpoint	OECD TG	Average cost (x €1,000) (Fleischer et al., 2007)	Pre-test	Full test average cost (lowest and highest cost)	Full test (extension)	Analytical determination of the dose
Eye Irritation (*in vitro*)	492	not yet available		4.2 (4.1-4.3)		not required
Skin Sensitization (LLNA)	429	3.29		4.7 (3.7-6.0)		not required
Skin Sensitization (modified LLNA)	442B	not yet available		4.3 (4.0-4.6)[a]		not required
Skin Sensitization	429 R	2		Never proposed		not required
Skin Sensitization (Guinea pig methods)	406	4		71.5 (6.8-75.5)	1.1	not required
Skin Sensitization (DPRA)	442C	not yet available		3.8 (2.2-6.8)		not required
Skin Sensitization (LuSens)	442D	not yet available		3.7 (3.0-4.3)		not required
Skin Sensitization (hCLAT)	442E	not yet available		6.9 (6.6-7.2)		not required
Oral Acute Toxicity (*in vivo*)	420, 423, 425	1.5		1.5	1.1	not required
Inhalation Acute Toxicity (*in vivo*)	403	11.7		3.9[b]		not required
Dermal Acute Toxicity (*in vivo*)	402	2		1.5 (1.0-2.0)	2	not required
Acute Toxicity (*in vitro*)	3T3 NRU	not yet available		4.5		not required
Repeated Dose Toxicity (28d oral)	407	49.4	11.5	46.5 (54.7-35.0)[c]	8.5	9.3
Repeated Dose Toxicity (28d, dermal)	410	49.6		42[d]		
Repeated Dose Toxicity (28d, inhalation)	412	105.5		64.5[e]		
Repeated Dose Toxicity (90d, oral)	408	115.7	10	105.6 (87.2-161.2)[f]		12
Repeated Dose Toxicity (90d, dermal)	411	135		120[g]	19.5	
Repeated Dose Toxicity (90d, inhalation)	413	250		137.6[h]		
Mutagenicity (Ames test, 5 strains)	471	3.2		3.8 (3.4-4.2)	0.5	not required
Mutagenicity (*In vitro* micronucleus test)	487			8.5 (6.6-11.0)	4.5	not required
Mutagenicity (*In vitro* mammalian chromosome aberration test)	473	11-19.2		13.2 (12.5-13.5)	7.3	not required
Mutagenicity (*In vitro* mammalian chromosome aberration test)	476	17.2		14.3 (11.9-17.8)	4.5	not required
Mutagenicity (*in vivo*)	475			62.5[d]		
Carcinogenicity	451	780.4		700		
Reproductive Toxicity	421	54.6	10.5	63.2 (55.5-78.5)		9.3
Reproductive Toxicity	422	92	18.8	112 (75-152.4)[i]		9.3
Reproductive Toxicity	414 (rat)	63.1	23	77.7 (75-90)[j]		12
Reproductive Toxicity	414 (rabbit)	92.5	40	126[d]		12
Reproductive Toxicity[k]	416	328				
Reproductive Toxicity[k]	416 (2nd species)	481				

Endpoint	OECD TG	Average cost (x €1,000) (Fleischer et al., 2007)	Pre-test	Full test average cost (lowest and highest cost)	Full test (extension)	Analytical determination of the dose
Extended one-generation reproductive toxicity study (EOGRTS)	443	not yet available	missing	566[l]	180.4	missing
Reproductive Toxicity[m]	426	1100				
Bioaccumulation (fish)	305	10		11.4[b]		missing
Growth inhibition study on algae	201	4.5		3.5 (2.5-6.0)[n]		6.5
Short term acute toxicity study on daphnia	202	3.7	0.5	3.2 (1.7-5.5)[n]	1.5	6.5
Long term toxicity study on daphnia (21 d)	211	13.4		11.4		missing
Short Term Toxicity (fish)	203	4.2	0.5	3.8 (2.0-6.5)[n]	1.5	6.5
Long Term Toxicity (fish)	210 (212, 215)	26.3		8.6[o]	5.4	5.1
Avian Toxicity[m]	205, 223	96.2				
Short term toxicity testing on earthworms	207	4.2		3.6[b]		missing
Short term toxicity testing on plants	208	7.6		18.4 (12.1-27.6)		5.6

[a]Lower price than some years ago; OECD TG 442A never proposed; [b]Chinese notification; [c]All oral, rat. 13,000€ (excluded) for Chinese notification. The lowest cost comes from a lab with an Indian facility; [d]Only one quotation; [e]Never requested. Cost from the price list of an Indian lab; [f]All oral, rat. Highest cost from 2018. Same lab in 2012, much lower price. 22,100€ (excluded) for a Chinese notification; [g]Never requested. Cost from the price list of an EU lab; [h]Never requested. Cost from the price list of an Indian lab; [i]All oral, rat. Highest cost from 2018. Same lab in 2012, much lower price; [j]No difference between gavage or diet; [k]Generally replaced by OECD TG 443; [l]Never requested. Cost from the price list of an EU lab; [m]Never requested; [n]No big difference since 2010 and no big difference to Chinese labs. More importance to the protocol (static, semi-static, WAF, etc.); [o]From a price list

In many cases, a pre-test is necessary to define suitable doses for the main experiments. It may happen that the pre-test needs to be repeated to refine these values, though this is fairly rare. The extra costs (Tab. 3, "Full test (extensions)" column) may arise as a follow up of the main study, for example to repeat an ambiguous experiment or to introduce a new biochemical endpoint or a new tested dose. The extent of this cost is variable as it strongly depends on the result of the main experiment. The standard acute aquatic tests usually begin with the limit dose and new experiments are added if some toxicity is recorded. Another high variable cost is the analytical determination of the doses, which is mandatory for GLP studies. This cost depends on the analytical technique that is used, ranging from ICP for metals to HPLC for standard organic substances. Many chemicals are UVCB (Unknown Variable Composition Biological materials) and the analytical determination requires the development of a customized method that can be very expensive. The costs reported in this column represent only the analysis performed during the main test. The analytical method requires a full validation according to the GLP requirements and this procedure may strongly increase the final costs.

The table also lists the average costs as reported by Fleischer (2007). A comparison with the new collected costs reveals that these numbers are still roughly valid. The only problem is that Fleischer did not consider the additional costs derived from the pre-tests and the need for the analytical determinations.

A list of study costs, apparently mostly from the US, was compiled by the Humane Society International[24] (Tab. 4), contrasting them with the respective *in vitro* alternatives.

The European REACH regulation (EC, 2006) was adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals while enhancing the competitiveness of the EU chemicals industry. REACH covers also older chemicals on the market. There now is an increasing number of new regulatory schemes / amendments of existing regulations in other countries (Canada, China, Korea, Malaysia, Russia, Taiwan, Turkey, USA, etc.). The different legislations and legislative proposals have some similarities with REACH

[24] http://www.hsi.org/issues/chemical_product_testing/facts/time_and_cost.html

Tab. 4: Prices for OECD TG studies and their alternatives compiled by the Humane Society International[24]

Type of Toxicity	Test Type	Study Cost (US$)
Genetic toxicity		
Chromosome aberration	animal test	$30,000
	in vitro test	$20,000
Sister chromatid exchange	animal test	$22,000
	in vitro test	$8,000
Unscheduled DNA synthesis	animal test	$32,000
	in vitro test	$11,000
Eye irritation/corrosion		
Draize rabbit eye test	animal test	$1,800
Bovine corneal opacity and permeability (BCOP) test	*in vitro* test	$1,400
Skin corrosion		
Draize rabbit skin test	animal test	$1,800
EpiDerm™ human skin model	*in vitro* test	$850
CORROSITEX® membrane barrier	*in vitro* test	$500
Skin sensitization		
Guinea pig maximization test	animal test	$6,000
Local lymph node assay (LLNA)	reduction alt.	$3,000
Phototoxicity		
Rat phototoxicity test	animal test	$11,500
3T3 neutral red uptake test	*in vitro* test	$1,300
Embryotoxicity		
Rat developmental toxicity test	animal test	$50,000
Rat limb bud test	*in vitro* test	$15,000
Non-genotoxic cancer risk		
Rat 24-month cancer bioassay	animal test	$700,000
Syrian hamster embryo (SHE) cell transformation test	*in vitro* test	$22,000
Pyrogenicity		
Rabbit pyrogen test	animal test	$475-$990
Limulus amoebocyte lysate (LAL)	1st gen. *in vitro*	$85-$160
Human blood method (Endosafe-IPT)	2nd gen. *in vitro*	$83-$100
Estrogen hormone interactions		
Rat uterotrophic assay (OVX)	animal test	$29,600
Subcellular receptor-binding assay	*in vitro* test	$7,200
Androgen hormone interactions		
Rat Hershberger assay	animal test	$37,000
Subcellular receptor-binding assay	*in vitro* test	$7,300

[24] http://www.hsi.org/issues/chemical_product_testing/facts/time_and_cost.html

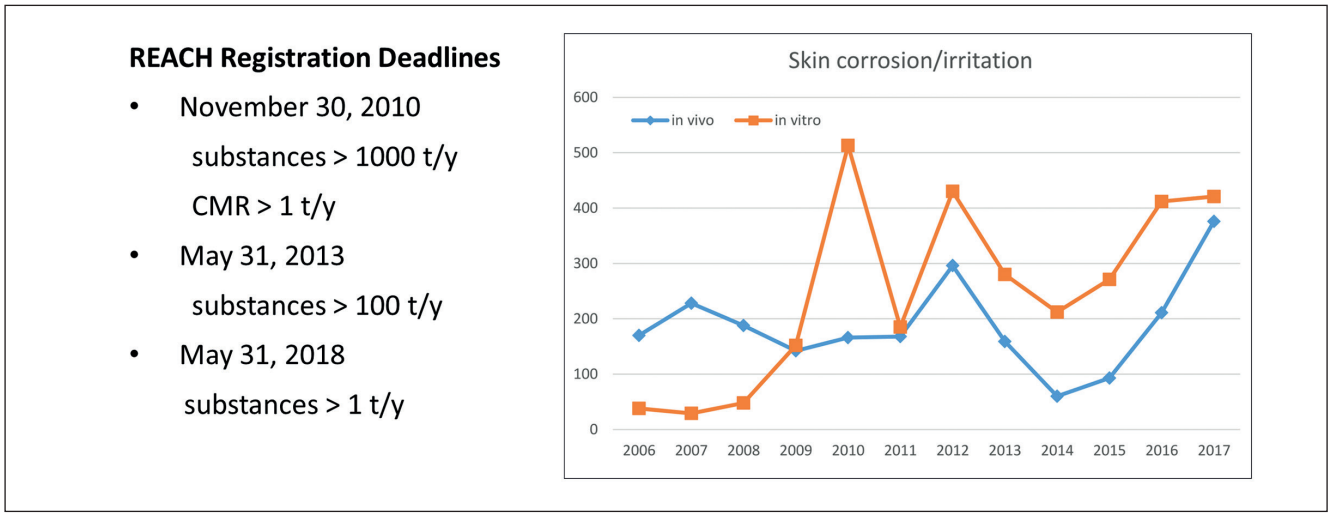

Fig. 3: Comparison of the number of *in vivo* and *in vitro* tests performed per year to assess skin irritation/corrosion of chemicals
Data were extracted from the e-ChemPortal of OECD[45].

but also many important differences as to timing for notifications or registrations, thresholds such as tonnages triggering registrations, availability of exemptions, data requirements (e.g., ecotoxicity testing), acceptance of QSAR and alternative methods, defining product composition / impurities, and communication in the supply chain. The new US legislation (Frank R. Lautenberg Chemical Safety for the 21st Century Act – TSCA 2016 update) and its implementation, with breakthroughs in the demand for alternative approaches, will be the subject of an upcoming paper in this series. Some examples of other legislations (China, Malaysia, Korea) have been summarized elsewhere[25]. The important conclusion is that there are enormous demands on test data. Regardless of the comprehensive registration of a substance in one country, this does not mean that testing is completed also for other registrations, since the data remain proprietary (in REACH, for example, data are owned by the mandatory consortia – SIEF – substance information exchange forum), there can be other companies registering the same compound without legitimate access to the earlier data, and information requirements can vary. Legitimate access to the information has to be clarified in each and every case. This is of critical importance as otherwise an enormous number of replicate studies becomes necessary. In fact, REACH has made test data a commodity. As the compensation for data-sharing has not been set by the legislation and a brokering mechanism is lacking, this has forced individual negotiations between the different parties. Often it is difficult to identify whether specific data is available as only the lead registrant of a SIEF is easily found on ECHA's website. Thus, a high number of additional experimental animal and resource use is to be expected from the mushrooming chemical legislations world-wide.

Reproductive toxicity triggers enormous demands for REACH (EC, 2006) compliance. We calculated earlier that in case REACH is implemented according to the valid ECHA guidance, this type of testing will contribute 70% of costs and 80% of animals (Hartung and Rovida, 2009; Rovida and Hartung, 2009) through the main driver – OECD Test Guideline (TG) 416 – the two-generation reproductive toxicity study. Thus, the discussion regarding the implementation of the extended one-generation reproductive toxicity study (now OECD TG 443) quickly became very important (Gilbert, 2010), as it can reduce the number of animals to 1,400 per chemical from up to 3,200 for TG 416. This is an interesting test case demonstrating the balance between testing demands, costs and test capabilities. CEHTRA (Consultancy for Environment and Human Toxicology and Risk Assessment), UK, prepared a report commissioned by ECHA "*Report on Survey of Worldwide CROs: Costs and Practicalities of Two New OECD Guidelines for Testing Chemical Substances OECD 443, Extended One-Generation Reproductive Toxicity Study, and OECD 488, Transgenic Rodent Somatic and Germ Cell Mutation Assay*"[26]. A total of 50 CROs were identified as being potentially able to perform the OECD 443 extended one-generation reproductive toxicity study. Based on the survey, it is estimated that the worldwide capacity for the OECD 443 test may be in the region of 63 studies per year. However, we showed that the first registrations for the first deadline alone suggested about 159 TG 416 studies would be needed (extrapolated from 400 randomly sampled dossiers) (Rovida et al., 2011), which represents 2.5 years of all capacity to run such studies. Studies from the 2nd deadline and those that requested waivers or category formation not accepted by ECHA must still be added.

The price range for testing one chemical in the two-generation OECD TG 416 (without formulation analysis) is between €141,000 and €408,000 (worldwide average €285,842; European average €318,295). For an extended-one-generation OECD

[25] http://www.cefic.org/Documents/IndustrySupport/REACH-Implementation/Workshops/RIEF-II-18-December-2013/Chemical%20legislation%20around%20the%20world%20(A.Jalba%20-%20Cefic).pdf

[26] https://echa.europa.eu/documents/10162/13628/survey_report_worldwide_cros_en.pdf

TG 443, the average price for the first-generation basic study (without second generation and extra cohorts) is €414,273; with inclusion of the second generation €469,778; with the extra neurotoxicity cohort €507,444; with the extra immunotoxicity cohort €440,414; with both cohorts €655,195. Notably, there is a three-fold difference between the minimum and maximum prices for the basic TG 416. But obviously, saving on animal numbers comes at a price: The European average for a full OECD TG 443 (including all cohorts but no 2nd generation) is €607,119, which is almost double the average European price for the OECD 416. On this basis it is clear that when comparing like for like (OECD TG 416 *vs* OECD TG 443 with 2nd generation), the extended one-generation study is 41% more expensive than the OECD 416.

But the impact of REACH on testing is also visible for the simpler tests. Figure 3 shows the example of skin irritation and corrosion. The three peaks of the graph are clearly related to the three REACH deadlines, the first at the end of 2010 and the second mid-2013 and the third, which is still clearly incomplete, in mid-2018. The number of *in vitro* tests is higher than *in vivo* tests, but the data clearly shows that the *in vivo* tests are still very much used despite the fact that these endpoints have been "fully" replaced.

In summary, CROs represent a sizable industry. Their economic interests can be in favor of animal testing and against transitioning to new and often cheaper alternatives. This can, for example, be witnessed in the ISO process for deriving safety standards. This decade-long process is based on volunteers and requires international travel, which is very costly and so typically weeds out those with no vested interest. Many CROs, who may be tempted to maintain or increase testing demands, are part of this process. As CROs often consult the customer in what tests should be done, they are key for any transition. Notably, some CROs have become part of the change: they aim to offer cutting-edge methods and establish them early under quality regimes – they thus often participate in validation studies of alternatives and their professional attitude in executing protocols is often an advantage over academic groups who are often tempted to vary protocols.

5 Chemical industry

Chemical safety testing and its alternatives has been addressed earlier in this series (Hartung, 2010b). The European trade association CEFIC (*Conseil Européen de l'Industrie Chimique* – European Chemical Industry Council) provides facts and figures for 2017[27,28,29] (numbers not referenced below come from these sources), some of which are extracted here: Chemical products are used in making 95% of all goods. World chemical turnover in 2016 was €3.36 trillion, which represents 0.4% growth from 2014. Global sales increased from 2006 to 2016 by 86.3%, to which China alone contributed 70.2% with an annual growth rate of 12.4%. Table 5 shows increase in world chemicals turnover, led by China with 39.6%, a three-fold increase from 2006, followed by the EU with 15.1% (17.8% including the non-EU European countries) and the US with 14.2%. Global R&D spending reached €39.4 in 2016 (up from €24.7 in 2006, a growth rate of 4.8% per year). In 2016, China contributed 30% to global R&D, Europe 23.2% and the US 20.0%. The major chemical companies in the world[30] are BASF ($78.7 billion in 2014), Dow Chemical ($58.2 billion), Sinopec ($58.0 billion), SABIC ($43.3 billion), ExxonMobil ($38.2 billion), Formosa Plastics ($37.1 billion), LyondellBasell Industries ($34.8 billion), DuPont ($29.9 billion), Ineos ($29.7 billion), Bayer ($28.1 billion), Mitsubishi Chemical ($26.3 billion) and Shell ($24.6 billion).

Tab. 5: World chemicals turnover (2016) – total €3.36 trillion

Country / economic region	Sales	%
NAFTA (US, Canada, Mexico)	528	15.7%
Latin America	127	3.8%
EU	507	15.1%
Rest of Europe (Switzerland, Norway, Turkey, Russia and Ukraine)	90	2.7%
South Africa	13	0.7%
China	1,331	39.6%
South Korea	113	3.4%
India	76	2.2%
Japan	140	4.2%
Rest of Asia	407	12.1%
Rest of World	28	0.8%

In the EU, according to Eurostat, chemical industry (excluding pharmaceuticals) is the fifth largest industry, contributing about 7% of total EU manufacturing added value. The European industry currently comprises approximately 29,000 companies (96% of which are Small Medium Enterprises (SMEs)), employing roughly 1.2 million people directly and a further 3-4 million people indirectly, generating a turnover of over €500 billion and net exports of nearly €50 billion. Germany (28.7%), France (13.9%), Italy (10.0%), The Netherlands (9.1%) and the UK (7.9%) are the largest producers within the EU. While total sales increased (€334 billion in 1996 to a reported maximum of €553 billion in 2012), they fell to just €408 billion in 2009 due to

27 http://www.cefic.org/Documents/RESOURCES/Reports-and-Brochure/Short-Introduction-To-the-European-Chemical-Industry-2014.pdf

28 http://fr.zone-secure.net/13451/451623/#page=1

29 https://www.chemlandscape.cefic.org/country/eu/

30 https://cen.acs.org/articles/93/i30/Global-Top-50.html

the financial crisis (from €530 billion in 2008), and after the recovery around 2012 declined to €507 billion in 2016. But, while European chemical sales have continued to grow over the past 20 years, Europe's share of global sales over the same period has declined from 32% to 15%. 30% of European production is sold outside the EU (22% to the US, 10% to China and 8% to Switzerland as the most important customers). The spread of products by sales in Europe shows 25.9% petrochemicals, 13.6% consumer chemicals (sold to final consumers such as soaps, detergents, perfumes and cosmetics), 11.7% basic inorganics (fertilizers, gases, etc.), 21.6% polymers; these 59.2% of chemicals are considered base chemicals to be distinguished from 27.2% specialty chemicals (paints and inks, crop protection, auxiliaries for industries, etc.). The costs of regulation for the industry were assessed in the REFIT program of the European Commission (EC), "*The total costs amount to €10 billion per year (33% for industrial emissions, 30% for chemicals and 24% for worker safety). These figures vary strongly by subsector amounting to 23.2% of value added for pesticides and only 2.7% for plastics; most other chemicals range from 12 to 17%, with a total average of 12% representing 30% of the gross operating surplus. Compared to 2004, by 2014 the costs of regulation have doubled*."

The North American chemical market according to Boston Consulting Group[31] generated sales of approximately $1 trillion in 2014 and is projected to grow at roughly 3.5% per year through 2020. Overall, it grew at an annual rate of 2.4% from 2011 through 2014. According to the American Chemistry Council (ACC)[32], the US trade association, with the development of shale gas and the surge in natural gas liquids supply, the U.S. has moved from being a high-cost producer of key petrochemicals and resins to among the lowest-cost producers globally. This shift in competitiveness is driving significant flows of new capital investment toward the U.S., with 317 natural-gas related projects already announced, valued at more than $185 billion (as of December, 2017). More than $88 billion in new projects have been completed or are currently under construction. It is one of the top US exporting industries, with $174 billion in annual exports, accounting for 14% of all U.S. exports in 2016. The industry employs 811,000 people, and of these jobs, more than 30 percent are export dependent. According to the U.S. Bureau of Labor Statistics, the chemical industry has an injury rate that is 55% lower than overall manufacturing. This contrasts impressively with Justus von Liebig's advice to August Kekulé "*If you want to become a chemist, you will have to ruin your health. If you don't ruin your health studying, you won't accomplish anything these days in chemistry*". According to the ACC[33], the U.S. chemical industry invested $14.3 billion in 2016 in environmental, health, safety, and security programs and $91 billion in research and development[34]. The business of chemistry accounts for 14% of US goods exports[35], $174 billion in 2016, and is among the largest exporters in the US.

The European REACH (EC, 2006) had a major impact on the field world-wide as discussed above. When REACH was published in 2006, the date of the final deadline of May 31, 2018 seemed far away. Now, we are there and can compare what was predicted and how it went in reality. First of all, the number of registered substances: 36,000 substances in the EINECS list and 4,000 in the ELINCS list made a total of 40,000 substances officially circulating in the EU market (2007), which appears still to be underestimated. In fact, at the end of the pre-registration period about 143,000 substances had their own pre-registration number (ECHA Press release, which is no longer available on the webpage, see Rovida and Hartung (2009) for details). Based on those numbers, the expected registrations could have been between 40,000 and 80,000. Now the ultimate deadline has passed, but the ECHA public database of registered substances counts only 20,920 records, according to the update of June 23, 2018[36], which represents only 14% of the pre-registered substances. At the moment it is not clear what has happened. We are tempted to sing following freely Pete Seeger's *Where Have All the Flowers Gone*:

Where have all the chemicals gone?
Long time passing
Where have all the chemicals gone?
Long time ago…

The difficulty in comparing the registered substances with the lists of the EINECS and ELINCS, lies also in the fact that in many cases the original EC number has been changed, and there is no way to understand what was lost in the process. It is unclear whether groups of substances were registered as categories, which might hold especially true for petrochemicals. Thus, currently, a high number of chemicals is not registered and their marketing in the EU will be forbidden. Even considering substances that are in the scope of other regulations (such as active biocides or plant protection products) and substances that do not pass the REACH registration threshold of 1 ton per year, the gap still remains large. Probably, in many cases companies preferred not to register and stop the manufacturing or the importation of a substance as the high costs of compiling the registration dossier made the investment unprofitable. BASF, the largest chemical company, ultimately registered about one third fewer substances than originally anticipated (Dr Robert Landsiedel, BASF, personal communication). There is also the possibility that some substances were simply forgotten or postponed and we will see the total number of registra-

31 https://www.bcg.com/publications/2016/supply-chain-management-specialty-chemical-distribution-north-america.aspx
32 https://www.americanchemistry.com/Pro-Growth-Pro-Competitiveness-Agenda-for-Chemical-Manufacturing.pdf
33 https://www.americanchemistry.com/Policy/Security/Chemical-Safey-and-Security-Fact-Sheet.pdf
34 https://www.americanchemistry.com/Jobs/EconomicStatistics/Industry-Profile/Industry-Facts/Chemistry-Industry-Facts.pdf
35 https://www.americanchemistry.com/Jobs/EconomicStatistics/Industry-Profile/Industry-Facts/Chemistry-Industry-Facts.pdf
36 https://echa.europa.eu/information-on-chemicals/registered-substances

tions increase in the coming months. This situation may cause trouble to the EU economy by moving the manufacturing and use of the orphan chemicals outside its border.

This "loss" of chemicals concerns only the last of the three deadlines. In 2009, we predicted the numbers of chemicals to be registered under REACH (Hartung and Rovida, 2009; Rovida and Hartung, 2009): In total, we suggested 68,000 registrations, which would lead to the use of 54 million animals if following ECHA's test guidance by the letter. Now, at least we know the number of registrations: Since 2008, 13,620 companies have submitted 88,319 registration dossiers in all tonnages to ECHA. For deadlines 1 and 2 we predicted a minimum of 12,007 and 13,328 were received[37]. For 2018, we predicted a minimum of 56,202 chemicals. Ironically, while the number of chemicals was way off, the number of registrations with about 60,000[38] was point on. As the 2018 registrations have to come with executed animal tests, to the extent the registrations are complete, the predicted number of necessary animal tests was correct. The submitted registrations cover 21,551 substances, which means that the portion of extensively tested chemicals in daily use rose from about 3 to 8% (though many tests are still at the proposal stage) and for the somewhat tested ones with public data from about 8 to 16%. Europe does not track new chemicals below an annual market or production volume of one ton, but the somewhat smaller US chemical industry brings about 1,000 chemicals to the market at this tonnage range each year.

It is too early also for a reasonable balance of the number of animals that were sacrificed in *in vivo* tests performed for the preparation of the REACH registration dossiers. REACH Annexes IX and X, i.e., those defining the information for substances manufactured or imported in quantities above 100 and 1000 tons per year, respectively, are the most demanding in terms of new *in vivo* tests. Deadline for registering chemicals in compliance with Annex X was November 30, 2010, while the deadline for Annex IX was May 31, 2013. However, any new *in vivo* tests described in those annexes need to go through the testing proposal procedure (Article 12), which takes some time, and many dossiers still need to be updated.

In addition, compared to the REACH requests, very few testing proposals were submitted and most of the registration dossiers claimed the waiving options as described in Annex XI of REACH. In the first three ECHA reports on the use of alternatives to animal tests for the REACH Regulation[39, 40, 41], the analyses of the registration dossiers revealed that there were very few testing proposals and in many cases the need for the information was fulfilled through alternative ways, such as read-across with other chemicals. Even though acceptable from a scientific point of view, ECHA noted that in many cases they were inadequately justified and contained deficiencies. As a consequence, in 2016 ECHA published a detailed document on Read-Across Assessment Framework (RAAF), updated in 2017[42], representing a very helpful guide to a successful application of the read-across approach. Simultaneously, in 2016, ECHA decided to introduce a manual check on all newly submitted dossiers and updates of the previously submitted dossiers controlling all main aspects that were often found to be problematic during the previous evaluations, including the possibility of waiving the most demanding *in vivo* tests. There is still no official data, but from personal experience (C.R.), we know that indeed many new *in vivo* tests will be performed or are currently running in this period. On average, considering the scheduling of the testing proposals plus the time necessary for running the tests, the new updates will be available in 2-3 years. Calculation of the number of animals used for REACH purposes should begin no earlier than 2020.

Another important aspect of REACH is that it has been a major boost for the use of novel technologies for regulatory purposes. It should be noted that REACH was the very first regulation that accepted adaptations to the standard set of toxicological information. Annex XI describes how to use alternative strategies to waive the requests for new *in vivo* tests. This opportunity was extensively described elsewhere (Rovida and Hartung 2009; Rovida et al., 2011). ECHA also published suitable guidance that helped submitters to dramatically reduce the number of new tests. This guidance (developed under the coordination of T.H. for the EC) was first published in 2008 and afterwards regularly updated as soon as new information was available[43]. ECHA hosted perhaps the majority of the discussion on this topic and in 2016 organized an important workshop to form the basis for the use of non-standard strategies for regulatory purposes. The report of that workshop still represents an important milestone in the field[44] and probably was key for the shift of the focus of discussion on non-animal methods from animal welfare organizations to regulatory toxicologists in general.

This was further improved by the amendments of the REACH Regulation that deleted the request for *in vivo* tests for skin sensitization (Regulation 2016/1688) and skin/eye irritation (Regulation 2016/863). Actually, the publication of those amendments would have been superfluous as the compulsory use of alternatives to *in vivo* tests as soon as validated methods are available was already foreseen in the text of REACH. However, the formal request for *in vitro* tests as the only way to fulfill a toxicological endpoint was very important to send the message that regulators

37 http://www.cefic.org/Documents/IndustrySupport/REACH-Implementation/Workshops/RIEF-IV-16-6-2015/12%20Reach%20and%20%20non-animal%20testing%20-%20Katy%20Taylor.pdf

38 https://www.echa.europa.eu/documents/10162/13609/work_programme_2018_in_brief_en.pdf/9412a2bd-64f1-13a8-9c49-177a9f853372

39 https://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2011_en.pdf

40 https://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2014_en.pdf

41 https://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2017_en.pdf

42 https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

43 https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf

44 https://echa.europa.eu/documents/10162/22931011/non_animal_approcches_en.pdf/87ebb68f-2038-f597-fc33-f4003e9e7d7d

are open to *in vitro* strategies. The best way to follow the true use of the official methods is through the e-ChemPortal[45], which is a portal coordinated by the OECD that allows simultaneous searching of reports and datasets by chemical name and number, by chemical property, and by GHS classification. In this portal it is very easy to count the number of times that a specific OECD TG was performed during a specific period of time. For example, the comparison between *in vivo* and *in vitro* testing for the determination of skin irritation potential is presented in Figure 3. The number is not absolute as it may include tests performed outside the EU and can also overestimate the ratio of *in vitro* to *in vivo* tests per chemical as substances are often tested twice *in vitro*. However, it may give an idea of the frequency of *in vitro* tests performed instead of *in vivo*.

An interesting economic transition in chemical industry is the adaptation of Green Chemistry, which includes many aspects of sustainability. Relevant for the discussion here is the aspect of frontloading toxicity (Green Toxicology) (Maertens et al., 2014; Crawford et al., 2017; Maertens and Hartung, 2018) to eliminate toxic substances early from the product development process – in ideal cases even before synthesizing them – which is possible by collaboration of chemists with toxicologists and recent advances in computational and *in vitro* toxicology. The impact of this movement on animal testing and alternative use cannot be assessed at this stage.

6 Agrochemical industry

Agrochemicals (excluding fertilizers, which typically need no animal testing), plant protection products, as they are called in European legislation, or pesticides are demanding developments of a magnitude that appears to be feasible only for an organization that includes specialists in many areas of scientific research, backed by large financial resources. The probable range confronting developers of new pesticide chemicals appears to be $750,000 to $3.25 million – but the trend is constantly upward[46]. On average it costs €2.2 billion and takes 10 years for a new active substance to be brought to market according to the European Trade Association[47].

Global chemical-based crop protection sales inched up by 0.2% to $53.7 billion at the distributor level in 2017, according to figures from Agrow[48]. The European crop protection market generated revenue of $13.5 billion in 2016[49] according to the European Crop Protection Association (ECPA). The agricultural biologicals market is projected at $6.75 billion in 2017[50] and with an estimated CAGR of 13.8% will reach $14.7 billion by 2023. The role of agricultural biological products has become a part of integrated pest management practices (IPM) in developed markets, wherein the biological products are used in combination with new synthetic crop chemistries.

Syngenta was the worldwide leader in agrochemical sales in 2013[51] at $10.9 billion, followed by Bayer CropScience ($10.4 billion), BASF ($6.9 billion), Dow AgroSciences ($5.6 billion), Monsanto ($4.5 billion), and then DuPont ($3.6 billion). All companies increased sales by on average 12.4%. Noteworthy, ongoing mergers of Dow and Dupont, Bayer and Monsanto, and the acquisition of Syngenta by ChemChina, a Chinese state-owned enterprise, are showing major concentrations of the market.

A study by the EC[52] analyzed the "agricultural input sector" including plant protection products: Consumption of plant protection agents in Europe increased in value until 2008, then declined for the following years. Quantity consumed has declined overall, suggesting an increase in unitary value of these products. Herbicides are the plant protection agents consumed in largest amounts, especially in northern Member States, followed by fungicides. Mediterranean countries show the largest consumption share of insecticides and the lowest share of herbicides over the total plant protection agents consumed. The total value of sales of plant protection agents' companies operating in EU Member States has seen a slight decline in the period 2003-2009, followed by a marked increase in the following years. The number of enterprises producing plant protection agents stayed relatively steady, varying between 630 and 655 in the period 2003-2012. Germany, France, UK, Italy and Spain are the countries where the highest values of plant protection agent turnover is realized, concentrating more than 80% of total sales.

The European plant protection agent industry appears concentrated, with an estimated range of the top-five companies spanning 79 to 83% of the market. The number of new active principles patented in the EU has declined considerably during the period 1980-2012, ranging between 3 and 8 per year. Investment costs for R&D and product development for companies operating in the EU crop protection agent industry are large and foster further consolidation. The value of the total European market is characterized first by a decline from €6.7 billion in 2000 to €6 billion in 2001, and then increasing to reach €7.7 billion in 2008 and declining to €7.2 billion in 2010. The European Crop Protection Association gives some information on the EU market 2017[53]: Total production was at 208 million tons; 26,000 people work in crop protection in Europe, and from 2011 to 2017, 54 new active agents were registered (i.e., average 8 per year), but only 4 have been accepted so far.

45 www.echemportal.org

46 https://pubs.acs.org/doi/pdf/10.1021/jf60089a603

47 http://www.ecpa.eu/sites/default/files/7450_Registration%20brochure_3.pdf

48 https://agrow.agribusinessintelligence.informa.com/AG029283/Global-crop-protection-market-flat-in-2017

49 https://www.inkwoodresearch.com/reports/europe-crop-protection-market/

50 https://www.marketsandmarkets.com/Market-Reports/top-10-trend-agricultural-biological-market-139215554.html

51 http://news.agropages.com/News/NewsDetail---11846.htm

52 http://www.europarl.europa.eu/RegData/etudes/STUD/2015/563385/IPOL_STU(2015)563385_EN.pdf

53 http://www.ecpa.eu/reports_infographics/ecpa-annual-review-2017

The crop protection industry is under strong pressure to use animal testing. A typical registration package includes about 30 animal tests produced at about €20 million. The reason for this outstanding demand has to do with the bad reputation of pesticides (starting with Rachel Carson's book *Silent Spring*), but also the fact that these are designed to be toxic, often neurotoxic, at least for insects. Notably, this safety testing package requires about 20 kg of the active agent (Dr B. van Ravenzwaay, BASF, personal communication) showing that this level of scrutiny cannot be achieved for many substances under development. The Agricultural Chemical Safety Assessment (ACSA) Technical Committee of the ILSI Health and Environmental Sciences Institute (HESI) (Doe et al., 2006) has shown that many animals can be saved by better integration, suggesting a tiered testing strategy. The extended one-generation study (TG 443) discussed above is more or less an outcome of these discussions. Some integrated animal tests have been adopted as OECD test guidelines:

- The use of transgenic rodents (TGR) might bear advantages for *in vivo* mutagenicity testing and OECD adopted TG 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays in 2013, which anticipates that in the future it may be possible to combine this with a repeat dose toxicity study (TG 407).
- Short-term toxicity tests with rodents (TG 407) can be combined with developmental toxicity screening assays (TG 422).
- The revised TG 453: Combined Chronic Toxicity/Carcinogenicity Studies of 2009 combines chronic toxicity (TG 452) with carcinogenicity studies (TG 451), which is preferred with respect to the number of animals used.

Noteworthy, the ACSA proposal also suggests to abandon the 12-month dog study and the mouse carcinogenicity study. The former has been done by the EU and US, and positive developments in Brazil and Korea let us hope that soon Japan and others will follow and the test will finally be dropped by the global industry.

Overall, the small number of new active substances per year consumed, despite the major registration demands, only 8.1% of animals for safety testing in Europe in 2011 (i.e., 0.7% of all animal use) for products or substances used or intended to be used mainly in agriculture. Two rather new legislations could impact on this:

- The Biocidal Products Regulation (BPR, Regulation (EU) 528/2012) concerns the placing on the market and use of biocidal products, which are used to protect humans, animals, materials or articles against harmful organisms like pests or bacteria, by the action of the active substances contained in the biocidal product.
- Regulation (EC) No 1107/2009 of the European Parliament and of the Council of October 21, 2009 concerning the placing of plant protection products on the market.

Both legislations, as detailed earlier for the first one (Ferrario and Rabbit, 2012), are hazard-based, i.e., substances will be banned not on the basis of risk (relevant exposures to hazard) but the presence of a hazardous property alone. Especially in conjunction with the emerging endocrine disrupter screening program, this can endanger many active agents on the market. Notably, quite to the contrary, RISK21[54], a HESI-managed initiative aimed at developing a scientific, transparent, and efficient approach to the evolving world of human health risk assessment, brings together international stakeholders from government, academia, industry, and some NGOs to work together collaboratively. RISK21 was created to address a needed transition in toxicology, exposure, and risk assessment methodology and communication. RISK21 is exposure-led, i.e., considers relevant exposure estimates upfront to prioritize and determine data needs. One of the authors (T.H.) is part of the consortium, which also aims to optimize use of resources and flexibility by tiered testing to allow making an informed decision on human health safety as soon as sufficient data are available.

An important discussion needed around these products is how to handle minimal formulation changes. These products are very complex in composition – they can contain over a hundred ingredients. Often minor reformulations take place without changing the active compounds. When this should trigger new testing, how to bridge to old studies, or which alternative methods are available impacts strongly on animal use. Unfortunately, many accepted alternative methods have only been validated (and often only work) for individual substances.

7 Food industry

The food industry[55,56] is a complex, global collective of diverse businesses that supplies most of the food consumed by the world population (Tab. 6): It is challenging to find an inclusive way to cover all aspects of food production and sale. The UK Food Standards Agency describes it thus as "*...the whole food industry – from farming and food production, packaging and distribution, to retail and catering*". From the perspective of animal testing, mainly food additives, food contact materials and contaminants are of interest. An earlier article in this series, also on food safety, was written mainly from a European perspective (Hartung and Koëter, 2008) and, more recently, the US system of the generally recognized as safe (GRAS) provisions was addressed (Hartung, 2018a).

The European Food Industry[57,58] achieves 1.1 trillion € turnover, which makes it the largest manufacturing sector in the EU, it adds 1.7% EU gross value and 14% of household expenditure are on food and drink products. The sector employs 4.24 million people, making it the leading employer in the EU. With

54 http://risk21.org

55 https://en.wikipedia.org/wiki/Food_industry

56 https://www.statista.com/outlook/40000000/102/food/europe?currency=eur#

57 http://www.fooddrinkeurope.eu/uploads/publications_documents/DataandTrends_Report_2017.pdf

58 http://www.fooddrinkeurope.eu/uploads/publications_documents/Data_and_trends_Interactive_PDF_NEW.pdf

Tab. 6: Structure of the food products and drink industry in 2012, growth 2008-2012 and market shares
Extracted from *The competitive position of the European food and drink industry Final report- Ref. Ares(2016)838411 - 17/02/2016*, which used data from EuroStat, AUSSTATS, CANSIM, CENSUS (USA) and IBGE (Brazil) as well UNComtrade for market shares.

Country / region	Turnover (in billion €)	Growth (% per year, 2008-2012)	Number of enterprises	Persons employed (x 1000)	Market share export (%)	Market share import (%)
EU28	1,061	1.5	288,655	4,515	12.1	11.3
USA	652	6.7	25,974	1,550	8.3	10.5
Australia	71	10.7	13,018	240	2.0	1.3
Brazil	186	13.6	4,959	1,615	6.2	0.9
Canada	73	7.5	8,318	266	5.8	3.0

€102 billion export (17.3% share of global exports) and €72 billion import, the trade balance is €30.1 billion. Other regions' food industry is characterized in Table 6.

EU R&D private investment in food and drink companies listed in the world's top 2,000 companies (by R&D 2015/2016) is €2.8 billion (31.2% of total) by 16 companies. In comparison, US R&D private investment totals €2.3 billion (25.5% of total) by 15 companies and Japanese R&D private investment totals €1.7 billion (18.9% of total) by 16 companies.

Regarding animal testing, food additives are the main focus in this sector. In 2012, the global food additives market was valued by Crystal Market Research[59] around $35.5 billion and is anticipated to reach approximately $61.6 billion while maintaining a CAGR of 5.09%. In 2014, the European regional food additives market reported for at around 30% of the global market owing to the rising significance of functional elements for incorporating characteristics like the extension of shelf life, emulsification, and flavor. In the same year, the Asia Pacific regional market reported for around 26% and is projected to experience significant growth during the next few years. Governmental initiatives to boost production yield in developing countries such as China and India are pushing expansion, and this is predicted to persist during the forecast years. The global food additives market is dominated by players like E. I. du Pont de Nemours and Company, Archer Daniels Midland Company, Novozymes, and Ingredion Incorporated. Other major market players are Tate & Lyle PLC, Chr. Hansen A/S, Koninklijke DSM N.V., Cargill, Ajinomoto Co., Inc., BASF SE, Adani Wilmar Ltd, Bio Springer S.A., Lonza Group, Givaudan and the Kerry Group.

In Europe, permitted food additives receive E number codes for use within the EU and European Free Trade Association (EFTA). The numbering scheme follows that of the International Numbering System (INS) as determined by the Codex Alimentarius committee, though only a subset of the INS additives are approved for use in the EU as food additives. The total approved number of food additives in the EU is 390[60]. Food additives permitted before 20 January 2009 are going through a new risk assessment by the European Food Safety Authority (EFSA) according to Commission Regulation (EU) No 257/2010 setting up a program in accordance with Regulation (EC) No 1333/2008[61]. As of March 5, 2018, scientific opinions covering 169 individual food additives were published by EFSA on their safety; the rest must be reevaluated by the end of 2020.

In the US, an enormous number of chemicals are added either directly or indirectly (as contaminants such as pesticides or contact materials) to food. Many of these have no (public) data as to their safety (Neltner et al., 2013): For chemicals added directly to food, 21.6% have feeding studies necessary to estimate a safe level of exposure and 6.7% have reproductive or developmental toxicity data in the FDA database. To market a new food or color additive, a manufacturer must first petition the FDA for its approval. Approximately 100 new petitions are submitted to the FDA annually, most of which are for indirect additives such as packaging materials. GRAS determination, in contrast, requires only a (voluntary) notification. Karmaus et al. (2016) found a total of 8,659 food-relevant chemicals including direct food additives, food contact substances, and pesticides, which is slightly below previous estimates of the food-use chemical universe; furthermore, only 3,888 were possible direct additives while 4,771 were food contact substances or pesticides. So, the US have about ten times more food additives than Europe. There are important efforts by both the industry, represented by the Grocery Manufacturer Association[62], and the FDA to revamp the GRAS system (Hartung, 2018a), which could impact on animal use in the US for food additives, which appears currently to be minor.

Animal use for food in Europe is only 0.6% of animals in toxicology (corresponding to the small number of new food additives) and for animal feed 5.2%[63]. Not included in these

59 https://www.crystalmarketresearch.com/report/food-additives-market

60 https://ec.europa.eu/food/safety/food_improvement_agents/additives/database_en

61 https://ec.europa.eu/food/safety/food_improvement_agents/additives/re-evaluation_en

62 https://www.gmaonline.org

63 https://eur-lex.europa.eu/resource.html?uri=cellar:4cdf6763-6714-4521-97d1-1cc8775e6432.0001.03/DOC_1&format=PDF

numbers are the animals used for the detection of marine biotoxins, mainly in shellfish. We highlighted the enormous numbers a decade ago (Hartung and Koëter, 2008). A CAAT workshop created a comprehensive overview with a landmark review (Daneshian et al., 2013). About 300,000 mice have been used per year for this type of safety testing alone[64]. The additional mice and rats employed for investigative toxicology with marine biotoxins are not included in this number. The 2013 report lists the shortcomings of this test, which has never been validated or systematically evaluated, as well as the increasingly used alternatives. Another area of concern is genetically modified plants (GMO), which we discussed at the time next to other animal uses related to food (Hartung and Koëter, 2008).

While ordinary food supplements such as vitamins and minerals are well known and should not require extensive testing, nutraceuticals represent a novel area that is expected to grow rapidly. At the moment the EU has no dedicated regulation and these substances are generally included in the area of food supplements even if the scope is different. More recently, the EU published a new Regulation on novel food (Regulation 2015/2283) defined as "*any food that was not used for human consumption to a significant degree within the Union before 15 May 1997*" including ingredients like insects, algae and so on. EFSA published the *Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources* on June 26, 2018[65]. The safety assessment is still based on the regular set of *in vivo* tests, but the dossier is prepared in a tiered way and a new *in vivo* test is performed only if deemed necessary.

8 Cosmetic industry

The cosmetic industry and its specific challenges has been the subject of an earlier paper in this series (Hartung, 2008). The 7th amendment (EC, 2003) of the cosmetics legislation (Cosmetics Directive 76/768/EEC) provided *inter alia* detailed provisions on the phasing out of animal testing. This was later transformed into a regulation (EC, 2009). A general testing ban on cosmetic ingredients from March 11, 2009, reinforced for 10 animal test requirements by an instant marketing ban, as well as a marketing ban for the more complex endpoints (those requiring repeated substance application, e.g., repeated dose toxicity, sensitization, reproductive toxicity, carcinogenicity and toxicokinetics), went into effect March 11, 2013. In many ways this is legally and economically unique:

- This industry has been singled out despite only minor animal use (0.03-0.05% of all animals in Europe before the ban).
- Test methods were banned before alternatives were available.
- The marketing ban applies also to tests carried out outside the EU and for products produced outside the EU.

So far, however, attempts to challenge or modify this have failed and the ban enjoys popularity in the public and serves as a model for other countries. The sale of cosmetic products containing new ingredients that have been tested on animals has been illegal in the EU since 2013, but the parliament now wants to expand this to a prohibition of all cosmetics testing on animals and wants countries around the world to join in this effort[66]. Currently, some 80% of countries around the world still allow animal testing and the sale of cosmetic products tested on animals. In addition, there are loopholes in the EU's regulation, allowing for products that are tested on animals outside the EU to be retested within the bloc's jurisdiction using different methods and then placed on the market. Most importantly, it is traditionally not the cosmetic industry who commissions the testing, but the chemical companies who offer their substances typically already with full safety datasheets. However, REACH might actually change this, as testing beyond standard requirements needs to be justified and with the production volume threshold raised to 1 ton per year and minimal test requirements up to 10 tons per year, it might be difficult to provide data for a comprehensive safety assessment of a potential new cosmetic ingredient. On the positive side, the ban has made cosmetics industry an engine of change for safety testing[67].

While cosmetics are perceived largely as reshuffling (reformulating) of existing ingredients and there is indeed in Europe a positive list of about 10,000 chemicals that can officially be used to do so, there are some areas that need new chemistries: Hair dyes, preservatives and UV filters are among these, as they require reactive chemistry, which is always problematic as this can be linked to skin sensitization and mutagenicity/cancer. Many of the now more broadly accepted alternative methods were driven by cosmetic industry. And even though many alternatives were not available by the deadline in 2013 (Adler et al., 2011; Hartung et al., 2011), the industry has apparently embraced this and pursues roadmaps[68,69] (Basketter et al., 2012; Leist et al., 2014) to achieve this goal ultimately.

Cosmetics is a very active area of development as European data shows[70,71]: On average, large industry companies have a product portfolio of around 10,000 different cosmetic products and they reformulate around 25% to 30% of their products every year. Out of these reformulations, about 10% depend on ingredients that are new to the market or to the cosmetics industry. Large companies introduce around 80 new ingredients to their product portfolio each year, while SMEs introduce on average 22 (with 40 to 160 products in their portfolio). In 2014/2015, the most active area for innovation in the cosmetics sector (globally)

64 https://www.foodnavigator.com/Article/2010/11/19/EU-unveils-change-of-test-method-for-marine-biotoxins
65 https://www.efsa.europa.eu/en/efsajournal/pub/5294
66 https://www.governmenteuropa.eu/ep-ban-cosmetics-testing-on-animals/87128/
67 https://www.cosmeticseurope.eu/how-we-take-action/promoting-science-research
68 https://www.cosmeticseurope.eu/how-we-take-action/promoting-science-research
69 https://www.cosmeticseurope.eu/files/1215/0245/3923/Non-animal_approaches_to_safety_assessment_of_cosmetic_products.pdf
70 https://ec.europa.eu/growth/sectors/cosmetics/cosing_en
71 https://www.cosmeticseurope.eu/cosmetics-industry/innovation-and-future-trends-cosmetics-industry/

was in shampoos, where 19% of all beauty innovation activity was focused. Total expenditure on R&D in Europe is estimated at 1.27 billion €. As examples of progress, it took 20 years of scientific advancement to remove the smell of ammonia from hair dye and there are at least 30 separate, scientific steps involved in the development of every new lipstick. Every year, 25% of all cosmetic products are improved or are completely new. For this reason, a large proportion of patents granted in the EU are for cosmetic industry's products (the record being 10% of all patents awarded in the EU in 2009)[72]. According to the European trade association (Cosmetics Europe)[73], over 100 companies manufacture ingredients, 4,600 manufacture cosmetic products and 20,100 are involved in wholesale. The industry employs 190,000 people (directly) and 1.58 million in the cosmetics value chain. Total expenditure on R&D in Europe is estimated at €1.27 billion[74]. The revenue in the cosmetics segment in Europe will amount to €10.8 billion in 2018[75].

In the US[76], 8.2 million people make their living by manufacturing, marketing, or selling cosmetics and personal care products, or by providing beauty services. The beauty industry employs more women and minorities, many in managerial positions, than the national average, and women make up 66% of the industry compared to 48% of the nation's overall workforce. 92% of industry jobs are provided by small businesses with less than 50 employees. The sector achieved, according to the US trade association PCPC (the Personal Care Product Council), a remarkable trade surplus in 2008 with more than $7 billion of cosmetics imported but more than $12 billion exported, opposite to trade in general. With a market volume of €12.4 billion in 2018, most revenue is generated in the US[77].

The largest cosmetics companies are[78] The L'Oréal Group ($28. billion in 2015), Unilever ($20.5 billion), The Procter & Gamble Company ($17.6 billion), Estée Lauder Companies ($11.1 billion), Shiseido Company ($7.1 billion), Beiersdorf ($5.9 billion) and Johnson & Johnson ($5.6 billion).

A strongly overlapping industry and main supplier to cosmetics is the fragrance industry. According to Euromonitor International[79], the global fragrance market was worth $48 billion in 2016. In the US, the market grew by 2.6% last year, to $7.9 billion. Statista[80] sees the world market growing from $72.2 billion in 2018 to $92 billion in 2024.The fragrance and perfume industry[81] is regulated by the FDA and GRAS (see food section), as well as being self-regulated by internal organizations like the Research Institute for Fragrance Materials (RIFM)[82] and the International Fragrance Association (IFRA)[83]. Both organizations are currently undertaking remarkable efforts to transition to alternative approaches. There are more than 3,000 chemicals on IFRA's Transparency List of chemicals used in the industry. The IFRA Code of Practice includes scientifically-based recommendations (usage standards), which are intended to ensure the safe use of fragrance materials in products, and there are currently 186 banned or restricted substances on the IFRA safety standards list. In Europe, fragrance products must comply with the provisions of EU Regulation 1223/2009 (the Cosmetics Regulation)[84].

A special dynamic came to the cosmetics sector because of the growth in the Chinese market. China has surpassed Japan and become the world's 2nd largest market in the total consumption of cosmetic products after the US[85]. With an estimated $50 billion in domestic sales in 2015 and 7% to 10% annual growth predicted in 2016 and beyond, China is projected to become the largest market for personal care and cosmetics products globally in the next five to ten years[86]. Despite its relatively large market size, merely 10% of the population uses cosmetics regularly. China is the 10th largest market for U.S. personal care and cosmetics exports[87], with US product exports totaling $392.6 million in 2015. Within the next decade, China has the potential to become the largest market for U.S. products, with U.S. exports to China growing 64% in the period between 2010 and 2015. While China's rate of economic growth has slowed over the past few years, this has not impacted the growth of U.S. personal care and cosmetics exports to China.

A peculiarity of the Chinese cosmetics market is that endproduct testing on animals carried out by Chinese authorities is required. People for the Ethical Treatment of Animals (PETA) estimated in 2013 that Chinese companies had tested products on more than 300,000 animals in the previous year[88]. This is very different to Europe (no endproduct testing since 1989, ban of ingredient testing in 2013) and the US (little testing under the responsibility of companies on ingredients) and in clear conflict

72 https://www.cosmeticseurope.eu/cosmetics-industry/understanding-cosmetics-regulation/
73 https://www.cosmeticseurope.eu/cosmetics-industry/
74 https://www.cosmeticseurope.eu/cosmetics-industry/innovation-and-future-trends-cosmetics-industry/
75 https://www.statista.com/outlook/70010000/102/cosmetics/europe?currency=eur#market-revenue
76 https://www.personalcarecouncil.org/about-us/creating-sustainable-future-economy
77 https://www.statista.com/outlook/70010000/109/cosmetics/united-states?currency=eur#market-revenue
78 https://pmcwwd.files.wordpress.com/2016/04/beautyinc0416web.pdf
79 https://www.fungglobalretailtech.com/research/reviewing-trends-global-fragrance-market/
80 https://www.statista.com/statistics/259221/global-fragrance-market-size/
81 https://www.fragrancex.com/fragrance-information/fragrance-industry.html
82 http://www.rifm.org
83 http://www.ifraorg.org
84 https://www.perfumerflavorist.com/fragrance/regulatory/EU-Regulations-Influencing-the-Fragrance-Industry-451639893.html?prodrefresh=y
85 http://www.cirs-reach.com/news-and-articles/Investigation-of-China-Current-Cosmetics-Market-and-Industry-Supervision-Analysis.html
86 https://www.trade.gov/industry/materials/AsiaCosmeticsMarketGuide.pdf
87 https://www.trade.gov/industry/materials/AsiaCosmeticsMarketGuide.pdf
88 https://www.bloomberg.com/news/articles/2018-01-16/ending-china-animal-tests-is-salve-for-big-beauty-quicktake-q-a

with the EU testing and marketing ban, which means that in principle, products tested in China may not be marketed in the EU. That has kept brands like The Body Shop, sold by L'Oréal to Brazil's Natura Cosmeticos SA last year, Clorox Co.'s Burt's Bees and Urban Decay out of China's $30 billion skincare and make-up market[88]. There is some movement on the Chinese side: The Chinese Food and Drug Administration in 2014 stated[89] it would educate and train provincial labs in alternative testing methods and waived animal testing on domestically produced non-special-use cosmetics, like nail care and perfume. In 2016, the regulator accepted data from the first non-animal test for phototoxicity, used to assess whether an ingredient damages skin after exposure to light. In September 2016, China's Zhejiang Institute for Food and Drug Control (ZJIFDC) opened a lab in collaboration with the Institute for In Vitro Sciences (IIVS), a U.S. non-profit research and testing laboratory based in Gaithersburg, MD, that has been training Chinese scientists in tests using reconstructed skin[90]. L'Oréal is the first cosmetics company to develop Chinese reconstructed skin, which it makes available to universities, scientists and competitors. CAAT is currently forming a coalition with IIVS, cosmetic companies led by Estée Lauder, and other stakeholders to enhance the discussion about international harmonization of regulatory testing requirements in China.

9 Tobacco industry

Tobacco products are not traditionally linked to major regulatory animal testing. Animal models are not even particularly suited, as it is quite difficult to produce lung cancer in mice and rats with cigarette smoke (Silbergeld, 2004). The interest into regulatory testing comes with increasing oversight by FDA: on July 28, 2017, the FDA announced a new comprehensive plan[91] that places nicotine and the issue of addiction at the center of the agency's tobacco regulation efforts. This plan will serve as a multi-year roadmap to better protect children and significantly reduce tobacco-related disease and death in the U.S. It is not expected that this will imply major animal testing. However, the surge of e-cigarettes[92] and other lower-risk tobacco products represents a challenge for industry and regulators with a possible impact on testing needs.

The global cigarette industry is one of the most profitable and deadly industries in the world[93]. Globally, cigarette consumption is growing in low- and middle-income countries and decreasing in high-income countries. Cigarette retail values in 2016 were worth $683 billion. In 2016, over 5.5 trillion cigarettes were sold to more than one billion smokers worldwide. Between 2002 and 2016, global cigarette volume sales increased by 1.3% while real retail values increased by 27.6%. Industry analysts predict that by 2021 the global cigarette volume will decline by 8.2%, and real value will decline by 1.1%.

The tobacco market was valued at €152 billion in Western Europe[94] and €51 billion in Eastern Europe[95]. In 2008, the industry employed 34,402 people in manufacturing, 117,372 in retail sale and 32,091 in whole sale[96]. Other numbers for 2009 note 251 enterprises with 50,338 employed[97]. The Confederation of European Community Cigarette Manufacturers (CECCM)[98] even claims 1.5 million jobs in 2016.

The number of cigarettes sold in the U.S. fell by 37% from 2001 to 2016[99], according to Euromonitor. Over the same period, though, companies raised prices, boosting cigarette revenue by 32%, to an estimated $93.4 billion last year. An average pack in the U.S. cost an estimated $6.42 in 2016, up from $3.73 in 2001, according to TMA, an industry trade group.

China National Tobacco Co. has become the largest tobacco company in the world by volume[100]. Following extensive merger and acquisition activity in the 1990s and 2000s, four firms dominate international markets – in alphabetical order: Altria (formerly Philip Morris International), British American Tobacco (BAT), Imperial Tobacco and Japan Tobacco. Large tobacco companies have entered the electronic cigarette market by either buying some of the small e-cigarette companies or by starting their own e-cigarette companies. Since 2012, BAT for example states[101] to have invested approximately $2.5 billion in developing and commercializing their range of vapor products and tobacco heating products (THPs).

With respect to testing needs, these lower-risk nicotine products change the game. On the one hand, the companies use them to optimize their products and convince regulators of their lower risk. On the other hand, regulatory tools are needed to control marketing. In 2014, already 460 brands were counted (Zhu et al., 2014) with 7,764 unique flavors available. Noteworthy, in the 17 months between their searches, there was a net increase

89 https://www.bloomberg.com/news/articles/2018-01-16/ending-china-animal-tests-is-salve-for-big-beauty-quicktake-q-a
90 https://www.cosmeticsandtoiletries.com/testing/animalalt/Animal-Testing-Alternatives-Reach-China-459092193.html
91 https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm568923.htm
92 https://www.fda.gov/TobaccoProducts/Labeling/ProductsIngredientsComponents/ucm456610.htm
93 https://www.tobaccofreekids.org/assets/global/pdfs/en/Global_Cigarette_Industry_pdf.pdf
94 https://www.statista.com/statistics/491553/tobacco-market-value-western-europe/
95 https://www.statista.com/statistics/491626/tobacco-market-value-eastern-europe/
96 https://ec.europa.eu/health//sites/health/files/tobacco/docs/tobacco_matrix_report_eu_market_en.pdf
97 http://www.cittadeltabacco.it/wp-content/uploads/2012/12/THE-EUROPEAN-TOBACCO-SECTOR-LIGHT.pdf
98 https://www.ceccm.eu/#The_industry
99 https://www.wsj.com/articles/u-s-tobacco-industry-rebounds-from-its-near-death-experience-1492968698
100 https://en.wikipedia.org/wiki/Tobacco_industry
101 http://www.bat.com/r%26d

of 10.5 brands and 242 new flavors per month. In the same year, the market was estimated at $3.5 billion[102] and for 2017 already at $10 billion[103]. China has been the forerunner in the regional e-cigarette industry, with an estimated 40.3% share in 2017[104]. After the U.S. and the U.K., China was estimated to be the third largest e-cigarette market globally in 2017, which is expected to grow further. Estimates from Wells Fargo suggest that sales might exceed those of traditional tobacco products by 2021[105]. BIS Research[106] estimates that the global electronic cigarette industry will exhibit a growth of over 22.36% (CAGR) from 2015 to 2025, to reach a total market value of $50 billion by 2025.

As discussed earlier in this series (Hartung, 2016a), especially the flavoring substances represent a toxicological challenge (Hartung, 2016b; Fowle et al., 2017). A systematic review of available data in 2014 (Orr, 2014) concluded that, overall, the limited toxicology data on e-cigarettes in the public domain is insufficient to allow a thorough toxicological evaluation of this new type of tobacco product. Others concluded after systematic review that currently available evidence indicates that electronic cigarettes are by far a less harmful alternative to smoking and significant health benefits are expected in smokers who switch from tobacco to electronic cigarettes (Farsalinos and Polosa, 2014).

Noteworthy, FDA is entering the regulation of flavors in tobacco products: FDA issued an advance notice[107] of proposed rulemaking to help determine how best to regulate flavors in tobacco products, including menthol. This action creates an opportunity for the public to comment on the role that flavors, including menthol, play in tobacco product use. We will have to wait and see whether this will create major animal testing needs.

10 Pharmaceutical industry

Animal studies play a critical role in preclinical testing of the safety and effectiveness of medical products and may be important for understanding the reasons for adverse events in marketed products and devising strategies to prevent them. Their development and quality control (including veterinary and dentistry) consumes 32.7% of animals in Europe[1].

EFPIA (European Federation of Pharmaceutical Industries and Associations) as the European trade association with direct membership of 33 national associations and 40 leading pharmaceutical companies, is the voice on the EU scene of 1,900 companies. The following information is extracted from their website[108,109,110,111]: Europe is the second largest pharmaceutical market in the world and accounts for 22.2% (€238 billion) of world pharmaceutical sales[112]. The EU pharmaceutical sector is considered to be the high-tech sector contributing the most to the EU trade balance with a trade surplus of €70.9 billion. The pharmaceutical sector sustains around 800,000 jobs throughout Europe.

Estimations show that up to 90% of the growth will be provided by emerging markets by 2020. Removing trade barriers and regulatory hurdles in third countries and increased market access enables the pharmaceutical industry to operate in a more transparent and predictable environment. These efficiencies reduce costs of production and generate cost savings, which can be used for increased investment in R&D, and an improved regulatory environment, which is key in order to advance innovation of new medicines and accelerate patient access to new treatments. The pharmaceutical industry is expected to invest close to €900 billion in research and development from 2015-2020[113].

Pharmaceutical Research and Manufacturers of America, formerly known as the Pharmaceutical Manufacturers Association, is a trade group representing companies in the pharmaceutical industry in the United States. They describe[114] America's biopharmaceutical companies investing about $75 billion annually in R&D in the U.S. and support 4.7 million jobs across the country (803,000 direct, 1.8 million vendors and suppliers and 2.2 million indirect). They count 7,000 medicines under development worldwide at any given time.

Pharmaceutical sales in Canada[115] have a 1.9% share of the global market, making Canada the 10th largest world market. Since 2011, compound annual growth has remained positive at 2.8% (IMS Pharmafocus 2021). 29,870 people are employed in manufacturing. From 2001 to 2016, total pharmaceutical sales in Canada doubled to $25.5 billion. $92 billion are spent on R&D.

Based on prescription sales, Pfizer is the world's largest pharmaceutical company. In 2012, the company generated $47 billion in pure pharmaceutical sales[116], while total revenue stood at nearly $60 billion. Other top global players from the US are

102 http://www.namastetechnologies.com/wp-content/uploads/2015/11/2.-WellsFargo-Vaporizer-Research-Report.pdf
103 https://www.statista.com/statistics/493214/global-e-cigarettes-dollar-sales/
104 https://www.researchandmarkets.com/reports/4457267/-global-e-cigarette-market-analysis-and-forecast
105 http://www.convenience.org/Media/Daily/Pages/ND0917134.aspx#.WzsovmZ7G52
106 https://bisresearch.com/industry-report/electronic-cigarette-market-size-forecast.html
107 https://www.federalregister.gov/documents/2018/03/21/2018-05655/regulation-of-flavors-in-tobacco-products
108 http://trade.ec.europa.eu/doclib/docs/2015/october/tradoc_153846.pdf
109 https://www.efpia.eu/about-medicines/development-of-medicines/trade/
110 https://www.efpia.eu/media/15535/efpia-contribution-european-commission-new-trade-strategy-july-2015.pdf
111 https://www.efpia.eu/media/219735/efpia-pharmafigures2017_statisticbroch_v04-final.pdf
112 https://www.efpia.eu/about-medicines/development-of-medicines/trade/
113 https://www.efpia.eu/media/219734/efpia_annual-report_2017_interactive.pdf
114 http://phrma.org/industryprofile/
115 https://www.ic.gc.ca/eic/site/lsg-pdsv.nsf/eng/h_hn01703.html
116 https://www.statista.com/markets/412/topic/456/pharmaceutical-products-market/

Johnson & Johnson, Merck, and Abbott. Novartis and Roche from Switzerland, GlaxoSmithKline and AstraZeneca from the United Kingdom, and Sanofi from France are the European big five.

In Europe, 75 new chemicals entered the market[117] between 2012 to 2016. A new report[118] published by the Tufts Center for the Study of Drug Development (CSDD) pegs the cost of developing a prescription drug that gains market approval at $2.6 billion, a 145% increase, correcting for inflation, over the estimate the center made in 2003. The center's analysis drew from information provided by 10 pharmaceutical companies on 106 randomly selected drugs first tested in humans between 1995 and 2007. The study concludes that another $312 million is spent on post-approval development - studies to test new indications, formulations, and dosage strengths - for a life-cycle cost of $2.9 billion. This development process takes on average 12 years to get to market[119].

A key figure for understanding the drug development process is its attrition. This describes how many clinical candidates make it to the market. Scannell et al. (2012) report that the number of new drugs approved per $1 billion spent on R&D has halved roughly every 9 years since 1950, falling around 80-fold in inflation-adjusted terms. The success rate in clinical testing was estimated at 19% (13% for small molecules) by DiMasi et al. (2010) and at 11% by Kola et al. (2004), who used 50 and 10 pharmaceutical company pipelines, respectively. The development process was analyzed more recently by Hay et al. (2014) and the UK Office of Health Economics[120] very similarly. For Phase I through III, costs were around $220 million in 2011. 9.6% of drugs made it to approval from 2006-2015. Failure rates in the clinical phase of development now reach 95% (Arrowsmith, 2012). This differs among indications (from 5.1% in oncology to 26.1% in hematology). The success rate of Phase I studies (healthy volunteers) was 63.2%, Phase II (small patient study for safety) 30.7%, Phase III (large patient study for efficacy) 58.1% and then to approval for 85.3%[120]. Others reported that the probability of products from a group of major companies making it to market from the initiation of Phase I trials fell steadily from 10% in 2002-2004 to 5% in 2006-2008 (Anon., 2012).

The British Office of Health Economics cites findings that the European Medicines Agency (EMA) approved 13 and 23 New Medical Entities (NME) in 2010 and 2011, respectively, while the US Food and Drug Administration (FDA) approved 19 NMEs in 2010 and 32 in 2011. The number of new entities launched has decreased from an average of 43 per year between 1990 and 1994 to around 30 or fewer per year over the last five years[120]. Hay et al. (2014), analyzing success rates of 835 drug developers including biotech companies, contrast this decline with the number of compounds in development, which has increased by 62%, and total R&D expenditures, which have doubled in the first decade of this century. This illustrates two things: There are drugs only going to the more lucrative US market (the US has 6% of the world population and consumes 65% of drugs under patent[121], data for 2011-2016). Second, there seems to be a trend that despite continuing or even increasing investment, the number of successful new drugs is declining. Noteworthy, a substantial number of drugs has had to be withdrawn from the market. Wikipedia now lists 174 such cases[122] – impressive in comparison to the number of accepted substances.

A special part of pharmaceutical industry is vaccine industry. With respect to animal testing, the vaccine industry used 15.3% of all animals in Europe in 2005 required for continuous efficacy and safety testing in animals of every batch for many old vaccines (Bottini and Hartung, 2009). The 2011 statistics do not allow derivation of a new number for comparison. The WHO states: "*Compared to the pharmaceutical market, the vaccine market is relatively small and concentrated on both supply and demand sides. It is highly regulated and largely dependent on public purchasers and donor policies. The vaccine market has very distinct features, which increase the complexity of assessing and understanding pricing and procurement. It is made up of individual markets for individual vaccines or vaccine types, each with their own specificities, particularly on the supply side*"[123]. The WHO itself has often been a hurdle for the introduction of alternatives for vaccine control: As typically the receiving countries want to be able, at least in principle, to control vaccine quality, they prefer to maintain the low-tech of animal studies and have objected to some of the more modern methods. To some extent this is understandable – it is not easy, for example, to run a cell culture laboratory at outside temperatures above 40°C.

Europe dominates the world vaccine production[124] (80% by doses produced, 11% North America, 4% Asia, 5% rest) with 23 production sites. 80% of vaccines are exported. About €2 billion were spent at 13 key R&D sites. It takes between 8 and 18.4 years to develop a new vaccine at average costs of €900 million. The world vaccine market was estimated at $21 billion in 2016 with a predicted growth to $61 billion by 2020[125]. Others saw the vaccine market size to reach $77.5 billion by 2024[126], with a CAGR of 10.3%. 45% of revenue is made in the US[125]. The report study indicates that the introduction of new products is fueling the growth of the market. Moreover,

117 https://www.statista.com/statistics/275262/pharmaceutical-industry-new-entities-by-region/

118 https://www.scientificamerican.com/article/cost-to-develop-new-pharmaceutical-drug-now-exceeds-2-5b/

119 https://www.medicinenet.com/script/main/art.asp?articlekey=9877

120 https://www.ohe.org/publications/rd-cost-new-medicine

121 https://www.efpia.eu/publications/data-center/the-pharma-industry-in-figures-economy/geographical-breakdown-of-sales-of-new-medicines/

122 https://en.wikipedia.org/wiki/List_of_withdrawn_drugs

123 http://www.who.int/immunization/programmes_systems/procurement/market/en/

124 https://www.vaccineseurope.eu/about-vaccines/vaccines-europe-in-figures/

125 https://www.globalresearch.ca/big-pharma-and-big-profits-the-multibillion-dollar-vaccine-market/5503945

126 https://www.grandviewresearch.com/press-release/global-vaccine-market

the significant expansion of the current product offerings is also expected to boost market growth. Due to the increasing prevalence rates of various infectious diseases such as diphtheria, influenza, hepatitis, pneumococcal diseases, and meningococcal diseases, there has been a notable increase in the use of vaccines across the globe. The WHO documents the enormous growth of the vaccine market from about $6 billion in 2000 to $33 billion in 2014[127]. They report a share of global market value of 33% for GlaxoSmithKline (GSK) / Novartis, 17% for Sanofi-Pasteur, 13% Pfizer, 12% Merck, 4% Merck Sharp & Dohme (MSD) / Sanofi-Pasteur and 21% other.

Notably, despite increasing R&D budget, pharmaceutical industry is continuously reducing animal testing in Europe: the share of relatively stable 12 million animals used in Europe dropped from 31% (2005) to 23% (2008) and to 19% (2011), clearly indicating that a substitution by other technologies is taking place. An earlier article in this series (Rovida et al., 2015) discussed the opportunities of alternative methods especially as they originate from the Toxicity Testing in the 21st Century movement in the US. EFPIA also reports on animal use and its alternatives[128, 129]. There was a large and steady increase in the use of *in vitro* tests by companies between 1980 and 2013; more than 20% of all *in vitro* tests reported were conducted in 2013, the last year of the survey period, and over 70% were conducted since 2010. *In vitro* assays were most widely used in genotoxicity, safety pharmacology, and drug metabolism studies.

As an interesting economical tool, with the Innovative Medicines Initiative (IMI)[130], the world's largest public-private partnership in the life sciences driving animal welfare and 3Rs was created in this European sector with so far €5.3 billion. IMI is pursuing the goal of developing the next generation of vaccines, medicines and treatments by improving research practice, getting new healthcare solutions to patients faster, and improving health outcomes thanks to new tools, methodologies, research infrastructure and big data. IMI claims so far 100 projects with 912 participants and 2,686 scientific papers as outcome. Established in 2009, and further expanded in 2014, the IMI consortia (involving industry, academia, SMEs, patients, regulators, etc.) have a direct or indirect impact on the use of animals and IMI projects are contributing to the 3Rs. IMI successes have brought results in 3Rs or new research paradigms (different ways of addressing scientific challenges) or more predictive testing tools that do not require – or require fewer – animals by removing from pipelines harmful molecules before animal studies are conducted.

11 Medical device industry

Worth globally $390 billion in 2012, the industry was expected to expand to over $476 billion in revenue by 2018[131]. The United States remains the largest medical device market in the world with a market size of around $140 billion, and the U.S. market represented about 40% of the global medical device market in 2015[132]. The US Government Accountability Office (GAO) 2014 statistics see the US medical device industry as the global leader with sales of around $136 billion, which represents approximately 45% of the global market[133]. According to Espicom's 2014 report, the US medical device market was projected to grow at a CAGR of 6.1% between 2014 and 2017. More than 7,000 medical device companies in the US, which are mostly SMEs, employ around 400,000 people directly and more than 2 million people indirectly. Thus, the industry is highly fragmented. Europe and China are the second and third largest medical device markets, respectively. U.S. exports of medical devices in key product categories identified by the Department of Commerce exceeded $44 billion in 2015.

R&D spending continues to represent a high percentage of medical device industry expenditures, averaging 6.7 percent of revenue from 2011 to 2016. Compared to several other industries including automotive, defense, and telecom, the medical device industry invests a higher percentage of yearly revenues into product innovation. This reflects the competitive nature of the industry, and constant innovation and improvement of existing technologies.

Recent studies[134] by the Congressional Research Service (CRS), BMI Research, and the Advanced Medical Technology Association (AdvaMed, the industry's main trade association) have estimated that total U.S. spending on medical devices was $172 billion in 2013. In 2013, medical device spending represented 5.9 percent of total national health expenditures. The share of total U.S. spending on health care devoted to medical devices has changed very little over time. Between 35 and 40% of domestic U.S. production is ultimately exported, and a similar share of domestic U.S. consumption is imported. Research by financial analysts suggests that large medical device companies typically spend between 5 and 15% of their revenues on research and development, with most companies somewhere in the middle of that range. The top five players in the US medical devices industry in terms of revenue are Johnson & Johnson ($28.7 billion revenue), General Electric ($18.1 billion), Medtronic ($17.1 billion), Baxter International ($16.4 billion), and Cardinal Health ($11.0 billion).

127 http://www.who.int/immunization/research/forums_and_initiatives/1_ABatson_Global_Vaccine_Market_gvirf16.pdf

128 https://www.efpia.eu/media/26001/discussion-paper-enhancing-implementation-of-3rs-provisions-of-directive-2010-63.pdf

129 https://www.efpia.eu/media/219744/putting-animal-welfare-principles-and-3rs-into-action.pdf

130 https://www.imi.europa.eu

131 http://www.frost.com/sublib/display-report.do?id=NC8C-01-00-00-00

132 https://www.selectusa.gov/medical-technology-industry-united-states

133 https://marketrealist.com/2015/11/must-read-overview-medical-device-industry

134 http://www.medpac.gov/docs/default-source/reports/jun17_ch7.pdf?sfvrsn=0

According to MedTech Europe[135], the industry is a major employer for more than 575,000 people in over 22,500 companies and has an annual turnover of €110 billion[136]. SMEs make up almost 95% of the industry. The sector has been growing on average by 4% a year over the past six years. 12,400 patent applications were filed with the European Patent Office (EPO) (40% from inside Europe)[137], which is more patents than in any other sector[138].

Canada has an estimated $6.7 billion (2016) medical device market[139] accounting for about 2% of the global market. From 2011 to 2016, Canadian medical device exports increased from $1.8 billion to $3.1 billion and imports increased from $6.5 billion to $8.6 billion. The trade gap increased from $4.7 billion (2011) to $5.5 billion (2016), an increase of 17%. Canada's largest trading partner for medical devices is the US. In 2016, medical device imports from the United States were $3.9 billion, representing 46% of Canada's total medical device imports. In 2016, Canada's medical device exports to the U.S. were $2.1 billion, or 67% of Canada's total medical device exports. In 2016, Netherlands (4%), Germany (4%), and China (3%) constituted the next three leading destinations for Canada's medical device exports and China (9%), Germany (7%) and Mexico (6%) constituted the next three leading sources of Canada's medical device imports after the U.S.

Since medical devices are often modified, the life cycles of individual products can be relatively short compared with prescription drugs; the industry has said that most medical devices are replaced by a newer version every 18 to 24 months[140] . The shorter life cycle means that the payback period for R&D is also shorter, and that successful medical devices are typically not as profitable as blockbuster prescription drugs (Seligman, 2013). This also strongly impacts on overall (animal) testing needs.

Two new European Regulations on medical devices entered into force on May 25, 2017[141]: Regulation (EU) 2017/745 on medical devices and Regulation (EU) 2017/746 on *in vitro* diagnostic medical devices. The new rules will only apply after a transitional period of 3 years (spring 2020) and 5 years (spring 2022) for *in vitro* diagnostic medical devices. Recital 73 of Regulation (EU) 2017/745 states, "*The principles of replacement, reduction and refinement in the area of animal experimentation laid down in the Directive 2010/63/EU of the European Parliament and of the Council (1) should be observed. In particular, the unnecessary duplication of tests and studies should be avoided*." However, the legislation itself refers mainly to animal testing and has little provisions for alternatives. Annex II stipulates testing needs such as biocompatibility; microbiological characterization; absorption, distribution, metabolism and excretion; possible interactions of those substances, or of their products of metabolism in the human body, with other devices, medicinal products or other substances, considering the target population, and its associated medical conditions; local tolerance; toxicity, including single-dose toxicity, repeat-dose toxicity, genotoxicity, carcinogenicity and reproductive and developmental toxicity, as applicable depending on the level and nature of exposure to the device. A lot will depend on the implementation by the individual notified body in the Member States, but altogether an extensive testing demand is shaping.

In Europe, the number of medical devices that received CE marking in 2015, i.e., marketed, is estimated to be around 4,500[142]. This illustrates the possibly enormous testing need with the new FDA and ISO guidance as well as European legislation and a backlog (see below) of adapting alternative approaches.

We recently published a report on a workshop on animal testing and medical devices (Kerecman Myers et al., 2017). At this point, biological testing of medical devices relies mostly on animal models according to an ISO standard though the standard states "*In vitro* test methods, which are appropriately validated, reasonably and practically available, reliable and reproducible shall be considered for use in preference to *in vivo* tests" (ISO 10993-1, 2009). ISO Technical Committee 194 (TC 194) on biological and clinical evaluation of medical devices is comprised of working groups that develop and maintain standards and technical reports. These are the ISO 10993 standards on biocompatibility and the ISO 14155 (2011) standard on clinical trials. From personal experience (T.H. 1999-2003), this standard generating process is very much dominated by CROs with respect to defining testing needs (see above). FDA has also published guidance on animal testing[143,144]. A few *in vitro* tests are starting to make it into this testing portfolio (biocompatibility[145], skin irritation, skin sensitization, pyrogenicity testing), but there is certainly room for improvement.

12 Animal test reproducibility and the costs of wrong decisions

It is fair to start from the assumption that no company wants to kill its customers. If toxic products are on the market, this is because of not knowing it, not knowing it in time, being uncertain about it or being deceived by wrong (false-negative) tests. In a

135 http://www.medtecheurope.org/index.php/node/659

136 http://www.medtecheurope.org/sites/default/files/resource_items/files/MedTech%20Market%20in%20Europe.pdf

137 http://www.medtecheurope.org/sites/default/files/resource_items/files/Innovation.pdf

138 http://www.medtecheurope.org/sites/default/files/resource_items/files/MEDTECH_FactFigures_ONLINE3.pdf

139 https://www.ic.gc.ca/eic/site/lsg-pdsv.nsf/eng/h_hn01736.html

140 https://www.ncbi.nlm.nih.gov/books/NBK209786/

141 https://ec.europa.eu/growth/sectors/medical-devices/regulatory-framework_en

142 http://www.medtecheurope.org/sites/default/files/resource_items/files/MedTech_FactsFigures2016_20160105.pdf

143 https://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM466358.pdf

144 https://www.meddeviceonline.com/doc/fda-issues-new-draft-guidance-for-animal-studies-conducted-for-medical-devices-0001

145 http://iivs.org/2017/08/28/non-animal-testing-approach-to-address-biocompatibility-testing-of-medical-devices-required-by-the-united-states-food-and-drug-administration-us-fda/

similar way, we have to assume that many innocent substances have been abandoned or withdrawn because of false-positive test results. Objective assessments for the extent of the different scenarios is not possible, but we might be able to start some educated guesses.

Not knowing about toxic substances
The main reason for this is not having tested for the respective hazard. How many (industrial) chemicals are there? Nobody knows exactly: 140,000 preregistered for REACH and 22,000 above 1 ton per year registered by May 2018, or about 85,000 in the US Toxic Substance Control Act Inventory[146] give us some indications. About 13,000 above 100 tons/year are now receiving in-depth assessments including the more relevant systemic toxicity testing (Smirnova et al., 2018). Adding to this about 800 pesticides and 3,000 drugs, it is probably fair to assume that even after completion of REACH only 20% of substances will have been extensively tested. In principle, every untested substance is a Damocles sword hanging over the company, a risk that can hit any time as a scandal or a liability. The Vioxx scandal cost Merck more than $8.5 billion in settlements alone[147]. This is not to say that the company could have prevented this by testing. Some side-effects will always escape detection and be found only upon widespread application in humans. We also cannot test for all possible threats. Computational (Hartung, 2017a) and biological profiling, however, might help to focus resources on "what smells like problem".

Not knowing it in time
Animal studies take time. Drawing on the standard example of the cancer bioassay, it takes 4-5 years to get results (at $1 million per chemical and animal species). This is not compatible with the development cycles, e.g., of medical devices or e-cigarettes as discussed here. And this is not even considering the high false-positive rate (53% of anything tested opposed to 5-10% true-positives, Basketter et al., 2012; Paparella et al., 2017). Receiving such results late, i.e., after market launch, makes it also even more tempting to challenge these results to keep a successful product in the market. The approaches of frontloading toxicity assessments and using fast tests to anticipate problems (Green Toxicology) (Maertens and Hartung, 2018) come to fruition here.

Being uncertain about it
Uncertainty in safety assessments comes from reproducibility issues, limited relevance of tests, discrepant information from different tests especially due to species differences, and general lack of predictive relevance. A lot of this is handled with uncertainty or safety factors (discussed in part in Hartung, 2018b). The difference between not knowing it (in time) and being uncertain is liability: If I have information, I might be found liable for not following up on it. This is why more predictive tests should appeal to managers and lawyers. Therefore, moving from opinion to evidence (Hartung, 2017b) and formal validation (Leist et al., 2012) are the tools needed to minimize uncertainty.

Deceived by wrong (false-negative) tests
Many more substances do not show a specific hazard than do, especially after the selection process of product development. We have shown, for example, that for the chemicals registered under REACH, no hazard label was given to more than 20% of chemicals (Luechtefeld et al., 2016a). However, it is particularly difficult to find rare exceptions, the few dangerous chemicals among the many harmless ones (Hartung, 2009), the black swans (Bottini and Hartung, 2009; Hartung, 2018b).

Abandoned or withdrawn because of false-positive test results
We have shown already in Hoffmann and Hartung (2005) that simple prevalence considerations imply that we have many more false- than true-positive results. We created the largest toxicological database (10,000 chemicals with 800,000 associated studies) by making ECHA's registration database machine-readable using natural language processing (Luechtefeld et al., 2016a). This laid the foundation now to objectively assess for the first time the reproducibility of the animal tests used (Luechtefeld et al., 2016b). Some chemicals were tested surprisingly often in the same animal test: For example, two chemicals were tested more than 90 times in rabbit eyes and 69 chemicals were tested more than 45 times in the infamous Draize rabbit eye test (Luechtefeld et al., 2016b). This enormous waste of animals allowed now to assess the reproducibility of these guideline studies. Using 350 to 750 chemicals, which were tested more than once for each guideline animal test, showed, for the test required for the 2018 deadline (57% of all animal use in toxicology in 2011), that a repeat study will find a toxic compound only in 70% of cases. Such a rate of reproducibility would not be acceptable for any alternative method. The somewhat better-balanced accuracy of 81% is owed to the fact that most chemicals are non-toxic in the animals, which is better reproducible than the toxic effect. And, it should be considered, that these are animal tests performed according to OECD test guidelines under Good Laboratory Practice, so to say, the best you can get. This clearly shows that the quality of these tests is over-estimated and agencies must lower the bar for accepting alternatives to them.

However, while these tests are frequently done – the six-pack of toxicology – they serve classification and labeling and thus especially worker safety and transport provisions. They will rarely lead to business decisions to abandon a substance. For this, systemic toxicities are key (Basketter et al., 2012; Smirnova et al., 2018). Arguably, carcinogenicity and developmental and reproductive toxicity (DART) are hardly assessed except for agrochemicals, so what remains are especially the repeat-dose organ toxicities. A study by Wang and Gray (2015) is therefore of critical importance: Using studies of the US National Toxicology Program, they show that these non-cancer endpoints are not at all reproducible, between rats and mice, between genders, and

146 https://www.epa.gov/tsca-inventory/about-tsca-chemical-substance-inventory
147 https://www.drugwatch.com/vioxx/lawsuits/

compared to historic controls. This means that substances are abandoned or delays are prompted for investigative toxicology to de-risk these compounds. In many cases, this will impair the economic viability of a product: Given the development costs of a drug, for example discussed above, and the development time of on average 12 years, the remaining time under patent is only 13 years. This means that each day lost to market, at least $0.5 million is lost ($2.9 billion divided by 13 years = 4,745 days), if the substance shall bring back its development costs. This is not even considering that revenue is typically increasing over time. Similarly, for pesticides, development costs are now $286 million[148] and it takes meanwhile 11 years to bring a product to market (up from 8 years in 1995). With 9 years of patent left, this means 3,285 days and $87,000 per day just to break even. Other estimates speak of costs of €2.189 million and 10 years to market[47], which would result in 600,000 € per day.

Another new type of systemic toxicity is coming to the foreground with respect to marketing barriers, i.e., endocrine disruption (Juberg et al., 2014). This is most evident for the hazard-based regulations in the EU for biocides and plant protection products (see above). Two animal tests, i.e., the uterotrophic and the Hershberger assay, represent the highest tier dedicated animal tests, only trumped by multi-generation reproductive toxicity studies. Recent evaluations of these assays were eye-opening: Browne et al. (2015) analyzed over 1,000 articles and identified 442 studies of 103 chemicals meeting their minimum criteria. The analysis found that for any single chemical often results differed with animal model, strain, dose of test chemical, and delivery route used in the study. The inherent variability in the uterotrophic "guideline" method became evident by the fact that for chemicals with more than one guideline-like study, 26% had contradictory results with at least one positive and one negative study. A similar analysis of Hershberger rodent bioassays for about 3,200 chemicals is in press with similarly sobering results. It is questionable whether such assays should be applied to decide about the fate of important active agents.

13 Conclusions

This study once more established that economical drivers in synergy with ethical and scientific arguments support the need for a change in the safety sciences (Hartung, 2017c). Figure 4 illustrates the basic concepts, i.e., the positive impacts of alternative / new approach methodologies for the regulated and biotech industry. A key aspect is the human relevance and predictive capacity of new methodologies.

As a summary of this survey of the landscape of animal testing, two updated tables similar to those in Bottini and Hartung (2009) were prepared. Table 7 summarizes the descriptive key data on the regulated industries. It presents a snapshot of very different areas, which are united by the regulated use of chemicals with traditional animal tests and their alternatives. Noteworthy, some

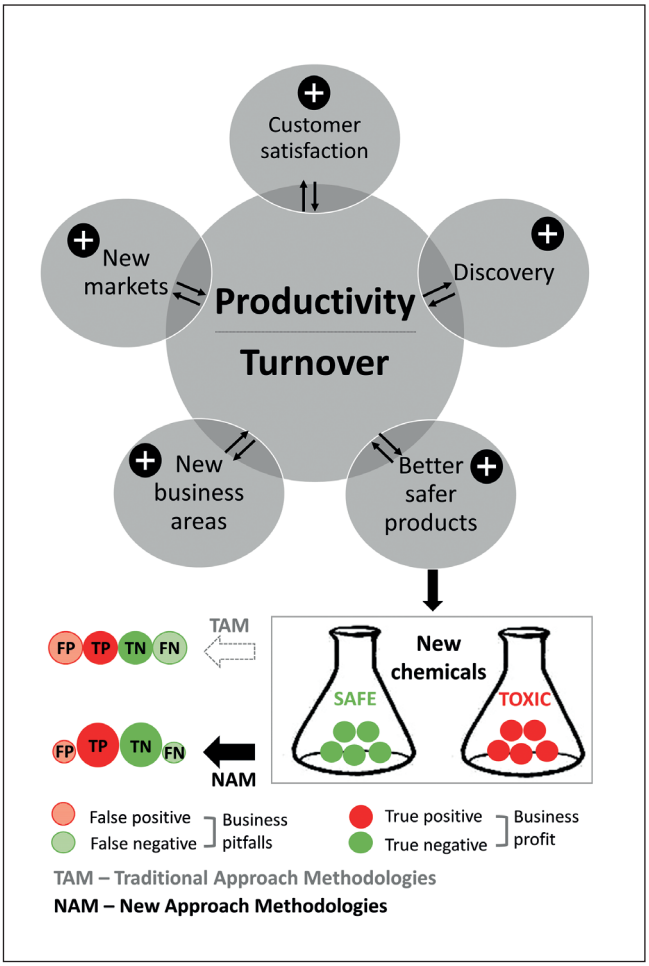

Fig. 4: The impact of NAM (new approach methods) on business success

Development of new predictive methods (NAM) leads to: (i) expansion of the business into new areas (e.g., production of cosmetic ingredients or production of medical devices), (ii) capture of new markets (e.g., establishing subsidiaries/spin-offs to produce organoids), (iii) more effective and rapid discovery of new chemical entities (NTE), (iv) increased satisfaction of the end users (also with regard to ethical concerns), and (v) manufacturing of better and safer products. All these together ultimately lead to increased productivity and turnover. Using NAMs over TAMs (traditional approach methodologies) for toxicity testing improves the capacity of companies to correctly predict the toxicity of chemicals in their products. The reduction of the number of false negatives (FN, products that are toxic, but are predicted to be safe) directly increases consumer safety. Decreasing the rate of false positives (FP, i.e., products that are in reality safe, but that are predicted to be toxic) has a direct effect on productivity and allows marketing of innovative products that would otherwise have been filtered out. The combined effect of allowing for safer products (low FN rate) and for more marketable products from the discovery process (low FP rate) leads to increased business profit.

[148] http://www.ecpa.eu/news/cost-crop-protection-innovation-increases-286-million-product

alternatives are also in their fifties already, such as mutagenicity tests or the Limulus pyrogen test.

In an abrogated form, we can summarize:

- *Contract research* is a flourishing industry expanding strongly as companies increasingly outsource both animal and alternative studies. Together with consultants as brokers in this process, they play a key role in any transition.
- The *chemical industry* has seen an enormous shift from European to Chinese dominance and recovery of the US. It is unlikely that this can be attributed to the REACH legislation, which probably represented more a revision and pruning of the inventory and possibly a cutting edge with better-tested chemicals in the future. Similar emerging legislations in many parts of the world will close the gap and reduce the toxicological ignorance – as even after REACH only 20% of chemicals will have (public) data.
- *Cosmetic industry* sees EU and US on a par and both trying to conquer the Chinese market. The rogue Chinese regulatory approach to cosmetic regulation is starting to open up, but it is still incompatible with the European testing ban, which is increasingly a model for other countries. The cosmetic industry is split – not European *vs* the rest but the big global companies *vs* the many small ones. The latter mainly reformulate substances without additional testing requirements. The big com-

Tab. 7: Overview of the landscape of industry regulated by animal testing
The different data sources and years (in the last decade) are referenced in the text.

Market	Number of companies (region)	Total industry sale (region)	World market	New substances per year	Animal use in 2011 in Europe (% all animals used)	Number of employees (region)
Pharmaceuticals • toxicology • R&D	1,900 (EU)	238 b€ (EU)	22,2%	19	~4.2 million (37%)* • ~400,000 • ~2.2 million R&D • ~1.6 million production and control	800,000 (EU)
Cosmetics	EU: 100+ ingredients 4,600 manufacturing 20,100 wholesale	10.8 b€ (EU) 12.4 b€ (US)	24%	2500-3000	90 (0.001%)	190,000 + 1.58 million (cosmetics value chain) (EU)
Food • additives	289,000 (EU)	1,1 t€ (EU) 0.65 t€ (US)	27.3 b€ (additives) 30% (EU)	~10-14 (EU)	~1000 (for human consumption) + ~4600 (for animal consumption) (.05%)	8.2 million (US) 4.2 million (EU)
Chemicals	28,329 (EU)	507 b€ (EU) 528 b€ (US)	3.36 t€ 15% (EU) 15.7% (US) 39.6% (China)	~1.000 (US)	~78,000 (used in industry) (0.7%)	1.2 million people directly 3-4 million indirectly (EU) 811,000 (US)
Plant protection products	22 (EU)	10.4 b€ (EU) 13.5 b€ (US)	41.3 b€ 25.1% (EU)	8 (EU)	~81,000 (for all agriculture) (0.7%)	26,000 (EU)
Tobacco (e-cigarettes) E-cigarettes	251 enterprises 460 brands	10 b€ (world)	19% (EU) 40% (China)	Up to 2400 (world)	None yet	1.5 million
Medical Technology • Toxicology • R&D	25,000 (EU)	110 b€ 140 m$ (US)	300 b€ (world) 40-45% (US) 16% (EU)	4500 (EU)	350,000 (3%)	575,000 (EU) 400,000 (US) (2 million indirectly)

Tab. 8: A calculation of animal testing costs in Europe for toxicological testing
EU animal use statistics from 2011 were used. Where the statistics aggregated several tests, a split according to the predicted REACH demands (Rovida and Hartung, 2009, Table 12) was assumed. The number of animals used for different types of test was divided by the number of animals foreseen in the respective OECD guideline studies and multiplied with the average costs of these tests by CRO from Table 3.

Animal test	Animal number per test	Costs per test (€)	Animal use in 2011	Total cost (in million €)
In vivo skin irritation	2	1,200	4,849	2.91
In vivo eye irritation	2.7	1,200	2,110	0.94
Skin sensitization	16	4,700	32,168	9.45
Further mutagenicity	64	62,500	21,288	20.79
Carcinogenicity	400	700,000	11,876	20.78
Two-generation reprotox	3,200	566,000	72,316	12.79
Acute oral tox.	8	1,500	51,362	0.97
Acute inhalation tox.	20	3,900	71,001	13.85
Acute dermal tox.	10	1,500	28,703	4.31
Short-term repeated dose tox.	50	46,500	268,562	268.36
Sub-chronic tox.	32	105,000	30,080	61.95
Long-term repeated tox.	160	372,000	26,674	4.49
Developmental tox. screening	560	63,200	39,778	5.41
Developmental tox. study	300	77,700	20,891	5.22
Short-term fish	42	3,800	57,676	1.07
Long-term fish tox.	108	8,600	13,370	0.23
Bioaccumulation (fish)	70	11,400	1,431	532.22
Total			**774,135**	**1,300**

panies need to comply with the different regulatory pressures and maintain an innovation pipeline. So far, the testing ban does not seem to have impacted very much (but the few years might have been buffered by the pipeline). However, the ban has made the cosmetic industry a driving force for alternative approaches, far more than proportional to their turnover or earlier animal use.

- The *food industry* is relevant with respect to animal testing only as to food additives and contaminants. We see on the one side a very restricted use of additives in Europe (390 substances), currently revisited by risk assessments by EFSA, and about 10 times more additives with very little data and only emerging revamping of the GRAS ("generally recognized as safe") assessment in the US. The latter could introduce testing needs, but the industry would be badly served with traditional methods and their notorious false-positive rate.
- *Agrochemicals* see a strong consolidation of the market with several mergers. The surging development costs make this a very small club anyway. The small number of new active agents limits animal use despite demanding testing requirements. A major threat to the market comes from the recent European legislation, which is hazard-based and includes endocrine disruption, which might mean a possible loss of a substantial number of active agents.
- The *tobacco industry* has somewhat surprisingly entered the field because of the new, lower-risk nicotine products. At the moment, animal use by the industry is more to show the advantage over traditional smoking and especially the US increasing tobacco control research fueled by the money FDA received from the industry after litigation. The critical aspect is the abundant use of flavors, likely about 10,000 by now. Their regulation might actually result in major testing needs in an industry largely untouched by this until now.
- *Pharmaceutical* testing is the mother of all traditional toxicology and, also due to time pressures and budget opportunities, the early adaptor of new technologies. Markets seem relatively stable. They are continuously reducing animal testing as indicated by European data, which is most remarkable as they are relatively free to choose the most efficient approach.
- *Medical devices* represent a very dynamic market with enormous product exchange. The FDA guidance and ISO standards could mean additional animal testing. There is a backlog of implementing alternative approaches compared to other sectors.

Last but not least, we tried to estimate the overall costs of regulatory animal testing in 2008 (Bottini and Hartung, 2009). Table 8 attempts this again on the basis of more recent figures. This table makes some rough assumptions: It assumes that all toxicology studies were done by the standards and costs of CRO. It simply divides the number of animals used by number of animals per test to arrive at the number of studies and then multiplies by average test costs. Noteworthy, with €532 million, numbers stayed roughly the same compared to 2005 (Bottini and Hartung, 2009). This final step rounds up our economic panorama of safety testing.

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Conflict of interest

The authors have no conflict of interest to state.