"Our scientific age demands that we provide definitions, measurements, and statistics in order to be taken seriously. Yet most of the important things in life cannot be precisely defined or measured. Can we define or measure love, beauty, friendship, or decency, for example?"
Dennis Prager[1]

"Descriptive statistics can be like online dating profiles: technically accurate and yet pretty darn misleading."
Charles Wheelan[2]

Food for Thought ...

New European Union Statistics on Laboratory Animal Use – What Really Counts!

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Abstract

Seven years after the last release, the European Commission has again collated and released data on laboratory animal use. The new report is the first to correspond to the requirements of the new Directive 2010/63/EU. Beside minor problems in reporting, the new reporting format is a major step forward, with additional new categories like severity allowing insight into animal use related questions that goes far beyond the previous reports.

An in-depth analysis confirms a slight decrease in animal use from 2015 to 2017, but also compared to the 2005, 2008 and 2011 reports, though the new reporting scheme makes this comparison difficult. Notable success is evident for replacing rabbit pyrogen testing but, in general, the implementation of accepted alternative methods lags behind expectations. Beside the roughly 10 million animals per year covered in the report, about 8 million animals were identified that fall under the Directive but are not included in this number. Their omission downplays the impact of REACH on animal use. The report, second to none in its detail internationally, represents an important instrument for benchmarking and strategically focusing activities in the 3Rs.

1 Introduction

These days, with a world in lockdown because of the COVID-19 crisis, show us impressively the importance of statistics for policy and public health decisions. Less acute, but a topic that is driving discussions in our part of the life sciences, after seven years of working somewhat in the dark, new statistics on animal use in Europe finally have been published (EC, 2020). Each and every statistic is problematic, as it condenses data, often with the intention to "illustrate" something, i.e., with possible bias. Andrejs Dunkel's quote "*It is easy to lie with statistics. It is hard to tell the truth without it*"[3] makes this point very nicely[4].

[1] Dennis Mark Prager is an American conservative radio talk show host and writer. Quote from https://libquotes.com/dennis-prager/quote/lbk7q8m

[2] In: Naked Statistics: Stripping the Dread from the Data

[3] https://libquotes.com/andrejs-dunkels/quote/lbn1b5k

[4] We apologize for using the quote a second time in this series (Luechtefeld and Hartung, 2017), but it drives home an important point.

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In 2010, the lengthy revision of the EU Directive on the protection of animals used for scientific purposes was finalized. We earlier provided a comparative evaluation of the new Directive 2010/63/EU (EU, 2010) with the former Directive 86/609/EEC (Hartung, 2010)[5]. Article 54 defined the new reporting requirements for the European Commission (COM) on the implementation of the Directive, i.e., publication of a statistical report every 5 years (formerly every 3 years) and the Member States (MS) were obliged to provide their annual statistical reports. As a consequence, the new report covers the years 2015-2017. New requirements include reporting on actual severity and additional categories of all uses including breeding. The exact content of the statistical reports was to be defined as part of the implementation. These requirements were largely changed by Regulation (EU) 2019/1010 on the alignment of reporting obligations (EU, 2019), which escaped the attention of many in the field. We will address this below.

In this series of Food for Thought … articles (Hartung, 2017), an earlier contribution investigated long-term trends of animal use in Europe (Daneshian et al., 2015) by analyzing the EU animal use statistics from the 15 countries that have been in the EU since 1995 plus respective data from Switzerland. The data up until 2011 suggested that the overall number of animals used for scientific purposes in these countries, i.e., about 11 million/year, remained relatively constant between 1995 and 2011, with net increases in Germany and the UK and net decreases in Belgium, Denmark, Italy, Finland, The Netherlands and Sweden.

According to the figures reported under the previous Directive's format (Directive 86/609/EEC), the total number of experimental animals used in the 27 MS of the EU in 2011 was 11.481,521 (EC, 2013). In Switzerland, 662,128 animals were used in the same year[6]. Now, this storyline can be continued. As always, the glass can be seen as half empty or half full… Most important is what we make of it, in the sense of "*Dear pessimist, optimist and realist, while you guys were busy arguing about the glass of water, I drank it. Sincerely, the opportunist*."[7]

Here we will try to distill what these new numbers tell us and put them into a broader context, following the motto of Henry Clay[8]: "*Statistics are no substitution for judgment*."

2 The altered legal situation: Regulation 2019/1010 on the alignment of reporting obligations in the field of environment policy

This new regulation (EU, 2019) has a direct impact on Directive 2010/63/EU (EU, 2010). It aims to take advantage of digitalization formats. The corresponding amendments (Tab. 1) are overall simplifying and facilitating: "*access for the scientific community, the general public and policy makers to essential information on the actual benefits, research outcomes and inflicted harm resulting from the use of live animals. The lack of systematic dissemination may also slow down the uptake of new ways to implement replacement, reduction and refinement, defeating the very reason for the obligation to carry out retrospective assessments*."

As a direct consequence, non-technical project summaries will be submitted in electronic format from 2021 onwards (new - article 43, paragraph 3). This will allow the COM to set up an open access searchable database (new - article 43, paragraph 4). Regarding statistics, in the same initiative, EU MS are asked to submit their data in electronic format by 2023 (new art. 54 paragraph 1). This will allow the COM to set up an open access searchable database (new - article 54, paragraph 2). However, one can expect the first consolidated EU statistics only in 2024, but then with annual updates. In the past, this was foreseen only every three years. Therefore, the perspective of having an open access, searchable, up-to-date database dwarfs any short-term inconveniences.

Previously, the COM had the obligation to publish a summary report of the statistics. This still seems to be the case, even if article 57 is now deleted. The corresponding new provision was added in article 54, paragraph 2. However, there is no longer an obligation to submit the report to the European Parliament (EP) and the Council, which may call its compulsory requirement into question.

The new report on animal use in the EU (EC, 2020) may be an example of what to expect as future summary reports on the animal statistics from COM. As a side note, a specific overview of the 28 MS is missing in the current statistics report compared to previous one (EC, 2013). In the authors' opinion, comparing MS should be encouraged since it may motivate sharing best 3Rs practices across the EU and beyond.

With this major revision of the format, the benefits in terms of transparency, access to data, and the potential to mine it are indisputable. One may regret the time shift of two years (2024 instead of 2022) until the next statistics report, which is a highlight for the 3Rs scientific community and allows retrospective assessment of the impact of 3Rs efforts. One can only commend the added value of COM as the honest broker providing the summary reports of the statistics and must hope that these remain as detailed as possible, even if no longer considered compulsory with an obligation to present the document to the Council and the EP.

3 The changing European political landscape

Since the implementation of Directive 2010/63/EU at MS level, stakeholders have initiated numerous activities (Tab. 2) that are durably impacting the 3Rs landscape.

5 An accompanying supplement reviewed all changes to the older Directive of 1986, available at doi:10.14573/altex.2010.4.285

6 https://www.tv-statistik.ch/de/statistik/

7 https://twitter.com/biiimurray/status/357218511577292801?lang=en

8 Banker, 1930, https://quoteinvestigator.com/2019/08/04/statistics/

Tab. 1: Comparison of the Articles of Directive 2010/63/EU after the implementation of Regulation 2019/1010

Directive 2010/63/EU	Amendments of Directive 2010/63/EU after Regulation 2019/1010
Article 43 Non-technical project summaries 1. Subject to safeguarding intellectual property and confidential information, the non-technical project summary shall provide the following: (a) information on the objectives of the project, including the predicted harm and benefits and the number and types of animals to be used; (b) a demonstration of compliance with the requirement of replacement, reduction and refinement. The non-technical project summary shall be anonymous and shall not contain the names and addresses of the user and its personnel.	Article 43 Non-technical project summaries 1. No amendments
2. Member States may require the non-technical project summary to specify whether a project is to undergo a retrospective assessment and by what deadline. In such a case, Member States shall ensure that the non-technical project summary is updated with the results of any retrospective assessment.	'2. Member States may require the non-technical project summary to specify whether a project is to undergo a retrospective assessment and, if so, set out the deadline. In such a case, **from 1 January 2021**, Member States shall ensure that the non-technical project summary is updated **within six months of the completion** of the retrospective assessment with the results thereof.
3. Member States shall publish the non-technical project summaries of authorised projects and any updates thereto.	3. Member States shall, until 31 December 2020, publish the non-technical project summaries of authorised projects and any updates thereto. **From 1 January 2021, Member States shall submit for publication the non-technical project summaries, at the latest within six months of authorisation, and any updates thereto, by electronic transfer to the Commission.';**
	'4. The Commission shall, by means of implementing acts, establish a common format for submitting the information referred to in paragraphs 1 and 2 of this Article. Those implementing acts shall be adopted in accordance with the examination procedure referred to in Article 56(3). The Commission services shall establish and maintain a searchable, open access database on non-technical project summaries and any updates thereto.';
Article 54 Reporting 1. Member States shall by 10 November 2018, and every 5 years thereafter, send the information on the implementation of this Directive and in particular Articles 10(1), 26, 28, 34, 38, 39, 43 and 46 to the Commission.	Article 54 "INFORMATION ON IMPLEMENTATION AND PROVISION OF STATISTICAL DATA " 1. Member States shall by 10 November 2023, and every five years thereafter, send the information on the implementation of this Directive and in particular of Article 10(1) and Articles 26, 28, 34, 38, 39, 43 and 46 to the Commission. **Member States shall submit and publish that data, by electronic transfer in a format established by the Commission in accordance with paragraph 4.** **No later than six months after the submission by Member States of the data referred to in the second subparagraph, the Commission services shall publish and regularly update a Union-wide overview on the basis of that data.**
2. Member States shall collect and make publicly available, on an annual basis, statistical information on the use of animals in procedures, including information on the actual severity of the procedures and on the origin and species of non-human primates used in procedures. Member States shall submit that statistical information to the Commission by 10 November 2015 and every year thereafter.	2. Member States shall collect and make publicly available, on an annual basis, statistical information on the use of animals in procedures, including information on the actual severity of the procedures and on the origin and species of non-human primates used in procedures. Member States shall submit that statistical information to the Commission, at the latest by 10 November **of the following year, by electronic transfer, in a non-summarised format established by the Commission in accordance with paragraph 4.** **The Commission shall establish and maintain a searchable, open access database containing that statistical information.** **On an annual basis, the Commission services shall make publicly available the statistical information submitted by the Member States in accordance with this paragraph and a summary report thereof.';**

Directive 2010/63/EU	Amendments of Directive 2010/63/EU after Regulation 2019/1010
3. Member States shall submit to the Commission, on annual basis, detailed information on exemptions granted under Article 6(4)(a). 4. The Commission shall by 10 May 2012 establish a common format for submitting the information referred to in paragraphs 1, 2, and 3 of this Article in accordance with the regulatory procedure referred to in Article 56(3).	3. No amendments
	'4. The Commission shall, by means of implementing acts, establish a common format and information content for submitting the information referred to in paragraphs 1, 2 and 3 of this Article. Those implementing acts shall be adopted in accordance with the examination procedure referred to in Article 56(3).';
Article 57 - Commission Report 1) By 10 November 2019 and every 5 years thereafter, the Commission shall, based on the information received from the Member States under Article 54(1), submit to the European Parliament and the Council a **report on the implementation** of this Directive 2) By 10 November 2019 and every 3 years thereafter, the Commission shall, based on the **statistical information** submitted by Member States under Article 54(2), submit to the European Parliament and the Council a **summary report** on that information.	deleted

Tab. 2: Non-exhaustive list on notable initiatives or actions taken by European 3Rs stakeholders since the last report in 2011

Year	Milestone
2013	Full ban on animal testing for cosmetics in EU[a]
2015	European Citizens' Initiative (Stop vivisection)[b]
2016	COM Scientific conference Non-Animal Approaches – The way forward[c]
2017	Dutch government plans to phase out the use of animals in certain areas by 2025 (namely regulatory testing of chemicals, food ingredients, pesticides and (veterinary) medicines, and biological products such as vaccines)[d]
2018	European Parliament's resolution on world ban for cosmetics[e]
2019 (May)	Closure of lab animal research facility in Wellcome Sanger in UK[f]
2019 (September)	US EPA's decision to eliminate animal testing for mammals by 2035[g]
2020 (February)	First COM report of strict legislation designed to protect research animals. The figures come from the first report on the state of animal research since the introduction of the new Directive.

[a] https://ec.europa.eu/growth/sectors/cosmetics/animal-testing_en
[b] https://europa.eu/citizens-initiative/stop-vivisection_en
[c] https://ec.europa.eu/environment/chemicals/lab_animals/3r/pdf/scientific_conference/non_animal_approaches_conference_report.pdf
[d] https://chemicalwatch.com/51958/dutch-government-plans-to-stop-animal-testing-by-2025
[e] https://www.europarl.europa.eu/doceo/document/B-8-2018-0217_EN.html
[f] https://www.nature.com/articles/d41586-019-01685-7
[g] https://www.sciencemag.org/news/2019/09/us-epa-eliminate-all-mammal-testing-2035

3.1 Activities of the European Parliament

Besides constant support of the COM directorates, in particular Environment, Research and Joint Research Centre, the commitment of the EP towards the protection of laboratory animals for scientific purposes is noticeable[9]. In the past (2014-2019) and current (2019-2024) terms, elected policy-makers have shown continued interest in this particularly challenging problem.

One can name a few reasons:

- *ethics:* "Dieselgate"[10] linked with "monkeygate"[11], the resolution to globally end animal testing in cosmetics[12] or, more recently, the allegations against the German LPT laboratory of cruelty towards laboratory animals[13] and falsifying GLP toxicological results[14]
- *conflict of interest:* The Monsanto papers[15] combined with the glyphosate renewal authorization procedure linked with results on carcinogenicity[16] in rodents. In two cases (dieselgate and Monsanto papers), the European Parliament set up a special committee with full power (president, budgets and hearing) in order to shed light on practices.
- *values:* Members of the European Parliament (MEPs) from different political groups have maintained one of the first-established and longest running intergroups[17] namely "Welfare and Conservation of Animals"[18] over the last two terms. This is one of the most popular and currently has a working group dedicated to animals in science led by MEP Tilly Metz (Greens, Luxembourg)
- *societal challenge:* The Stop *Vivisection* European citizen initiativ[19] and COM response[20]
- *legislative:* Multiple opportunities in the past term allowed MEPs to amend testing requirements (e.g., medical device regulation[21]) and facilitate the use of alternative methods such as but not limited to the 7th environment action plan[22], pilot projects on 3Rs, in particular in education and training[23], as well as a *Feasibility Study on a Common Open Platform on Chemical Safety Data* for ECHA and EFSA[24].

The current legislative term also promises to further enhance alternatives to animals. In particular with regard to:

a) European Green Deal[25]: Currently, it is the most promising and relevant policy file to pursue 3Rs efforts. In particular, the zero-pollution environment as stated in EP resolution[26] of January 15, 2020 on the European Green Deal includes: "*A zero-pollution ambition for a toxic-free environment [..] [which] underlines the need to reduce animal testing in risk assessments and calls for increased efforts and funds to this end*."

b) Europe's Beating Cancer Plan[27]: With cancer identified as a leading cause of death in Europe, most of the efforts have been focused on its treatment rather than its prevention. As stated in the roadmap, besides personal lifestyle changes, cancer risk can be reduced by "*reducing environmental risk factors (such as air and water pollution or exposure to carcinogenic chemicals, be it at the workplace, via the environment or in products)*." This can be an opportunity to perform selected 3Rs efforts in relation to cancer and combine them with the cancer mission[28].

c) A chemicals strategy for sustainability[29]: This current draft motion of resolution summarizes the objectives that EP wants COM to achieve. It can be seen as a "sub-product" of the zero-pollution ambition of the European Green Deal. It provides a list of things to consider such as endocrine disrupters, neurotoxicants, plant protection products, etc. This includes reduction of animal testing but also a concomitant increase in product safety.

d) Declaration of intention of the MEPs coordinated by the Intergroup on the Welfare and Conservation of Animals[19]. Besides the call for action to COM, what is of particular interest is the list of 59 MEPs, who have signed the declaration so far. To put the number of MEPs in perspective, this corresponds to 8.3% of the 704 MEPs in the post-Brexit scheme. The Greens, Socialists and Democrats are the best represented political groups that have signed the declaration (Fig. S1[30]).

In Figures S1 and Figure S2[30], three MS feature most prominently in the collected signatures, i.e., France, Italy and Poland, and three MS do not have an MEP representative for this partic-

9 http://epthinktank.eu/2020/03/20/replying-to-eu-citizens-campaign-against-animal-experimentation/
10 https://www.politico.eu/tag/dieselgate/
11 https://www.politico.eu/article/lead-scientist-in-monkey-tests-automakers-fully-aware-of-trials/
12 https://www.europarl.europa.eu/doceo/document/B-8-2018-0217_EN.html
13 https://www.the-scientist.com/news-opinion/german-lab-faces-criminal-charges-after-undercover-investigation-66579
14 https://www.pan-europe.info/press-releases/2020/02/fraud-german-laboratory-casts-additional-doubts-2017-re-approval-glyphosate
15 https://www.politico.eu/article/glyphosate-monsanto-accused-of-ignoring-relevant-scientific-data/
16 https://ec.europa.eu/info/research-and-innovation/strategy/support-policy-making/scientific-support-eu-policies/group-chief-scientific-advisors/glyphosate_en
17 https://www.europarl.europa.eu/about-parliament/en/organisation-and-rules/organisation/intergroups
18 https://www.animalwelfareintergroup.eu/
19 https://europa.eu/citizens-initiative/stop-vivisection_en
20 https://ec.europa.eu/transparency/regdoc/rep/3/2015/EN/3-2015-3773-EN-F1-1.PDF
21 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0745
22 https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32013D1386
23 https://ec.europa.eu/environment/chemicals/lab_animals/related_topics_en.htm
24 https://etendering.ted.europa.eu/cft/cft-display.html?cftId=5516
25 https://www.europarl.europa.eu/doceo/document/TA-9-2020-0005_EN.pdf
26 https://www.europarl.europa.eu/doceo/document/TA-9-2020-0005_EN.pdf
27 https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/12154-Europe-s-Beating-Cancer-Plan
28 https://ec.europa.eu/info/horizon-europe-next-research-and-innovation-framework-programme/mission-area-cancer_en
29 https://www.europarl.europa.eu/cmsdata/198020/envi-work-in-progress-16.03.2020.pdf
30 doi:10.14573/altex.2003241s

ular question (Cyprus, Croatia and Romania). Last, most of the MEPs (75%), who signed the declaration, are also part of the intergroup, which covers more than laboratory animals as a topic. Therefore, without belittling the MEPs' commitment, the number of signatures matters, but a signature does not automatically mean full dedication of the MEP to the topic. Moreover, since this is only a snapshot of the situation, it does not preclude stronger or weaker MEP commitment on *ad hoc* basis.

3.2 European Chemical Agency (ECHA), European Food Safety Agency (EFSA) and European Medicines Agency (EMA)

These three agencies play a pivotal role in the application and enforcement of alternative methods for testing requirements. Multiple initiatives and official reports already exist describing their efforts[31,32,33] or commenting on their absence of commitment[34] towards the 3Rs. Over the last years, a certain 3Rs mindset has emerged from the different agencies, even though not set in stone:

a) Under ECHA, data is the new 3Rs *El Dorado*. According to the last ECHA report in 2017[35]:
   - Existing information was used in 89% of the dossiers.
   - Read-across (information derived from similar substances) was used in 63% of the dossiers.
   - Weight of evidence (combining information from different sources) was used in 43% of the dossiers.
   - QSAR (quantitative structure-activity relationship) prediction was used in 34% of the dossiers.
b) Under EFSA, *in vitro* approaches seem to focus on developmental neurotoxicity testing[36] with the OECD[37].
c) Under EMA, 3Rs activities[38] are organized around a group of external experts that review guidance and provide reflections papers on the 3Rs principles.

The agencies' efforts may appear divided; however, they do collaborate regularly on joint tasks, e.g., endocrine disrupters[39] or a common work program[40] via the European Union Agencies Network (EUAN)[41]. Another example of joint collaboration is the EP-funded Pilot Project on the Feasibility Study on a Common Open Platform on Chemical Safety Data[42]. This was initiated by CAAT's European policy program, working with MEPs toward a broader release of registration data by agencies. The European Parliament Committee on the Environment, Public Health and Food Safety adopted this position unanimously in fall 2017 and held a hearing with the heads of the relevant agencies in early 2018.

The process has not been completed but, following some positive responses, this pilot project is currently in preparation. ECHA, EFSA and EMA have, among their many responsibilities, a strong role to play in collecting safety data for all manufactured goods placed on the EU market. They have to gather similar types of information (toxicological endpoints) but they handle them differently (dissemination/confidentiality/format). This difference is mostly linked to internal EU agency policies. However, it has multiple consequences, in terms of the costs of implementing EU regulations, levelling the playing field for the different industrial sectors, and redundancy of animal testing, which hamper the efficacy of the agencies. The pilot project aims to improve the transparency, digitalization and harmonization of data formats between the agencies.

In 2015, the chemical trade association CEFIC[43] signed a memorandum of understanding (MoU) with ECHA in which the agency agreed to share the collected safety data set. On this basis, CEFIC LRI developed an open software tool named AMBIT[44], which uses the ECHA data set, to facilitate high-quality chemical safety prediction. Similarly, the OECD QSAR toolbox[45] makes use of the dataset. The opportunities for predictive toxicology that arise from such large datasets have been discussed earlier in this series (Hartung, 2016; Luechtefeld and Hartung, 2017), indicating that a virtuous effect could be triggered by higher transparency. In the same period, CAAT-Europe (University of Konstanz) was in discussions to sign a similar MoU to use and share ECHA's data set with scientific partners. CAAT had already published their results earlier in 2016 (Luechtefeld et al., 2016a-d). They performed retrospective assessments of some of the most commonly used, standardized test guidelines and provided recommendations to improve their use and *in fine* EU consumer protection. In 2017, ECHA opened its dataset to the public and foresaw to update it periodically; however, there has been no update so far. Similarly, EFSA released endpoint data (e.g., OPENFOODTOX[46]) in 2016.

The European Parliament ENVI committee, which is the contact point for the three agencies, requested a "*common agency safety data initiative*" in 2018 to pave the way for this pilot project. However, EMA decided not to be involved because of

31 https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/ar2018.pdf
32 https://echa.europa.eu/animal-testing-under-reach
33 https://www.ema.europa.eu/en/committees/working-parties-other-groups/chmp/expert-group-3rs
34 https://www.ombudsman.europa.eu/en/decision/en/60909
35 https://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2017_en.pdf/075c690d-054c-693a-c921-f8cd8acbe9c3
36 https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2018.EN-1410
37 http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2017)4&doclanguage=en
38 https://www.ema.europa.eu/en/documents/report/biennial-report-joint-cvmp/chmp-working-group-application-3rs-regulatory-testing-medical-products-2016/2017_en.pdf
39 https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5311
40 https://euagencies.eu/sites/default/files/euan_wp_2019_2020_0.pdf
41 https://euagencies.eu
42 https://ec.europa.eu/transparency/regdoc/rep/3/2019/EN/C-2019-4121-F1-EN-ANNEX-1-PART-1.PDF
43 http://www.cefic.org
44 http://cefic-lri.org/toolbox/ambit/
45 https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
46 doi:10.2903/sp.efsa.2018.EN-1438

Brexit and their relocation to The Netherlands. The goal of the project, worth €600 000, consists of: "*facilitating seamless sharing of data between authorities and provide public access to researchers, regulators, industry and the citizen at large. This will promote: a) transparency and trust in EU decision making, b) research and data analytics, c) innovation d) less animal testing & more predictive toxicology, and e) better regulatory decision making and informed consumer choices. A common portal could provide:*

- *A registry of toxicological studies for chemical substances and regulated products. Industry-sponsored studies are available for regulatory assessment by the respective authority, but they are currently not always available to other authorities, industrial actors, the research community or the public at large.*
- *A repository for research and scientific data. Peer-reviewed studies are not always used to the extent that they could be in regulatory assessments as searching for, and getting access to, studies is resource demanding. Scientists as well as publishers of their studies lack the fundamental interest to share data with the aim to address regulatory questions*.
- *A platform for data analytics, predictive toxicology (i.e. avoidance of animal testing), better environmental monitoring, better study design, development of artificial intelligence and machine learning applications*."

It was adopted at the end of 2018, and the call for tender was released in 2019. The work is currently ongoing. All these key activities contribute to the elaboration of a sustainable approach to releasing data in a systematic and repetitive manner, independent of the *ad-hoc* support of individuals heading agencies and their counterparts in civil society, which could fade away over time. EMA is nevertheless following in the footsteps of ECHA and EFSA. In January 2020, the Court of Justice of the European Union confirmed the right to access to documents contained in the marketing authorization applications, namely toxicology reports and a clinical study report, for two medicinal products, one for human use (Case C-175/18 P) and the other for veterinary use (Case C-178/18 P)[47]. One particular aspect should be mentioned: "*The Court of Justice has thus concluded that the application of a general presumption of confidentiality is merely an option for the institution, body, office or agency concerned*." This gives EMA much more leeway and capacity to handle this valuable data and may allow consolidation of the chemical's common open platform.

3.3 Member State questionnaires

One of the novelties in the new statistics report (EC, 2020) is a questionnaire sent to each MS asking them to provide comments and contextualize the annual statistics submitted to COM. The six questions are listed in Table S1[30]. Question 4 is of particular interest as it analyzes the voluntary efforts by MS to further incorporate the 3Rs into daily and scientific practice. Based on MS responses to question 4, a set of criteria developed by the authors was used to score efforts at MS level. Four aspects were considered:

1. Education and training
2. Communication/promotion/dissemination/workshops/conferences/database
3. Funding/awards
4. National/local 3Rs center

With one point allocated per criterion, the maximum score was 4. Of the 28 MS (Fig. 1 and Tab. S2[30]), United Kingdom, Netherlands and Lithuania were excluded since their responses either referred to other documents (LT) or were misleading as they referred to existing national efforts (UK, NL). Eight MS scored no points, reporting no voluntary 3Rs activities since they were solely implementing the Directive *stricto sensu* or did not fill in the question (MT, PT, BG, CY). Four MS scored one point, with their main activities falling under communication/promotion (IE, ES, AT) or education and training (GR). Seven MS scored two points, all having education and training in common but differing in the second criterion, with FR hosting a national/local 3Rs center (Francopa) whereas the others (RO, SK, ES, HR, LU, PL) reported efforts in communication/promotion. The five MS that scored three points each had funding of 3Rs research at national level in common (IT, BE, DK, FI, DE, IT). Sweden (SE) was the only MS to score the full four points. Looking at the overall voluntary 3Rs activities of the 25 MS, i) 56% are actively communicating and promoting 3Rs principles for end-users, ii) 44% are coordinating education and training, iii) 24% offer funding and awards for 3Rs work at a national level, and iv) 24% have established a national or regional 3Rs center.

This interpretation is based on a qualitative, subjective analysis from partial information collected under a specific context. The authors are well aware that other national initiatives do exist, even if not reported under question 4, e.g., 79% of MS do have a 3Rs center[48] – some even have more than one – whereas this is only reported in question 4 in 24% of the cases. Why is this information missing? Lack of communication between national stakeholders? Lack of manpower and too many reporting obligations? Lack of interest? Difficult to say. Nevertheless, beyond the scoring aspect, this should be seen as an attempt to start a constructive discussion about quantitatively describing and ranking MSs' efforts towards the 3Rs beyond trends in statistics. There is clearly room for improvement, and Article 47.4 of Directive 2010/63/EU (EU, 2010) can show the way forward: "*Member States shall, at national level, ensure the promotion of alternative approaches and the dissemination of information thereon*." The COM website[49] publishes related MS efforts; however, until now, only 15 MS have published their efforts to promote the 3Rs at least once (see Tab. S3[30]). Without discussing the content of the MS submission, only Belgium and Slovakia have regularly and continuously reported their 3Rs-related efforts. Only one fifth of the MS complied with Article 47.4 in 2017 and 2020. Of note, the three MS that currently use the most animals in the EU (France, Italy and Germany) only submitted a single report during the past 10 years.

47 https://curia.europa.eu/jcms/upload/docs/application/pdf/2020-01/cp200006en.pdf
48 https://norecopa.no/media/8321/3r-centre-map-201119.pdf
49 https://ec.europa.eu/environment/chemicals/lab_animals/3r/advance_en.htm

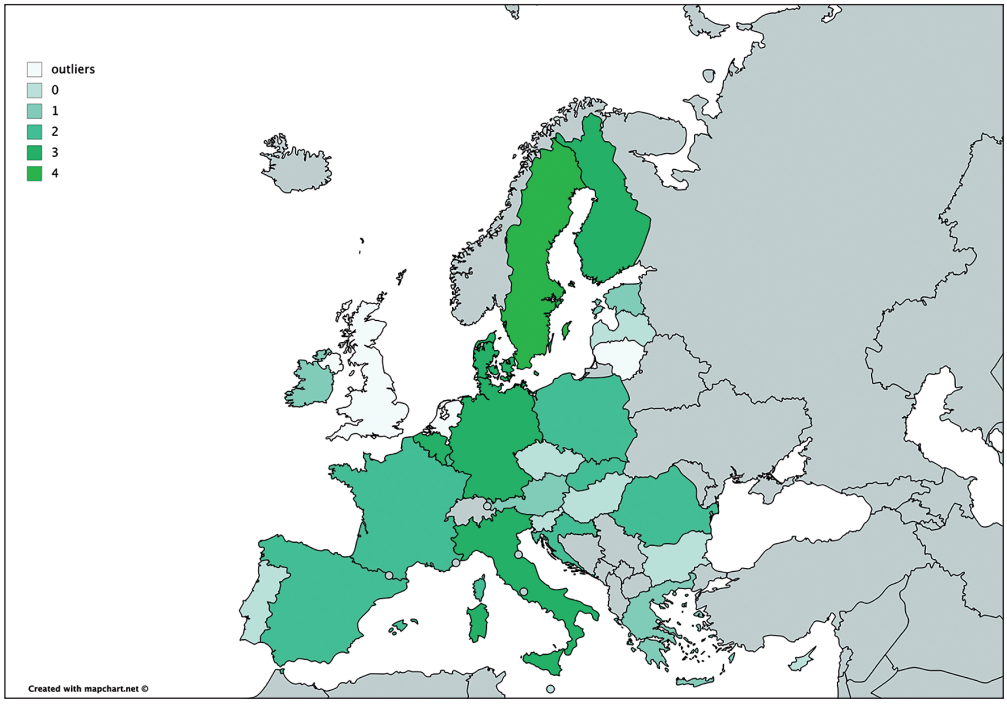

Fig. 1: Scoring 3Rs activities as provided by the MS to COM

Similar investigations were performed elsewhere. For example, Taylor (2014) took the first look at MS efforts towards 3Rs based on Article 47.4 (EU, 2010). In 2016, the European Federation of Pharmaceutical Industries Association (EFPIA) focused on other key performance indicators (13 in total)[50] such as evidence of senior executive ownership of 3Rs; new technologies; examples of investment in enrichment; number, subject and impact of internal awards, etc. In its latest report[51], EURL ECVAM mentioned a *Feasibility Study on Indicators of Alternative Methods or Approaches to Animal Experimentation*. The results of the study are – to the authors' knowledge – not published. Recently, Hawkins and Bertelsen (2019) provided 3Rs indicators to help assess the culture of care at the level of a facility. Those separate initiatives can pave the way to further actions and can be used as a baseline for future comparisons when the next statistics are released. This may counterbalance a reductionist analysis based only on the trends in animal use. This "draft" scoring system could be consolidated by incorporating the number of publications per MS using a specific set of 3Rs key words and be updated accordingly before each statistical report. National competent authorities and policy makers could take advantage of this scoring system.

Altogether, this shows the European political environment as very supportive of alternatives to animal experimentation. This is supported also by polls (Eurobarometers) on animal welfare[52] and more specific animal experimentation[53]: "*The majority (66%) find that scientists should be allowed to do research on animals like mice if it produces new information about human health problems, while only 18% of respondents disagree. As seen earlier only 44% of respondents find animal testing acceptable when larger animals such as dogs and monkeys are the subject.*" We see reporting and the resulting transparency as an important sparring partner for these efforts. There are limitations in some details, but altogether the consistent support is appreciated.

4 Authorization of animal experiments

Directive 2010/63/EU (EU, 2010) requires a more stringent authorization process for animal experiments. Before going into the details of the numbers of animals used, it is interesting to consider the number of approved and rejected projects. There are differences between MS in the management of the authorization process. In many cases, non-compliant projects are adjusted through a dialogue with the competent authority or withdrawn before they are rejected. There are large discrepancies among MS regarding the project authorization decisions, with the majority of them generally accepting all projects (Tab. 3).

Directive 2010/63/EU (EU, 2010) requires that non-technical summaries of authorized projects are published to inform the public on live animal use. These summaries are published in the

50 https://www.efpia.eu/media/25626/3rs-posters-05122016-2.pdf
51 https://op.europa.eu/en/publication-detail/-/publication/6e340c15-a2f6-11e9-9d01-01aa75ed71a1/language-en/format-PDF
52 http://eurogrourb.cluster020.hosting.ovh.net/wp-content/uploads/Eurobarometer-2016-Animal-Welfare.pdf
53 https://ec.europa.eu/commfrontoffice/publicopinion/archives/ebs/ebs_340_en.pdf

Tab. 3: Numbers of animal research projects finally accepted, submitted, rejected and percentage rejected of those submitted

MS	submitted	accepted	rejected	% rejected
Austria	721	717	4	0.60%
Belgium	1,621	1,605	16	1.00%
Bulgaria	23	23	0	0.00%
Croatia	50	47	3	6.40%
Cyprus	6	6	0	0.00%
Czechia	528	528	0	0.00%
Denmark	269	269	0	0.00%
Estonia	17	17	0	0.00%
Finland	124	124	0	0.00%
France	3,708	3,708	0	0.00%
Germany	3,800	3,800	0	0.00%
Greece	183	175	8	4.60%
Hungary	271	206	65	31.60%
Ireland	120	120	0	0.00%
Italy	1,264	1,005	259	25.80%
Latvia	15	13	2	15.40%
Lithuania	24	24	0	0.00%
Luxemburg	22	22	0	0.00%
Malta	1	1	0	0.00%
Netherland	440	431	9	2.10%
Poland	774	774	0	0.00%
Portugal	56	56	0	0.00%
Romania	114	114	0	0.00%
Slovakia	93	92	1	1.10%
Slovenia	28	18	10	55.60%
Spain	1,569	1,569	0	0.00%
Sweden	662	657	5	0.80%
United Kingdom	587	587	0	0.00%
Total	**17,090**	**16,708**	**382**	**2.30%**

national language of the MS and lack standardized key words or other features by which they could be analyzed and compared. Therefore, the anticipated open search database would be a major improvement. Justifications of the absence of alternatives are commonly generic, often referring directly or indirectly to tradition rather than a failure to find an option after a serious commitment to look for other possibilities. For example, the list of Italian projects contains only information regarding basic or applied research; all other information is probably not public. It is also not transparent when a retrospective analysis is requested (in addition to the mandatory requirements laid out in Art. 39, paragraph 2) and if so, where this analysis is made publicly available. The authors' recommendation is that both non-technical summaries and retrospective evaluations are incorporated in the proposed EU-wide database in a way that allows consultation and statistical analysis.

5 The key differences in reporting on numbers of animals

The 2015-2017 data of the report (EC, 2020) are significantly different from the previous data and cover areas of animal use that were not included under the previous legislation. A somewhat limited comparison may be possible for the numbers of animals used for the first time for the purposes of research and testing.

However, even there, until 2011:

a) Invertebrate species were not included (cephalopods with very small impact).
b) The previous numbers partly included (depending on the MS) animals that were used for the creation of genetically altered animal lines, which are now separate.
c) Reuse was not accounted for before (which has a small impact of 2%)
d) Some bias in reporting over time is noted: The transition to the changed reporting criteria did not happen simultaneously, with data from 2017 being most reliable. Discussion should thus best be based on 2017 data.

The new report separately publishes the number of animals used for the creation of a new, genetically-altered animal line and the maintenance of an existing genetically altered animal line. These aspects are covered below.

6 Where is the beef? Numbers of animals used

The COM is requested to publish a report on the use of animals for scientific purposes in the EU, according to Directive 2010/63/EU (EU, 2010), though this was somewhat weakened by Regulation 2019/1010 on the alignment of reporting obligations in the field of environment policy (EU, 2019). The Sixth Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union was published in 2010 (EC, 2010) and the Seventh in 2013 (EC, 2013); both still corresponded to the previous Directive 86/609/EEC. The current report is the first that is entirely based on Directive 2010/63/EU, which all MS were required to transpose into national legislation by January 1, 2013. As stated above, it is difficult to compare this latest report with previous ones. Our attempts are given below. The COM explains that this situation is due to a large difference between the two Directives and the previous reporting requirements and concludes that the data presented in this report are not, in general, comparable with the information presented in reports published under Directive 86/609/EEC.

Nevertheless, some adaptations were applied here to try to reduce the differences by excluding some categories of animals, even though they are covered by the scope of Directive 2010/63/EU:

a) Fetal forms of mammals;
b) Animals killed solely for organs and tissues, and sentinels, unless the killing is performed under a project authorization using a method not included in Annex IV of Directive 2010/63/EU;
c) Animals bred and killed without being used, apart from genetically altered animals with intended and exhibited harmful phenotype, and those having been genotyped with an invasive method before being killed.

It is clear that in order to compare the new data with the previous data, only animals that underwent a treatment are counted; new animals (cephalopods), animals at the early stage of development (fetus and embryo) and animals that were bred for the purpose of an experiment were not considered here. Genetically altered animals are counted separately. Figure 2 shows the results, including the earlier statistics, supporting a trend towards reduced animal numbers over these twelve years.

For 15 of the 28 MS, 2018 data are already available[54]. They represent 69% of animal use in 2017 (major ones that are missing are France, Italy and The Netherlands); these numbers suggest a further decline of 3.4% from 2017 to 2018 (Tab. S4[30]), continuing this trend.

However, the reporting by the COM has a big impact on toxicological tests. Even disregarding sentinels, all developmental and reproductive toxicity studies use many more animals than are reported (Knight and Rovida, 2014). Therefore, the new Directive has a much broader scope of applicability compared to the previous Directive 86/609/EEC, but this is not reflected in the new analysis, because the newly reported categories of animals are kept separate.

The number of animals that were bred in 2017 was 12.6 million, thus about 2.8 million were not used in procedures. Ultimately, these animals were killed without gaining any information for science and society. Data for 2015 and 2016 are not available. Even disregarding animals that are part of an experiment but are not treated with the test substance, this number should include animals that are used for the production of fetal calf or bovine serum (FCS or FBS) or S9 fraction (organ tissue homogenate used in biological assays – the S9 fraction is most frequently used in assays that measure the metabolism of drugs and other xenobiotics[55]), which are ingredients widely used in many biological laboratories.

For farm animals, probably the only justified use is that they are necessary to test new veterinary drugs. However, they are also used to analyze metabolites and residues from feed additives.

The data reported on 2015-2017 (EC, 2020) show:

- The totals of the three years are similar with no clear trend, maybe a small decrease toward 2017, but an increase over 2015 and 2016.

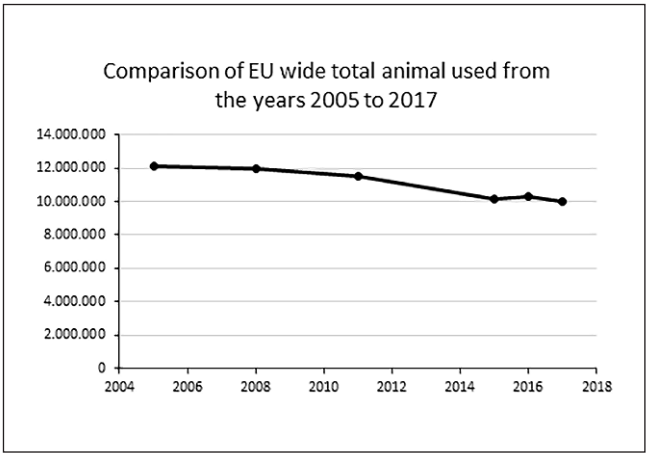

Fig. 2: Comparison of EU-wide total animal use from 2005 to 2017

Numbers from 2015 to 2017 are first time use corrected for country Croatia, cephalopods and new genetically altered animal lines (assuming all countries included them in their previous reports) to make them comparable with numbers reported in 2005 to 2011. Data from 2005 reflects only 25 MS and not 27.

- The use of birds (6%) and amphibians, cephalopods & reptiles (0.3%) decreased.
- The use of mammals stayed approximately the same, mainly mice (61%) with stable use, followed by rats 12%, rabbits and others 8%, with dogs/cats/non-human-primates at 0.3%.
- Fish use was stable (13%).
- There was a large increase in % for other rodents in 2017 (mainly bats studied for human infectious disorders).
- Non-human primates increased by 15%, with cynomolgus monkey use (representing 88% of non-human primates in 2017) increasing by 16%.
- No Great Apes were used for scientific purposes in the EU.
- 90% of animals were born at a EU-registered breeder, with the notable exception of non-human primates. In 2017 only 1.1% were from the rest of the world; Numbers born in the EU but not at a registered breeder slightly decreased by 2%. In 2017, the origin of non-human-primates was 87% outside EU driven by cynomolgus monkeys almost entirely from outside of the EU; all other > 50% from EU registered breeder. No non-human primates were sourced from the wild in 2017.

Reuse of animals remains rare and stable at 2%. When the term "details of all uses of animals" is used, this means that if an animal is used twice, it is also counted twice. Otherwise, the table is named "number of animals." The difference is about 200,000 animals per year in total and is probably not relevant in the analyses of regulatory tests, whose protocols always end with the sacrifice of the animals. Large mammals are more often reused, such as horses, donkeys and cross-breeds (82%), sheep (71%), cats (44%), dogs (36%) and cynomolgus monkeys (28%). Rep-

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

55 It is defined by the U.S. National Library of Medicine's "*IUPAC Glossary of Terms Used in Toxicology" as the "Supernatant fraction obtained from an organ (usually liver) homogenate by centrifuging at 9000 g for 20 minutes in a suitable medium; this fraction contains cytosol and microsomes*".

tiles (55%) and xenopus (37%) amongst amphibians were also often reused. Main areas of reuse are routine production (12%) and higher education and training (8%). When including reuse:

- No clear trend in total number over time (2015-2017)
- 68% research purposes, 23% regulatory use to satisfy legislative requirements (2017)
- Higher education or training for the acquisition, maintenance or improvement of vocational skills was stable with 1.6-1.7%
- Basic research-related uses by type of research: nervous system, immune system and oncology account for about half of the uses. Increases in nervous system and oncology.
- Testing for regulatory purposes (79% account for batch potency testing purposes) decreased by 7% between 2015 and 2017; 39% related to toxicity and other safety testing; reproductive toxicity increased by 45%; use in the food and feed area by 28%; several areas decreased significantly: carcinogenicity (-48%), target animal safety (-42%), neurotoxicity (-72%) and eye irritation/corrosion (-46%); > 90% of regulatory experiments were triggered by EU requirements; regulatory uses by type of legislation (main drivers medical: 61% medicinal products for human use, veterinary 15%, industrial chemicals legislation 11%, 52% quality control including batch safety and potency testing).
- For routine production uses total numbers are stable, however, monoclonal antibody production by the mouse ascites method almost doubled from 2015 to 2017, which is very relevant since this method involves mostly severe uses (70%), see below.

Interestingly, a similar trend was seen in Switzerland's statistics[56]: Compared to 2011 with 662,128 animals, there was a small peak in 2015 with 682,332 animals, then a decrease in 2018 to 586,643 animals. It is tempting to attribute the peak to REACH requirements, with which Switzerland complies.

7 Regulatory use and routine production

In our earlier analysis up to 2011 (Daneshian et al., 2015), we noted the relatively low and constant numbers of experimental animals used for safety assessment (toxicology, 8%) and attributed them to the particularly intense research on alternative methods in this area. The new report (EC, 2020) provides more details on the specific legislations that triggered the demand for new tests: The report names the use of animals (total 2.19 million) in 2017 for different legislations, i.e., human medicinal products (61%), veterinary products (15%), industrial chemicals (11%), feed (4%), plant protection products (3%), medical devices (3%), food (2%), biocides (0.2%%) and others (1%) (Fig. 9 of EC, 2020).

The report claims a 7.2% reduction in the number of animals used for regulatory purposes, whose numbers dropped from 2,356,352 in 2015 to 2,186,859 in 2017. This number is encouraging, so we wanted to understand which MS contributed most, hoping to correlate the reduced number of tests with a national policy of promoting alternative methods. Unfortunately, the numbers fluctuated too much to indicate a trend. We did not further investigate the reasons for this, which would have involved a detailed analysis of the industrial settings of the specific MS, including information such as the number of contract research organizations (CROs), manufacturing sites, etc., which is beyond the scope of this paper.

Tab. 4: Regulatory use of animals for pyrogenicity testing (2015-2017) by Member States

EU MS	2015	2016	2017
Austria	14,794	13,157	9,125
Belgium	0	0	0
Bulgaria	150	0	0
Croatia	63	67	0
Cyprus	0	0	0
Czechia	72	51	81
Denmark	0	0	0
Estonia	0	0	0
Finland	0	0	0
France	5,981	7,689	6,191
Germany	6,992	347	5,591
Greece	0	0	0
Hungary	952	646	29
Ireland	570	506	312
Italy	4,007	4,352	2,717
Latvia	0	0	0
Lithuania	0	0	0
Luxemburg	0	0	0
Malta	0	0	0
Netherland	0	0	0
Poland	234	202	236
Portugal	0	0	0
Romania	58	27	234
Slovakia	0	0	0
Slovenia	111	40	59
Spain	9,960	9,878	9,472
Sweden	0	0	0
United Kingdom	2,609	2,472	1,125
Total	**46,553**	**39,434**	**35,172**

[56] https://www.tv-statistik.ch/de/statistik/

Tab. 5: Number of animals used in the EU for monoclonal antibody production by mouse ascites between 2015 to 2017
Only countries reporting any animal use for monoclonal antibody production are listed.

MS	2015	2016	2017	Total	%
Czechia	0	0	230	230	0.2%
France	24,200	46,128	44,198	114,526	94.4%
Germany	894	2,147	384	3,425	2.8%
Hungary	0	350	157	507	0.4%
Italy	1,520	0	0	1,520	1.3%
Spain	719	309	55	1,083	0.9%
Total	**27,333**	**48,934**	**45,024**	**121,291**	**100.0%**

Another important consideration is that the time of reporting has changed: under Directive 2010/63/EU, animals are now counted at the end of the experiment, while under Directive 86/609/EEC, they were counted at the start. In the area of regulatory testing there are many studies that last one or two years, generating an important gap between the time when animals were counted before the experiment and when this was switched to counting them at the end. There is no information on when this happened in the different MS, and this may explain the fluctuation of numbers recorded in the period 2015-2017 (Tab. S5[30]).

Within the area of regulatory use, we will focus our attention on two types of testing, i.e., animals used for pyrogenicity tests or to produce antibodies and animals used in toxicity safety assessment. The former because there are validated alternatives and there is no reason why labs are still authorized to perform the test and the latter because there is sufficient information in the document for further analyses.

Regarding pyrogenicity tests, there was a decrease in the number of animals used, from 46,553 in 2015 to 35,172 in 2017 (Tab. 4). In total, 14 countries reported animal-based pyrogenicity testing, which accumulated to 121,159 animals between 2015 and 2017. Main MS drivers of these numbers are Austria and Spain (54.8% 2015-2017) followed by Germany, France and Italy. All five countries together issued > 90% of quality control pyrogenicity testing (Tab. 4). In many cases, the numbers dropped over the three years, while France, Poland and Spain had more or less constant numbers. The absolute numbers are even more remarkable in light of about 170,000 animals estimated in 2011 and a notorious resistance to the acceptance of new approaches in this field (Hartung, 2015). However, a significant number of animals (25,172 in 2017) are still used for quality control pyrogenicity testing despite internationally harmonized and accepted alternatives having been available for many years. The decrease compared to a decade ago is a positive outcome, but this number really should be zero, as to the best of our knowledge no product has not been testable after some modifications of the novel methods. Strangely, mice were also reported for pyrogenicity testing: 10 mice in 2015 and 1050 in 2016. The mouse is not a standard animal for this test – actually mice develop hypothermia instead of fever in response to microbial pyrogens – but it is not possible to discover in which MS this occurred and why.

Another, very surprising outcome was that a substantial number of animals still are used in the EU for monoclonal antibody production from mouse ascites. This is especially relevant because the mouse ascites method involves mostly severe animal uses (70% in 2017). Moreover, instead of decreasing, the numbers almost doubled between 2015 and 2017. Six countries reported monoclonal antibody production using this method, and France accounts for almost 95% of animals used while the other five MS reported only small numbers of animals used (Tab. 5).

The fraction of animals used in the category "toxicity and other safety testing including pharmacology" has remained stable at 8-9% between 2005-2017 (Tab. 6). Surprisingly, a REACH-induced increase in animal use cannot be seen. Positively noted, quality control testing has been slightly but steadily decreasing over the years, which indicates that 3Rs-motivated reduction and waiving of tests is having a positive impact. It should be noted that more animals are used for quality control testing than for toxicity and other safety testing (Bottini and Hartung, 2009).

The category "toxicity and other safety testing including pharmacology" represents more than 840,000 uses of animals in 2017, which corresponds to 9% of all uses of animals in the EU. Most of the uses in this area were related to reproductive toxicity, repeated dose toxicity, pharmaco-dynamics, developmental toxicity, ecotoxicity, and acute and sub-acute toxicity. It is not clear how combined studies like the OECD TG 422, which is

Tab. 6: EU wide absolute numbers of animals used and for regulatory toxicity and quality control testing in the years 2005 to 2017

	2005	2008	2011	2015	2016	2017
Toxicity and other safety testing including pharmacology	1,026,286	1,042,153	1,004,873	873,587	831,683	843,375
% of total animal use	8.5%	8.7%	8.8%	8.9%	8.3%	8.8%
Quality control (incl. batch safety and potency testing)	1,854,553	1,790,043	1,597,809	1,332,536	1,218,170	1,131,580
% of total animal use	15.3%	14.9%	13.9%	13.6%	12.1%	11.8%
Total use animals	**12,117,583**	**12,001,022**	**11,481,521**	**9,782,570**	**10,028,498**	**9,581,741**

Tab. 7: EU-wide regulatory toxicity and other safety testing for endpoints in which replacements to animals testing are available or in development in the years 2005 to 2017

Toxicity endpoint	2005	2008	2011	2015	2016	2017
Skin sensitization	43,889	38,437	32,168	49,549	51,645	47,341
Carcinogenicity	42,024	20,807	11,876	24,023	5,328	12,493
Genotoxicity	35,483	26,922	21,288	12,405	9,597	10,303
Skin irritation/corrosion	12,243	7,310	4,849	4,773	3,222	4,120
Eye irritation/corrosion	4,208	2,284	2,110	1,518	1,075	814
Phototoxicity	NA	NA	NA	596	469	525

Tab. 8: Laboratory animals used 2008-2017 for developmental toxicity testing

	2008	2011	2015	2016	2017
mice	3,744	1,188	644	164	977
rats	20,263	20,189	104,091 as	101,403	70,778
rabbits	6,047	2,560	6,961	9,678	14,910
guinea pig	120				
Total	**32,182**	**25,948**	**113,711**	**113,261**	**88,682**

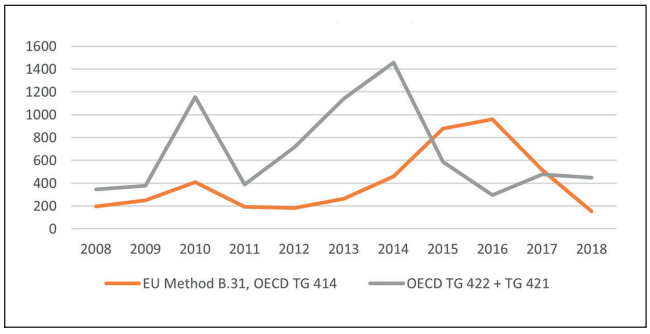

Fig. 3: Developmental toxicity studies (EU Method B.31, OECD TG 414) carried out from 2008 to 2018 compared to the combined screening test for repeated dose toxicity study with the reproduction/developmental toxicity (OECD TG 422 + TG 421)

Data from echem.portal (https://www.echemportal.org/echemportal/).

one of the most commonly used tests for REACH purposes, are counted. Reproductive toxicity related uses saw a large increase (+45%) as did safety testing in the food and feed area (+28%).

Regarding the data on toxicological tests for regulatory purposes, there are some anomalies. Unfortunately, these numbers are reported only in the summary of the COM, and there is no correspondence with the tables in the document containing data from the individual MS. For example, regarding skin and eye irritation and skin sensitization, it should be considered that in 2016, REACH was amended to first ask for *in vitro* methods and move to *in vivo* testing only after demonstrating that *in vitro* testing is not applicable. Table 7 includes data from the previous reports related to the years 2008 (EC, 2010) and 2011 (EC, 2013). For those three endpoints, there is a decrease in the number of animals in 2016 and 2017 compared to the previous years, but not as dramatically as expected. Another interesting observation is that there was no substantial decrease in the years before, demonstrating once more that a validated alternative method is not *per se* used until there is a corresponding change in the regulatory request, in contrast to the clear requirement of Directive 2010/63/EU that asks for an immediate stop of the *in vivo* method as soon as an alternative is validated. The animal species reportedly used for these endpoints is also puzzling (Tab. S6[30]), as these endpoints are ruled by precise guidelines that admit rabbits for skin/eye irritation, and mice or guinea pigs for skin sensitization. It is difficult to explain the use of the others animals, particularly hamsters. Another consideration regards the use of guinea pigs for skin sensitization, which has increased compared to a steady number of mice, demonstrating that the Buehler test or the Guinea Pig Maximization Test (GPMT) (OECD TG 406) are still in use in spite of the general recommendation to use the validated Local Lymph Node Assay (LLNA) (OECD TG 429 and 442A/B), which is done in mice. The ratio of skin sensitization tests using guinea pigs to mice significantly increased over time from a ratio of 0.87 to 1.56, showing that the relative use of the mouse LLNA is strongly decreasing (Tab. S6, Fig. S3[30]).

For regulatory toxicity and other safety testing endpoints in which replacement alternatives to animal testing are available and accepted, animal use has significantly decreased over the last 15 years, i.e., for the endpoints genotoxicity, skin irritation/corrosion, and eye irritation/corrosion testing (Tab. 7). In contrast, for endpoints in which international regulatory acceptance of alternative testing strategies has been achieved only recently, like skin sensitization, and for carcinogenicity, where the problem of the animal test is largely accepted but alternative testing strategies (especially for drugs) are only emerging, the trends are not as obvious.

Analyses of other endpoints are more difficult because too many variables make comparison impossible. There are many tests for repeated dose toxicity, with varying treatment durations or exposure routes. There are combined tests, where we do not know in which category they are counted, and regarding reproductive toxicity, there are two tests, the two-generation study (OECD TG 416) and the extended one generation study (OECD TG 443) that

differ greatly. Looking at the numbers, no clear change is visible over the past 5 years. However, it would be false to conclude that there is no higher demand for *in vivo* testing for regulatory purposes. In fact, the number of facilities in the EU has not changed, so when capacity is reached, new studies are performed outside the EU, even though commissioned to EU CROs.

EU-wide reproductive toxicity testing increased from 0.53% of total animal use in 2008 to 1.47% in 2017. To better understand the situation, we analyzed developmental toxicity (Tab. 8) in more detail. This endpoint should represent a good example, as it is less affected by external variabilities. The standard method is the OECD TG 414, which corresponds to EU method B.31, usually performed on rats as a first species and then confirmed on rabbits, even though different protocols can be applied in the pharmaceutical area. The number of animals used for developmental toxicity testing (Tab. 8) fluctuated, probably due to REACH, owing to which many new tests were requested, with a five-fold increase around 2015 over pre-REACH values a decade earlier. However, the large majority of these tests are still at the stage of (accepted) testing proposals and will only be executed in the coming years. Some experts predict at least as much new animal use for REACH in the coming years as have been used so far. To try to confirm this, the universal website of eChemPortal[57] was accessed to count the number of published studies mentioning a developmental toxicity protocol, the OECD TG 414 or method EU B.31. Figure 3 includes OECD TG 422/421 (screening test for repeated dose toxicity and reproductive toxicity) as it was the most used method for REACH purposes and can provide a hint on the effect of this regulation on the use of animals for scientific purposes. We predicted earlier that reproductive toxicology would be most often demanded by REACH (Rovida and Hartung, 2009; Hartung and Rovida, 2009), i.e. 90% of animal use.

The two highest peaks for OECD TG 422 (Fig. 3) are clearly related to the REACH deadlines of 2010 and 2013 (Rovida and Hartung, 2009). OECD TG 414 had the highest number in 2016, followed by a decrease in 2017 and 2018. The increase in 2015 is due to the REACH registration dossiers contained testing proposals submitted in 2010. Considering that the evaluation usually takes two years and performing the test also takes a couple of years, it is not surprising that the number started to increase in 2015. We have no explanation why there was a decrease in 2017 and 2018. The number of tests requested for REACH purposes have been dramatically increasing in the latest years. In fact, in addition to the regular evaluation procedure, ECHA has now implemented an additional technical completeness check, which evaluates all read-across predictions in the dossier, likely rejecting the majority (Ball et al., 2016).

While the total number is decreasing, the number of animals used to fulfil the requirements in the area of industrial chemical legislation is increasing. This number will increase further in the coming years because many new tests have been just conpleted or are ongoing. In fact, the most demanding tests have to await authorization by COM, and in many cases the regulators asked for new tests after the evaluation procedure. ECHA's plan is to analyze all the submitted dossiers by 2027, so we should expect a high number of test requests also in the coming years.

Animal use for medical device testing is increasing; the latter is expected to increase even more when the new Regulation 2017/745 is fully implemented, with deadline in 2024.

8 Severity of animal use in research and testing

For the first time, the severity of procedures was included in the report. However, the report states that there have been issues with respect to the reporting of the *actual* severity of procedures due to a confusion between prospective severity rating of entire groups of animals and actual severity classifications, for which every animal is supposed to be assessed and ranked individually for adverse health effects. COM thus discourages conclusions from these early days of reporting, as there is currently no consistent way of assessing severity among institutions and MS.

A study assessing the severity rating by Germany-based animal researchers as part of their project license applications in 2010 revealed that prospectively the severity of pain and suffering inflicted by the experimental procedures was frequently underestimated. In almost 60% of cases, researchers' severity estimates were lower than those given in international guidance documents, e.g., *Bundesamt für Veterinärwesen* (1995), which are in line with current guidance given in Annex VIII of Directive 2010/63/EU. Only surgical interventions under general anesthesia, i.e., at least of moderate severity according to guidance, were included in the analysis, but almost 40% of the over 600 surgical procedures were rated as "mild" by the experimenters (Herrmann and Flecknell, 2018). The study results as well as the inconsistent assessments among research establishments found in the new report highlight that more guidance needs to be provided by the COM's Expert Working Group on how to assess and categorize the severity of procedures. In addition, the external validity of animal models that are known to cause severe suffering should be scrutinized, and only models that show sufficient validity justifying their severity then should be refined in an effort to reduce their severity, including the search for earlier experimental ("humane") endpoints. Severe models without sufficient validity should be banned.

What the report does show is a trend of increasing severity with regulatory purposes being the most severe, followed by translational and applied research, routine production and basic research. Batch potency testing constituted the most severe category, representing more than 25% of all severe uses in the EU. In 2017, 892,723 animals were utilized for this purpose and according to the users, 264,633 animals endured severe pain and suffering. The report does not go into particulars as to how many animals were used for the various batch tests of vaccines for humans (e.g., pertussis and polio) and animals (e.g., tetanus and rabies) or for botulinum toxin (Botox). Taylor et al. (2019) estimated that, based on official statistics and non-technical summaries, at least 400,000 animals per year are used for Botox testing in the EU alone. An estimated 50% of Botox may be used for aesthetic (cosmetic) purposes. This should fall un-

[57] https://www.echemportal.org/echemportal/

der the ban on testing cosmetics and thus should be illegal or, when considered under Directive 2010/63/EU, the harms clearly outweigh the benefits (aesthetics) and thus its testing in animals should be prohibited. Since validated animal-free tests exist (Bitz, 2010), the use of the mouse bioassay must finally be removed from the European Pharmacopeia.

The statistics also revealed that the production of monoclonal antibodies by the ascites method is not only the highest in severity but they also show an increase by 65% in the use of animals between 2015 (27,333 animals used) and 2017 (45,024 animals used); 70% of uses for monoclonal antibody production were rated as being "severe" (Fig. 10 in EU, 2020). However, the production of monoclonal antibodies using mice can be replaced by non-animal derived antibodies (Gray et al., 2016). The EURL ECVAM Scientific Advisory Committee (ESAC) stated already back in 1998 that *in vivo* production of monoclonal antibodies by ascites was no longer scientifically necessary, except in rare cases[58]. EURL ECVAM is currently preparing a recommendation with the ESAC Opinion and ESAC workgroup report (Zuang et al., 2019) that shall be published shortly, emphasizing that the production of monoclonal antibodies by the ascites method is not justifiable, and countries need to do stricter monitoring to prevent its use.

9 Genetically altered animals

Already our earlier analysis (Daneshian et al., 2015) highlighted the continuously increasing impact of genetically altered animals on animal use statistics: "*Although the total numbers have remained relatively constant, consumption of transgenic animals has increased drastically; in Germany transgenic animals accounted for 30% of total animal use in 2011. Therefore, more focus on alternatives to the use of animals in biomedical research, in particular on transgenic animals, will be important in the future. ...Not only the absolute numbers of such animals are increasing, but also their relative contribution to all animals has reached levels of over 20% in Switzerland, 30% in Germany (BMEL, 2014), and over 40% in the UK ... Notably, direct comparisons of countries have to be taken with some care, as the statistical rules may differ (these are national statistics, not EU statistics)*." The previous numbers included partly those animals that were used for the creation of genetically altered animal lines, which are now separate. In 2017, 1,276,587 animals were used for the provision of genetically altered animals for the purpose of scientific research. This included 634,705 animals used for the first time for the creation of new genetically altered animal lines, and 641,882 animals used for the first time for the maintenance of colonies of established genetically altered animal lines.

According to the report, 2.57 million of the 9.38 million animal uses involved genetically altered animals in 2017, and 17% of these animals showed a harmful phenotype. Between 2015 and 2017, there was a slight increase in the number of genetically modified (GM) animals (25% to 27%), with mice being the major species used (64%), followed by zebrafish (38%). Basic research used 75% of the GM animals, followed by applied research using 21%. Besides genetically altering mice and fish, the genetic modification of other species is also evolving. The report lists a number of other species whose genome has been altered, e.g., rats, other fish, domestic fowl, rabbits, xenopus and marmosets. The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technique seems to be the driver, as this technique is much easier and cheaper to use, predicting a further rise in the number of species as well as the overall number of altered animals (Bailey, 2019). There has been controversy about the specificity of the CRISPR technique (Peng et al., 2016). Since GM animals constitute the bulk of overall animals used, a critical evaluation of their indispensability, including a harm-benefit analysis is imperative.

10 Pharmaceutical industry and animal use

Over the decades, the reduction of animal use by pharmaceutical industry is most impressive. This was fueled by the use of new technologies already in the 80's and 90's[59]: "*The use of these methods was one factor that contributed to the decrease in animals involved in commercial research during the same period, from 60 percent (or 2.1 million) of the total number of procedures in 1987, to 36 percent (or 1 million) of the total in 2003*." This continued as we noted earlier (Meigs et al., 2018): "*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 offered by alternative methods, especially as they originate from the *Toxicity Testing in the 21st Century* movement in the US.

The new report no longer allows us to identify the number of animals used by the drug industry. The category "translational and applied research", which includes these but also respective academic research and other product developments, accounted for about 2.2 million uses (22%) of animals in 2017.

EFPIA, 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. They provide data on pharmaceutical R&D in Europe over time[60]: From €7.6 billion in 1990, this increased to €17.9 billion in 2000, €27.9 billion in 2010, €33.4 billion in 2015, and €36.5 billion in 2018. This makes the concurrent decline in animal numbers even more impressive. Not surprisingly, EFPIA welcomed the report[61].

A special part of pharmaceutical industry is vaccine industry (Meigs et al., 2018): Europe dominates the world vaccine produc-

58 https://tsar.jrc.ec.europa.eu/test-method/tm1998-04

59 http://nuffieldbioethics.org/wp-content/uploads/Animals-Chapter-8-The-Use-of-Animals-for-Research-in-the-Pharmaceutical-Industry.pdf

60 https://www.efpia.eu/about-medicines/development-of-medicines/

61 https://efpia.eu/news-events/the-efpia-view/blog-articles/animal-use-statistics-europe-s-proactive-approach-in-funding-alternatives

tion with 80% by doses produced. 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. Vaccine testing represents a large part of quality control (including batch safety and potency testing), and certainly a major part of the reduction of animal use in this category is in this area.

11 REACH and animal use

In 2009, we called attention to what REACH could mean for animal numbers (Hartung and Rovida, 2009; Rovida and Hartung, 2009). This was based on the surprisingly high number of pre-registrations and the testing guidance for industry, which one of the co-authors coordinated on behalf of the COM. At the time, in 2007, the number of registered substances officially circulating in the EU market was 40,000 (36,000 substances in the EINECS list and 4,000 in the ELINCS list). This was considered an underestimation. 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, between 40,000 and 80,000 registrations were expected. From 2008 until today (March 24, 2020), 15,418 companies have submitted 99,268 registrations on 22,877 substances in all tonnage categories to ECHA[62]. It is not clear why the "one substance, one registration" principle of REACH did not work out in practice. The number of registered chemicals represents only 16% of the pre-registered substances and even includes all new registrations. We analyzed this earlier (Meigs et al., 2018): "*For deadlines 1 and 2 we predicted a minimum of 12,007 and 13,328 were received. 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 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%.*"

Why do the statistics not show a major increase in animal numbers due to REACH? There are a number of possible explanations:

- Companies were prompted to clean up their inventory and some substances were dropped, which explains the discrepancies mainly occurring in the lowest tonnage band.
- There are still non-registered substances on the market, e.g., in imported goods or in cases where different producers/importers are not aware that they jointly meet the minimum tonnage band.
- Many substances are "hiding" under grouping; this especially holds true for many petrochemicals where the acceptance of grouping by industry is still pending. This could dramatically alter the testing needs for REACH, as these are all high-production volume chemicals, which would sum up to enormous test demands if considered individually.
- Major parts of the first phase of testing occurred before the second deadline in 2013, i.e., when there were no statistics. Albert Bertilsson pointedly said "*Lack of statistics is to hide inconvenient facts*."[64]
- Laboratory capacity in Europe has been reached, explaining the astonishingly stable numbers, and additional testing has been exported to other countries, especially China and India, and is thus not accounted for in the statistics.
- A lot of testing is still to come: This holds true as completeness checks by ECHA, which shall be launched only in April 2020[65], and the majority of accepted testing proposals (Taylor et al., 2014) have yet to be executed. Our own analysis of the testing proposals for developmental and reproductive toxicology for the first deadline alone (Rovida et al., 2011) suggested up to 1.6 million animals just for these endpoints.
- Probably the main reason is that "*What remains outside of the scope of annual statistical reporting, even if covered by the scope of the Directive, are: a) Foetal forms of mammals*." Reproductive and developmental toxicity include far more pups than adult animals, e.g., a two-generation study treats only 20 male and 20 female, but in total on average 3,200 animals are involved in case of rats (factor 80) and 2,100 in case of rabbits (factor 53). Similarly, the one generation study OECD TG 414 treats 40 animals but 784 rats (factor 20) or 560 rabbits (factor 14) are involved. The developmental toxicity screening test OECD TG 422 treats 20 animals but involves on average 412 (factor 21). Applying this to 140,513 animals for reproductive toxicity testing or 97,671 animals for developmental toxicity in 2017, several million animals would need to be added. Our analysis from 2009 (Rovida and Hartung, 2009) suggested 90% of REACH animal use in this field.

This shows that our earlier predictions were not very far off, which notably included the fetal life forms as covered by Directive 2010/63/EU (EU, 2010).

11 Brexit and animal welfare

Brexit impacts are likely to be negative at UK and EU level. The UK Withdrawal Act 2018, due to come into force upon the UK's exit from the EU, introduces a statutory instrument[66] removing all mention of the EU from the Animals (Scientific Procedures) Act 1986, as amended[67]. A regrettable aspect is that UK experts will

62 https://echa.europa.eu/registration-statistics-infograph#
63 https://www.echa.europa.eu/documents/10162/13609/work_programme_2018_in_brief_en.pdf/9412a2bd-64f1-13a8-9c49-177a9f853372
64 http://dtprinciples.blogspot.com/2018/05/alberts-law.html
65 https://echa.europa.eu/-/revised-completeness-check-to-be-launched-in-april-2020
66 http://www.legislation.gov.uk/uksi/2019/72/contents/made
67 http://www.legislation.gov.uk/ukpga/1986/14/contents

no longer participate in EU exchanges and sharing of best practice, so that the PARERE network (Preliminary Assessment of Regulatory Relevance), the EU Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL), and meetings of the MS authorities for Directive 2010/63/EU National Contact Points, will take place without UK representatives.

Aside from these structural differences, Brexit will not affect UK provisions concerning the protection of animals used in laboratories, as the legislation has not been weakened, and although the UK will no longer contribute to EU statistical reports, the UK statistics will be published as before.

While there are fears that separate UK sector-specific legislation could cause duplicate animal testing, for example, if a new UK chemical regulation is introduced without provisions for REACH data to be shared or otherwise accepted by UK regulators, this is still under discussion, and the majority of government statements indicate duplicate animal testing should be avoided[68].

The UK's National Centre for the 3Rs (NC3Rs) may still collaborate at EU level although past involvement, such as through ESTAF (the ECVAM Stakeholder Advisory Forum), is unlikely to continue as before. UK scientists are still eligible to receive EU grant funding for the lifetime of individual Horizon 2020 projects, including projects finishing after December 31, 2020, when the transition period ends. However, the future participation of UK institutions and 3Rs experts in projects funded under Horizon Europe will depend on terms that are yet to be agreed.

The loss of UK MEPs to the EP – and the weight of UK citizens lobbying for higher animal welfare standards – is keenly felt by animal welfare advocates, but there will be continuity in the campaigning and regulatory efforts of formerly UK-based international animal protection organizations that have relocated legal registrations to EU MS.

12 Conclusions – Is the glass half full or half empty?

There is a sometimes justified skepticism about statistics, especially in the political arena. Famous quotes like Benjamin Disraeli: "*There are three types of lies – lies, damn lies, and statistics*" or Mark Twain "*Facts are stubborn things, but statistics are pliable*," capture this. This is not the case here. Carefully curated data are presented in a very objective way, leaving the interpretation largely to those willing to mine it or, as Luis Alberto Urrea phrased it, "*Numbers never lie, after all: they simply tell different stories depending on the math of the tellers*."[69] The report (EC, 2020) already received some praise (Abbott, 2020[70]).

Table 9 shows recent laboratory animal use statistics for a number of non-EU countries. The high number of animals in Norway is always surprising – to the best of our knowledge due to large-scale vaccination trials in fish.

Tab. 9: Laboratory animals used in non-EU countries
Data taken from Speaking-of-Research (https://speakingofresearch.com/facts/animal-research-statistics/). For our own estimates, see text.

Country	Year	Number of animals / procedures (vertebrates)	Number of mammals / procedures excluding mice and rats*
Canada*	2018	3,832,817	2,132,069
Israel	2018	428,993	130,672
New Zealand	2016	254,453	175,647
Northern Ireland	2018	28,790	6,324
Norway	2018	1,686,658	1,618,494
South Korea	2018	3,727,163	593,236
Switzerland	2018	586,643	119,328
United States (US)	2018	~ 11-23 million**	780,070

* These figures are only for CCAC members, which account for most major research institutions in Canada.

** This estimate is based on the number of animals being 15-30 times higher than the number of mammals excluding mice and rats.

We have argued earlier in this series (Leist et al., 2008) that animal use statistics can be misleading: "*What is more problematic than just the technical problems described above, is the conceptual error of using animal statistics to define the success of alternative methods. Let's assume a constant number of EU member countries and clear statistical rules for all. Would then constant numbers of animal experiments indicate that alternative methods have not been successful in a given period? No! Scientific research is expanding, and the number of scientists and publications is exploding*." Let's explore this thought with the current data. For the pharmaceutical sector we have done this above already.

The numbers of articles, journals and researchers is continuously increasing[71]: "*The number of peer reviewed journals published annually has been growing at a very steady rate of about 3.5% per year for over three centuries …. Taken over similar timescales, the number of articles has also been growing by an average of about 3% per year. The reason for this growth is simple: the growth in the number of scientific researchers in the world*." The report shows the close correlation. So, using the 2005 data as baseline, i.e., already 25 MS, about 3% growth over 13 years until 2017 means a 47% increase in articles, journals and researchers. In fact, the publication numbers in the EU (science & engineering total)[72], increased from 461.700 in 2005 to 701.437 in 2017, i.e., by 52%. The annual growth of R&D expenditure in the EU

68 https://hansard.parliament.uk/Commons/2018-02-01/debates/F120E1AB-D8B7-4ECD-9EE3-30E988B4E1BC/LeavingTheEUChemicalsRegulation?highlight=animal#contribution-1AFA3C16-47D7-4249-9359-8F7638D89A3C

69 The Devil's Highway: A True Story

70 https://www.nature.com/articles/d41586-020-00352-6

71 Ware, Mark and Mabe, Michael (2015). The STM Report: An overview of scientific and scholarly journal publishing. Copyright, Fair Use, Scholarly Communication, etc. 9. http://digitalcommons.unl.edu/scholcom/9

72 https://ncses.nsf.gov/pubs/nsb20206/publication-output-by-region-country-or-economy

between 2000 and 2017 was around 5%[73]; this corresponds to an increase of 89% between 2005 and 2017.

In the US, no comparable laboratory animal use statistics are available. Actually, there is no record of the use of mice, rats and birds, as they are exempt from the Animal Welfare Act of 1967. The last available statistics from 2018 thus list only 780,070 animals used[74]: "*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 11 and 23 million, however, there are no published statistics to confirm this*."

Taylor et al. (2008) showed reasonably close correlations of animal use for different countries with the number of publications in biomedicine (PubMed) or R&D spending. We built on this, showing that a similar correlation exists with GDP (Gross Domestic Product, i.e., a measure of the value of economic activity within a country) (Bottini and Hartung, 2009). Using this, rough calculations of the total US animal numbers can be made. Based on R&D spending (2018: US 511.1 and EU 379$[75], i.e., 135%), science & engineering publications[76] (2018: US 16.54% and EU 24.34% of world, i.e., 68%), and corrected for biomedical publications 83%, GDP (2018: EU 16.3% vs. US 15.2% of world, i.e., 93%), and number of researchers[77] (2013: EU 22.2% vs. US 16.7% of world, i.e., 75%), the US should use 68 to 135% of the EU animal numbers. Using only the animals in the US statistics, however, (2017 EU vs. 2018 US: EU 2,534,392 vs. US 780,070, i.e., 31%) would suggest far smaller numbers, but this is likely due to the much broader reporting requirements in the EU. This shows the limitations of such indicators for extrapolation.

Taylor and Rego Alvarez (2019) updated their estimates recently based on 2015 figures from 37 countries. They suggest 14.6 million animal experiments in the United States and world-wide 79.9 million. For the US, noteworthy, this is also a decline compared to their 2005 estimate of 17.3 million.

Noteworthy, the animal use in the US reported by the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) is showing a strong trend to decline: 1,177,566 (2005), to 1,134,693 (2010) to 953,007 (2012) to 891,196 (2013) to 834,453 (2014) to 767,622 (2015) to 820,812 (2016) to 780,070 (2018). This suggests a 34% decrease in larger animals over 14 years, even exceeding the European decline. Still, the estimations by Speaking of Research of a total laboratory animal use of 11 to 23 million animals in the US or by Taylor et al. (2008, 2019) of 15 to 17 million seem more realistic, but in the absence of reporting this is impossible to verify. This shows quite clearly how important actual statistics are to evaluate animal use in a country and its different sectors.

It might, however, easily escape the attention that even in the EU, substantial numbers of animals were not counted, as stated correctly in the report (EC, 2020):

"*Data outside of the scope of the report*

What remains outside of the scope of annual statistical reporting, even if covered by the scope of the Directive, are:

a) Foetal forms of mammals;

b) Animals killed solely for organs and tissues, and sentinels, unless the killing is performed under a project authorisation using a method not included in Annex IV of Directive 2010/63/EU;

c) Animals bred and killed without being used, apart from genetically altered animals with intended and exhibited harmful phenotype, and those having been genotyped with an invasive method before being killed."

We discussed the impact of this above, i.e., 2.8 million for breeding only. Taylor et al. (2008) found the average percentage of animals killed for their tissues for the six countries reporting them was 21.1%, which would add up to about 2 million. A very rough estimate of 2 million fetal rats and rabbits for developmental and reproductive toxicity was shown above. In addition, animals used to produce genetically modified strains accounted for 1.3 million. In total, these are more than 8 million animals, almost doubling the total count of the report.

Overall, the COM and the MS must to be commended for this effort and the improvement of the statistics. "*If the statistics are boring, then you've got the wrong numbers*." (Edward Tufte, Yale University[78]). There might be some minor problems, which can certainly be mitigated. The fact that there is no longer an obligation for such reports is regrettable. We will have to see how well the planned database serves the same purposes. The regular stock-taking of the reports represented an important moment of visibility and heightened awareness. Let us hope that the high interest of stakeholder including the EP and NGOs will urge the COM to maintain this tradition. This article hopefully shows how much information can be gained from such reports, even scratching only on the surface of this treasure trove of data.

13 Outlook

The flash image of animal use numbers in 2017 can only please to some extent. Progress is being made, even though it is slow. It is satisfying to see that areas like pyrogenicity testing are finally making major steps toward replacement: While about 170,000 rabbits were used here annually in the previous decade, numbers are down and continuously falling now. However, if we think that the methods replacing them have been around for more than twenty years, it is difficult to understand why it took so long and why a total of more than two million rabbits were unnecessarily used in this time. And it is not understandable why any rabbit is still used today. Please take these comments with a grain of salt

[73] https://abm-website-assets.s3.amazonaws.com/rdmag.com/s3fs-public/Tim%20Studt%20GS%203%20GFF.pdf
[74] https://speakingofresearch.com/facts/statistics/
[75] https://en.wikipedia.org/wiki/List_of_countries_by_research_and_development_spending
[76] https://ncses.nsf.gov/pubs/nsb20201/downloads
[77] https://en.unesco.org/sites/default/files/usr_1-7_share_gdp_gerd_researchers_publications.pdf
[78] https://www.goodreads.com/quotes/582717-if-the-statistics-are-boring-then-you-ve-got-the-wrong

as they are close to the heart of the corresponding author (Hartung, 2016, see also CoI statement).

At the same time, there are disappointments such as the slow uptake of some other alternatives. A key example is the LLNA, a better animal test developed to replace guinea pig testing. Because of the use of a different species, it is easy to assess implementation. The method was validated in 1999, and accepted in 2001. Until 2011 despite even being prescribed in the REACH legislation, only slightly more than 50% of testing was done in mice, but the new statistics show that the guinea pig assays are now returning, representing more than 60%. Another example shown above is the production of monoclonal antibodies in mouse ascites, where methods using bioreactors are available at all production levels (Gray et al., 2016).

The recent advances in organoids, organ-on-chip and microphysiological systems is a major hope for advancing animal replacement (Marx et al., 2016, 2020; Smirnova et al., 2018). Francis Collins, Director of the U.S. National Institutes of Health testified in US Congress already in 2016 "*I predict that 10 years from now, safety testing for newly developed drugs…will be largely carried out using human biochips…This approach…will mostly replace animal testing for drug toxicity and environmental sensing, giving results that are more accurate, at lower cost and with higher throughput*."[79] Taking an example from our own research might illustrate the potential: 969,275 animals used in 2017 for basic research on the nervous system, 305,782 for translational research on human nervous and mental disorders and 2,769 for neurotoxicity show the potential numbers of animal experiments that organoids such as human mini-brains (BrainSpheres) (Pamies et al., 2017) could replace.

It is not an easy or a short journey toward animal replacement, but it is done not only for animal welfare but for better science and economic reasons. Statistical reports like the one provided now by the COM and the MS are important benchmarks to show where we are on the road. But a good warning about analyzing the data from three years ago comes from Phil Dourado[80], "*By the time your perfect information has been gathered, the world has moved on*."

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Conflict of interest

Thomas Hartung is named inventor on Johns Hopkins' patent application for a BrainSphere model, licensed to AxoSim Inc., New Orleans, LA, where he serves as Consulting Vice President of Scientific Affairs, holding shares of the company. He consults AstraZeneca, Cambridge, UK, in the field of organotypic cultures. He holds patents as inventor of the whole blood pyrogen test and the use of cryopreserved blood, which are licensed to Merck-Millipore; he receives royalties from Merck-Millipore from sales of the kit version.

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