



Food for Thought...

Animal Use for Science in Europe

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Summary

To investigate long-term trends of animal use, the EU animal use statistics from the 15 countries that have been in the EU since 1995 plus respective data from Switzerland were analyzed. 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. The relatively low and constant numbers of experimental animals used for safety assessment (toxicology, 8%) may be due to the particularly intensive research on alternative methods in this area. The many efficiently working NGOs, multiple initiatives of the European Parliament, and coordinated activities of industry and the European Commission may have contributed to keeping the animal numbers in this field in check.

Basic biological science, and research and development for medicine, veterinary and dentistry together currently make up 65% of animal use in science. 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. One initiative designed to provide inter-sector information exchange for future actions is the “MEP – 3Rs scientists pairing scheme” initiated in 2015 by CAAT-Europe and MEP Pietikäinen.

Keywords: animal testing, animal statistics, stem cells, alternative methods, replacement

1 Introduction

The use of animals for scientific purposes has been a topic of political, ethical and scientific discussions for decades. The issue is still topical due to large numbers of animals still being used and killed in research laboratories, in industrial production control, and for safety and quality control purposes. Moreover, the legal and scientific environments are continuously changing, so that it is important to review the current situation from time to time, and to provide topical information to all stakeholders and decision takers. Such stock-taking is an essential basis for planning of future activities, and for rational and responsible handling of the current situation. In this context, an important new activity has been the “MEP-3Rs scientist pairing scheme” that brings together Members of the European Parliament (MEP) that feel responsible for good political decisions in the area of experimental animal use and cognate scientists from the respective same countries that are involved in active

research on methods to substitute animal testing. The first meeting, organized by CAAT-Europe and chaired by MEP Ms. Sirpa Pietikäinen from Finland (Vice-President of the European Parliament intergroup on the welfare and conservation of animals (<http://www.animalwelfareintergroup.eu/>)) was held on January 27, 2015 in Brussels on “*Safety Testing, 3Rs and Policy Making: Challenges & Opportunities for the Scientific Community*.”

Despite large successes in the field of alternative methods during the last 20 years (Leist et al., 2008b), the consumption of animals in the EU still exceeds 11 Mio per year (EC, 2013), and also still includes dogs (Box and Spielmann, 2005; Dellarco et al., 2010; Hasiwa et al., 2011), and non-human primates (Burm et al., 2014; Bailey and Taylor, 2009). A re-evaluation of animal experimentation has become necessary, as drastic changes have taken place during the last decade, concerning the i) legal background (Hartung, 2010a), ii) scientific and technological opportunities and developments (Hartung, 2011) and iii) societal demands (Bottini and Hartung, 2009).

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2 Altered legal situation

The altered legal situation comprises on the European level i) Directive 2010/63 on the use of animals for scientific purposes (Hartung, 2010a; Lindl et al., 2012); ii) REACH Regulation 1907/2006: this is on the one hand a gigantic re-testing program of industrial chemicals produced or marketed in the EU (Hartung and Rovida, 2009a,b; Rovida, 2010; Rovida and Hartung, 2009; Hartung, 2010b), and on the other hand a modern legislation that favors the use of alternative methods over animals for providing safety data (EC, 2008; ECHA, 2014); iii) biocide legislation 528/2012 (Ferrario and Rabbit, 2012): this deals with, e.g., insecticides and herbicides (EC, 2012); iv) Cosmetics Regulation 1223/2009 which entered into force in 2013 and has completely phased out animal testing in the cosmetics field (EC, 2009; Hartung, 2008). A whole industry sector has needed revise its research and development strategy concerning new products and hazards (e.g., nanoparticles), and there is still an ongoing debate on whether sufficient alternative methods are already available for this¹ (Adler et al., 2011; BUAV, 2011; Hartung et al., 2011; Taylor et al., 2011). It is also not yet clear, how the politically motivated ban on using a certain technology (animal testing) is balanced by public investments into alternative technologies that are now urgently required; and v) ongoing discussions on the regulation of endocrine disruptors, such as the question whether risk assessment and its further legal/regulatory use should continue to be based on the established scientific, and in particular toxicological, principles of a careful evaluation of exposure and hazard (Dietrich et al., 2013; Juberg et al., 2014) or rather on other types of concepts (a purported mode-of-action) not otherwise used in toxicology.

Moreover, several large national changes have taken place, such as the adoption of animal rights into the constitution in Germany (§20a of the German Grundgesetz (Constitution)) and the civil code in France (Neumann, 2015).

3 Altered scientific situation

Concerning changes in research and development, many new and powerful technologies have dramatically changed the way scientific questions can be approached. The type/amount of information that can be provided in a given time has grown vastly. This has, for instance, led to considerations of leading scientists that non-animal methods would allow more realistic and feasible predictions of safety concerns of environmental chemicals to humans than classical animal testing (Collins et al., 2008).

A landmark event towards a new toxicological approach was the 2007 publication of the report of the National Research Council (NRC) on "Toxicity Testing in the 21st Century: A Vision and a Strategy" (NRC, 2007), which suggested a mechanism-based toxicology with elements of systems biology incorporated (Leist et al., 2008a). The approach has led to the ToxCast™ program of the United States Environmental Protection Agency (US

EPA). In this context, an initial set of about 400 toxicants, well characterized by classical animal-based methods, was used for measurements in a battery of biochemical/cell biological assays yielding more than 700 endpoints (Dix et al., 2007; Judson et al., 2014; Kavlock and Dix, 2010). Similar concepts have been embraced by other major US regulatory/research authorities that then formed the Tox21 consortium (<http://tox21.org> (Knudsen et al., 2013)) to join forces along these new technologies. The NRC report also sparked large European research initiatives, such as the SEURAT-1 consortium (Gocht et al., 2015) or the ESNATS (Zimmer et al., 2014; Leist et al., 2013; Kuegler et al., 2010; Bolt, 2013; Krug et al., 2013) and ChemScreen projects (Krug et al., 2013; Bolt, 2013; Rovida et al., 2014; Piersma, 2015; van der Burg et al., 2015; Wedebye et al., 2015). In the years to follow, also other areas, such as countermeasures to chemical and biological warfare (Hartung and Zurlo, 2012) have chosen similar new strategies to suggest animal-free research strategies.

Besides the development of new technologies (Leist et al., 2012b), such as metabolomics (Ramirez et al., 2013; Bouhifd et al., 2015), high-content imaging (van Vliet et al., 2014) or epigenetic profiling (Balmer et al., 2012, 2014; Balmer and Leist, 2014), the most important new developments in the field are high throughput assays (Judson et al., 2014) of 3D models (Alepee et al., 2014) and of stem cell-derived human non-transformed cells. Concerning the evaluation of toxicological data, two major scientific principles are being developed (Daston et al., 2015; Gocht et al., 2015): i) the improvement of read-across and rational toxicant grouping to incorporate biological data in addition to (or even instead of) chemical structure data (Kleinstreuer et al., 2014; Patlewicz et al., 2014); and ii) systems toxicology approaches that are rather qualitative, such as adverse outcome pathways (AOP), or that try to use more quantitative systems biology information, like pathways of toxicity (Hartung, 2012; Rovida et al., 2015; Hartung et al., 2012; Sauer et al., 2015; Bouhifd et al., 2013, 2014, 2015; Kleinsang et al., 2014; Whelan and Andersen, 2013; Sturla et al., 2014; Sturla and Hollenberg, 2014). The latter two may eventually be combined to one unified system in various ways (Bal-Price et al., 2015). On the basis of such new technologies, roadmaps have been defined on how to approach an evaluation of toxicological hazard and risk employing mainly animal-free methods (Basketter et al., 2012; Leist et al., 2014; Embry et al., 2014; Pastoor et al., 2014). Importantly, methods are increasingly combined in integrated testing strategies (ITS) or integrated approaches to testing and assessment (IATA) (Hartung et al., 2013; Rovida et al., 2015; Tollefsen et al., 2014). It will be important that these efforts are met with adaptations to the validation process (Hartung, 2007; Judson et al., 2013; Leist et al., 2012a).

The new technologies affect not only toxicology, but also all other areas of animal use, including, for example, teaching (Daneshian et al., 2011), the lot control of biotech products such as Botox (Fernandez-Salas et al., 2012) or the control of seafood for accumulated marine biotoxins (Daneshian et al., 2013). Most importantly, animal-free basic biomedical research possibilities

¹ <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52013DC0135&from=EN>

also have been transformed dramatically. The most important new trends comprise the advent of human stem cell technology (Corti et al., 2015; Singh et al., 2015; Schadt et al., 2014; Giri and Bader, 2015; Kim et al., 2014; Lancaster and Knoblich, 2014; Karakikes et al., 2014; Inoue et al., 2014; Ko and Gelb, 2014), the option to introduce defined genetic changes into such cells (Li et al., 2014), and the construction of microphysiological systems based on human cells (Materne et al., 2015; Fabre et al., 2014; Sung et al., 2014; Hartman et al., 2014; Marx et al., 2012; Andersen et al., 2014; Hartung, 2014).

Such new scientific developments may be better suited to meet societal demands of safety from long-term chemical effects which is hard to judge from animal experiments. Important areas of concern are the effects of chemical mixtures, alterations of neurodevelopment, and currently controversially-discussed delayed effects during an individual's lifespan or even across generations (Quinnies et al., 2015; Szyf, 2015; Klip et al., 2002; Schneider et al., 2008; Anway et al., 2005; Smirnova et al., 2014).

4 Altered societal demands

The societal demands regarding the use of animals for scientific purposes have been subjected to continuous analysis. Important neutral feedback tools are the surveys commissioned by the Research Directorate-General of the European Commission from 2001, 2005 and 2010 (EC, 2001, 2005, 2010). The analysis of these European attitude data towards animal research (von Roten, 2009, 2013) shows clearly that a large fraction of Europeans refuses animal experimentation (56%), and that this fraction increased over time in almost all member states. In addition, a questionnaire of the European Commission from 2006 regarding the revision of the Directive 86/609/EEC revealed that 86% of the general public care about the needs for improvement of the level of protection of animals used for scientific purposes. Another indicator of societal demands is the recent "Stop vivisection initiative." This is a European Citizens' Initiative (ECI) that asks the European Commission to "consider the solid scientific principles that invalidate the animal model" and thus to ban animal use in research and testing in the EU. This initiative, registered in June 2012 (ECI(2012)000007), had by November 2013 collected over 1.17 million signatures across 26 of the EU's 28 member states. These were presented in March 2015 to the Commission. The European Commission rejected the petition on June 3, 2015.

5 The status of animal experimentation in Europe

The EU publishes reports on the use of laboratory animals every three years, and the preparation of the report takes about two years. For instance, the 7th report on the use of animals for scientific purposes was published in 2013 and contains the animal

numbers for 2011. The numbers for 2014 are expected to be available in 2016.

According to the latest figures reported under the previous directive's format (Directive 86/609), the total number of experimental animals used in the 27 member states of the EU in 2011 was 11,481,521 (EC, 2013). 761,675 animals were used in Switzerland for scientific purposes.²

For many other non-EU countries, such detailed numbers are difficult to obtain, and there have been several attempts to estimate them on the basis of available information and mathematical models. A comprehensive treatise of the world-wide use of animals was compiled by the British Union for the Abolition of Vivisection – BUAV (Taylor et al., 2008), and estimates a range of 58 million to about 115 million. There are also non-EU countries in Europe with high animal consumption, such as Norway with a high rate of fish testing. These are not considered in this manuscript.

In the absence of further information, a model calculation may help to predict animal use in a country or region from the gross national product. The two parameters have been found to be highly correlated (> 90%) (Bottini and Hartung, 2009). For more detailed analysis, the numbers need to be handled with care, and additional information is advisable, as the rules for inclusion into the statistics may vary, and also may have changed over time (for instance for the inclusion of animals killed for removal of tissues, or for the counting of fetuses in developmental toxicity studies). Moreover, only few national statistics to date, among them the Swiss statistics, report information on stress levels for animals, another important parameter besides the sheer numbers (Leist et al., 2008b). This situation will improve EU statistics in the future.

To investigate long-term trends of animal use in Europe, the 15 EU countries that were already EU members in 1995 plus Switzerland were chosen as statistical basis. In these countries the animal consumption totaling about 11 million/year remained constant over 15 years (Fig. 1A), with net increases in Germany and the UK and net decreases in Belgium, Denmark, Italy, Finland, The Netherlands and Sweden. While toxicological testing contributed to about 8% of animal use (Fig. 1B), the number of animals used for basic biological science, and research and development for medicine, veterinary and dentistry amounted to about 9 million yearly. Future efforts to reduce animal consumption will thus need stronger efforts in the non-toxicological domains. Here, the availability of human cells and tissue-like constructs may play a particularly large role, in addition to the recognition that animal data have often been misleading or have been of little help to solve human health problems (Leist and Hartung, 2013); possibly due to large differences of mice and humans (Cunningham, 2002; Hartung and Leist, 2008; Olson and Ley, 2002; Cavanaugh et al., 2014; Chandrasekera et al., 2014) on a basic genetic level (Diede et al., 2013; Lin et al., 2014; Yue et al., 2014).

Besides the technical options and the scientific situation, probably large changes in the mindset of researcher, journal

² <http://tv-statistik.ch/de/erweiterte-statistik/index.php>

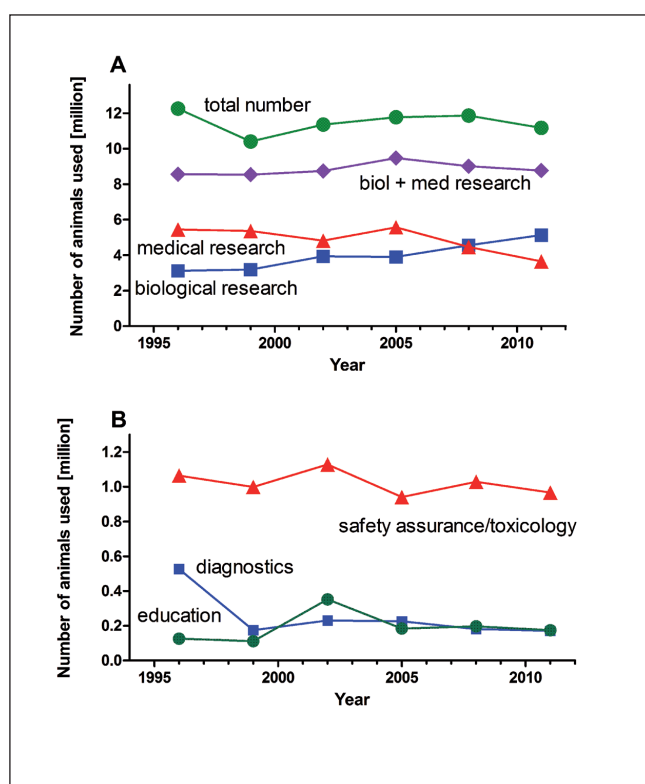


Fig. 1: Numbers of animals used for scientific purposes in 16 core European countries

Data obtained from European Commission reports on the statistics on the number of animals used for experimental and other scientific purposes in Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, Portugal, Spain, Sweden, Switzerland, The Netherlands and United Kingdom for the years 1996, 1999, 2002, 2005, 2008 and 2011; corresponding data from Switzerland were obtained from the Swiss federal food safety and veterinary office; (A) Total number of animals used (green circles), and detail numbers for research and development for medicine, veterinary and dentistry, summarized as medical research (red triangles), biological research, which refers to basic biological research (blue squares) and medical + biological research combined (purple diamonds). (B) The proportion of animals used for the purpose of safety assurance, i.e., toxicological testing (red triangles), for diagnostic (blue squares) and education purposes (green circles).

editors and granting agencies will be required if this situation is to be changed. An example is the highly topical field of stem cell and pluripotency research. Even in this highly dynamic field, the old method of measuring teratoma formation in animals to ascertain pluripotency is hardly ever given up, even though modern (genetically-based) alternatives are available that work better than this animal experiment, in the sense that they provide richer and more quantitative data (Buta et al., 2013; Muller et al., 2010, 2008). For cases that require in vivo data in this area, a pluripotency test method is available that does not require growth of teratomas in mice. This alterna-

tive approach is used still rarely, although it has the potential to reduce suffering, and it has a higher scientific validity (Li et al., 2015; De Los Angeles et al., 2015). This situation is exemplary for many areas of biomedical research, in which old and traditional animal experimentation is still performed, although suffering could be reduced, or the experiment may be entirely replaced. Two important factors that stabilize the traditional animal experimentation system are journal requirements for publication (some journals do not allow publication at all without animal data), and the strong financial support for animal experimentation in academic institutions. However, there are also opposite trends of increasing use of refinement and replacement options, especially in industry, where rational decisions can be taken free of publication and career-pressure, and with a clear view of the overall budget.

Decisive change in academia can only occur when animal users and specialists for alternative methods collaborate to find new solutions. Changes will not happen by themselves, as they require work and funding. If research in alternative methods is not funded, it will not happen. At present, an extremely small percentage of R&D expenditure (far below 0.1%) is used to fund alternatives to animal testing in the biomedical field (Taylor, 2014), and the animal lobby uses the arguments that alternatives are not available to continue with animal experimentation. At present there are only few attempts ongoing to break this vicious circle. On the contrary, the people benefiting from the present situation and from a high number of animal experiments mostly dominate funding decisions, and they are reluctant to let even small proportions of the large finances invested in this sector (Bottini and Hartung, 2009) be diverted to support research on alternative methods. An example of this state of mind is the “Basel declaration,” in which supporters of animal experimentation demanded a continuation of the status quo and purposefully neglected the chance to define a constructive and joint way forward (Gruber, 2011).

Fortunately, also more positive examples are found on how the responsible and refined use of animal models can go hand in hand with the development of alternatives. For instance, the many large researching European companies involved in the chemical, pharmaceutical, food, pesticide or cosmetics sector invest considerable resources into alternative methods research. One of the best approaches to develop better alternative methods, and to create confidence in their performance, is such a type of interaction between the traditional approach and more modern approaches. National funding efforts for alternatives are almost non-existing, except in the UK under NC3Rs. Therefore, resources for alternatives to animal testing derive almost exclusively from EC level and the private sector.

6 Use of genetically-modified animals

It is a conspicuous finding that the numbers of experimental animals used in some countries are now increasing after they remained constant (or slightly decreased) over several years. A closer look at the statistics shows that this is not due to higher demands in safety or quality testing.

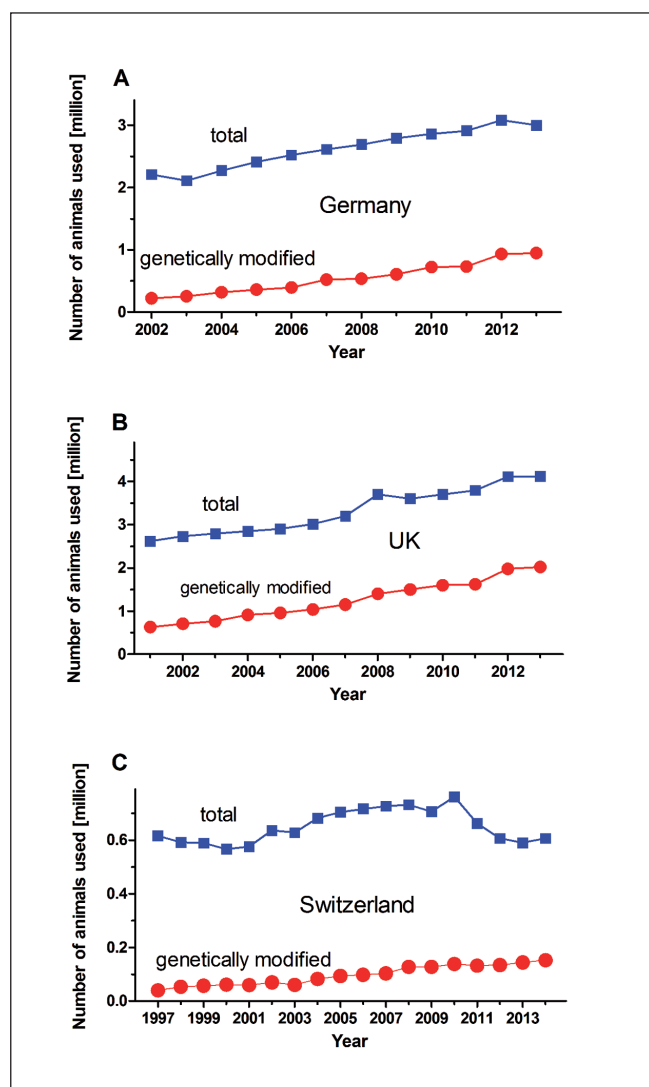


Fig. 2: National examples for the number of transgenic and non-transgenic animals used for scientific purposes

The total annual number of animals (blue squares) and genetically-modified animals (red circles) in (A) Germany, (B) UK and (C) Switzerland. Data are from annual publications on statistics on animals used for scientific purposes from the German Federal Ministry of Food and Agriculture (BMEL), the UK Home Office and the Swiss Federal Food Safety and Veterinary Office (BLV).

A lot of the increase can be explained by the still increasing use of genetically-altered animals in basic biomedical research. These are mostly mice that were manipulated to lack some normal genetic information or that express additional genes, for instance human genes known to be involved in disease or genes isolated from jelly fish that allow easy recognition of certain cells. The use of this technology has skyrocketed, so that more than 1 million such mice are used annually in Germany alone (Fig. 2A), nearly 2 million in the UK (Fig. 2B), and also, e.g., Switzerland, the numbers are rising continuously (Fig. 2C). Not all countries offer statistics on the use of genetically-modified

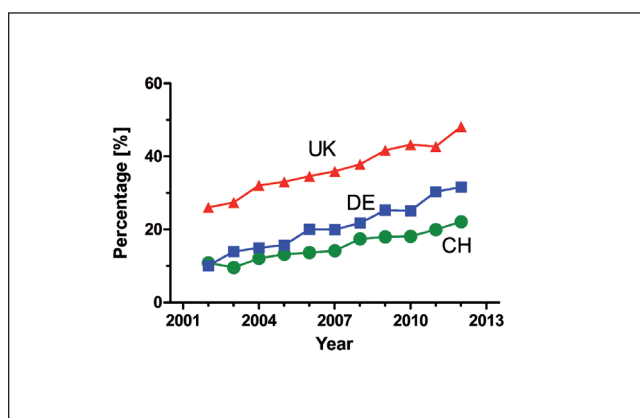


Fig. 3: Proportion of genetically modified animals of the total number of animals

The graph shows the annual percentage of transgenic animals of the total animals used for scientific purposes from 2002 to 2012 in the United Kingdom (UK, red triangles), Germany (DE, blue squares) and Switzerland (CH, green circles). Data are calculated from annual publications on statistics on animals used for scientific purposes from the German Federal Ministry of Food and Agriculture (BMEL), the UK Home Office and the Swiss Federal Food Safety and Veterinary Office (BLV).

animals. Nevertheless, the fact that three exemplary countries offering such statistics use already 3 million such animals together show that this makes for a large proportion of the overall animal consumption in Europe. 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 (Fig. 3). 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). For instance, animals produced during the breeding process but not used for experiments are counted in some countries (UK) as experimental animals, but not in others, e.g., Germany.

7 Numbers of experimental animals in relation to biomedical progress

In order to better understand the implication of the statistical numbers on the use of experimental animals over time, it is helpful to view them in light of the overall scientific developments happening during the same time period. A basic unit to measure the output of research is the number of publications produced. To get an overview on how the publication activity developed over the last 30 years, the examples of Alzheimer's disease and Parkinson's disease were chosen (Fig. 4A). They show a pronounced rise in research output during that time, with the output more than doubling within the last 15 years. This trend, though exemplary, is typical for many biomedical fields, also including, e.g., cancer research, asthma or investigation of heart disease. If this is related to the overall relatively constant animal consump-

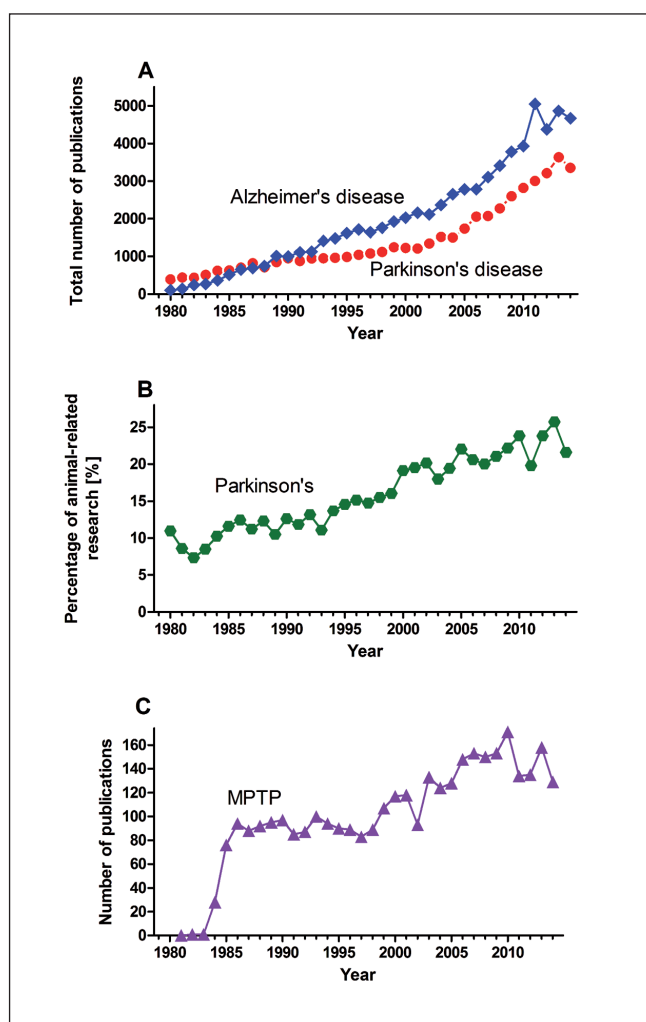


Fig. 4: Publication activity and animal consumption in exemplary areas of neurodegenerative research

(A) Total number of publications in the field of Alzheimer's disease and Parkinson's disease research (using the search terms "Alzheimer" or "Parkinson" and limiting publications to "Journal Article", "Other Animals", i.e., non-human animals and searching for every year individually using the "Publication dates" interface of PubMed). (B) Calculated percentage of publications in the field of Parkinson's disease involving animals (using the search terms "Parkinson" and limiting publications to "Journal Article", "Other Animals", i.e., non-human animals and searching for every year individually using "Publication dates" interface of PubMed). (C) Total number of publications in one exemplary field of experimental Parkinson's disease research using the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP) animal model.

tion, it appears that fewer animals are being used per research output unit, i.e., per publication.

The reasons for this may be complex. There are at least some fields in which the percentage of publications that use animals is increasing, i.e., there is more experimental research compared to clinical research (Fig. 4B). On the other hand, there is an increas-

ing trend to combine animal research with animal-free methods (e.g., cell cultures or molecular biology studies), in parallel with a trend to obtain much more data from one given animal. This would mean that many publications that do involve animal experimentation also use refinement (e.g., non-invasive imaging methods that allow longitudinal study designs), reduction and replacement methods, and the number of animals used for one given publication is therefore falling. This is altogether a promising trend that could be further promoted (Gruber and Hartung, 2004).

The potential for substitution of animal experiments by modern approaches is shown by the example of one single defined animal model often used in Parkinson's disease research. The toxicant 1-methyl-4-phenyl-tetrahydropyridine (MPTP) was discovered in the early eighties as a contaminant in illicit recreational drugs that triggered Parkinsonism in users (Schildknecht et al., 2013a). It has since been used to trigger a Parkinson-like state in experimental animals. Since this compound has no other known use or purpose, it makes literature searches for the specific animal experiment in which this toxicant is used, particularly easy. Since its discovery, the compound has been used for more than a hundred publications per year, with a rather increasing frequency over time (Fig. 4C). This implies that 2,000-10,000 animals are being used every year, just for this single model, assuming that 15-70 animals (these are very conservative estimates) have been used per publication. A recent *in vitro* model based on the use of human nerve cells (Scholz et al., 2013; Schildknecht et al., 2013b) in combination with glial cells (supporting brain cells, known to be important from the *in vivo* experiments), has reproduced the main features of the MPTP model seen in animals (Efremova et al., 2015), and may thus contribute to a large reduction of the use of experimental animals in biomedical research.

8 The "MEP – 3Rs scientists pairing scheme" as an example of novel European inter-sector collaboration in the fields of chemical safety, animal welfare and research effectiveness

The fields of animal welfare, animal-free research, promotion of the 3Rs and improved safety testing have been approached from many angles in Europe (Box 1). Projects of large industry and the European Commission have advanced 3Rs approaches, and in particular animal-free testing methods. In parallel, non-government organizations (NGO), small and medium enterprises (SME) and scientific societies have done, and are doing, important work in the field. Together with valuable input from regulators and (inter)national authorities, this has already led to important changes in legislation and daily practice. Although a lot has been achieved, further progress and modifications are necessary, to implement, for instance, roadmaps on animal-free toxicity testing (Basketter et al., 2012; Leist et al., 2014), on quality control of seafood/shellfish (Daneshian et al., 2013), to provide the missing tools (Adler et al., 2011) required for toxicity assessment of cosmetics and to address the large future challenges, such as the introduction of more replacement methods

Box 1: Examples of European institutions and projects focusing on alternatives to animal experimentation**A-cute-Tox:**

This FP6 project “An *In-Vitro* Test Strategy for Predicting Human Acute Toxicity” ran 2005 - 2010.

CAAT-Europe:

founded 2009 as a joint venture between the Bloomberg School of Public Health at the Johns Hopkins University, USA, and University of Konstanz, Germany, to form a transatlantic bridge for knowledge and information transfer on alternatives to animal experimentation; acts as an information hub and honest broker for further development, evaluation and optimization of alternative approaches to animal testing in toxicology and other biomedical fields.

ChemScreen:

this FP7 project stands for “Chemical substance *in vitro* / *in silico* screening system to predict human and ecotoxicological effects” ran 2010 to 2014.

ECEAE:

The European Coalition to End Animal Experiments was created in 1990 by national organizations to campaign to ban animal testing in the cosmetics sector.

ECHA:

European Chemicals Agency, regulatory agency of the European Union formed June 2007; ECHA manages REACH and the Biocides registration; Helsinki, Finland (<http://echa.europa.eu>).

ECOPA:

European Consensus Platform for Alternatives, founded in 1997, brings together all national consensus platforms on alternative methods; each platform represents animal welfare, industry, academia and governmental institutions.

EPAA:

European Partnership for Alternative Approaches to Animal Testing, created in 2005 to promote the application of 3Rs; the EPAA board, as a public-private partnership, represents 5 European Commission Directorate Generals, 7 industry sectors and 37 companies.

ESNATS:

the FP7 project “Embryonic Stem cell-based Novel Alternative Testing Strategies” aimed at developing a novel toxicity test platform based on embryonic stem cells, ran 2008 to 2013.

ESTIV:

European Society of Toxicology *In vitro* (ESTIV), founded in 1994, strengthens and promotes *in vitro* toxicology, both scientifically and educationally across Europe.

EURL ECVAM:

The European Centre for the Validation of Alternative Methods (ECVAM) was established in 1991 to actively support the development, validation and acceptance of 3Rs methods. The activities of ECVAM were taken on by the European Union Reference Laboratory on Alternatives to Animal Testing (EURL ECVAM), formally established in 2011; EURL ECVAM, located in Ispra, Italy, belongs to the Joint Research Centre (JRC) of the European Commission; **EU-NETVAL**, the European Union Network of Laboratories for the Validation of Alternative Methods, comprising for instance **ZEBET** (Center for evaluation of test methods at the German authority for risk assessment (BfR) in Berlin) in Germany, supports EURL ECVAM in validation studies for assessment of the reliability and relevance of alternative methods.

Eurogroup for Animals:

was established as a non-governmental organization in 1980 as the first coalition of European animal welfare groups. It is well recognized by the European Parliament and Commission as the leading animal welfare organization at EU level and represents animal welfare interests on many EU advisory committees and consultation bodies. It

also holds the secretariat of the European Parliament intergroup on the welfare and conservation of animals.

EUSAAT:

The European Society for Alternatives to Animal Testing was founded in 1994 (as MEGAT, the Middle European Society for Alternatives to Animal Experiments). It aims to disseminate information on alternatives to animal testing, and it is responsible for the annual organization of the European Congress on Alternatives to Animal Testing in Linz, Austria.

EU-ToxRisk:

a Horizon2020 project, endowed with EUR 30 million and starting in 2016; the project will focus on repeated dose systemic toxicity, with liver, kidney, lung and nervous system as well as developmental/reproduction toxicity as targets. Both read-across and the AOP concept will be promoted.

INVITROM:

The International Society for *In vitro* Methods promotes the development, application and acceptance of *in vitro* models in biomedical research.

IVTIP:

The *In vitro* Testing Industrial Platform gathers companies (worldwide) in an informal platform founded in 1993. Currently it comprises 46 companies from different sectors (assay developers, technology providers, chemical, pharmaceutical and cosmetics companies) with significant *in vitro* testing activities.

LUSH:

Public limited company; since 2012 LUSH tenders a prize for animal-free methods research and policy support; with £250,000 it is by far the biggest award in the non-animal testing area.

MEP – 3Rs scientists pairing scheme:

This platform, created in 2015, brings together Members of the European Parliament (MEP) interested in alternative approaches to animal testing with relevant experts from corresponding member states. The first meeting was held in January 2015 in the facilities of the European Parliament in Brussels and involved MEPs and scientists from 17 European countries.

Predict-IV:

The FP7 project “Profiling the toxicity of new drugs: a non animal-based approach integrating toxicodynamics and biokinetics” ran from 2008 to 2013.

ReProTect:

This integrated FP6 project intended to develop a novel approach in hazard and risk assessment of reproductive toxicity; ran from 2004 to 2009.

Society ALTEX Edition:

publishes ALTEX – Alternatives to Animal Experimentation – the only open source journal entirely dedicated to 3Rs.

SEURAT-1:

This FP7 Research Initiative running 2011 - 2015 was funded with € 50 million by Cosmetics Europe and the European Commission. It intends to accelerate the development of the complex area of repeated dose toxicity.

Stop vivisection initiative:

An European Citizens’ Initiative (ECI) asking the European Commission to “consider the solid scientific principles that invalidate the animal model” and thus to ban animal use in research and testing in the EU. This initiative, registered in June 2012 (ECI(2012)000007), had by November 2013 collected over 1.17 Million signatures across 26 of the EU’s 28 member states. These were presented as a petition in March 2015 to the Commission. The European Commission rejected this petition on June 3rd 2015.



Fig. 5: Participants of the first meeting of the MEP- 3Rs scientist pairing scheme

Members of the European Parliament (MEP, bold) interested in alternatives to animal testing from 17 European member states (depicted white on the map) were paired with scientists from corresponding countries (in *italics*) involved in research in the field of alternatives to animal testing (yellow rectangles).

in basic biological research and R&D. This requires a reciprocal understanding of needs, issues and opportunities relevant to various stakeholders. The bases for this are contact interfaces and platforms that catalyze easy contact and information exchange. A particular gap was identified by CAAT-Europe concerning the contact of MEPs and scientists, in particular scientists from the home country of the respective MEPs. Since these two groups have a lot of interesting information and experiences to exchange, the “MEP – 3Rs scientists pairing scheme” was created to provide a suitable platform.

Vice-President of the Intergroup on animal welfare at the European Parliament Ms. Sirpa Pietikäinen volunteered to host the first meeting of the program “MEP – 3Rs scientists pairing scheme.” MEP and scientists from 17 European member states (Tab. 1, Fig. 5) participated in this networking event for information exchange on January 27, 2015 at the European Parliament in Brussels. Coupled to the networking event, a workshop was held by the scientists. This event, “*Science Communication in Safety Testing & 3Rs: Challenges & Opportunities for the Scientific Community and on EU Policy Makers*” informed about

how the EU institutions perceive science, toxicology and 3Rs, and about different approaches at member state level. The keynote lectures were “3Rs and policy making at the European Parliament” by Francois Busquet, CAAT Europe; “Strategy from the Animal Welfare point of view” by Kirsty Reid, Eurogroup for animals; “Decision processes in policy making at the European Commission and the EU agencies” by David Demortain, INRA; “A new Swedish national research center based on 3R principles” by Ian Cotgreave, SWETOX; “Francopa, the french case” by Philippe Hubert, INERIS, and “The Finnish Centre for Alternative Methods, FICAM” by Timo Ylikomi, School of Medicine University of Tampere.

9 Outlook

To consolidate the MEP pairing, and to provide information for the stakeholders, a website was created (<http://caat.jhsph.edu/programs/MEP/index.html>). Due to the large success of the event in Brussels, a second round is planned, and the organizers

Tab. 1: Paired scientists and MEP from corresponding countries

Country*	MEPs	Scientists
Austria	Karin Kadenbach, Joerg Liechtfried	Prof. Walter Pfaller (Medical University Innsbruck)
Czech Republic	Pavel Poc	Prof. Ludek Blaha (Recetox, Masaryk University)
France	Pascal Durand	Mr Philippe Hubert (Director of Ineris), Prof. Robert Barouki (Universite Paris Descartes)
Finland	Sirpa Pietikainen	Prof. Timo Ylikomi (University of Tampere)
Germany	Susanne Melior, Stefan Eck	Prof. Thomas Hartung (University of Konstanz (CAAT)) Dr Mardas Daneshian (University of Konstanz (CAAT Europe))
Greece	Eva Kaili, Mitiliadis Kyrkos	Prof. Dimosthenis Sarigiannis (Aristotle University)
Ireland	Mairead McGuinness	Dr Rex FitzGerald (SCAHT)
Italy	Simona Bonafe's office, Fabio Castaldo's office, Marco Zullo	Prof. Anna Bassi (LARF, University of Genoa), Dr Laura Calvillo (Istituto Auxologico Italiano), Dr Susanna Allosio (National Research Council, Genova)
Luxemburg	Georges Bach, Claude Turmes	Dr Valerie Zuang (European Commission)
Poland	Roza Thun's office, Janusz Wojciechowski's office	Prof. Leonora Buzanska (Polish Academy of Sciences)
Portugal	Liliana Rodrigues's office	Prof. Nuno Franco (Institute for Molecular and Cellular Biology)
Romania	Daciana Sarbu, Claudiu Tanasescu's office	Dr Lucian Farcal (Biotox SRL)
Slovenia	Alojz Peterle, Ivo Vajgl	Dr Martina Klaric (Cosmetics Europe)
Spain	Pilar Ayuso	Prof. Guillermo Repetto (University Pablo de Olavide)
Sweden	Fredrik Federley	Prof. Ian Cotgreave (Swetox)
The Netherlands	Anja Hazenkamp	Prof. Coenraad Hendriksen (Institute for Translational Vaccinology), Dr Marie-Jeanne Schiffelers (Utrecht University)
United Kingdom	Julie Girling, Keith Taylor	Prof. George Loizou (The Health and Safety Laboratory)

* MEPs from Belgium (Bart Staes) and Denmark (Jeppe Kofod) also showed interests to join but the corresponding scientists were not available at the time of the event.

agreed to admit also additional interested MEP, and to mediate the contact to suitable scientists from their home countries. This long-term sustainable activity will complement the traditional approach of small scientific workshops organized at the European Parliament to inform MEP and their staff of current issues important for legislation under discussion. It is expected that the contacts from the pairing scheme will help MEP, NGOs and other institutions involved in the organization of such workshops to find the most knowledgeable experts.

Another important interface requiring a neutral platform independent of lobbying interests is the contact of MEP to SME working in the 3Rs field. Animal-free test methods are an emerging business sector with particularly strong roots in Europe. For instance, various companies offer skin models, testing services, cells as basis for *in vitro* testing and analytical methods that add value to animal-free testing approaches. In the last years, this market has been largely expanding, without the public being aware of it. A pairing scheme of MEP and key people from SME

from this dynamic field will provide exciting opportunities for both sides involved, and preparations for a kick-off event in October 2015 in collaboration with IVTIP (*In vitro* Testing Industrial Platform) are ongoing.

In view of the above analysis of the use of experimental animals for different purposes, it is important that these pairing schemes are sector-independent. At present, safety testing plays a strong role, but also scientists and organizations interested in efficacy testing and broad biomedical research are involved. With all the past success of 3Rs in the field of safety testing (Kandarova and Letasiova, 2011; Bouvier d'Yvoire et al., 2012; Leist et al., 2012a) it is important that the experience gained there is leveraged to the basic research and R&D field. Over nine million animals are used there per year, and very few coordinated research activities are ongoing to reduce this number. This should be a high incentive to work hard on alternative systems that replace animals and produce more human-relevant data.



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Conflict of interest

The authors declare no conflict of interest.

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