STOP VIVISECTION

EUROPEAN CITIZENS' INITIATIVE

1,173,131 Signatures to Phase Out Animal Experimentation

DOSSIER

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Stop Vivisection European Citizens' Initiative
1,173,131 Signatures to Phase Out Animal Experimentation

Our requests for a scientific research truly devoted to human health

The task of the legislator is to pass laws that will lead to a better future for all. We citizens launched the initiative Stop Vivisection because we believe that there is a crucially important area of our lives and the lives of our children, where the seeds for a better future were neglected, misunderstood, or discarded.

We Europeans live in wealthy societies, which are getting increasingly sick. Cancer, diabetes, Alzheimer's, Parkinson's and other neurodegenerative diseases, allergies, endocrine disorders, autism: all these illnesses are on the rise, often in an alarming way, according to statistics of the World Health Organization and other public and private study centers. They are on the rise everywhere, in all age groups (*see Annex I on disease incidence*).

This phenomenon - the increasing incidence[1] of all these serious diseases - is not due to an ageing society. Nor is it due, as far as we know, to an inadequacy of investments. Immense resources are consumed in fact every year, every day, every second, in medical research. *(American President Nixon declared "war on cancer" back in year 1971. And the forecasts annnounced they would have defeated it in ten years. It's been almost 50, and the goal is still far away).*

But these immense resources, of which no one can keep track, are being wasted and will end up in nothing if we do not make ourselves aware of a crucial issue: animal testing does not find the remedies we are looking for because it is not capable itself of responding to the challenge. Because the opposite is true: experimenting on animals makes the solution more distant with every passing day. Experimenting on animals imperils multiple fields of our lives addressed by the Treaty on the Functioning of the European Union (TFEU) which are relevant to Stop Vivisection: health, environment, transparency in our society, consumer protection, scientific development. Not to mention the fact that it cruelly wipes out the very concept of animal welfare and true protection we are to grant to animals *(see Annex II on the legal framework to which Stop Vivisection refers).*

[1] *Incidence*: the rate of occurrence of new cases of a particular disease in a population being studied. *Prevalence*: the percentage of a population that is affected with a particular disease at a given time.

The public has little idea of how challenging it is to launch an ECI, from the moment ECIs have become an institutional reality[2]. Yet despite all the obstacles along the way, last month (March 2015), Stop Vivisection delivered to the European Commission 1,173,131 certified signatures (those collected and then discarded even for small mistakes were many more). Well, along with us, these 1,173,131 citizens made a request of momentous importance: they ask the legislators to take note that Directive 2010/63/EU is a legislation outstandingly behind the times, anchored to the past, with little or no relevance to current scientific knowledge, and with no relevance to the extraordinary developments of science and technology that have to lead us to a better future.

There are several glaring contradictions in this law. We note, for example, that it is entitled "Directive on the protection of animals used for scientific purposes" while there is really very little protection of animals in it *(see Annex III on Directive 2010/63/UE).* But the principal contradiction, the one on which the whole construction of the law relies and therefore mostly deserves to be taken into consideration, is Recital 10, which states that "the use of live animals continues to be necessary to protect human health."

The statement that vivisection is necessary for human health, is a concept underlied by all legislation related to medical and toxicological European research *(see Annex IV on European legislation involving animal experiments). It* contradicts reality; it has no scientific basis whatsoever; it is, to all intents and purposes, an “orphan” statement. Not a single researcher, not a single scientific analysis in the world can substantiate such a statement. The real truth, as some of you certainly know, is that animal testing has never been validated. The truth is that the more studies of the past made on animals are reviewed, the more we discover that they have no scientific validity.

Animal models are not predictive for humans. Thirty years ago this was supported by few isolated voices. If you seek now, you find dozens of such voices in the heart of the most advanced medical research and toxicology, in the heart of the most prestigious public and private institutions in the world. Now, hundreds of scientific

[2] *After more than three years since april 2012, the starting date on which the European Union made possible the submission of an European Citizens' Initiative (ECI), the number of ECIs that were submitted has reached a total of 39. Of these, as far as 05/02/2015, 3 have reached the milestone of one million signatures: One of us, that received a final negative reply from the European Commission; Right to water, that received only a partially positive reply from the Commission (no concrete policy proposal); Stop Vivisection, whose encounter with the European Commission and public hearing in the Parliament are scheduled Monday May 11, 2015. Moreover, 20 were refused for registration by the Commission; 10 have been withdrawn for various reasons (mostly due to the burdensome administrative procedure); 3 are currently collecting signatures; 3 have the procedure under way, with the collection of signatures completed. FINAL RESULT: out of 39 ECIs that have been submitted the result has been (only partially) positive for only one of them, while 27 of them have no longer any chance to be heard, which means that ECI rules have to be reviewed*

studies and reviews, surveys, statements and reports are available to anybody interested in seeing, knowing and understanding *(see Annex V on animal models).*

Which tools can lead us to a better future?
Their name is alternative methods. In fact, if you look on the web, if you consult the pages of the countless public and private organizations that deal with alternative methods, you will find a wealth of data and of amazing news *(see Annex VI on alternative methods).* What is the problem then? What is not working? Why was Stop Vivisection born? Why do citizens need to get mobilized on an issue that on paper, apparently, legislators also seem to have at heart? The problem is that these alternative methods are not mandatory. They are used only by those who want to use them, without fear of penalties or negative judgments of any kind if they are not used. And this happens even with laws that appear to favour alternative methods, such as the REACH regulation[3]. It is an extreme, offensive waste of resources and lives, both human and animal.

Requests by Stop Vivisection:
In the light of the above, we demand:

1. An EU Legislation to phase out animal experiments

More than 1 million of EU citizens claimed that they want EU legislation evolving towards the full abolition of animal experiments for scientific and ethical reasons. The European Commission shall clarify:

- in which way it is going to respect the citizens' will and reach the target of a European Union without animal experiments;
- what legislative acts it is going to change/abrogate;
- with which deadlines.

The change of cultural horizon needs very committed work to be done during a transitory period, by means of a strong collaboration between politicians and involved scientists. Old beliefs are often very slow to eradicate and the mentioned belief is causing too heavy a damage in our society for us not to induce the very beneficial change that we are promoting, for the sake of human health. This change will lead to the "pivotal event" described in the Report of the National Academy of Sciences of the United States called "Toxicity testing in the 21st century: a vision and a strategy".

The transitory period, starting now, has to end as quickly as possible, in no case after year 2020. After this transitory period, starting January 1st, 2021, animal experiments made for the knowledge of human responses have to be outlawed, with strong fees for contravention of the law.

[3] Costanza Rovida: *Food for Thought - Why No New In Vitro Tests Will Be Done for REACH by Registrants*, ALTEX, 2010/3, http://www.altex.ch/resources/altex_2010_3_175_183_FFT_Rovida.pdf.

2. **The statement "*the use of live animals continues to be necessary to protect human health*" shall be removed from all EU legislation regarding medical and toxicological research**

   This statement is the basis of existing EU legislation and policies but its lack of effectiveness it's demonstrated by scientific evidences and results. Many recent studies that were published have demonstrated this belief to be wrong. There is abundant documentation in this dossier, also about the very relevant damages to prevention, due to flawed toxicity testing, as well as damages to medical research, because of the unreliable "animal model".

3. **A permanent conference every 2 years**

   The above mentioned belief that *"the use of live animals continues to be necessary to protect human health"*, and subsequent Stop Vivisection request of removing it from our cultural horizon in the name of science (requests 1 and 2), shall become the subject of a major EU Conference, organised for the first time by the end of 2016, with the following key characteristics:

   - it should be organised at EU level;
   - it should be attended by the figures of scientific excellence worldwide;
   - it should have the scope of debating at the highest scientific level the origins, the nature, the scope, the results, the unvalid status of animal experimentation and what the major alternatives are;
   - it should be public.

4. **All available alternative methods shall be mandatory by law**

5. **Alternative methods as an EU priority (policies, funds and accountability)**

   The European Union shall adopt policies with the aim of strengthening the teaching and the research of alternative methods in all European universities of medicine and related sciences. At least 50% of EU funds for research shall be delivered on the development of alternative methods and the use of these funds shall be fully transparent and shall permit civil society to control that they are properly invested on alternatives methods. The European Commission should provide yearly a public communication:

   - to describe policies, results and the roadmap of future activities (by indicating specific deadlines);
   - to demonstrate the effective use of the funds to develop alternative methods.

   The European Parliament should prepare a yearly report on this communication.

6. **Validation as soon as possible of specific alternative methods already existing**

   We demand the European authorities to urgently intervene in the field of alternative methods, notably in the area of quality control for products for human and veterinary medicine, for which reliable new methods exist or can be developed and validated in short time.

   In particular:

- the Botuline toxins, for the safety testing of which an alternative method has been developed and patented, and yet 300 millions of rats are still being killed every year;
- the vaccine quality testing, for the safety of which many tests are already available;
- the detection of shell fish biotoxins, where excellent in vitro tests have been approved, but still the mouse bioassay is widely used.

7. Different way of validating alternative methods

We demand that new alternative methods be not tested nor compared with animal data, not to invalidate the whole process. They must be compared to retrospective meta-analysis based exclusively on already known human data, such as the effects of well-known molecules already on the market or epidemiological data. Priority in validation must be given to methods which fall into the *"replacement"* category (as opposed to *"reduction"* or *"refinement"*); to methods whose action spans several experimental fields and to methods which make exclusive use of human tissues and materials (as opposed to the ones that use animal tissues or material).

8. EU transnational engagement on the necessity to phase out animal experiments and on the mandatory nature of alternative methods

These issues need a worldwide engagement and the European Union shall be the leader in promoting this kind of scientific and research policies.

9. Validation of alternative methods must be at the expense of the European Union, not of the researchers

Currently validation of alternative methods is at the expense of the researchers who discovered them. We demand the EU to finance the European Centre for the Validation of Alternative Methods, absorbing the cost of the entire validation process, so that it could be possible, even by researchers not funded by large companies, to have the alternative methods they discovered to be validated, in order to have more and more validated alternatives and to ensure the research for new methods.

10. Annual report for alternatives in applied research

Currently the use of alternative methods in applied research is at the discretion of the researcher who can choose between animals and alternatives. We demand that the European Centre for the Validation of Alternative Methods do annual reports listing the main techniques of animal testing for applied research and the main alternatives to each of them. Where there are alternatives to a particular technique, they must be mandatory and animal data based on such technique should not be accepted anymore as evidences for following clinical studies.

IMPORTANT NOTE: wherever the term "alternative methods" appears in the text, it refers to "non-animal replacement methods".

Annex I – Graphs and statistics on disease incidence

Countless documents prove we are facing a dramatic rise in all types of illnesses due to the action of chemicals, and our inability to tackle them with adequate scientific methods of research.

1 – A most comprehensive WHO-UNEP study on Endocrine Disrupting Chemicals and their effects on human health and wildlife was published in 2012

The title of the WHO-UNEP report is "State of the science of endocrine disrupting chemicals – 2012 – *An assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme (UNEP) and WHO"*[1].

Quotes from the "Summary for Decision Makers"

Introduction, page 2:

Many endocrine-related diseases and disorders are on the rise:

- large proportions (up to 40%) of young men in some countries have low semen quality, which reduces their ability to father children;
- the incidence of genital malformations, such as non-descending testes (cryptorchidisms) and penile malformations (hypospadias), in baby boys has increased over time or levelled off at unfavourably high rates;
- the incidence of adverse pregnancy outcomes, such as preterm birth and low birth weight, has increased in many countries;
- neurobehavioural disorders associated with thyroid disruption affect a high proportion of children in some countries and have increased over past decades;
- global rates of endocrine-related cancers (breast, endometrial, ovarian, prostate, testicular and thyroid) have been increasing over the past 40–50 years;
- there is a trend towards earlier onset of breast development in young girls in all countries where this has been studied. This is a risk factor for breast cancer;
- the prevalence of obesity and type 2 diabetes has dramatically increased worldwide over the last 40 years. WHO estimates that 1.5 billion adults worldwide are overweight or obese and that the number with type 2 diabetes increased from 153 million to 347 million between 1980 and 2008.

Key Concerns, page 3:

- **Internationally agreed and validated** (sic) **test methods for the identification of endocrine disruptors capture only a limited range of the known spectrum of endocrine disrupting effects. This increases the likelihood that harmful effects in humans and wildlife are being overlooked;**
- for many endocrine disrupting effects, agreed and validated test methods do not exist, although scientific tools and laboratory methods are available;
- for a large range of human health effects, such as female reproductive disorders and hormonal cancers, there are no viable laboratory models. This seriously hampers progress in understanding the full scale of risks.

Quotes from the **Full Report**

Executive summary, page XV

There is an increasing burden of disease across the globe in which EDCs are likely playing an important role, and future generations may also be affected. A focus on

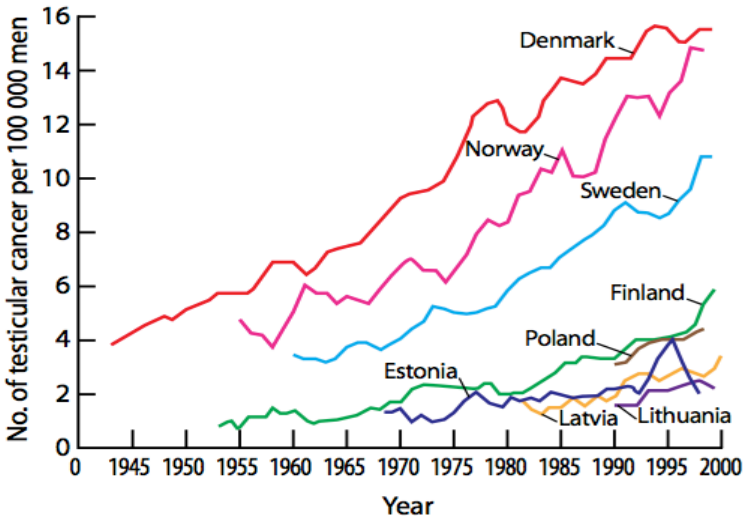
[1] *A* Full report and a Summary for decision makers are available here: http://www.who.int/ceh/publications/endocrine/en/

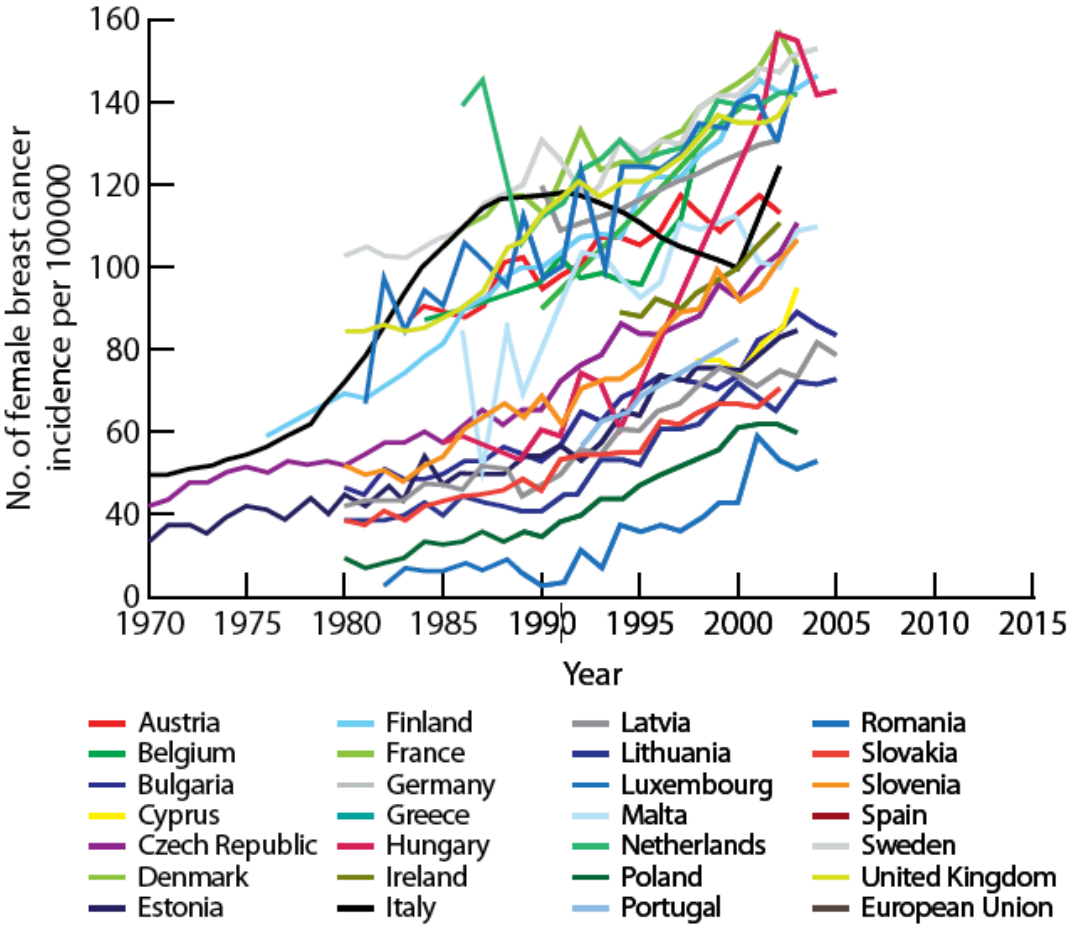
linking one EDC to one disease severely underestimates the disease risk from mixtures of EDCs. We know that humans and wildlife are simultaneously exposed to many EDCs […]. Despite substantial advances in our understanding of EDCs, uncertainties and knowledge gaps still exist that are too important to ignore. These knowledge gaps hamper progress towards better protection of the public and wildlife.

WHO – UNEP Press Release
http://www.who.int/mediacentre/news/releases/2013/hormone_disrupting_20130219/en

Endocrine Disrupting Chemicals and related links
http://www.who.int/ceh/risks/cehemerging2/en/
DATA AND FIGURES from the WHO-UNEP Report on the State of the science of endocrine disrupting chemicals -2012

Figure 7. Testicular cancer rates across northern Europe (from Richiardi et al., 2004; used with permission of the publisher).




No. of female breast cancer incidence per 100000
160
140
120
100
80
60
40
0
1970
1975
1980
1985
1990
1995
2000
2005
2010
2015
Year
Austria
Belgium
Bulgaria
Cyprus
Czech Republic
Denmark
Estonia
Finland
France
Germany
Greece
Hungary
Ireland
Italy
Latvia
Lithuania
Luxembourg
Malta
Netherlands
Poland
Portugal
Romania
Slovakia
Slovenia
Spain
Sweden
United Kingdom
European Union

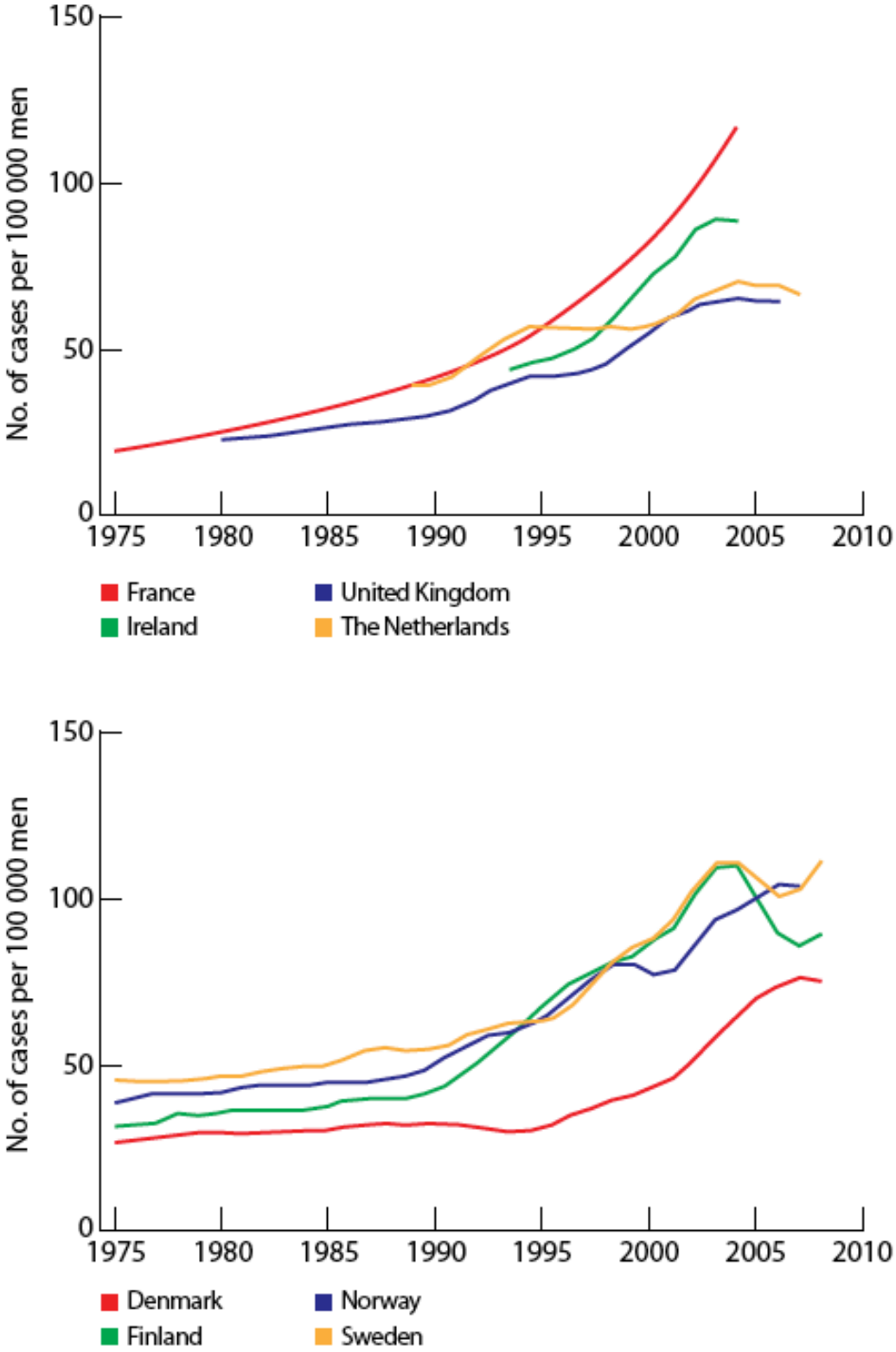


Figure 2.18. Trends in the incidence of prostate cancer in selected countries: age-standardized rate (W) per 100 000.
Source: http://globocan.iarc.fr/factsheets/cancers/prostate.asp

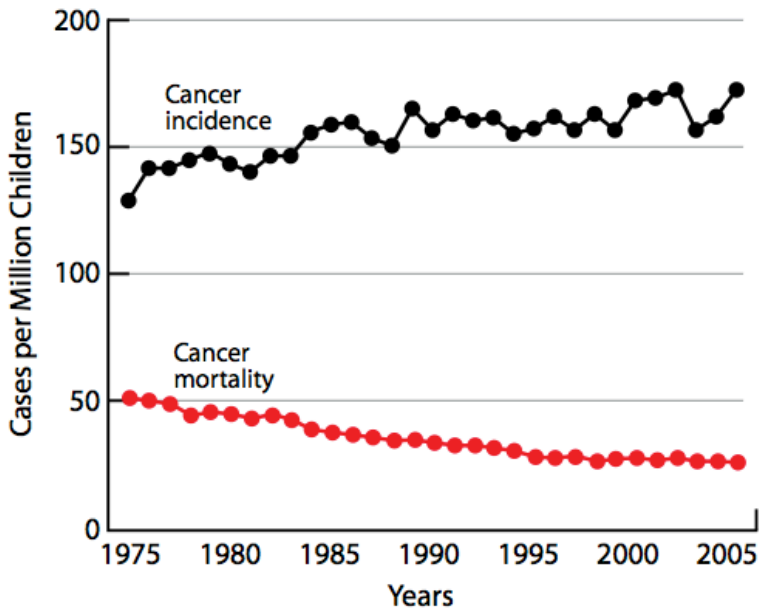


Figure 6. Children are among the most vulnerable humans. The figure shows cancer incidence and cancer mortality among children under 20 years of age in the USA (based on data from the United States National Cancer Institute's Surveillance, Epidemiology and End Results Program).

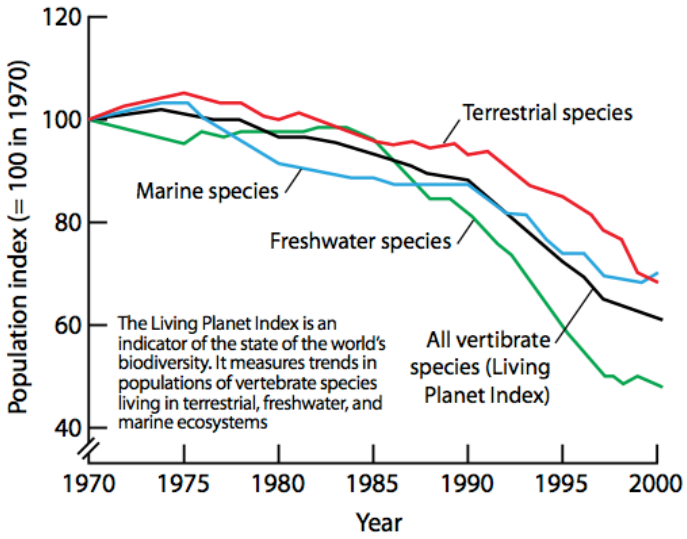


Figure 12. Population declines in wildlife (vertebrates) over 30 years, 1970–2000 (source: World Wide Fund for Nature [WWF] and the World Conservation Monitoring Centre of UNEP, used with permission).

2 – Cancer trends in the United Kingdom

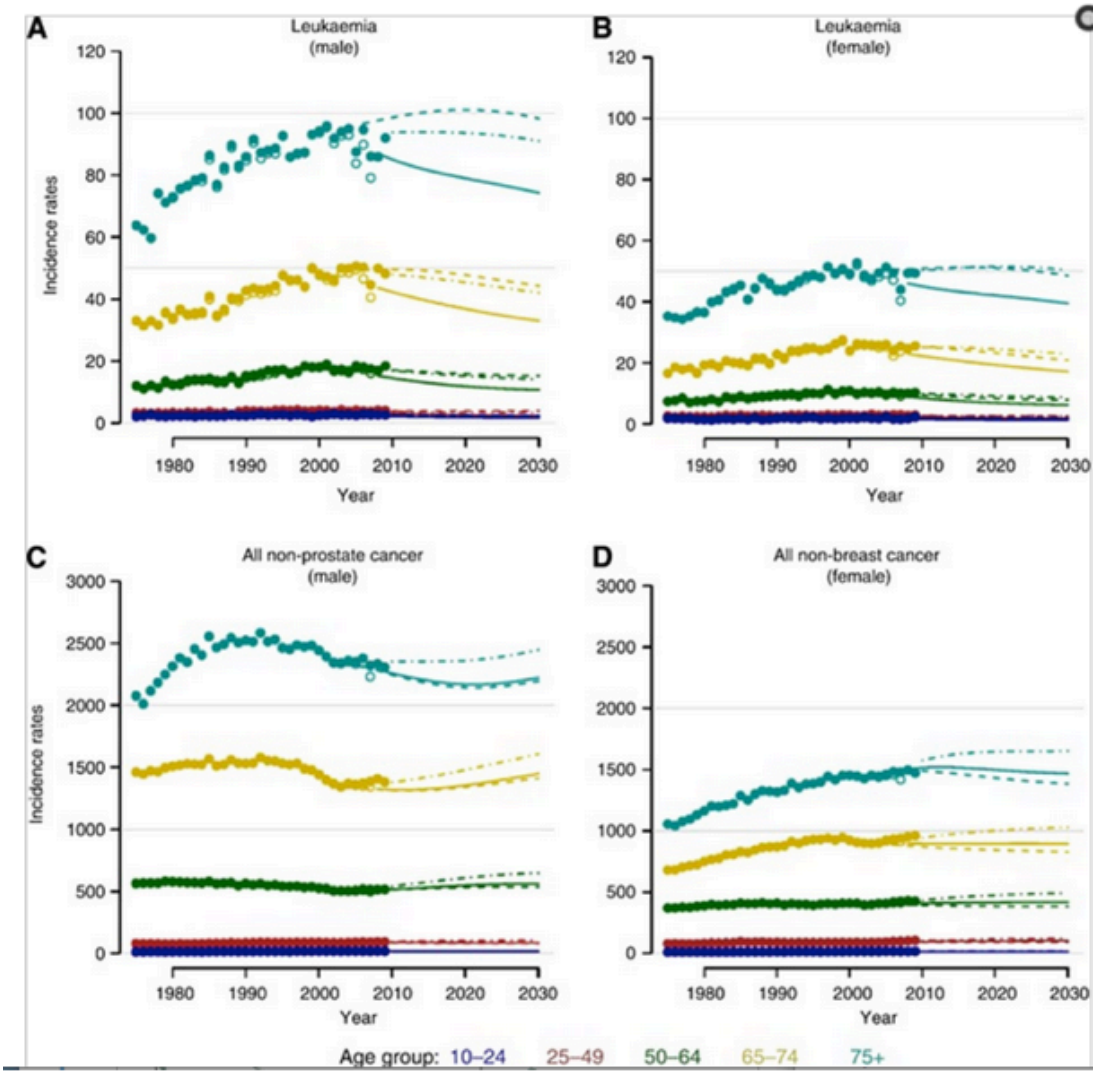
A recent study published by the *British Journal of Cancer* showed statistics that were deeply criticised: as a matter of fact a tight analysis of data highlights that the incidence of cancer is destined to rise much more than expected in the original study. The authors recognized they committed a few errors. To be noticed: the expected rise in cancer incidence cannot be merely attributed to population ageing.

1 (Article) **Cancer incidence in the United Kingdom: projections to the year 2030**
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3242594/

2 (Critical review of the study) **Comment on "Cancer incidence in the United Kingdom: projections to the year 2030"**
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3619081/

3 Reply to **"Comment on cancer incidence in the United Kingdom projections to the year 2030"**
http://www.ncbi.nlm.nih.gov/pubmed/23429208

DATA AND FIGURES from the WHO-UNEP Report on the State of the science of endocrine



3 – More on the impact of chemicals and endocrine disrupters on human and environmental health

"The impacts of endocrine disrupters on wildlife, people and their environments - The Weybridge+15 (1996-2011)" is a report published by the **European Environment Agency**, 10 may 2012:
http://www.eea.europa.eu/publications/the-impacts-of-endocrine-disrupters
http://www.eea.europa.eu/media/newsreleases/increase-in-cancers-and-fertility

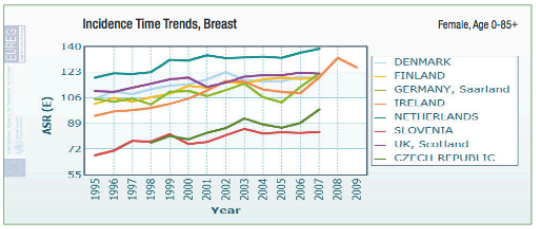
3 – More on cancer trends

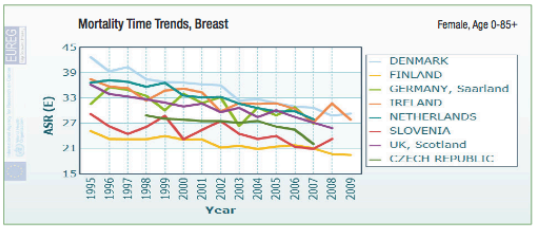
http://eco.iarc.fr/
http://eco.iarc.fr/EUREG/AnalysisG.aspx

http://eco.iarc.fr/EUROCIM/Default.aspx

http://eco.iarc.fr/EUREG/Default.aspx

http://www.encr.eu/images/docs/factsheets/ENCR_Factsheet_Breast_2014.pdf



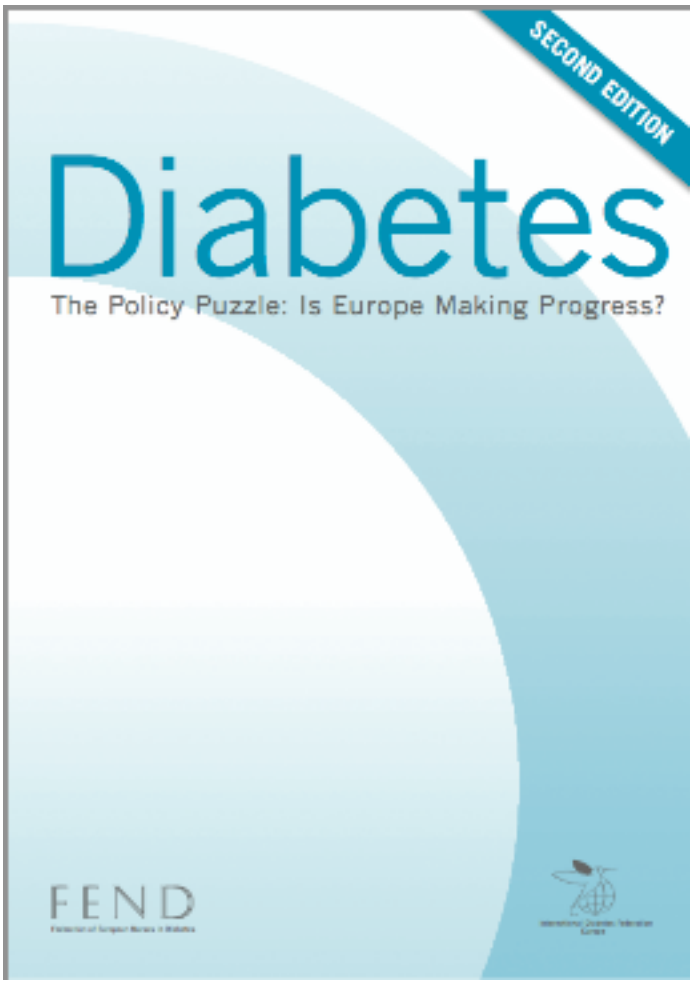


4 – The burden of diabetes

"In several EU countries, diabetes and its complications are the cause of death which has shown the greatest increase over the past 20 years".

Federation of European Nurses in Diabetes

http://www.idf.org/sites/default/files/EU-diabetes-policy-audit-2008%20-2nd%20edition.pdf



From the Executive Summary of Diabetes – the Policy Puzzle: is Europe Making Progress?[2]

The increasing prevalence of diabetes mellitus, a chronic metabolic disease resulting in serious complications, ranging from cardiovascular disease to kidney failure,

[2] http://www.idf.org/sites/default/files/EU-diabetes-policy-audit-2008%20-2nd%20edition.pdf

therapeutic amputation and blindness, shows no signs of slowing down. Now a global epidemic, the situation in Europe has continued to deteriorate over the last three years, further exacerbated by the growing obesity problem across the region.

- In the European Union (EU), there are now over 31 million people living with diabetes aged between 20-79. This signifies an average EU prevalence rate of 8.6% of the adult population – up from 7.6% in 2003 – a figure which is expected to grow to over 10% by 2025.
- Diabetes prevalence rates in the EU vary widely from 4% in the UK to 11.8% in Germany. There are at least 13 countries with rates of over 9% of the adult population, the majority of which are new EU Member States.
- The average prevalence of diabetes in the EU has risen from 7.6% of the adult population (aged 20-79) in 2003, to 8.6% in 2006. This represents over 31 million people across the 27 EU Member States. This prevalence rate is forecasted to rise to 10.3% by 2025.
- In several EU countries, diabetes and its complications are the cause of death which has shown the greatest increase over the past 20 years".
- The average prevalence of diabetes in the EU has risen from 7.6% of the adult population (aged 20-79) in 2003, to 8.6% in 2006. This represents over 31 million people across the 27 EU Member States. This prevalence rate is forecasted to rise to 10.3% by 2025.

5 – On autism and Alzheimer's Disease

The global prevalence of autism has increased twentyfold to thirtyfold since the earliest epidemiologic studies were conducted in the late 1960sand early 1970s[3]

A major increase is also observed in the diagnosis of Alzheimer's.

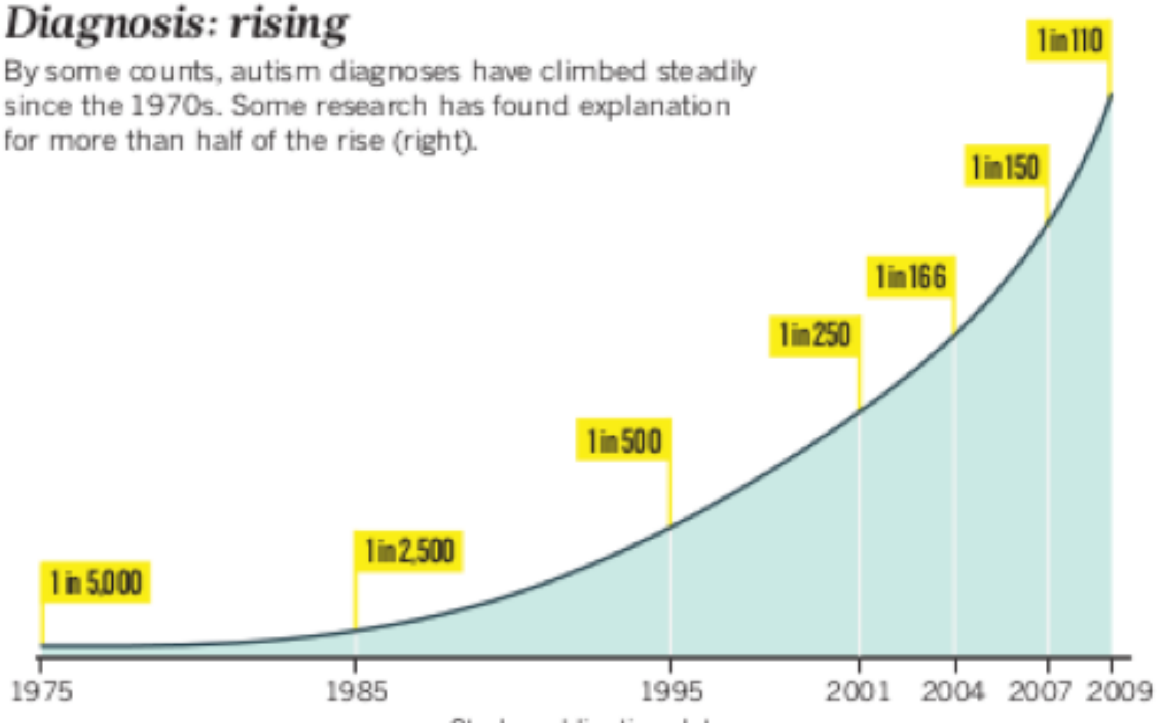
Alzheimer's Disease International (ADI) published global prevalence data on dementia in the World Alzheimer Report 2009 based on a systematic review of 154 studies conducted worldwide, and United Nations population projections through to the year 2050.

"We estimated 36 million people with dementia in 2010, nearly doubling every 20 years to 66 million by 2030 and to 115 million by 2050"[4].

[3] http://www.cdc.gov/mmwr/preview/mmwrhtml/ss6302a1.htm?s_cid=ss6302a1_w

Diagnosis: rising

By some counts, autism diagnoses have climbed steadily since the 1970s. Some research has found explanation for more than half of the rise (right).



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2) *Prostate Cancer. Estimated Incidence, Mortality and Prevalence Worldwide in 2012* (IARC) http://globocan.iarc.fr/old/FactSheets/cancers/prostate-new.asp

3 a)*Données épidémiologiques sur le diabète de type 2,* D. Simon, Service de diabétologie, Hôpital de la Pitié, Paris - Inserm U-258, Villejuif, E. Eschwege, Inserm U-258, Villejuif, 2002 http://opac.invs.sante.fr/doc_num.php?explnum_id=1809

3b) *How can we change the future for diabetes in Europe?* European Diabetes Leadership Forum, Copenhagen 2012 http://www.oecd.org/els/health-systems/50080632.pdf

3c) *Diabetes. The policy Puzzle: Is Europe Making Progress?* http://www.idf.org/sites/default/files/EU-diabetes-policy-audit-2008 -2nd edition.pdf

4a) *Autism Spectrum Disorder (ASD), Centres for Disease Control and Prevention* http://www.cdc.gov/ncbddd/autism/data.html

4b) *Autism counts,* Nature 2011 http://www.nature.com/news/2011/111102/full/479022a.html

5) Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. *Evidence for decreasing quality of semen during past 50 years* BMJ. 1992 Sep 12;305(6854):609-13., http://www.ncbi.nlm.nih.gov/pubmed/1393072

6a) *The Global Impact of Dementia 2013-2050* , Alzheimer's Disease International http://www.alz.co.uk/research/GlobalImpactDementia2013.pdf

6b) Liara Rizzi, Idiane Rosset and Matheus Roriz Cruz *Global Epidemiology of Dementia: Alzheimer's and Vascular Types*,, june 2014 http://www.hindawi.com/journals/bmri/2014/908915/

6c) *La maladie d'Alzheimer et les troubles apparents*, ORS Limousin, 2007 http://www.orslimousin.org/publications/synthese/2009/fiche_alzheimer_2009_2p.pdf;

6d) *I nuovi numeri della demenza in Europa*, http://www.alzheimer.it/numeri_eu.html

7) http://www.artac.info/fr/appel-de-paris/presentation_000074.html - contenu_000324

8a) *Joint Meeting of the Chemicals Committee and the working Party on Chemicals,* Pesticides and Biotechnology Validation Report (phase2) for the Zebrafish Embryo Toxicity Test, Series on Testing Assessment, The OECD observer. Organisation for Economic Cooperation and Development 08/2012 http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282012%2925&doclanguage=en;

8b) CHE, The collaborative on health and environment, http://www.healthandenvironment.org/diseases

8c) http://earthweareone.com/mit-researchers-new-warning-at-todays-rate-half-of-all-u-s-children-will-be-autistic-by-2025/

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http://msherzan.pbworks.com/f/Animal+Research+is+Wasteful+and+Misleading.pdf

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9d) Thomas Hartung, *Toxicology for the twenty-first century*, (Nature 460, 208-212, 9 July 2009), http://www.nature.com/nature/journal/v460/n7252/full/460208a.html

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Annex II – STOP VIVISECTION Legal Framework

Treaty on the Functioning of the European Union

PART ONE / TITLE II

Article 9

In defining and implementing its policies and activities, the Union shall take into account requirements linked to the promotion of a high level of employment, the guarantee of adequate social protection, the fight against social exclusion, and a high level of education, training and protection of human health.

Article 13

In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage.

Article 15 (ex Article 255 TEC)

1. In order to promote good governance and ensure the participation of civil society, the Union's institutions, bodies, offices and agencies shall conduct their work as openly as possible.

2. The European Parliament shall meet in public, as shall the Council when considering and voting on a draft legislative act.

3. Any citizen of the Union, and any natural or legal person residing or having its registered office in a Member State, shall have a right of access to documents of the Union's institutions, bodies, offices and agencies, whatever their medium, subject to the principles and the conditions to be defined in accordance with this paragraph.

PART THREE / UNION POLICIES AND INTERNAL ACTIONS

TITLE I / THE INTERNAL MARKET

Article 26 (ex Article 14 TEC)

1. The Union shall adopt measures with the aim of establishing or ensuring the functioning of the internal market, in accordance with the relevant provisions of the Treaties.

2. The internal market shall comprise an area without internal frontiers in which the free movement of goods, persons, services and capital is ensured in accordance with the provisions of the Treaties.

3. The Council, on a proposal from the Commission, shall determine the guidelines and conditions necessary to ensure balanced progress in all the sectors concerned.

TITLE II / FREE MOVEMENT OF GOODS

CHAPTER 1 / CUSTOMS COOPERATION

Article 32 (ex Article 27 TEC)

In carrying out the tasks entrusted to it under this Chapter the Commission shall be guided by:

(d) the need to avoid serious disturbances in the economies of Member States and to ensure rational development of production and an expansion of consumption within the Union.

CHAPTER 3 / PROHIBITION OF QUANTITATIVE RESTRICTIONS BETWEEN MEMBER STATES

Article 36 (ex Article 30 TEC)

The provisions of Articles 34 and 35 shall not preclude prohibitions or restrictions on imports, exports or goods in transit justified on grounds of public morality, public policy or public security; the protection of health and life of humans, animals or plants; the protection of national treasures possessing artistic, historic or archaeological value; or the protection of industrial and commercial property. Such prohibitions or restrictions shall not, however, constitute a means of arbitrary discrimination or a disguised restriction on trade between Member States.

TITLE III / AGRICULTURE AND FISHERIES

Article 38 (ex Article 32 TEC)

1. The Union shall define and implement a common agriculture and fisheries policy. The internal market shall extend to agriculture, fisheries and trade in agricultural products. "Agricultural products" means the products of the soil, of stockfarming and of fisheries and products of first-stage processing directly

related to these products. References to the common agricultural policy or to agriculture, and the use of the term "agricultural", shall be understood as also referring to fisheries, having regard to the specific characteristics of this sector.

2. Save as otherwise provided in Articles 39 to 44, the rules laid down for the establishment and functioning of the internal market shall apply to agricultural products.

3. The products subject to the provisions of Articles 39 to 44 are listed in Annex I.

4. The operation and development of the internal market for agricultural products must be accompanied by the establishment of a common agricultural policy.

(animals bred for laboratories are not "agriculture")

TITLE XIV / PUBLIC HEALTH

Article 168 (ex Article 152 TEC)

1. A high level of human health protection shall be ensured in the definition and implementation of all Union policies and activities.

Union action, which shall complement national policies, shall be directed towards improving public health, preventing physical and mental illness and diseases, and obviating sources of danger to physical and mental health. Such action shall cover the fight against the major health scourges, by promoting research into their causes, their transmission and their prevention, as well as health information and education, and monitoring, early warning of and combating serious cross-border threats to health.

The Union shall complement the Member States' action in reducing drugs-related health damage, including information and prevention.

2. The Union shall encourage cooperation between the Member States in the areas referred to in this Article and, if necessary, lend support to their action. It shall in particular encourage cooperation between the Member States to improve the complementarity of their health services in cross-border areas.

Member States shall, in liaison with the Commission, coordinate among themselves their policies and programmes in the areas referred to in paragraph 1. The Commission may, in close contact with the Member States, take any useful initiative to promote such coordination, in particular initiatives aiming at the establishment of guidelines and indicators, the organisation of exchange of best practice, and the preparation of the necessary elements for periodic monitoring and evaluation. The European Parliament shall be kept fully informed.

TITLE XV / CONSUMER PROTECTION

Article 169 (ex Article 153 TEC)

1. In order to promote the interests of consumers and to ensure a high level of consumer protection, the Union shall contribute to protecting the health, safety and economic interests of consumers, as well as to promoting their right to information, education and to organise themselves in order to safeguard their interests.

2. The Union shall contribute to the attainment of the objectives referred to in paragraph 1 through:

(a) measures adopted pursuant to Article 114 in the context of the completion of the internal market;

(b) measures which support, supplement and monitor the policy pursued by the Member States.

3. The European Parliament and the Council, acting in accordance with the ordinary legislative procedure and after consulting the Economic and Social Committee, shall adopt the measures referred to in paragraph 2(b).

4. Measures adopted pursuant to paragraph 3 shall not prevent any Member State from maintaining or introducing more stringent protective measures. Such measures must be compatible with the Treaties. The Commission shall be notified of them.

TITLE XIX / RESEARCH AND TECHNOLOGICAL DEVELOPMENT AND SPACE

Article 179 (ex Article 163 TEC)

1. The Union shall have the objective of strengthening its scientific and technological bases by achieving a European research area in which researchers, scientific knowledge and technology circulate freely, and encouraging it to become more competitive, including in its industry, while promoting all the research activities deemed necessary by virtue of other Chapters of the Treaties.

2. For this purpose the Union shall, throughout the Union, encourage undertakings, including small and medium-sized undertakings, research centres and universities in their research and technological development activities of high quality; it shall support their efforts to cooperate with one another, aiming, notably, at permitting researchers to cooperate freely across borders and at enabling undertakings to exploit the internal market potential to the full, in particular through the opening-up of national public contracts, the definition of common standards and the removal of legal and fiscal obstacles to that cooperation.

3. All Union activities under the Treaties in the area of research and technological development, including demonstration projects, shall be decided on and implemented in accordance with the provisions of this Title.

Article 180 (ex Article 164 TEC)

In pursuing these objectives, the Union shall carry out the following activities, complementing the activities carried out in the Member States:

(a) implementation of research, technological development and demonstration programmes, by promoting cooperation with and between undertakings, research centres and universities;

(b) promotion of cooperation in the field of Union research, technological development and demonstration with third countries and international organisations;

(c) dissemination and optimisation of the results of activities in Union research, technological development and demonstration;

(d) stimulation of the training and mobility of researchers in the Union.

Article 181 (ex Article 165 TEC)

1. The Union and the Member States shall coordinate their research and technological development activities so as to ensure that national policies and Union policy are mutually consistent.

2. In close cooperation with the Member State, the Commission may take any useful initiative to promote the coordination referred to in paragraph 1, in particular initiatives aiming at the establishment of guidelines and indicators, the organisation of exchange of best practice, and the preparation of the necessary elements for periodic monitoring and evaluation. The European Parliament shall be kept fully informed.

TITLE XX / ENVIRONMENT

Article 191 (ex Article 174 TEC)

1. Union policy on the environment shall contribute to pursuit of the following objectives:

- preserving, protecting and improving the quality of the environment,

- protecting human health,

- prudent and rational utilisation of natural resources,

- promoting measures at international level to deal with regional or worldwide environmental problems, and in particular combating climate change.

2. Union policy on the environment shall aim at a high level of protection taking into account the diversity of situations in the various regions of the Union. It shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should as a priority be rectified at source and that the polluter should pay.

In this context, harmonisation measures answering environmental protection requirements shall include, where appropriate, a safeguard clause allowing Member States to take provisional measures, for non-economic environmental reasons, subject to a procedure of inspection by the Union.

3. In preparing its policy on the environment, the Union shall take account of:

- available scientific and technical data,

- environmental conditions in the various regions of the Union,

- the potential benefits and costs of action or lack of action,

- the economic and social development of the Union as a whole and the balanced development of its regions.

4. Within their respective spheres of competence, the Union and the Member States shall cooperate with third countries and with the competent international organisations. The arrangements for Union cooperation may be the subject of agreements between the Union and the third parties concerned.

The previous subparagraph shall be without prejudice to Member States' competence to negotiate in international bodies and to conclude international agreements.

Annex III – Stop Vivisection on Directive 2010/63/EU

Executive Summary

Whilst conceived and finalised to satisfy relevant economic and financial needs[3], Directive 2010/63 not only proves to be out of step with modern scientific knowledge but also fails to meet general, political and ethical assumptions formally stated by legislators[4]

Directive 2010/63/EU on the protection of animals used for scientific purposes is an updated version of Directive 86/609/EEC, whose chief aim is to reduce the numbers of animals used for experiments by requiring that animal experiments should not be performed when an alternative method exists, and by encouraging the development and validation of alternative methods to replace animals. The latter served as the basis for the Commission to set up ECVAM, the European Centre for the Validation of Alternative Methods, in 1991.

Neither of these Directives has succeeded in achieving meaningful reduction in the numbers of animals used for experiments. Indeed, in some areas, there has been a **marked increase in animal use.** This is particularly significant and of great concern with respect to the numbers of animals used and killed in the breeding of genetically modified lines.

The use of the term "alternative methods" has led to much confusion and has misled the public. In particular, there is no legal definition of what constitutes an

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[3] Directive 86/609/EEC was revised with the stated aim of harmonising animal research legislation across EU countries to ensure a level playing field throughout the EU for industry and the research community. In fact, as outlined in recital 1 of Directive 2010/63/EU "Certain Member States had adopted national measures ensuring a high level of protection of animals used for scientific purposes while others only applied the minimum requirements laid down in Directive 86/609/EEC", so that "Such disparities had to be eliminated in order to ensure a proper functioning of the internal market" (i.e. as animal testing is more expensive in European Countries with higher standards of animal welfare, the research labs of those richer countries badly needed to reduce their competitive disadvantages to hold their ground against other competitors in the EU).

[4] Recital 2 states: *Animal welfare is a value of the Union that is enshrined in Article 13 of the Treaty on the Functioning of the European Union (TFEU).*
Recital 6 states: *New scientific knowledge is available in respect of factors influencing animal welfare as well as the capacity of animals to sense and express pain, suffering, distress and lasting harm. It is therefore necessary to improve the welfare of animals used in scientific procedures by raising the minimum standards for their protection in line with the latest scientific developments.*
Recital 12 states: *Animals have an intrinsic value which must be respected. There are also the ethical concerns of the general public as regards the use of animals in procedures. Therefore, animals should always be treated as sentient creatures and their use in procedures should be restricted to areas which may ultimately benefit human or animal health, or the environment. The use of animals for scientific or educational purposes should therefore only be considered where a non-animal alternative is unavailable. Use of animals for scientific procedures in other areas under the competence of the Union should be prohibited*.

"alternative method" and in the absence of a legal precedent, the meaning of the term will continue to mislead and confuse the public. This is of particular concern with respect to **replacement methods** (versus the reduction of animal numbers or the refinement of animal procedures).

The European Centre for the Validation of Alternative Methods (ECVAM) is not adequately able to fulfill its role to encourage the development and validation of alternative methods to replace animal methods, for several reasons. It is seriously understaffed and under funded. Perhaps worst of all, is that it was given a scientific mission that is impossible to achieve. ECVAM's terms of reference are based on historical animal data that have never been formally validated, against which it must compare modern, evidence-based non-animal test methods. The absurdity of the situation is made obvious when attempting to compare historical animal data against results obtained using human material, much like trying to compare apples and oranges.

In the 23 years since its inception, ECVAM has validated fewer than 40 alternative test methods, which translates into fewer than two validated test methods per year. In addition, the vast majority (around 80%) of these "alternatives" still use animals or animal tissues. These facts translate into a betrayal of public trust and a lack of transparency.

Directive 2010/63/EU is out of step with modern scientific knowledge. The result has been the unnecessary use and killing of millions of animals in the EU every year. There is also an indirect negative impact on human health and the environment because of an unscientific reliance on a methodology that is not evidence based (animal tests) and that is not capable of providing safety data relevant to the human species.

>> Introduction to Directive 2010/63/EU recital (6) states:

New scientific knowledge is available in respect of factors influencing animal welfare as well as the capacity of animals to sense and express pain, suffering, distress and lasting harm. It is therefore necessary to improve the welfare of animals used in scientific procedures by raising the minimum standards for their protection in line with the ***latest scientific developments***

Stop Vivisection Comment:

New scientific knowledge is available not only in respect of factors influencing animal welfare but also of factors on whether animals should be considered as having predictive value with respect to the human species. This new scientific knowledge includes advances in evolutionary developmental biology ("evo-devo"), complexity theory and personalised medicine [see references in Annex I].

Simply put, Directive 2010/63/EU is out of step with the latest scientific developments and current knowledge on species differences between humans and animals.

>> Introduction to Directive 2010/63/EU recital (10) states:
While it is desirable to replace the use of live animals in procedures by other methods not entailing the use of live animals, ***the use of live animals continues to be necessary to protect human and animal health*** *and the environment. However, this Directive represents an important step towards achieving the final goal of full replacement of procedures on live animals for scientific and educational purposes as soon as it is scientifically possible to do so. To that end, it seeks to facilitate and promote the advancement of alternative approaches. It also seeks to ensure a high level of protection for animals that still need to be used in procedures.* ***This Directive should be reviewed regularly in light of evolving science*** *and animal-protection measures.*

Stop Vivisection Comment:
The above phrase ***"the use of live animals continues to be necessary to protect human and animal health"*** is both confusing (it lumps human and animal health in the same category) and more importantly, it is out of step with modern knowledge of species differences between the workings of the human and the animal body [see references in Annex I]. This directive should therefore ***be reviewed in light of evolving science, which can be summed up as follow.***

No animal species is a reliable model for another

First and foremost, all species can be defined in terms of their reproductive isolation. That is to say, two different species cannot interbreed, with very rare exceptions, which invariably produce sterile progeny. This is due to the fact that, in order to produce a fertilised egg, the genetic material contained in the ovum and sperm that are to unite, must complement each other. This can only occur if the female and male animal are both of the same species. It cannot happen where the individuals are of different species, as the genetic material would be incompatible.

The second point of note is that all biological functions are determined by the genes of the individual. Overall, these biological functions are controlled chiefly by proteins, including enzymes, which help with functions such as the digestion of food, muscle contraction, the transport of oxygen in the blood, and so on. All of these proteins are unique and are geared to perform specific tasks. Their unique structure is determined, in turn, by the genes responsible for their synthesis. Two almost identical genes may produce two completely different proteins.

To sum up, every species of animal has a unique genetic code. The genetic code determines the structure of the proteins that ensure the biological activities associated with that species. Two different species will thus produce different proteins, which will be evident in terms of their biological activity.

>> Introduction to Directive 2010/63/EU recital (11) states:
The care and use of live animals for scientific purposes is governed by internationally established principles of replacement, reduction and refinement. To ensure that the way in which animals are bred, cared for and used in procedures within the Union is in line with that of the other international and national standards applicable outside the Union, the principles of replacement, reduction and refinement should be

considered systematically when implementing this Directive. ***When choosing methods, the principles of replacement, reduction and refinement should be implemented through a strict hierarchy of the requirement to use alternative methods.*** *Where no alternative method is recognised by the legislation of the Union, the numbers of animals used may be reduced by resorting to other methods and by implementing testing strategies, such as the use of in vitro and other methods that would reduce and refine the use of animals*.

Stop Vivisection Comment:
Since no animal species is a reliable model for another, the only methodologically relevant alternative to an animal experiment with respect to human health is full replacement with test methods that are relevant to the species in question, namely humans.

>> Introduction to Directive 2010/63/EU recital (12) states:
Animals have an intrinsic value which must be respected. ***There are also the ethical concerns of the general public as regards the use of animals in procedures.*** *Therefore, animals should always be treated as sentient creatures and their use in procedures should be restricted to areas which may ultimately benefit human or animal health, or the environment. The use of animals for scientific or educational purposes should therefore only be considered where a non-animal alternative is unavailable. Use of animals for scientific procedures in other areas under the competence of the Union should be prohibited*.

Stop Vivisection Comment:
In the contentious world of animal research, one question surfaces time and again: how useful are animal experiments as a way to prepare for trials of medical treatments in humans? ***The issue is crucial, as public opinion is behind animal research only if it helps develop better drugs*****.** Consequently, scientists defending animal experiments insist they are essential for safe clinical trials, whereas animal-rights activists vehemently maintain that they are useless [Giles J. Animal experiments under fire for poor design. *Nature* 2006].
A majority of public opinion is opposed to the use of animals in "curiosity-driven" research (also known as "basic research" or "fundamental research"). A survey funded by the European Commission shows that 68% of EU citizens are opposed to this kind of research and yet this strong message has not been translated into meaningful action by the European Commission. On the contrary, basic research is on the increase.
http://ec.europa.eu/environment/chemicals/lab_animals/pdf/results_citizens.pdfhttp://ec.europa.eu/environment/chemicals/lab_animals/questionnaire1.htm

>> Introduction to Directive 2010/63/EU recital (17) states:
Having regard to the present state of scientific knowledge, the use of non-human primates in scientific procedures is still necessary in biomedical research […]

Stop Vivisection Comment:
This assertion is no longer accurate in light of current knowledge of species differences with respect to humans and non human primates. The use of the chimpanzee, our closest living relative in evolutionary terms, is now under question [see references in Appendix I].
According to the Committee on the Use of Chimpanzees in Biomedical and Behavioral Research of the US National Research Council, "Recent advances in alternative research tools have rendered chimpanzees largely unnecessary as research subjects". This conclusion was based on an in-depth analysis by the Institute of Medicine, in collaboration with the National Research Council of the scientific necessity for chimpanzees in NIH-funded biomedical and behavioral research [National Research Council. Chimpanzees in Biomedical and Behavioral Research: Assessing the Necessity. Washington, DC: The National Academies Press, 2011].
Indeed, the chimpanzee is immune to HIV/AIDS, hepatitis B and common malaria and suffers different cancers to humans.
The corollary of the above is that genetically more distant non human primates (such as macaque and marmoset monkeys) will be ***even less relevant*** than the chimpanzee with respect to the study of human disease.

>> Introduction to Directive 2010/63/EU recital (17) continues:
Therefore the use of non-human primates should be permitted only in those biomedical areas essential for the benefit of human beings, for which no other alternative replacement methods are yet available. Their use should be permitted only for basic research, the preservation of the respective non-human primate species or when the work, including xenotransplantation, is carried out in relation to potentially life-threatening conditions in humans or in relation to cases having a substantial impact on a person's day-to-day functioning, i.e. debilitating conditions.

Stop Vivisection Comment:
The above is not rooted in science. Animal models either are predictive of human outcome or they are not. Based on empirical evidence, evolutionary biology and complexity theory, animals are not predictive of human outcome [see references in Appendix I]. Therefore, the use of non human primates is not predictive for human outcome.
<u>Consensus that animals are not predictive of human outcome:</u>
Our position, and apparently the position of scientists calling for standardization of animal protocols and Systematic Reviews, that animal models do not currently qualify as predictive modalities for human response to drugs and disease is supported by experts in various fields of science.

For example, Alan Oliff, then-executive director for cancer research at Merck Research Laboratories stated: "The fundamental problem in drug discovery for cancer is that the [animal] model systems are not predictive at all" [210].
An editorial in *Nature Reviews Drug Discovery* states: "Clearly, one part of the problem [of drug research] is poorly predictive animal models . . ." [211]. Ellis and Fidler echo this stating: "Preclinical models, unfortunately, seldom reflect the disease state within humans" [212].
Horrobin addressed the use of animal models stating: "Does the use of animal models of disease take us any closer to understanding human disease? With rare exceptions, the answer to this question is likely to be negative" [98].
Fliri pointed out that: "Currently, no method exists for forecasting broad biological activity profiles of medicinal agents even within narrow boundaries of structurally similar molecules" [213].
Speaking of toxicity trials for new drugs in humans, an unnamed clinician was quoted in *Science* as stating: "If you were to look in [a big company's] files for testing small-molecule drugs you'd find hundreds of deaths" [214].
Frances Collins, director of NIH, has also spoken out on the poor predictive value of animal models [215, 216].

Reference for above:
Ray Greek & Andre Menache "Systematic Reviews of Animal Models: Methodology versus Epistemology" *Int J Med Sci* 2013; 10(3):206-221

>> Introduction to Directive 2010/63/EU recital (27) states:
Animal tissue and organs are used for the development of in vitro methods. To promote the principle of reduction, Member States should, where appropriate, facilitate the establishment of programmes for sharing the organs and tissue of animals that are killed.

Stop Vivisection Comment:
The use of animal tissues and organs are appropriate for the development of *in vitro* methods in the field of veterinary medicine. In the field of human disease research, ethically sourced tissues and organs of human and not animal origin would be methodologically more relevant to the species in question, namely humans.

>> Article 4 of **Directive 2010/63/EU states:**

Principle of replacement, reduction and refinement

Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure.

Stop Vivisection Comment:

In the absence of a definition of "alternative methods" in *Article 3* of the Directive, the concept of what constitutes a "scientifically satisfactory method or testing strategy" is open to vastly different interpretations.

This lack of clarity in the Directive has given animal researchers almost unlimited scope to conduct invasive animal research. For example, a researcher can justify a task-reward study in monkeys, in which the animal is deprived of water and food and is forced to sit for hours in a restraining chair looking at images on a screen while electrodes implanted in its brain record data. These studies are categorised as "basic research", which by definition, make no claim to applicability in either animal or human medicine. Often, such studies are repetitive, with minor variations and conducted by the same team of researchers for many years, without any clinical application in sight.

The animal researcher can justify the monkey studies by pointing out that there is no correlating brain area in humans (e.g. the V1 area in the visual cortex differs significantly between monkeys and humans). Although non-invasive imaging studies in humans would provide information that is directly relevant to humans, the Directive allows the animal researcher to pursue the invasive monkey experiments, on the grounds that a human study would not provide the data that the monkey study will provide. It should be evident that the monkey data is relevant to monkeys, not humans.

Although monkeys and humans may share conserved processes, the presence of conserved processes is insufficient for inter-species extrapolation when the trait or response being studied (e.g. cognitive brain function) is located at higher levels of organization, is in a different module, or is influenced by other modules.

Reference: Greek R & Rice MJ. Animal models and conserved processes *Theor Biol Med Model.* 2012 Sep 10;9:40.

>> Article 5 **Directive 2010/63/EU states:**

Purposes of procedures

Procedures may be carried out for the following purposes only:

(a) basic research;

(b) translational or applied research with any of the following aims:

(i) the avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality or their effects in ***human beings****, animals or plants;*

(ii) the assessment, detection, regulation or modification of physiological conditions in ***human beings****, animals or plants;*

Stop Vivisection Comment:

The above is not rooted in science. Animal models either are predictive of human outcome or they are not. Based on empirical evidence, evolutionary biology and complexity theory, animals are not predictive of human outcome [see references in Appendix I].

>> Article 27 of **Directive 2010/63/EU states:**

Tasks of the animal-welfare body

1. The animal-welfare body shall, as a minimum, carry out the following tasks:
(b) advise the staff on the application of the requirement of replacement, reduction and refinement, and keep it informed of technical and scientific developments concerning the application of that requirement;

***Stop Vivisection Comment*:**
Animal welfare bodies are invariably weighted in favour of animal research over non animal methods. The status quo, in which an animal researcher is challenged by a philosopher or ethicist who sits on the ethics committee, does not constitute a level playing field. It is also a betrayal of public confidence in the ethical review process.

>> Article 49 of **Directive 2010/63/EU states:**

National committees for the protection of animals used for scientific purposes

Each Member State shall establish a national committee for the protection of animals used for scientific purposes. It shall advise the competent authorities and animal-welfare bodies on matters dealing with the acquisition, breeding, accommodation, care and use of animals in procedures and ensure sharing of best practice.

Stop Vivisection Comment:
Animal welfare bodies are invariably weighted in favour of animal research over non animal methods. The status quo, in which an animal researcher is challenged by a philosopher or ethicist who sits on the ethics committee, does not constitute a level playing field. It is also a betrayal of public confidence in the ethical review process.

>> About "PROTECTION OF ANIMALS":

Finally, Directive 2010/63/EU contradicts its very title "**On the protection of animals used for scientific purposes")** as there is no protection ("*the state of being kept from harm*") for animals that are the object of procedures where researcher are entitled:

- to lock in complete isolation for prolonged periods animals of social species such as dogs and primates (Annex VIII);
- to make use of metabolic cages involving severe restriction of movement over prolonged periods (Annex VIII);
- to reuse in subsequent procedures animals that have or will endure experiments classified as "mild", "moderate" or "non recovery" (such for instance surgery associated with post surgical pain, suffering or impairment of general conditions: thoracotomy, craniotomy, laparotomy, orchidectomy, orthopaedic surgery, organ transplantation… (Article 16, Annex VIII);

- to carry out procedures that are expected to result in persistent impairment of the general condition, production of unstable fractures, thoracotomy without adequate analgesia, or trauma to produce multiple organ failure (Annex VIII);
- to administer neuromuscular blocking agents with analgesics instead of general anaesthesia (Article 14);
- to force animals to swim with exhaustion as the end-point, to immobilise them to induce gastric ulcers or cardiac failure, to administer them inescapable electric shock to produce learned helplessness (Annex VIII);
- to carry out experiments on stray cats and dogs, a typology of experiments that was forbidden by Directive 1986. MEPs that passed the 1986 Directive on the use of lab animals did consider that the phenomenon of stray dogs was to be addressed by eliminating it, via sterilisation and public education. MEPs that passed the 2010 directive, oblivious of the above mentioned considerations, didn't bother voting article 11, that allows stray dogs and cats be taken from shelters and streets and taken to the laboratories. (Article 11).

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Annex IV – EU legal framework governing medicinal products, plant protection products, biocidal products, food additives and chemicals

Medicinal products

- Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use

- Council Recommendation 87/176/EEC of 9 February 1987 concerning tests relating to the placing on the market of proprietary medicinal products

Plant protection products

- **Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market**

- Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC

- Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances
- Commission Regulation (EU) No 545/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for plant protection products

Biocidal products

- **Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market**

- **Regulation (EU) No 528/2012 of the European parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products**

Food additives, food enzymes and food flavourings

- **Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings**

Chemicals – REACH

- **Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and subsequent changes.**

Annex V – STOP VIVISECTION on animal models. Why they are not predictive for the human species

Animal experimentation stems from a reductionist and mechanistic vision of nature, which treats people and animals like machines, that you can get to know by studying mechanical relationships between their different parts. In this light, the machine-animal becomes a model for the machine-man. This logic should be based on precise matches between man and animal; every biologist knows however that different animals may have similar anatomical and physiological characteristics, but many other are partially or totally different; this consideration also makes the animal model completely unreliable, since each animal is only a model of himself.

An article by Pandora Pound and Michael Bracken - British Medical Journal, 30 MAY 2014

Is animal research sufficiently evidence based to be a cornerstone of biomedical research?

Public acceptance of the use of animals in biomedical research is conditional on it producing benefits for humans. Pandora Pound and Michael Bracken argue that the benefits remain unproved and may divert funds from research that is more relevant to doctors and their patients

Pandora Pound medical sociologist[1], Michael B Bracken Susan Dwight Bliss professor of epidemiology[2]

[1]Bath, UK; [2]Yale University Schools of Public Health and Medicine, New Haven CT, USA

Proponents of animal research claim that the benefits to humans are self evident.[1] However, writing in The BMJ 10 years ago we argued that such uncorroborated claims were inadequate in an era of evidence based medicine.[2] At that time over two thirds of UK government and charitable investment was going into basic research,[3] perhaps creating an expectation that such research was highly productive of clinical benefits. However, when we searched for systematic evidence to support claims about the clinical benefits of animal research we identified only 25 systematic reviews of animal experiments, and these raised serious doubts about the design, quality, and relevance of the
included studies. As our colleagues had done earlier,[4] we argued the case that systematic reviews should be extensively adopted within animal research to synthesise and appraise findings, just as they are in clinical research.

Poor quality and reporting of animal studies

The overall number of systematic reviews of animal studies remains lamentably low, with the ratio of reviews to total
number of publications being about 10-fold higher in human studies.[5] In 2011 Korevaar and colleagues identified 244
systematic reviews of preclinical studies up until 2010, estimating that the number was doubling every three years.[6]

As the number of systematic reviews increased, the poor quality of much preclinical animal research became increasingly apparent.[7] Evidence accumulated that many animal studies failed to address important threats to internal and external validity, making prediction to humans tenuous at best.[8 9] For example, the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) surveyed 271 animal studies conducted between 1999 and 2005 and found that only 32 (12%) reported using random allocation to treatment or control and that investigators were blinded to the allocation in only 14% (5/35) of studies that used qualitative scoring.[10]

Systematic reviews of animal studies also revealed evidence of selective analysis and outcome reporting bias[11] as well as publication bias[12] leading to overstatement of the validity of entire bodies of research.[13]

The Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) has been at the forefront of conducting systematic reviews of animal studies. Initially focusing on stroke, it later expanded to include neurological disease, bone cancer, multiple sclerosis, and Parkinson's disease. By 2012 John Ioannidis, professor of health research and policy at Stanford, concluded that CAMARADES had found consistent suggestions of serious bias in animal studies, making it: "nearly impossible to rely on most animal data to predict whether or not an intervention will have a favourable clinical benefit-risk ratio in human subjects."[14]

Lack of benefit for humans

Concerns have been raised that compounds with little or no therapeutic potential could proceed to clinical trials because overoptimistic conclusions are drawn about their efficacy as a result of flaws in experimental design and inadequate control of bias.[15-19] Several studies have shown that even the most promising findings from animal research often fail in human trials and are rarely adopted into clinical practice.[20-22] For

example, one study found that fewer than 10% of highly promising basic science discoveries enter routine clinical use within 20 years.[23] In stroke medicine, despite decades of immense human, animal, and financial investment, animal models have failed to yield a single neuroprotective treatment for humans.[24 25] Similarly, none of more than 100 drugs studied in an established mouse model of amyotrophic lateral sclerosis, many of which had been reported to slow down the disease, was ultimately found to be beneficial after more rigorous experiments. Eight of these drugs had been used in thousands analysis of patients who participated in failed clinical trials.[26] A similar lack of translation has become apparent in inflammation.[27]

Falling investment in basic and animal research

Public funding bodies are becoming aware of the lack of return on investment, and public and charitable spending on basic research has decreased in the UK from 68.3% in 2004-5 to 59.4% in 2009-10.[28] This seems wise since retrospective analysis of the payback from research is beginning to suggest that it is clinical rather than basic research that has most effect on patient care.[29 30] Almost half of all research involving animals in the UK in 2012 was conducted by universities (48%), the remainder occurring in commercial organisations (27%), public bodies (13%), and non-profit organisations (9%).[31] The drug industry is also beginning to decrease its reliance on animal research because each translational failure represents huge losses of invested capital.[21 32] In Europe drug companies have reportedly decreased their use of animals by more than 25% from 2005 to 2008.[33]

A broken model?

The animal research community continues to cite selected instances of how research on animals has resulted in medical advances, or will one day do so (see www.understandinganimalresearch.org.uk/resources/animal-researchnews-feed/). However, these convey little confidence about the overall reliability and success of animal models, taking into account the total evidence. Given the large amount of animal research being undertaken, some findings will extrapolate to humans just by chance. Understanding Animal Research, a British organisation financed mainly by those conducting or funding animal research, highlights four reports purporting to support the validity of animal research,[34] all of which rely solely on expert opinion, one of the weakest forms of evidence according to widely agreed standards.[35]

Would improvements in preclinical experimental procedures and research reporting enhance the prediction from animals to humans and provide greater benefits for humans? In an article reviewing developments in the field of stroke, Sutherland and colleagues note that despite researchers adhering to recommendations intended to improve the quality of preclinical stroke studies for over 10 years, there is no evidence of an increased rate of successful translation.[25] Others argue that animal models will always fail to predict human outcomes reliably because humans and animals are such complex interactive systems with different evolutionary trajectories that even small differences between species could be important.[36] The genomic and inherent differences between rodent and human physiology are increasingly acknowledged,[37] and even non-human primates have many differences in the epigenome that fundamentally affect the functionality of the genome[38] and may account for their lack of success in predicting clinical response.[39-41] Even if the research was conducted faultlessly, animal models might still have limited success in predicting human responses to drugs and disease because of inherent inter-species differences in molecular and metabolic pathways.[42]

The use of transgenic animals, in which the genome has been changed by insertion of foreign genetic material, attempts to increase the validity of animal models by making them more closely resemble human phenotypes of interest. Yet transgenic models, where genes are regarded as operating largely independently of each other, have been criticised as limited,[43] oversimplistic, and, at least to date, as contributing more to an idea of therapeutic promise than actual clinical outcomes.[21 36] Furthermore, it has been observed that transgenic animals do not always produce the desired phenotype after cross breeding several generations, thereby undermining the rationale for this research strategy.[26]

Attempts to improve animal research and reporting

In response to the serious deficiencies found in the conduct and reporting of animal studies the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines [44] were produced in 2010. Over 300 journals and the major UK funding agencies have endorsed these guidelines, but a recent survey of papers published in Nature and PLoS found little improvement in reporting standards.[45] A Gold Standard

Publication Checklist has also been developed by SYRCLE (Systematic Review Centre for Laboratory Animal Experimentation) in the Netherlands to encourage more rigour in the conduct, not just reporting, of animal research.[46]

Michael Festing, a retired Medical Research Council scientist, recently acknowledged that few basic scientists receive any formal teaching, most relying on what they learn from their supervisor.[47] Similarly, the leadership of the National Institutes of Health in the US recognises that poor training may in part be responsible for the lack of reproducibility of animal models.[48]

The UK Fund for the Replacement of Animals in Medical Experiments now offers voluntary workshops in experimental design and statistical analysis, and an online course in experimental design (www.3rs-reduction.co.uk) has been developed. Training is also available for preclinical investigators to learn how to conduct systematic reviews (www.syrcle.nl).

In 2008 the Medical Research Council (MRC) funded a pilot "research translator" at an English university hospital site to try to facilitate the translation of findings from bench to bedside. One of the findings from a qualitative study investigating this initiative was that basic scientists' motivation came from scientific discovery rather than the application of their findings to medicine.[49] Recent attempts to improve translation within the animal research community include the "co-clinical trial" in which preclinical trials explicitly parallel ongoing human phase I and II trials[50] and the development of a scoring system to identify biomarkers that better predict therapeutic success.[51]

Time for change

The culture within research is shifting, and animal research is no longer as immune from challenge or criticism as it once was. Nonetheless, although science is more self critical, in practice it can be difficult to achieve change because stakeholders (governments, funders, universities, allied research industries, and researchers) may all have interests, not infrequently financial,[52] in continuing to do things as they have always been done. Although there are also valid criticisms of clinical research,[53] urgent attention needs to be paid to the quality of animal research for important reasons. Much clinical research follows on from animal research. If the foundations of the biomedical research enterprise are unsound, then whatever is built on these foundations will be similarly precarious.

The current situation is unethical. Poorly designed studies and lack of methodological rigour in preclinical research may result in expensive but ultimately fruitless clinical trials that needlessly expose humans to potentially harmful drugs or may result in other potentially beneficial therapies being withheld. Moreover, if poorly conducted studies produce unreliable findings, any suffering endured by animals loses its moral justification because their use cannot possibly contribute towards clinical benefit. Non-publication of animal studies is similarly unethical because the animals involved cannot contribute towards the accumulation of knowledge and because non-publication may result in further, unnecessary animal and human experiments.[13]

In addition to intensifying the systematic review effort, providing training in experimental design and adhering to higher standards of research conduct and reporting, prospective registration of preclinical studies,[54] and the public deposition of (both positive and negative) findings would be steps in the right direction.[18] Greater public accountability might be provided by including lay people in some of the processes of preclinical research such as ethical review bodies[55] and setting research priorities.[28] However, if animal researchers continue to fail to conduct rigorous studies and synthesise and report them accurately, and if research conducted on animals continues to be unable to reasonably predict what can be expected in humans, the public's continuing endorsement and funding of preclinical animal research seems misplaced.

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Contributors and sources: PP has conducted research in the sociology of medicine for over two decades and has a particular interest in evidence based medicine in animal research. MB is an epidemiologist who teaches and has considerable experience in evidence based medicine. He has been an active member of the Cochrane Collaboration from its inception and has a particular interest in research methods. PP conceived the idea for this article and wrote the first draft. MB contributed his knowledge, expertise, and critical eye to subsequent drafts. PP is the guarantor.

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Introduction to **"The Nuremberg Code subverts human health and safety by requiring animal modeling",** a report by **Ray Greek, Annalea Pippus** and **Lawrence A Hansen**

Modern vivisection was born in 1865 in France with physiologist **Claude Bernard**, who, to the horror of his wife and daughter, also experimented on their family dog. But two crucial dates have to be kept in mind: **1937 and 1947**: it was then, in those two years at the turn of Second World War, that animal testing found the political push it needed to take root extensively and deeply in **laboratory practice**, in the budgets of large companies and in the common sense of western societies. And those are the years that we have to investigate to understand how and why medical research and toxicology inadvertently drove into the dead end where they languish now.

This is clearly described in this far reaching report, entitled "**The Nuremberg Code subverts human health and safety by requiring animal modeling** with a rich bibliography accompanying it. It is signed by two medical doctors and researchers and a lawyer: Ray Greek (president of "Americans for Medical Advancement"), Annalea Pippus (graduate in law and psychology) and Lawrence Hansen (in the top list of the "Journal of Alzheimer's Disease" for his contribution to research in the field of neuroscience, the subject he teaches at the University of California-San Diego, School of Medicine in La Jolla).

The story uncovered by Greek and his colleagues starts in 1937, when one one sulfa drug dissolved in ethylene glycol was administered to children and adults, resulting in the death of 107 people (ethylene glycol is well known today as an ingredient in antifreeze products). The scandal and the fear were such that within a few months Washington passed **a new law**, the US Federal Food, Drug and Cosmetics Act, which prescribed to test drugs on animals before they are marketed.

Theater of the events of year 1947 was - instead - the courtroom in Nuremberg, where the US conducted a **trial against 23 leaders of the Nazi concentration camps**, including **20 medical doctors**, who were called to the bar not only for having run the concentration camps in the way that we all know, but also for having performed a frightful series of experiments on the camp prisoners: to study the effects of cold temperatures, altitude, burns from phosphorus, of typhoid, malaria, transplantation of bone, sulfa drugs.

This "**Doctors' Trial**" (no to be mistaken with the first and most famous process against Goering, Hess, and other Nazi leaders, also held in Nuremberg a few months before) ended with 7 acquittals, 9 sentences to prison and 7 sentences to death by hanging. But the more substantial and lasting fruit of this trial was a **code of ethics called the "Nuremberg Code"**, which indicates what criteria should be used or not used in medical experimentation on humans. The underlying assumption of this code of ethics, very similar to the one behind the US Federal Food, Drug and Cosmetics Act passed ten years before, is that experiments on animals are a winning alternative.

Not true. But it was not easy to realise in those years. "At the time of the Nuremberg trials" - writes Greek - "**medical science was very different than it is now**. The structure of DNA had not been elucidated. the notion of a magic bullet (that for every disease, or at least every infectious disease, a chemical existed that could interact with the single site causing the malady and thus cure the disease without harming the restof the body) was foremost in the minds of drug developers, the modern synthesis inevolution was brand new, and **animals and humans seemed to be more or less the same** except for humans having a soul.

There were no organ transplants, infectious diseases were still a major killer in the developed world, the fields of cognitive ethology and animal cognition were unheard of, and differences between ethnic groups and sexes in terms of disease and drug reactions had not yet been discovered". Physics was beginning to break free from the chains of determinism and reductionism, but the theories of chaos and complexity were yet to come. In short: "It was a different world and people in the 1940s are to be excused for thinking that animals and humans would react more or less the same to drugs and disease".

Today, these excuses are no longer valid. The new knowledge in the field of evolutionary biology, physics, ethology, theories of chaos and complexity, the critique of determinism and reductionism made a clean sweep of those certainties. Prior to the Human Genome Project (HGP), for instance, scientists thought the

number of genes was proportional to the complexity of the organism. The number of genes in some organisms was known or approximated; therefore, the scientists involved in the HGP were looking for an estimated 100,000+ genes in humans. As the project advanced, it became clear that humans had nowhere near this many genes. This was perplexing. But because of the new division of evolutionary biology (known as evolutionary developmental biology or evo devo), of the HGP and its spinoffs, and because of speculation by King and Wilson in the 1970s, scientists now know the following: all mammals have more or less the same genes. Some species have a few genes that other species do not have, but one could more or less build any mammal using the genes from another.

The differences among species lie, in large part, in the regulation and expression of the same genes, resulting **in very large and unpredictable differences between one species and another**, starting with the enzymes that metabolize drugs: "different enzymes metabolize different drugs, metabolize the same drugs at different rates, and form different metabolites, all of which influence toxicity and dosing." This is why **of all experimental drugs that are successful on animals, 96% must be discarded in subsequent human clinical trial because toxic or ineffective**, or both (yes, you read that right: the ninety-six per cent). The rationale of Greek, Pippus and Hansen Hansen is full of ideas, examples and explanations of great interest and refer to its pages those who want to know more.

The Nuremberg Code subverts human health and safety by requiring animal modeling

Ray Greek[1], **Annalea Pippus**[1] and **Lawrence A Hansen**[2]

[1] Americans For Medical Advancement, 2251 Refugio Rd, Goleta, CA 93117, USA
[2] Department of Neurosciences and Pathology, University of California, San Diego, Mail Code 062, 9500 Gilman Drive (MTF 351), La Jolla, CA, 92093-0624, USA
The electronic version of this article is the complete one and can be found online at:
http://www.biomedcentral.com/1472-6939/13/16

Abstract

Background
The requirement that animals be used in research and testing in order to protect humans was formalized in the Nuremberg Code and subsequent national and international laws, codes, and declarations.

Discussion
We review the history of these requirements and contrast what was known via science about animal models then with what is known now. We further analyze the predictive value of animal models when used as test subjects for human response to drugs and disease. We explore the use of animals for models in toxicity testing as an example of the problem with using animal models.

Summary
We conclude that the requirements for animal testing found in the Nuremberg Code were based on scientifically outdated principles, compromised by people with a vested interest in animal experimentation, serve no useful function, increase the cost of drug development, and prevent otherwise safe and efficacious drugs and therapies from being implemented.

The fallacy of vivisection as biomedical research method
by Pietro Croce in **"Vivisection or Science"**

* Do we want to show that the deadly Amanita phalloides is an excellent edible mushroom? Then we have only to feed it to the rabbit.

* Do we want to ruin the trade of citrus-fruit growers? Let us poison cats and rabbits with the lemon juice we add as flavouring to our food.

* Do we want to make someone fall asleep? Let's give them morphine. But do we want to send a cat into frenzy of excitement? Let's give it morphine too.

* If we wish to convince the consumers of tinned food that botulin poison is harmless, let's give it to the cat and it will lick its lips. Then lets give it instead to the cat's traditional prey, the mouse, and that animal will die as if struck by lightning.

* If we want to demonstrate that prussic acid (whose fumes can kill a human) is an excellent aperitif, let us give it to toads, sheep and hedgehogs.

* Do we want to discourage people from eating parsley? Let's give it to the parrot, which will probably be found lying stone-dead next morning.

* Strychnine, like arsenic, a favourite weapon of murderers in crime novels) is harmless to guinea-pigs, chickens and monkeys in amounts capable of causing convulsions in an entire human family.

* Should we wish to rule out penicillin as a therapeutic drug, we have only to give it to a guinea-pig or a hamster, which will be dead in a couple of days.

* Chloroform, used successfully for decades in human surgery,, is poisonous for dogs, catas and rabbits, causing loss of muscular coordination and convulsions.

* To show that vitamin C is useless, we can withhold it from the diet of the dog, the rat, the mouse and the hamster. They will continue to thrive because their bodies produce vitamin C of their own accord. However we must certainly not eliminate it from the diets of guinea-pigs, humans and other primates,or they will die of scurvy.

These are only 10 examples out of many dozens given by prof. Pietro Croce (who headed the Research Laboratory of the L. Sacco Hospital in Milan for many years, in addition to working in many Research departments in US and Spanish hospitals, and being a member of the College of American pathologists).

Scientific reports

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Inflammatory findings on species extrapolations: humans are definitely no 70-kg mice
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The Nuremberg Code subverts human health and safety by requiring animal modeling
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Are animal models predictive for humans?
(Philos Ethics Humanit Med. 2009 Jan 15; 4:2) *Shanks N, Greek R, Greek J.*

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(ALTEX. 2008;25(3):213-31) *Knight A.*

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(BMJ USA 2005; 331: E389-E391) *Knight A, Bailey J, Balcombe J.*

Where is the evidence that animal research benefits humans?
(BMJ. 2004 February 28; 328(7438): 514–517) *Pandora Pound, research fellow,1 Shah Ebrahim, professor,1 Peter Sandercock, professor,2 Michael B Bracken,professor,3 Ian Roberts, professor,4 and Reviewing Animal Trials Systematically (RATS) Group*

Laboratory routines cause animal stress
(Contemp Top Lab Anim Sci. 2004 Nov;43(6):42-51) *Balcombe JP, Barnard ND, Sandusky C.*

Are animal tests inherently valid?
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(ATLA 28, 315–331, 2000, 315) *Report and Recommendations of a Volunteers in Research and Testing Workshop Gill Langley, Graham Harding, Penny Hawkins, Anthony Jones, Carol Newman, Stephen Swithenby, David Thompson, Paul Tofts and Vincent Walsh*

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The US National Academy of Sciences

Report of the National Research Center (NRC) Committee on Toxicity Testing and Assessment of Environmental Agents, prepared in response to EPA's request

Toxicity Testing in the 21st Century: A Vision and a Strategy

Advances in molecular biology and toxicology are paving the way for major improvements in the evaluation of the hazards posed by the large number of chemicals found at low levels in the environment. The National Research Council was asked by the U.S. Environmental Protection Agency to review the state of the science and create a far-reaching vision for the future of toxicity testing. The report finds that developing, improving, and validating new laboratory tools based on recent scientific advances could significantly improve our ability to understand the hazards and risks posed by chemicals. This new knowledge would lead to much more informed environmental regulations and dramatically reduce the need for animal testing because the new tests would be based on human cells and cell components. Substantial scientific efforts and resources will be required to leverage these new technologies to realize the vision, but the result will be a more efficient, informative and less costly system for assessing the hazards posed by industrial chemicals and pesticides.

Executive Summary

Change often involves a pivotal event that builds on previous history and opens the door to a new era. Pivotal events in science include the discovery of penicillin, the elucidation of the DNA double helix, and the development of computers. All were marked by inauspicious beginnings followed by unheralded ad- vances over a period of years but ultimately resulted in a pharma- copoeia of life-saving drugs, a map of the human genome, and a personal computer on almost every desk in today's workplace.

Toxicity testing is approaching such a scientific pivot point. It is poised to take advantage of the revolutions in biology and bio- technology. Advances in toxicogenomics, bioinformatics, systems biology, epigenetics, and computational toxicology could trans- form toxicity testing **from a system based on whole-animal testing to one founded primarily on in vitro methods** that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin. Anticipating the impact of recent scientific advances, the U.S. Environmental Protection Agency (EPA) asked the National Research Council (NRC) to develop a long-range vision for toxicity testing and a strategic plan for implementing the vision.

This report of the NRC Committee on Toxicity Testing and Assessment of Environmental Agents, prepared in response to EPA's request, envisions a major campaign in the scientific community to advance the science of toxicity testing and put it on a forward-looking footing. The potential benefits are clear. Fresh thinking and the use of emerging methods for understanding how environmental agents affect human health will promote beneficial changes in testing of these agents and in the use of data for decision-making. The envisioned change is expected to generate more robust data on the potential risks to humans posed by exposure to environmental agents and to expand capabilities to test chemicals more efficiently. A stronger scientific foundation offers the pros- pect of improved risk-based regulatory decisions and possibly greater public confidence in and acceptance of the decisions.

With those goals in mind, the committee presents in this re- port a vision for mobilizing the scientific community and marshalling scientific resources to initiate and sustain new approaches, some available and others yet to be developed, to toxicity testing. This report speaks to scientists in all sectors: government, public interest, industry, university, and consulting laboratories, who design and conduct toxicity tests and who use test results to evaluate risks to human health. The report also seeks to inform and engage decision-makers and other leaders who shape the nature and scope of government regulations and who establish budgetary priorities that will determine progress in advancing toxicity testing in the future. The full impact of the committee's wide-ranging recommendations can be achieved only if both scientists and nonscientists work to advance the objectives set forth in the vision.

Quotes by Experts

Jeremy Rifkin:*"Anti-vivisection societies and animal rights organizations have been making this argument for a long time, only to be scorned by scientific bodies, medical associations, and industry lobbies who accuse them of being anti-progress and caring more about animals than people. Now, it is the scientific establishment that has come to the very same conclusions. Toxicity testing in animals is bad science."*

Nature 10/11/05:*"Scientists at the European Centre for the Validation of Alternative Methods (ECVAM) in northern Italy — which was set up by the European Commission to develop alternatives to animal testing — argue that animal tests are badly flawed. They say the new drive for alternative methods will improve the science of toxicity testing. And public safety demands that the new tests are shown to be better predictors of toxicity than the existing methods."*

Lancet 04/06/2011:*"A fundamental problem is that a rat is not a human. They are different sizes, have different metabolisms and have different diets so using animals to predict effects on humans is difficult. Fifty percent of compounds that prove to be safe in rats prove not to be safe in humans so it really is the toss of a coin," Dexter told Sky News."*
"It is increasingly clear that an important factor contributing to these problems is the over-reliance of the pharmaceutical industry on the use of animals to predict drug behaviour in man. The stark differences, not only in the diseases of different animal species, but also the ways that they respond to drugs, are now well known. Many studies have shown that animal tests frequently fail to translate to the clinic, with estimates of their ability to predict effects on people as low as 37—50%, or no better than the toss of a coin."

Thomas Hartung: *"But the toxicology tests on which regulators rely to gather this information are stuck in a time warp, and are largely based on wasteful and often poorly predictive animal experiments".*
The toxicity tests that have been used for decades are "simply bad science", he explains. "We now have an opportunity to start with a clean slate and develop evidencebased tests that have true predictive value."
"To test a chemical for its potential to cause cancer takes five years and involves 400 rats. More than 50% of the results are positive, of which 90% are false positives.

David Biello in Scientific American (13.10.2011): *"We are screening 10,000 chemicals using these rapid tests to characterize the bioactivity of the chemicals to predict their hazard and to use that information to prioritize for further screening and testing," says biologist David Dix, deputy director of EPA's National Center for Computational Toxicology. "We can test a lot of chemicals with a lot of repetitions at a lot of different concentrations."*
The program, initially started at EPA as ToxCast to assess 1,000 chemicals (and known as Tox21 in its expanded form), employs a robot to speed chemical screening. On plastic plates filled with 1,536 tiny wells, the robot drops varying amounts of different chemicals onto human cells and human proteins. Essentially, each plate has 1,536 experiments underway at the same time. "In a stack of 100, we have 150,000 combinations of chemicals and targets," Dix says.
The robot arm and its numerous five- to 10-microliter wells replace the old standby of toxicology—animal testing. In addition to being slow and controversial, animal tests do not reveal how a chemical might impact humans, nor do they deliver any insight into the mechanisms by which a given chemical produced toxic outcomes. Simply by running the robotic tests, the EPA and its partner agencies will generate more information on chemical toxicity in the next few years than has been created in the past century. The effort has already screened more than 2,500 chemicals, including the dispersants employed to clean up BP's 2010 oil spill in the Gulf of Mexico.
The new information may allow toxicology to evolve from a reactive science to a predictive one; models of liver toxicity based on chemical testing, for example, could predict how new chemicals would interact with the liver, based on molecular structure and other information. Already, ToxCast scientists have made such a predictive model for liver toxicity: It forecast accurately tumor formation in rats and mice that had been exposed for two years to certain chemicals. A similar effort proved accurate for reproductive toxicity, including vascular development and endocrine disruption - an area of keen interest for human exposure to chemicals such as bisphenol A (BPA).
In addition, the high-speed robotic testing will allow toxicologists to better understand mixture and low-

dose effects by testing both combinations of chemicals for additive damage as well as how, for example, 15 different concentrations of a given chemical impact human cells. "We suspect that when we look at 10,000 chemicals we'll see a lot of activity that we didn't know about," Dix says of the two-year effort, in which the EPA has partnered with a handful of federal health agencies.
"For a lot of chemicals, there's no requirement for animal toxicity testing or any other type of testing," Dix notes. "Tox21 is going to provide information where there is no information."

Vittorio Prodi: *"Toxicity testing is not delivering what safety of products demands nor is it sufficiently relying upon the most advanced technologies. It typically involves studying adverse health outcomes in animals subjected to high doses of toxicants with subsequent extrapolation to expected human responses at lower doses. But we are not 70kg rats feeding largely on chemicals. The system is expensive, time-consuming, low-throughput and often provides results of limited predictive value for human health. The toxicity testing methods are largely the same for industrial chemicals, pesticides and drugs, and have led to a backlog of more than 80,000 chemicals to which humans are potentially exposed but whose potential toxicity remains largely unknown.*
In the US, a new toxicity testing plan has been launched which includes the use of predictive, high-throughput cell-based assays (of human origin) to evaluate perturbations in key pathways of toxicity, and to conduct targeted testing against those pathways. Mapping the entirety of these pathways (hence the 'Human Toxome Project') could be a large-scale effort, perhaps on the order of the Human Genome Project. It could develop tremendous opportunities for REACH, the testing ban for cosmetics, the pesticide regulation, and the endocrine disruptor screening, while reducing animal suffering. How can Europe contribute to this goal?"

Francis Collins, director, NIH's National Human Genome Research Institute, 2008: *"Animal experimentation is "expensive, time-consuming, uses animals in large numbers, and it doesn't always work."*

Samuel Wilson, acting director of the National Institute of Environmental Health Sciences and NTP: *"The new research model would allow scientists to test 100,000 compounds in 1,500 different concentrations in about two days compared with years if the testing was done on animals."*

Francis Collins in The Scientist: *"With earlier and more rigorous target validation in human tissues, it may be justifiable to skip the animal model assessment of efficacy altogether".*

Science 15-02-2008, *Francis S. Collins, George M. Gray and John R. Bucher: "We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments."*

Allison Abbott in Nature 10/11/2005: *"Most animal tests overor underestimate toxicity, or simply don't mirror toxicity in humans very well."*
"Commercial and political pressures are pushing for a halt to the use of animals in toxicology tests in Europe. This change will also mean a move towards better science, says Alison Abbott."

Horst Spielmann:
"Animal embryotoxicity tests are not reliably predictive for humans," says Horst Spielmann, a toxicologist at the Federal Institute for Risk Assessment in Berlin. "When we find that cortisone is embryotoxic in all species tested except human, what are we supposed to make of them?"

Pandora Pound in British Medical Journal: *"Ideally, new animal studies should not be conducted until the best use has been made of existing animal studies and until their validity and generalisability to clinical medicine has been assessed."*

John Prineas and Michael Barnett in New Scientist: *"Their findings back the view that the reason for the lack of progress in this field is that most Multiple Sclerosis research is done on mice with a disease that is actually quite different".*

National Institute of Environmental Health Sciences: *"A second argument against selection bias is that*

knowledge to predict carcinogenicity in rodent tests is highly imperfect, even now, after decades of testing results have become available on which to base prediction."

Robert Sharpe: *"Most adverse reactions which can occur in patients cannot be demonstrated, anticipated or avoided by the routine subacute and chronic toxicity experiment" (Zbinden 1966)*

Honess et al 2004: *"More long-tailed macaques (Macaca fascicularis) than any other primate are imported into the UK for research, and journey times may be of up to 58 h."*

Erwin, Drake and Deni – 1979: *"The subjects were housed individually 1-m3 wire cages. All were kept in the same colony room and were exposed to identical environmental conditions."*

X.S. Puente 2006: *"Despite the high conservation of cancer genes between both species, we identified 20 genes containing several codon insertions or deletions in their protein coding regions, although the functional significance of these differences, including their putative association with cancer, will require further studies"*

Yasuhiro 2009: *"Animals captured and bred in Vietnam for instance may respond differently in toxicological or immunological studies to those originating in the Philippines or in Mauritius"*

7th World Congress on Alternatives & Animal Use in Life Sciences (Conclusive Press Release): *"Participants agreed that current knowledge of the human genome and the genomes of many animal species have resulted in such a level of scientific progress in the area of gene mapping and expression (genomics) that it will make it possible in the near future to apply these tools, together with current computational technologies (linking and analysing massive data bases) and sophisticated second generation in vitro test systems, to assess the hazards and risks of chemical and microbiological substances without the use of experimental animals."*

Robert Matthews 2008:*"It is crucial to know how and why such tests fail to predict what happens in humans". That can happen in two ways: firstly, where animals fail to warn of real toxic effects in humans - as in thalidomide - and secondly, where they give false alarms, with the animals falling victim to drugs that would be fine in humans."*

Quotes from Scientific Articles

"Proponents of animal research claim that the benefits to humans are self evident. However, writing in The BMJ 10 years ago we argued that such uncorroborated claims were inadequate in an era of evidence based medicine. At that time over two thirds of UK government and charitable investment was going into basic research, perhaps creating an expectation that such research was highly productive of clinical benefits. However, when we searched for systematic evidence to support claims about the clinical benefits of animal research we identified only 25 systematic reviews of animal experiments, and these raised serious doubts about the design, quality, and relevance of the included studies." *[Dr Pound P, dr. Bracken MB. Is animal research sufficiently evidence based to be a cornerstone of biomedical research? BMJ, May 2014, 30;348:g3387. doi: 10.1136/bmj.g3387.]*

"If there was an animal model good enough to substitute for people, we would not have a 92% failure rate in clinical trials."*[Dr. Thomas Hartung quoted in Nature Medicine]*

"The chimpanzee is our closest living relative. The early genome comparison by DNA hybridization techniques suggested a nucleotide difference of 1-2%. However, if one looks at proteins, which are mainly responsible for phenotypic differences, the picture is quite different, and about 80% of proteins are different between the two species."*[Dr. Glazko, Dr. Veeramachaneni, Dr. Nei, Dr. Makałowski. Eighty percent of proteins are different between humans and chimpanzees. Gene. 2005 Feb 14;346:215-9.]*

"Although acute inflammatory stresses from different etiologies result in highly similar genomic responses

in humans, the responses in corresponding mouse models correlate poorly with the human conditions"*[Dr. Seok et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. PNAS 2013]*

"The low predictivity of animal experiments in research areas allowing direct comparisons of mouse versus human data puts strong doubt on the usefulness of animal data as key technology to predict human safety."[…] Can one show, or reasonably assume, that the predictivity of animals for man does not differ fundamentally in different fields of biomedical research? The answer from screening the scientific literature must be clearly 'yes'."*[Dr. Hartung & Dr. Leist. Inflammatory findings on species extrapolations: humans are definitely no 70-kg mice. Arch Toxicol. 2013 Apr;87(4):563-7.]*

"We conclude that even if legitimate criticisms of animal models were addressed, through standardization of protocols and systematic reviews, the animal model would still fail as a predictive modality for human response to drugs and disease."*[Dr. Greek & Dr. Menache. Systematic Reviews of Animal Models: Methodology versus Epistemology. Int J Med Sci. 2013; 10(3): 206–221.]*

"Despite the lack of systematic evidence for its effectiveness, basic animal research in the United Kingdom receives much more funding than clinical research."*[Dr. Pound, Dr. Ebrahim, Dr. Sandercock, Dr. Bracken, Dr. Roberts et al. Where is the evidence that animal research benefits humans? BMJ. 2004 February 28; 328(7438): 514–517.]*

"Changes from baseline or control measures typically ranged from 20% to 100% or more and lasted at least 30 min or longer. We interpret these findings to indicate that laboratory routines are associated with stress, and that animals do not readily habituate to them. The data suggest that significant fear, stress, and possibly distress are predictable consequences of routine laboratory procedures, and that these phenomena have substantial scientific and humane implications for the use of animals in laboratory research."*[Dr. Balcombe, Dr. Barnard, Dr. Sandusky. Laboratory routines cause animal stress. Contemp Top Lab Anim Sci. 2004 Nov;43(6):42-51.]*

"We conclude that the use of sentient animals in basic research cannot be justified in light of society's priorities."*[Dr. R. Greek, Dr. J. Greek. Is the use of sentient animals in basic research justifiable? Philos Ethics Humanit Med. 2010 Sep 8;5:14. doi: 10.1186/1747-5341-5-14.]*

"Laboratory animal models are limited by scientific constraints on human applicability, and increasing regulatory restrictions, driven by social concerns. However, a range of non-animal methodologies is available within biomedical research and toxicity testing."*[Dr. Knight. Non-animal methodologies within biomedical research and toxicity testing. ALTEX. 2008;25(3):213-31.]*

"The ability of animal studies to detect serious post marketing adverse events is limited."*[Dr. van Meer, Dr. Kooijman, Dr. Gispen-de Wied, Dr. Moors, Dr. Schellekens. The ability of animal studies to detect serious post marketing adverse events is limited. Regul Toxicol Pharmacol. 2012 Dec;64(3):345-9.]*

"Replacing animal procedures with methods such as cells and tissues in vitro, volunteer studies, physicochemical techniques and computer modelling, is driven by legislative, scientific and moral imperatives. Non-animal approaches are now considered as advanced methods that can overcome many of the limitations of animal experiments." *[Dr. Langley, Dr. Evans, Dr. Holgate, Dr. Jones. Replacing animal experiments: choices, chances and challenges. Bioessays 2007; 29(9): 918-26.]*

"Animal experiments scrutinised: systematic reviews demonstrate poor human clinical and toxicological utility." *[Dr. Knight. ALTEX. 2007;24(4):320-5.]*

"The results of drug tests in mice have never translated perfectly to tests in humans. But in recent years, and especially for neurodegenerative diseases, mouse model results have seemed nearly useless." *[Dr. Schnabel. Neuroscience: Standard model. Nature. 2008 Aug 7;454(7205):682-5.]*

"The toxicology tests on which regulators rely to gather this information are stuck in a time warp, and are largely based on wasteful and often poorly predictive animal experiments." *[Dr. Abbott. Animal testing: more than a cosmetic change. Nature 2005 Nov 10;438(7065):144-146.]*

"There is a great deal of often overlooked data showing non-human primates research to be irrelevant, unnecessary, even hazardous to human health and to have little or no predictive value or application to human medicine." *[Dr. Bailey. Non-human primates in medical research and drug development: a critical review. Biogenic Amines 2005; 19(4-6): 235–255.]*

"The proposition that animal tests are inherently valid, merely because they are animal tests, is discussed and is rejected. It is concluded that there is no justifiable reason for subjecting new or substantially modified animal test procedures or testing strategies to a validation process that is any less stringent than that applied to non-animal tests and testing strategies." *[Dr. Balls (2004). Are animal tests inherently valid? ATLA: Alternatives to Laboratory Animals, 32(Suppl. 1B), 755–758.]*

"We believe that although animal experiments are sometimes intellectually seductive, they are poorly suited to addressing the urgent health problems of our era, such as heart disease, cancer, stroke, AIDS and birth defects. Even worse, animal experiments can mislead researchers or even contribute to illnesses or deaths by failing to predict the toxic effects of drugs. Fortunately, other, more reliable methods that represent a far better investment of research funds can be employed."*[Dr. Barnard and Dr. Kaufman. Animal research is wasteful and misleading . Scientific American, 00368733, Feb97, Vol. 276, Issue 2]*

"Although the mouse provides the most common model for many aspects of the human immune system, the 65 million years of divergence has introduced significant differences between these species, which can and has impeded the reliable transition of pre-clinical mouse data to the clinic." *[Dr. Brady. Of mice and men: the potential of high resolution human immune cell assays to aid the preclinical to clinical transition of drug development projects. Drug Discovery world 2008/9:74-78.]*

"When one empirically analyzes animal models using scientific tools they fall far short of being able to predict human responses. This is not surprising considering what we have learned from fields such evolutionary and developmental biology, gene regulation and expression, epigenetics, complexity theory, and comparative genomics." *[Dr. Shanks, Dr. R. Greek, Dr. J. Greek. Are animal models predictive for humans? Philos Ethics Humanit Med. 2009 Jan 15;4:2.]*

"The value of animal experiments for predicting the effectiveness of treatment strategies in clinical trials has remained controversial, mainly because of a recurrent failure of interventions apparently promising in animal models to translate to the clinic." *[Dr. van der Worp, Dr. Howells, Dr. Sena, Dr. Porritt, Dr. Rewell, Dr. O'Collins et al. Can animal models of disease reliably inform human studies? PLoS Med 2010, 7: e1000245]*

"Patients and physicians should remain cautious about extrapolating the findings of prominent animal research to the care of human disease. […] Poor replication of even high-quality animal studies should be expected by those who conduct clinical research." [Dr. Hackam & Dr. Redelmeier. Translation of research evidence from animals to humans. JAMA 2006;296(14):1731-2.]

"Six volunteers became critically ill during the phase-one test of TGN1412, developed by now-defunct drug firm TeGenero. Although preclinical research on monkeys had shown no sign of danger, the drug provoked devastating immune reactions in the human subjects." *[Dr. Hopkin. New test could weed out dangerous drug trials. Published online 7 December 2006. Nature]*

"Several investigations have revealed animal carcinogenicity data to be lacking in human predictivity. […]The likely causes of the poor human predictivity of rodent carcinogenicity bioassays include: 1) the profound discordance of bioassay results between rodent species, strains and genders, and further, between rodents and human beings; 2) the variable, yet substantial, stresses caused by handling and restraint, and the stressful routes of administration common to carcinogenicity bioassays, and their effects on hormonal regulation, immune status and predisposition to carcinogenesis; 3) differences in rates of absorption and transport mechanisms between test routes of administration and other important human routes of exposure; 4) the considerable variability of organ systems in response to carcinogenic insults, both between and within species; and 5) the predisposition of chronic high dose bioassays toward false positive results, due to the overwhelming of physiological defences, and the unnatural elevation of cell

division rates during ad libitum feeding studies. Such factors render profoundly difficult any attempts to accurately extrapolate human carcinogenic hazards from animal data." *[Dr. Knight, Dr. Bailey, Dr. Balcombe. Animal carcinogenicity studies: 2. Obstacles to extrapolation of data to humans. Altern Lab Anim. 2006 Feb;34(1):29-38.]*

"An emerging body of evidence indicates that there are fundamental differences in how the process of tumorigenesis occurs in mice and humans." *[Dr. Rangarajan & Dr. Weinberg. Comparative biology of mouse versus human cells: modelling human cancer in mice. Nature Reviews Cancer 3, 952-959 (December 2003)]*

"Although these approaches are without exception deemed "very promising" in the literature, it cannot be expected that research on GMO will make any contribution to a new therapeutic strategy in the near future." *[Dr. Stingl, Dr. Völkel & Dr. Lindl. 20 years of hypertension research using genetically modified animals: no clinically promising approaches in sight. ALTEX 2009; 26(1): 41-51.]*

"By using in vitro laboratory tests, dangers for patients and unnecessary animal experiments can be avoided."*[Dr. Müller. In vitro biocompatibility testing of biomaterials and medical devices. Med Device Technol. 2008 Mar-Apr;19(2):30, 32-4.]*

"Our reliance on animals to establish safety results in the exposure of clinical volunteers and patients to many treatments that are at best ineffective and at worst dangerous. Take for example the notorious Northwick Park clinical trial drug, TGN1412, that left six young men in intensive care in 2006. This drug was demonstrably safe in monkeys at doses 500 times higher than those that nearly proved fatal to the volunteers. Soon after the disastrous trial, an assay that used human cells was developed to predict such an immune system over-reaction." *[Dr. Archibald, Dr. Coleman, Dr. Foster. Open letter to UK Prime Minister David Cameron and Health Secretary Andrew Lansley on safety of medicines. Lancet. 2011 Jun 4;377(9781):1915.]*

"The assumption that gene functions and genetic systems are conserved between models and humans is taken for granted, often in spite of evidence that gene functions and networks diverge during evolution. […] Therefore, animal models of gene function and human disease may not provide appropriate information, particularly for rapidly evolving genes and systems." *[Dr. Vincent Lynch. Use with caution: Developmental systems divergence and potential pitfalls of animal models. Yale J Biol Med. 2009 June; 82(2): 53–66.]*

"For new oncology drugs, only about 5% of investigational new drug applications submitted progress beyond the investigational phase due to a general lack of preclinical systems that can accurately predict efficacy and toxicity of new agents." *[Dr. Wittenburg & Dr. Gustafson. Optimizing preclinical study design in oncology research. Chem Biol Interact. 2011 Apr 25;190(2-3):73-8.]*

"The complexity of human metastatic cancer is difficult to mimic in mouse models. As a consequence, seemingly successful studies in murine models do not translate into success in late phases of clinical trials, pouring money, time and people's hope down the drain." *[Dr. Ellis & Dr. Fidler. Finding the tumor copycat: Therapy fails, patients don't. Nature Medicine 16, 974–975 (2010)]*

"Animal testing is not ideal either, as the predictive value of such tests is limited owing to metabolic differences between humans and animals, and many ethical issues are raised by the testing." *[Dr. Neuzil, Dr. Giselbrecht, Dr. Lange et al. (2012) Revisiting lab-on-a-chip technology for drug discovery. Nature Reviews. Drug Discovery 11:620-632. 10.1038/nrd3799.]*

"Species, and even individual humans, can differ in genetic composition. For example, there may be differences in the presence (or absence) of certain genes. The presence (or absence) of certain alleles. The background genes and modifier genes that influence the genes being perturbed by drugs or disease. The regulation and expression of genes. Gene networks. Alternative splicing, which allows one gene to form or be part of forming many different proteins. Proteins and protein–protein interactions. Gene–protein interactions. Old genes evolving to perform new functions. Horizontal gene transfer (HGT). HGT occurs when genes from one organism are incorporated into another organism without the recipient organisms

being the offspring of the donor. For example, resistance to anti-bacterial drugs can occur through HGT. Epigenetics. Epigenetics is the relatively new field that studies changes in gene expression that can be inherited and that occur without changing the underlying DNA sequence. For example, because of environmental influences, a regulatory gene may be changed such that it is turned on or off thus allowing a disease to manifest. The presence of gene and chromosomal mutations such as single nucleotide polymorphisms (SNPs), copy number variants (CNVs), duplications, inversions, deletions, and insertions. In response to a perturbation to the system, such as a drug or disease, even one of the above differences can result in life or death consequences. Furthermore, convergent evolution can result in the same trait being present but being mediated by very different pathways in different species. Different molecules can also perform the same function. All of these types of differences are present in every species." *[Dr. Greek R, Dr. Pippus A, Dr. Hansen LA. The Nuremberg Code subverts human health and safety by requiring animal modeling. BMC Med Ethics. 2012 Jul 8;13:16. doi: 10.1186/1472-6939-13-16.]*

"Over 90% of phase 3 clinical trials in oncology fail to meet their primary endpoints despite encouraging preclinical and even early-stage clinical data. This staggering and sobering figure underscores the limitations of existing animal models for the evaluation of potential anticancer agents. The paucity of models is especially apparent with the advent of drugs that target the tumor milieu, or microenvironment, such as antiangiogenics […] immunotherapies and compounds directed against tumor-associated fibroblasts." *[Dr. Singh M, Dr. Ferrara N. Modeling and predicting clinical efficacy for drugs targeting the tumor milieu. Nat Biotechnol. 2012 Jul 10;30(7):648-57. doi: 10.1038/nbt.2286.]*

"Dr. Richard Klausner, then-director of the National Cancer Institute: "The history of cancer research has been a history of curing cancer in the mouse […] We have cured mice of cancer for decades—and it simply didn't work in humans.""
[Dr. Cimons, Dr. Marlene, Dr. Josh Getlin, and Dr. Thomas H. Maugh_II. 2010. Cancer Drugs Face Long Road From Mice to Men 1998]

"Many are now coming to the realization that, as in other therapeutic areas, the greatest limitation for identifying new drugs for treating cancer are the deficiencies in the animal models used for testing NCEs [new chemical entities, also referred to as new molecular entities or NMEs]"
[Dr. Enna SJ, Dr. Williams M. Defining the role of pharmacology in the emerging world of translational research. Adv Pharmacol. 2009;57:1-30.]

"The difficulties in predicting drug efficacy from preclinical models have been of concern for more than two decades […] Thus, novel findings apparently related to the systems and targets involved in disease causality; the delineation of the efficacy, selectivity and safety of NCEs; and the predictive relevance of biomarkers and animal model data to the human disease state, even when there is evidence for target engagement in humans, all frequently fail to enhance the success rate for new drug applications (NDAs)."*[Dr. Mullane K, Dr. Williams M. Translational semantics and infrastructure: another search for the emperor's new clothes? Drug Discov Today. 2012 May;17(9-10):459-68. doi: 10.1016/j.drudis.2012.01.004. Epub 2012 Jan 16.]*

"We conclude that even the presence of conserved processes is insufficient for inter-species extrapolation when the trait or response being studied is located at higher levels of organization, is in a different module, or is influenced by other modules." *[Dr. Greek R, Dr. Rice MJ. Animal models and conserved processes. Theor Biol Med Model. 2012 Sep 10;9:40. doi: 10.1186/1742-4682-40.]*

Annex VI – STOP VIVISECTION on alternative methods

Research & Innovation/HEALTH web page of the European Commission states that "the Health Programme funds research projects in the field of 'integrated testing strategies', cell-based technologies, "omics", bioinformatics and computational biology, computational modelling and estimation techniques, and high throughput techniques in order to develop **test methods which are better, faster, cheaper, and which have a higher predictive value than currently used animal tests".**
http://ec.europa.eu/research/health/biotechnology/alternative-testing-strategies/index_en.html
Of course, you'd think that a method which is "better", "faster", "cheaper" and with a "higher predictive value than "currently used animal tests" would be made legally mandatory as soon as available: for the sake of human health, of national budgets, of the national health services, for the sake of the animals themselves. Not so. Not even when the better, faster, cheaper, predictive methods are within reach.
This is the case for quality control testing for products for human and veterinary medicine, for which reliable new methods can be developed and validated in short time. In particular:

- the **Botuline toxins** for the safety testing of which an alternative method has been developed and patented, and yet at least 300 millions of rats are still being killed every year (a fact politically quite disturbing, given the recognised cosmetic use of the Botox product);
- the **vaccine quality testing**, for the safety of which many tests are already available;
- the **detection of shell fish biotoxins**, where excellent in vitro tests have been approved, but still the mouse bioassay is widely used.

Countless institutional and private web sites are devoted to informing both the public and the researchers on the use of alternative methods, and on the 3Rs, to which principles Directive 2010/63/EU affirms to be firmly committed (one R is for Reducing the number of animals used; the second R is for Refining techniques so the animals suffer less; the third R is for Replacing animal-based tests as alternatives are invented). A short and forcefully incomplete list of such points of reference must count:

- the European Commission Environment page on Animal used for scientific purposes http://ec.europa.eu/environment/chemicals/lab_animals/links_en.htm;
- the European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM) online page https://eurl-ecvam.jrc.ec.europa.eu/;
- the European consensus-platform for alternatives (ECOPA) http://www.ecopa.eu/;
- the European Society for Alternatives to Animal Testing (EUSAAT) http://www.eusaat.org/;
- the International Network for Humane Education (INTERNICHE) http://www.interniche.org;
- The Johns Hopkins CAAT US and CAAT EU resources page http://caat.jhsph.edu/resources/Hopkins;

A wealth of news and data is to be found and used to phase out animal experiments. But they are not. Quite the opposite, where information is made available, as in

Germany and the UK[1], they appear to be on the rise, and even more on the rise they will appear when all Genetically Modified Animals will begin to be counted (if ever) in official statistics.
Some reasons for this failure were already stated by first head of ECVAM (the European Union Reference Laboratory for alternatives to animal testing) Professor Michael Balls in 1995: "*Validation of alternative methodologies is handicapped by "excessive politeness towards vested interests which want to hang onto animal tests. The most important thing, as far as I'm concerned, is that we stop talking and get on with the job...and that we do not rest until the undoubted potential of replacement alternatives becomes a reality in practice, for the benefit of humans, as well as animals.*"

"We have not (yet) succeeded in persuading others just how awful (scientifically) most of the animal tests are, and how very unscientific is the way the data they provide are used in risk assessment. We must do better, but others must also be made to face up to reality as well".
Other reasons are being clearly stated today in European Commission "Press Release Database: questions and answers on the new directive for the protection of animals used for scientific purposes".
To the question "Why has the obligation to use alternatives where practicably available been removed from the revised text?" the answer is:

Directive 86/609/EEC states in Article 7 that '*an experiment [involving an animal] should not be performed if another scientifically satisfactory method, not entailing the use of an animal, is reasonably and practicably available'.* This lead to misinterpretation and ambiguity resulting in court cases in various Member States. The new text obliges users to choose an alternative method should it be recognised by Community legislation (Article 13).

Which means that a scientific, political, cultural and moral principle as crucial as the one demanding that "alternatives be mandatory where practicably available" has been sacrificed on the altar of legal distinctions, whose rationale seems rather specious and not so difficult to overcome, when political will is at work.
Perhaps worst of all, is that ECVAM was given a scientific mission that is impossible to achieve. ECVAM's terms of reference are based on historical animal data that have never been formally validated, against which it must compare modern, evidence-based non-animal test methods. The absurdity of the situation is made

[1] http://www.aerzte-gegen-tierversuche.de/en/frontpage/98-ressources/1476-statistics-2012; https://www.gov.uk/government/statistics/statistics-of-scientific-procedures-on-living-animals-great-britain-2012; http://www.altex.ch/News.17.html?ncat=1&eid=324; http://www.theguardian.com/news/datablog/2012/jul/10/animal-testing-risk-suffering# ; http://www.theguardian.com/science/2013/jul/16/research-animals-rises-4m-procedures.

obvious when attempting to compare historical animal data against results obtained using human material, much like trying to compare apples and oranges.

In the 23 years since its inception, ECVAM has validated fewer than 40 alternative test methods, which translates into fewer than two validated test methods per year. In addition, **the vast majority (around 80%) of these "alternatives" still use animals or animal tissues.**
These facts translate into a flagrant betrayal of public trust and a lack of transparency.

More references
Lab-on-a-Chip: they are microfluidic chips that mimic the activity, the mechanical and physiological response of whole organs and systems. Useful in drug development and basic research[5].

Multi-Compartmental modular Bioreactors (MCmB): they are innovative systems for dynamic cell cultures and co-cultures, which through different modular chambers connected together, in series or parallel, can replicate the organ-organ interactions.
The Kirkstall **"Quasi-Vivo"®** system is an example of it, enabling multiple cell types to be cultured in inter-connected culture chambers and interconnected to allow the simulation of various metabolic pathways, through a nutrient flow which pass across the chambers, to investigate and test multi-compartmental biological models in vitro.
Among the biopharmaceutical applications we report: interactions between organs and systems, drug development, research on nanoparticles, regenerative medicine, security and toxicity screening, ADME studies, models of disease (such as glaucoma, hypertension or diabetes) and research on stem cells[6 7 8 9 10].

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Pharmacogenomics: it is the branch of pharmacology that deals with the genetic variation on the drug response by patients, correlating gene expression or changes in single nucleotides to the efficacy, the toxicity and drug interactions. Its use can significantly reduce the adverse effects to drugs [11].

The **Integrated Discrete Multiple Organ Co-culture (IdMOC)** is a novel in vitro experimental system that allows the evaluation of biological effects of chemicals, with interactions between multiple cell types including endocrine, paracrine, and metabolic interactions. The system uses a 'wells-within-a-well' concept for the co-culturing of cells or tissue slices from different organs as physically separated (discrete) entities in the small inner wells. These inner wells are nevertheless interconnected (integrated) by overlying culture medium in the large outer containing well. The IdMOC system thereby models the in vivo situation, in which multiple organs are physically separated but interconnected by the systemic circulation, permitting multiple organ interactions. One specific application of IdMOC is the evaluation of metabolism-dependent chemical properties such as metabolism-dependent toxicity and pharmacology [12] [13].
The greater problems for the transplants are the individual immune reactions and the mechanisms of rejection. The animal is not always able to predict human immune responses, however there are several alternative methods, such as **limiting dilution assays**, the **ELISpot**, **flow cytometry**, in addition **to in vitro methods that use T cells** to predict rejection or tolerance for transplantation [14] [15] [16] [17]. Also useful

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in this field are **microarrays** [18] and mathematical models as the **ODE** (**ordinary differential equations**, which can denote, using variables, the effectiveness of antiviral and immunosuppressant drugs) and the **MPC** (**model predictive control**, on which the actual feasibility of the post transplant immunosuppression control is based) [19].

An in vitro model, termed **"MIMIC" (Modular IMmune In vitro Construct)**, was designed and developed to reflect the human immune system in a well-based format, can be used to simulate a clinical trial for a diverse population, without putting human subjects at risk, uses the circulating immune cells of individual donors to recapitulate each individual human immune response by maintaining the autonomy of the donor. Thus, an in vitro test system has been created that is functionally equivalent to the donor's own immune system and is designed to respond in a similar manner to the in vivo response. This system is also useful for the assessment of vaccines [20] [21].

In the neuroscience field there are different types of alternatives to animals:

- **Functional neuroimaging techniques** that allow to analyze the functions of certain brain areas, such as: **PET** (Positron Emission Tomography), **fMRI** (functional magnetic resonance imaging), **EEG** (electroencephalography), **SPECT** (single-photon emission computed tomography), **MEG** (magnetoencephalography), **NIRSI** (near-infrared spectroscopy), **DSI** (diffusion spectrum imaging), **DTI** (diffusion tensor imaging), **DWI** (diffusion weighted imaging) and **DfMRI** (diffusion functional MRI).
- **TMS** (Transcranial Magnetic Stimulation): it creates temporary and fully reversible brain lesions and can therefore replace the lesion studies in primates where the

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candidates, increases T-cell activation markers and T-cell proliferation. Transpl Immunol. 2008 May;19(2):112-9. doi: 10.1016/j.trim.2008.03.001. Epub 2008 Apr 3.

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brain region of interest is close to the surface. Considering that the TMS creates short durated and reversible lesions, it has the added advantages that the brain doesn't reshape itself to compensate for the injury, as it is the case in animal studies, and then the same individual can be studied repeatedly before and after the cerebral "lesion".

- **Brain-to-Brain Interface**: through the **integrated use of EEG and TMS**, researchers at the University of Washington put in direct communication two human brains, allowing a person to play a video game with the fingers of another person [22].
- **Single-neuron recording:** patients with intractable epilepsy or other severe disorders sometimes undergo elective surgery to remove the affected brain area. During this surgery the patient is conscious in order to guide the surgeon, and some voluntarily participate in studies involving the recording of direct field potentials from the brain. In this way, researchers have undertaken studies of visual processing for episodic memory using direct recordings from the hippocampus and they have discovered that neurons within the hippocampus are directly linked to visual memory performance [23]. Studies of Brain-Computer Interface (whose main goal is the neural control of artificial limbs, wheelchairs, etc.) used the **intracranial electrocorticography (ECoG or iEEG)** on the same type of patients [24].
- **Microstimulation electricity:** while the TMS can non-invasively inhibit or stimulate the cerebral cortex, for subcortical areas it is possible to use time-limited and reversible electrical microstimulations. Often it is demanded to volunteers who have to undergo neurosurgery such as, for example, patients suffering from Parkinson's disease[25].
- The **biocompatibility** of medical devices (pacemakers, implants, etc.) is the ability of these materials to be well tolerated by the host organism in which they must operate and to determine a proper response. It could be studied using **in vitro tests for cell compatibility (cytotoxicity) and blood compatibility (haemocompatibility)**, in order to avoid hazards for patients and unnecessary experiments on animals. The results obtained using this methodology are more reproducible and more predictive than those obtained from animal studies [26] [27].

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- To establish the effectiveness of medical devices for surgical exercises and the development of additional techniques in this field, anatomical models such as those developed by **SynDaver™** are available. These models are in possessing a level of complexity that allows them to be substituted for either a live animal, an animal cadaver, or a human cadaver in the testing of these devices and in the surgical context [28].
- The **"Tox-Test Dummy"** system combines different **3D organotypic in vitro models**, in silico systems of **physiologically based toxicokinetic (PBTK) modeling**, **cellular and biochemical assays** and **toxicogenomics** and it is able to replace animals in systemic toxicity (such as in the detection of repeated dose toxicity, in reproductive toxicity, in carcinogenicity and in toxicokinetics) [29].
- Regarding genetics, it is possible to perform gene knockout experiments using **3D in vitro models of stem cells** [30]. There are techniques such as the **iCRISPR** that allow the genome editing on human stem cells [31], it is possible to study genetic diseases (as ADA-SCID, Shwachman-Bodian-Diamond syndrome (SBDS), Gaucher disease (GD), Duchenne (DMD), Becker muscular dystrophy (BMD), Parkinson's disease (PD), Huntington's disease (HD), juvenile-onset, type 1 diabetes mellitus (JDM), Down syndrome (DS)/trisomy 21 and Lesch-Nyhan syndrome) on **induced pluripotent stem cells of patients** [32] and it's possible to do researches on gene expression [33], as well as on function, regulation and interactions of proteins [34] using **microarrays**.

With regard to the psychiatric and neurological (including neurodegenerative) diseases, we have the following alternatives:

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- **Disease-in-a-dish:** the skin cells of patients with these disorders are reprogrammed to an embryonic state to become neurons, which reproduce the disease and on which it is possible to study its molecular processes [35].
- **Brain on Chip:** the use of 3D cell cultures combined with microfluidics in the study of neurodegenerative diseases. It's also useful to test the neurotoxicity of drugs [36].
- **Cerebral Organoids:** mini-brains created from human stem cells, thanks to which it is possible to perform research on neurological diseases [37].

We can study the teratogenicity through **human pluripotent stem cells** [38], as well as on **organoids** [39], while neural teratogenicity tests can be performed on **human embryonic stem cells** [40].

For toxicity and developmental neurotoxicity it is possible to use - in addition to the methods already mentioned - **human stem cells** accompanied by the use of **transcriptomics** and **epigenetics** [41], human neural progenitor cells that grow as **neurospheres** [42] and the integration of **human embryonic stem cells** with **genomics**[43].

Useful to test the reproductive toxicity are the **in vitro methods** derived from the **ReProTect** project [44].

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With regard to pharmacokinetics and pharmacodynamics, there are many alternatives: the already mentioned **IdMOC**, able to evaluate the ADMET, metabolism and drug-drug interactions [45]; **small intestine and liver "on-chip" models** [46]; the above mentioned **Quasi-Vivo**; **human hepatocytes cultures** [47]; models for extrapolation of pharmacokinetic data in humans starting from in vitro data, such as **PopGen** [48]; ADME simulators such as **Simcyp** [49]; **mathematical models** to identify drug-drug interactions and pharmacodynamics [50]; **co-culture models of human intestinal and liver lines** (Caco-2 and HepaRG) [51]; **"Cells-on-a-chip"** [52]; **ADME "on-chip"** [53]; **three-dimensional co-cultures of primary liver cells** [54]; **microfluidic liver co-cultures** [55]; **Datachip** and **MetaChip** [56]; **metabolomics** and **bioartificial**

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organs [57]; **PBPK (Physiologically based pharmacokinetic) models** [58]; further **in vitro** [59] and **in silico** [60] methods.
It's demonstrated that through the **Microdosing** associated to the **Accelerator Mass Spectrometry (AMS)** it's possible to determine the human therapeutic dose of a drug in a better way than using animal models[61].
Finally, for cancer research, there are alternatives such as **3D co-cultures**, thanks to which it's also possible to recapitulate the progression of tumors [62]; **organs-on-chips**, that model the tumor and the metastasis[63]; and **microarrays** [64]. Instead, for the detection of carcinogenicity, there are **in silico models** [65], **structural alerts** and in **vitro cell transformation assays** [66].

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