



Re-Evaluation of Animal Numbers and Costs for *In Vivo* Tests to Accomplish REACH Legislation Requirements for Chemicals – a Report by the Transatlantic Think Tank for Toxicology (t4)*

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Abstract

The EU REACH legislation for chemicals of 2006 represents the largest investment into consumer product safety ever. A reanalysis of cost and animal use estimates was carried out based on the final legislation, test guidance for industry published by the European Chemical Agency, and the preregistration completed in December 2008. The new estimates for the number of substances falling under REACH range from 68 to 101,000 chemicals, substantially exceeding the earlier estimates of 29,000 substances. The latter estimates were, however, based on data before 1994 and both expansion of the EU and growth of the chemical industry since have contributed to higher numbers today.

The lower estimate of 68,000 chemicals was carried through current testing requirements with due regard to emerging alternative approaches, using in all cases the most optimistic assumptions (minimal animal numbers per test and neglecting most triggering of additional tests and confirmatory (re-)tests as well as tests requested but not yet defined for endocrine disruption, respiratory irritation, respiratory sensitization and developmental neurotoxicity). The most demanding studies are in the area of reproductive toxicity testing with about 90% of all animal use and 70% of the required costs for registration. The overall result suggests a demand of 54 million vertebrate animals and testing costs of 9.5 billion euro. This clearly challenges the feasibility of the program without a major investment into high-throughput methodologies.

Keywords: REACH, chemicals, animal use, economics, statistics, policy

1 Introduction

REACH (Registration, Evaluation, Authorization and restriction of CHEMicals), the new EU chemicals regulation, is the largest ever investment into the safety of chemicals and consumer products. Acknowledging the fact that we lack about 86% of the safety testing data for existing chemicals (White Paper, 2001, Roe et al. 1997), the EU legislation from 2006 aims to collect such data for all chemicals produced or marketed in quantities of more than one ton per year (REACH, 2006). The legislation lists the specific information requirements in its annexes for a given chemical depending on the overall production/marketing volume per year. During the political decision process, several attempts were made between 2001 and 2005 to estimate the costs of REACH, both financially (see for example Pedersen et al., 2003, RPA and Statistics Sweden, 2002) and with regard to animal numbers re-

quired (IEH Report, 2001, van der Jagt et al., 2004, Höfer et al., 2004). Since then, no such effort has been undertaken.

Several sources of information now allow the estimates to be reassessed:

- 1) The legislation has been finalized, changing some testing requirements (REACH, 2006).
- 2) The EU has been enlarged and now includes 27 instead of 12 countries (3 countries on 1st January 1995, 10 on 1st May 2004 and 2 more on 1st January 2007) compared to the data from before 1994 used in earlier studies.
- 3) Progress has been made with regard to the availability of alternative methods.
- 4) Testing guidance on information requirements has been developed for industry (ECHA, 2008a-c, Regulation 440/2008).
- 5) The preregistration of substances by industry ended in December 2008.

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6) Other high production volume chemicals safety programs have advanced, giving indications of already existing data.

Taking these information sources into account, a re-evaluation of estimates was carried out for *in vivo* test requirements.

2 Considerations from preregistration of substances

REACH requires the registration of individual chemical substances, those combined in preparations, and those intentionally released from articles. Registrations are submitted to the European Chemicals Agency (ECHA) and apply to substances manufactured or imported to the EU in annual quantities of one ton or more. However, substances related to medicinal products for human or veterinary use, food or feedstuff, and a few other specific cases – such as elemental substances (O₂, N₂, etc.) and substances occurring in nature if they are not chemically modified – are exempted from registration (Article 2, Annexes IV and V).

Enforcement of REACH began in June 2007, with the setting of deadlines for the registration of substances (Tab. 1). REACH also differentiates between phase-in and non phase-in substances. “Non phase-in” refers to all substances that have been notified according to Dangerous Substances Directive 67/548/EEC and to those newly introduced to the market after the implementation of REACH. It is often overlooked that REACH, with its new testing requirements, is also applied to all new chemicals to be notified. Phase-in substances are all substances that were on the EC market before 18 September 1981 (“existing chemicals”) and those previously designated as polymer and no longer considered as such. These three categories, excluding newly introduced chemicals, are classified in the ESIS (European Chemical Substances Information System) as ELINCS (European List of Notified Chemical Substances), EINECS (European Inventory of Existing Commercial Chemical Substances), and NLP (No Longer Polymer), respectively.

REACH has planned a registration deadline for substances according to the correspondent circulating volume (Tab. 1).

Abbreviations and glossary

CAAT – Center for Alternative to Animal Testing at the Johns Hopkins University, Baltimore, USA

CAS number – Chemical Abstracts Service registry numbers assigned by American Chemical Society as unique numerical identifiers for chemical elements, compounds, polymers, biological sequences, mixtures and alloys.

CEFIC – European Chemical Industry Council, trade association

CMR – Carcinogen / Mutagen / Reproductive toxicant

CRO – Contract Research Organisation

CSA – Chemicals Safety Assessment in REACH

CSR – Chemical Safety Report in REACH

DNT – Developmental Neurotoxicity

EEA – European Economic Area established 1994 between three member states of the European Free Trade Association (EFTA), and all member states of the European Union (EU); allows EFTA countries to participate in the European single market without joining the EU.

EFTA – European Free Trade Association

ECETOC – European Centre for Ecotoxicology and Toxicology of Chemicals

ECHA – European Chemicals Agency, Helsinki, Finland

EC number – European Commission number is a seven-digit code that is as-

signed to chemical substances that are commercially available within the European Union

EINECS – European Inventory of Existing Commercial Chemical Substances

ELINCS – European List of Notified Chemical Substances

EPA – US Environmental Protection Agency

ESIS – European Chemical Substances Information System

EU-12, EU-15, EU-25, EU-27 – the European Union with 12 member states (before 1995), 15 member states (before 2004), 25 member states (before 2007) and 27 member states (since 2007), respectively

HPV – High Production Volume chemicals

HPVIS – High Production Volume Information System, database of US EPA

IEH – Institute for Environment and Health, Cranfield University, UK

ITS – Integrated Testing Strategy

IUCLID – International Uniform Chemical Information Database

NLP – No Longer Polymer

Non phase-in substances – all substances that have been notified according to Dangerous Substances Directive 67/548/EEC and to those newly introduced to the market after the implementation of REACH

NTP – US National Toxicology Program

OECD TG – OECD (Organisation for Economic Co-operation and Development) Test Guideline

PBT – Persistent Bioaccumulative and Toxic chemicals

Phase-in substances – all substances that were on the EC market before 18 September 1981 (“existing chemicals”) and those previously designated as polymer and no longer considered as such (NLP)
(Q)SAR – Quantitative Structure Activity Relationship (computational toxicology, “*in silico*” method)

REACH – EU legislation from 2006 on Registration, Evaluation, Authorization and restriction of Chemicals

RPA – Risk & Policy Analysts Ltd., London, Norfolk, UK

SME – Small Medium Enterprise

t4 – Transatlantic Think Tank for Toxicology formed between CAAT, and the three toxicological chairs for alternatives to animal experiments endowed by the Doerenkamp-Zbinden-Foundation, Switzerland, at University of Konstanz, Germany, University of Utrecht, The Netherlands, and Johns Hopkins University, Baltimore, USA
TSCA – US Toxic Substances Control Act, legislation from 1981

UVCB – Substances of Unknown or Variable Composition, Complex Reaction products and Biological materials

The preregistration phase is now over and the preparation of a registration dossier should be ongoing for each preregistered substance.

On 18 December 2008 (ECHA press release, 2008) ECHA published the first list of preregistered substances in accordance with the preregistration date. Some 65,000 companies have made as many as 2,750,000 preregistrations, far exceeding the 180,000 preregistrations from 27,000 companies originally expected (ECHA Memo, 2008). By suggesting registration deadlines, companies indicate the likely trade and production volume of their chemicals. ECHA has further screened all these entries by combining compounds with the same EC number or CAS number and by deleting some articles. The latest published list (ECHA Press Release, 2009) now contains 143,835 substances. Among those, 54,686 have a 2010 deadline for registration, 59,599 a 2013 deadline, and 29,550 a 2018 deadline. These numbers are quite different from those foreseen in previous assessments for REACH implementation. In particular, most of the work done to prepare for REACH was based on a report written by Pedersen et al. (2003). Their estimation (Tab. 2) was based on data reported in the IUCLID (International Uniform Chemical Information Database), which contains information on substances manufactured in the EU in the years 1991-1994. Even if no official numbers are available it appears that a large abuse of preregistration occurred from contract labs, consultants, and other companies who intended to exploit

this tool to obtain business information. However, this element alone cannot explain the great number of preregistrations received.

It is noteworthy that the 2008 preregistrations are much closer to an earlier estimate made by RPA and Statistics (RPA and Statistics Sweden, 2002). RPA and Statistics had performed a survey of industry, competent authorities, and associations through a questionnaire about manufactured, imported, or used chemical substances. This questionnaire was able to record EINECS chemicals, plus nearly 30,000 new chemicals placed on the market annually as well as about 100,000 intermediates isolated annually in the EU. Intermediates can be classified in ESIS but without information about quantity. Most uncertainty in the RPA and Statistics report concerned the unknown amount of each chemical produced. About 23% of intermediates that are effectively placed on the market generate some degree of double counting. For these reasons, the RPA report assumes the possibility of four scenarios as depicted in Tab. 3. Numbers depicted in Scenarios 3 and 4 are now closer to the real number of chemicals that have been preregistered.

The previous underestimation of substances preregistered has several reasons:

- 1) The Pedersen et al. (2003) report is based on IUCLID, which contains information on substances manufactured in the EU in volumes ≥ 10 tons/year in the period 1991-1994.

Tab. 1: List of REACH deadlines

CMR = carcinogenic, mutagenic and reproductive toxicants

Date	Event
30 December 2006	Publication in the Official Journal (OJ)
1 June 2007	Entry into Force
1 June 2008	Starting of preregistration of phase-in substances
1 December 2008	End of preregistration phase
1 December 2010	Deadline for Registration of substances under the provision of Annex X: - ≥ 1000 t/y - ≥ 100 t/y (substances which may cause long-term adverse effects in the environment and classified as R50/53 according to Directive 67/548/EC) - ≥ 1 t/y (substances classified as CMRs Category 1 or 2 according to Directive 67/548/EC)
1 June 2013	Deadline for Registration of substances under the provision of Annex IX: - ≥ 100 t/y
1 June 2018	Deadline for Registration of substances under the provision of Annex VII and VIII: - ≥ 1 t/y

Tab. 2: Comparison of number of preregistered substances and estimation done by Pedersen et al. (2003)

Data from ECHA Press Release (2008) and ECHA Press Release (2009) as well as Pederson et al. 2003.

Registration date	Preregistered	From Pedersen et al.	
01 December 2010	54,686	2,704	≥ 1000 t/y
01 June 2013	59,599	2,461	≥ 100 t/y
01 June 2018	29,550	24,177	≥ 1 t/y
Total	143,835	29,342	



- 2) The report includes no substances which are present in IUCLID but classified as NLP (No Longer Polymer). Eventually, NLP also have to be preregistered. Within the scheme of the notification of new substances (Directive 67/548/EEC) polymers are subject to special rules. The term polymer was further defined in the 7th Amendment of the Directive 67/548/EEC (Directive 92/32/EEC, 1992). This change means that some substances which were considered to be polymers under the reporting rules when the European Inventory of Existing Commercial Chemical Substances (EINECS) was established are no longer considered to be polymers under the 7th Amendment. NLP substances require the same preregistration as EINECS substances. This adds about 700 substances to the preregistration, even though this number is still an underestimation since notification of NLPs was on a voluntary basis.
- 3) Both reports were made before the official publication of REACH based on draft versions of the regulation. For example, none of them include released substances from articles, even though released substances will reduce the possibility of exposure based waiving rather than increasing the overall number of circulating chemicals.
- 4) Both reports were based on information collected within the borders of the EU at a time when it was composed of only 12 Member States before 1995. At the moment of REACH implementation, the EU included 27 Member States and the

regulation is now also enforced in the EEA (European Economic Area), i.e. in three more countries. At the moment, REACH has been therefore implemented in 30 countries.

- 5) According to the European Chemical Industry Council (CEFIC, 2009), during the years 2002-2007, chemicals sales grew slightly more slowly than consumption (4.5% versus 5.2%). The average growth rate of imports over the last five years (7.0%) considerably exceeded that of exports (3.6%). A growth rate of about 5% annually translates to 97% growth from 1994 to preregistration in 2008, based on the IUCLID data.

2.1 Analyses of preregistered substances

The list provided by ECHA includes, for each preregistered substance, the EC number, the CAS number, the chemical name, and the earliest registration date. This allows a more detailed analysis so we can determine whether all of the listed substances will be fully registered and how many tests will be required to fill the Chemical Safety Report (CSR), as required in Annexes VII-X. The initial digit of the EC number gives indication about whether the chemical is in the EINECS, the ELINCS, or NLP lists. EINECS contains all substances that were reported to be on the market in Europe between 1 January 1971 and 18 September 1981. The total number of substances classified in EINECS is 100,204. All substances placed on the EU market after 18 September 1981 are classified as “new” chemicals and listed in the ELINCS database. The ELINCS database includes

Tab. 3: Total number of chemicals requiring registration according to REACH estimated by RPA & Statistics, 2002

Foreseen Total number of registrations		
33,865	Scenario 1	Low number of chemicals and no intermediates
76,365	Scenario 2	Low number of chemicals and low number of intermediates
93,365	Scenario 3	Mid range number of chemicals and mid range number of intermediates
118,865	Scenario 4	High number of chemicals and high number of intermediates

Tab. 4: List of preregistered substances according to their EC classification

Data from ECHA Press Release (2008) and ECHA Press Release (2009).

Registration date		Number of preregistered substances
01/12/2010	EINECS	47,166
	ELINCS	1,730
	NLP	692
	Other	5,098
01/06/2013	EINECS	53,038
	ELINCS	2,097
	NLP	10
	Other	4,454
01/06/2018	EINECS	-
	ELINCS	-
	NLP	-
	Other	29,550



4,381 substances. ELINCS substances have been already notified under Directive 67/548/EEC and the dossier can be accepted as registration for REACH without requiring further in vivo testing.

From the EC number, it is possible to identify to which category a substance belongs, since all EINECS chemicals start with “2,” all ELINCS chemicals start with “4,” and all NLP chemicals start with “5.” Following these assumptions, the pre-registered substances are divided and sorted as listed in Tab. 4. Tab. 4 gives the numbers for all substances previously classified in the ESIS database that have been preregistered, plus some others that have no EC number and thus were not considered in the previous evaluations.

Considering EINECS and NLP as Phase-in substances, ELINCS as non phase-in substances, and unclassified substances as “others,” the preregistered substances can be further grouped (Tab. 5).

Most ELINCS substances have been preregistered (3,827 non-phase in substances vs. 4381 substances in ELINCS). The high number of preregistrations in this class can be easily explained as a precautionary measure adopted mainly by downstream users handling those chemicals. Non-phase-in substances will need extra assessment only in the case of a new use resulting in a new exposure scenario requiring evaluation. Reasonably, this is the case only for a limited number of substances. However, if production has increased since original notification within the last 27 years, additional information requirements might also emerge. This might hold especially true for new chemicals that are now suggested for the 2010 deadline, which means the notifier is expected to have larger production volumes or specific concerns, triggering testing demands typically not met for new chemicals.

Phase-in substances are exactly the same as the number of substances present in the EINECS and the NLP, with only one

NLP missing. It’s surprising that none of them is in the lower tonnage level that has a 2018 deadline.

“Other” substances are those that had no EC number during preregistration and now have been given a new list number in the EC format. In order to make registration easier, ECHA assigned new EC numbers starting with “6” to substances without previous EC number but with a CAS number (6-EC class), and starting with “9” to substances that previously had neither an EC number nor a CAS number (9-EC class). The numbers of these substances are listed in Tab. 6.

The presence of 22,015 substances with no previous classification (neither EC number nor CAS number) is astonishing, even more so because 4,333 of those seem to be on the EU market at a tonnage level higher than 1000 tons/year, which means a 2010 registration deadline. A representative sample of such compounds has been analysed below.

2.2 Qualitative assessment

Following the assessment of the number of substances that have been preregistered in REACH, some more qualitative considerations are required about what exactly is present in this list.

As stated in REACH definitions (Article 3), a substance is “a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used”. According to this definition, the same chemical entity can be included in multiple registrations, possibly explaining the high number of preregistered substances.

Certainly, various individuals and companies abused the preregistration tool put into effect, since preregistration gives the right to participate into the forum responsible for the registration of that substance. These include Contract Research Organisations (CROs) that want to propose new tests for the substance, consultants who want to be involved in the registration process,

Tab. 5: List of preregistered substances to registration dates

Data from ECHA Press Release (2008) and ECHA Press Release (2009). This list is not including late preregistered substances.

Registration date	Non phase in substances	Phase in substances	Unclassified substances	Total
30/11/2010	1,730	47,858	5,098	54,686
31/05/2013	2,097	53,048	4,454	59,599
31/05/2018	---	---	29,550	29,550
Total	3,827	100,906	39,102	143,835

Tab. 6: Preregistered Substances not previously classified in ESIS by registration date

Data from ECHA Press Release (2008) and ECHA Press Release (2009).

Registration deadline	With CAS number	Without CAS number	Total without regular EC number
01/12/2010	765	4,333	5,098
01/06/2013	1,394	3,060	4,454
01/06/2018	14,928	14,622	29,550
Total	17,087	22,015	39,102



competitors who want to acquire information about the substance, and many others.

However, the main impact on the number of preregistrations is attributable to mistakes made by registrants. In fact, there is a high level of fear that REACH can close the commercial activity of companies that are not compliant with the new regulation. In the EU most companies of chemical industry are small and medium-sized (SME); most of them only deal with preparations and articles and lack specific chemical toxicology competence. At the moment there are no official data, but it is well known that many downstream users have registered important substances they are using, even if purchased in Europe. Another indication of misunderstanding is the presence of all substances that are already classified in the ELINCS database when it was clearly stated that non phase-in substances (i.e. substances already present in the ELINCS database) did not require preregistration.

On its website ECHA also considers possible reasons for mistakes in 14,000 entries, including difficulties with English (the only language admitted for preregistration) and the classification of mixtures (ECHA Press release, 2009). ECHA has already skimmed some of the initial registrations (ECHA Press Release, 2008). Nevertheless, ECHA has no authority to automatically delete a registration without properly communicating with the registrant.

In order to better understand the false entries, some of them have been randomly selected from the 39,102 substances that have no EC number. Reasoning about each single entry is difficult. Only legal entities that have preregistered have full access to the ECHA database, which may contain further explanations. Some randomly selected illustrative examples include:

1) No previous EC classification, but with CAS number (6-EC class):

a. EC 606-203-6, CAS # 1902-01-8 *Butanoic acid, 2,4-dihydroxy-3,3-dimethyl-, monosodium salt*: The acid form of this chemical is Pantoic acid which is a component of Coenzyme A (Prebiotic syntheses of pantoic acid and the other components of coenzyme A). Pantoic acid itself has no preregistration record. Even if there is no evidence, presumably it is a pharmaceutical intermediate that needs preregistration if manufactured in quantity ≥ 1000 t/year.

b. EC 616-851-1, CAS # 8005-02-5 C.I. *Solvent Black 7*: This is a food dye and it is out of the scope of REACH (article 2). There are about other 20 substances recorded as “Solvent Black,” all of them missing the EC number.

c. EC 618-464-3, CAS # 9011-05-6 *Urea, polymer with formaldehyde*: Common name is Polynoxylin and it is used as a topical antiseptic. As medicinal product is out of the scope of REACH (article 2).

2) Neither CAS nor EC number (9-EC class):

a. EC 921-055-5, *Pig iron*: Pig iron is the intermediate product of smelting iron ore with coke, producing a purer form of iron. This is already present with another name and proper CAS and EINECS numbers (CAS 65996-67-0, EINECS 265-998-4, Iron, furnace)

b. EC 923-825-6, *Pépins de raisin*: It is the French name for grapeseed oil, which is the oil extracted from grapeseeds. There are many other entries about the processing of grape, some of them differing only in the spelling, such as Pulpe de raisin (EC 924-336-0) *Pépin de raisin* (EC 920-787-2), *Pépin de raisin* (EC 920-890-2) and some others. According to Annex V of REACH all natural extracts and products are exempted from registration.

c. EC 924-108-0, *Reaction product of cement clinker, calcium sulphate, water and either ashes (residues or slags, ferrous metal, blast furnace)*: Understanding the meaning of this entry is impossible. Presumably it deals with a recovering process. This section of REACH is still vague and a new guideline about waste and recovered substances is expected shortly. There are about 20 entries with very similar substance names, differing only in typos of the substance name, indicating that they all refer to the same product.

d. EC 917-706-8 *Eisen*: Totally inadequate substance definition.

Among the substances within the EN 9-class there are 13,692 entries with the substance name starting with “reaction mass.” On the other hand, the query in the list of the field “reaction product” returns 4,468 entries. These entities are considered as *Substances of Unknown or Variable Composition, Complex Reaction products* and *Biological materials* (UVCB). UVCBs require that components as high as 10% are identified and characterized. Moreover, the source of the material should be very well described. It can be that this data is already present in the full record, which is accessible only to ECHA and the registrants.

2.3 Further considerations on number of preregistrations

As listed in Tab. 5, the 100,906 substances from EINECS plus 39,102 non-classified substances results in a total of 140,008 substances that may require extensive testing for registration. This number is substantially larger than the previously foreseen number of 29,342 and understanding the reason for this enormous discrepancy is of utmost importance.

REACH originated from the white paper “Strategies for a future Chemicals Policy,” presented in 2001 (White paper, 2001). This first document mentioned only chemicals in the EINECS and ELINCS lists. Today, REACH includes isolated intermediates, chemicals in any preparations, as well as chemicals released from articles. Previous assessments foreseeing the impact of REACH on the EU economy were based on the original White Paper and on other draft versions that were in general very similar. The final regulation was published on 30th December 2006.

This is probably why earlier estimates foresaw only 29,342 substances for registration (Tab. 2). All NLP must be immediately added. There are only 703, increasing the estimate only slightly, to approximately 30,000 substances.

In addition to the evaluation of 30,000 substances, isolated intermediates can be very important, even though the exact type



and amount are recorded nowhere. RPA and statistics (2002), in its survey, counted as many as 85,000 possible intermediates that need to be registered. The addition of this contribution adds up to approximately 115,000 substances, still far from the final number of preregistered substances. More inputs should be found elsewhere. Even if preregistration of isolated intermediates is mandatory, when they are not marketed they are exempted from the preparation of a dossier and, where strictly controlled conditions of use apply, they have very reduced data needs. The number of intermediate chemicals in REACH is difficult to estimate but, especially at higher tonnages, this will apply to many basic chemicals used to synthesize more specialized products.

Phase-in substances are considered by REACH as the chemical substances circulating in the EC before 1981 and registered in IUCLID. That is why previous estimations consider the substances that were present in IUCLID in the period 1991-1994. Even though this estimate is correct from a qualitative point of view, since EINECS contains today the same number of chemicals, from a quantitative point of view it completely underestimates the quantity of each chemical currently circulating in the EU. In theory, the picture recorded by IUCLID in years 1991-1994 should be consistent with today, since new chemicals should all have been recorded in ELINCS. However, it does not consider the discrepancy between the increasing rate of chemical industry growth and the EU enlargements that occurred in 1995 with the accession of Austria, Sweden, and Finland to the EU, the 2004 accession of Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Slovakia, Slovenia, Malta, and Cyprus, and the latest enlargement in 2007, which included the accession of Bulgaria and Romania. Moreover, REACH is now also enforced in the countries of the European Economic Area (EEA), which incorporates Norway, Liechtenstein, and Iceland.

The precise quantification of the contribution from these countries is very difficult as source information is hardly accessible. However, it is well known (Angerer et al., 2007, CEFIC, 2009) that the chemical industry in some of the new member states plays an important role in the economy. Commercial activities in most of these countries were mainly with Russia and Eastern countries, rather than EU-15; i.e. they contributed little to the EU-12 chemical commercial business in 1991. Moreover, most of the production was focused on "basic chemicals" rather than "specialty chemicals." The difference between the two is in both volume and function, as specialty chemicals are manufactured in lower volumes and used for specific purposes. It is reasonable to assume that basic chemicals are all present in EINECS, leading to a much higher volume of those chemicals circulating in the EU today.

Last, but not least, the average annual growth rate of the chemical industry was much higher in most of the new member states (e.g. Poland, Czech Republic, Estonia) than in the old member states (Angerer et al., 2007, CEFIC, 2009). According to CEFIC (2009) in 2007, EU-15 sales in the chemical sector represent 501 billion euro versus 94.5 billion euro in the rest of Europe. Considering that in 1993 Austria, Finland, and Swe-

den were not yet counted and that some of the countries were not part of REACH, the 18% increase of the chemical business seems to be realistic.

2.4 Conclusion on number of expected full registrations

Current information is inadequate to provide a precise account of the number of substances that will require further *in vivo* testing for preparation of the registration dossier. However, an approximation can be attempted.

All substances that have ELINCS number have been notified and, in general, they already have what is needed to fulfill REACH requirements. That is why they are not counted for this study, even though some may require more testing. Substances belonging to the "6" and "9" EC categories are also excluded, because our samples showed that most of them are either out of the scope of REACH or already preregistered with another name and proper EN number. Of course, the elimination of both ELINCS and 6/9 EC classes from the category of chemicals requiring further *in vivo* testing is not fully correct. However, these omissions should be counterbalanced by the number of substances considered in this study that will either quit registration or will be registered in a lower tonnage level. The omission of all substances not previously classified in ESIS surely leads to a large underestimation of the total number of registrations that will be required.

All preregistered phase-in substances, i.e. substances classified in EINECS and NLP database, are included in the estimate, assuming that companies know their trade and production volumes. This total of 100,906 substances will be split as:

- 47,858 present in the EU in quantity higher than 1000 tons/per year and under the provision of ANNEX X of REACH
- 53,048 present in the EU in quantity higher than 100 tons/per year and under the provision of ANNEX IX of REACH

This can be considered a quite realistic scenario for the number of chemicals that need full registration with CSA/CSR.

Another approach is to start from the hypothetical number of chemicals in the Pedersen et al. (2003) estimation, which is based on a realistic picture of the situation in the EU for the years 1991-1994. These numbers are increased by a factor derived from the growth of the chemical industry as depicted by CEFIC (2009), i.e. by a factor of 1.97 due to the general increase of chemical industry plus a factor of 1.18 due to the enlargement of the EU (see above). This approach still represents an underestimation as isolated intermediates and released substances from articles are not taken into consideration. UVCBs are substantially excluded in all these calculations. Presumably, most of them are mixtures of other known chemicals and a maximum exploitation of read-across and grouping opportunity is expected.

All these assumptions are summarized in Tab. 7. Presumably, the real situation will lie between scenarios 2 and 3 of this table, with a total number of chemical to be registered within 68-100,000.



3 Number of *in vivo* toxicity tests

The types of *in vivo* toxicology tests required for filling the CSR of a substance are described in different REACH Annexes according to Tab. 8, i.e. total production or marketing volume in Europe determines the testing requirements which are then modified by specific toxicity and use profiles of the substances. REACH immediately asks for the results of tests described in Annexes VII and VIII, while the tests under the provisions of Annexes IX and X are proposed by the submitter and performed only after receiving the authorization by ECHA.

Annex VII requires only skin sensitization and acute toxicity as *in vivo* tests and no CSR. Acute toxicity study by oral route is implemented in Annex VIII by another route, either dermal or inhalation. Moreover, the short term repeated dose toxicity study can trigger further studies if there are indications of toxicity of particular concern. The screening developmental toxicity

study is waived when a full study is required. A developmental toxicity study is performed on two species and an expert decision is based on the outcome of the first test and all other relevant available data. *In vivo* skin irritation is still written in the Annex although it is no longer obligatory as an *in vitro* alternative is now fully accepted.

Actually, REACH requires many more tests, such as those for the characterization of physicochemical properties, *in vitro* tests, and *in vivo* tests on plants and invertebrates. All these tests remain out of the scope of the present study. REACH also refers to hazards such as respiratory sensitization, endocrine disruption, and neurodevelopmental toxicology, but these are not reflected in the Annexes and therefore are also not considered.

If all preregistered substances required all *in vivo* testing, the final costs and the number of sacrificed animals would be enormous. The real number of tests will be the result of several factors, including:

Tab. 7: Foreseen number of expected full registration dossiers

Different scenarios by marketed quantities (i.e. basic testing demands), with (1) considering all preregistrations; (2) excluding likely mistakes as explained in the text; (4) estimates by Pederson et al. (2003) and (3) correcting scenario 4 with 97% growth of chemical industry in 14 years and 18% by expansion of the EU.

Scenario	Chemical marketed in quantity:				Total
	≥ 1 t/y	≥ 10 t/y	≥ 100 t/y	≥ 1000 t/y	
1 Preregistered substances	29,550		59,599	54,686	143,835
2 Considering only Phase-in substances			53,048	47,858	100,906
3 Considering market increase 1994 to 2008	44,632	11,570	5,721	6,286	68,208
4 Pedersen et al. (2003) Estimation	19,200	4977	2,461	2,704	29,342

Tab. 8: *In vivo* tests required in Annexes VIII through X of the REACH legislation

REACH §	Information	ANNEX VII ≥1t/y Deadline 2018	ANNEX VIII ≥10t/y Deadline 2018	ANNEX IX ≥ 100 t/y Deadline 2013	ANNEX X ≥ 1000 t/y Deadline 2010
8.2.1	<i>In vivo</i> eye irritation		X	X	X
8.3	Skin sensitisation	X	X	X	X
8.4	Further mutagenicity			X	X
8.5.1	Acute oral tox	X	X	X	X
8.5.2	Acute inhalation tox		X	X	X
8.5.3	Acute dermal tox				
8.6.1	Short-term repeated dose		X	X	X
8.6.2	Sub-chronic tox			X	X
8.6.3	Long-term repeated tox				X
8.7.1	Developm. tox screening		X	X	
8.7.2	Developm. Tox study			X	X
8.7.3	Two-generation reprotox			X	X
8.9.1	Carcinogenicity				X
9.1.3	Short-term fish		X		
9.1.6	Long-term fish tox			X	X
9.3.2	BioAccumulation (fish)			X	X
9.6.1	Long-term or reproductive toxicity to bird				X

- Existing, available information: Some of the substances have already been tested and/or covered by other legislation that provides for an equivalent level of protection (e.g. Biocidal Products Directive, Medicinal Products Directive, US High Production Volume chemical program). In REACH there is the obligation to make use of any available information and share any available result if obtained from an *in vivo* test.
- Waiving: Sometimes the applicability of a test depends on the physicochemical properties of the substance. Some other tests are triggered by the response of another test.
- Exposure based waiving: Some of the testing for the higher annexes may be omitted based on a comprehensive exposure assessment developed in the Chemical Safety Report. Provisions regarding this opportunity have been recently updated (Regulation 134, 2009).
- (Q)SAR (Quantitative Structure Activity Relationship), grouping, read across: A theoretical model can predict the property of a substance (QSAR, *in silico* approach, see also article by Hartung and Hoffmann in this issue of ALTEX), or a group of substances belonging to the same chemical class can be grouped and only one of them is tested (grouping), or a property of a substance can be derived by the existing information for a similar substance (read across).
- *In vitro* methods and other alternative approaches are accepted when suitable, as defined in Annex XI of REACH.

The strategy for reducing the number of tests performed for each substance is well accepted by REACH. Annex XI outlines

the general rules for adaptation of the standard testing regime as set out in Annexes VII to X.

For each of these factors, Pedersen et al. (2003) have tried to estimate the weight in the final count. In the present study, the same approach is applied to the number of preregistered substances that will probably undergo a full registration process, i.e. 47,858 substances under Annex X and 53,048 substances under Annex IX provisions. However, new testing strategies derived from the guidance to industry for endpoint specific information requirements and published by ECHA (2008a-c), offer a re-analysis of the available data and the state of the art of *in silico* and *in vitro* alternatives, including novel reduction and refinement alternatives. Precise quantification of these factors is not possible at the present level of implementation of REACH because of the lack of knowledge concerning the acceptance of alternative approaches.

3.1 Existing and promised data

Pedersen et al. (2003) have considered both the availability of data as recorded in the HPV (High Production Volume) database and the data collected by RPA and Statistics through a survey conducted on industries and other organizations. RPA and Statistics (also found in Tab. 2 in Pedersen et al., 2003) grouped tests in Base set, Level 1, and Level 2 data, as described in Directive 67/548. This classification now approximately corresponds in REACH to Annex VII and VIII, Annex IX and Annex X, respectively.

Tab. 9 reports the percentage values of available data. The percentage values include both existing data and other informa-

Tab. 9: Existing data for each endpoint as foreseen by Pedersen et al. (2003), based on data from RPA & Statistics (2001)

Noteworthy, long-term bird studies were not included in the previous reports.

	Information	REACH Annex		Annex VIII	Annex IX	Annex X
8.1.1	<i>In vivo</i> skin irritation	VIII	Base set	17%	22%	22%
8.2.1	<i>In vivo</i> eye irritation	VIII	Base set	17%	22%	22%
8.3	Skin sensitisation	VII	Base set	17%	22%	22%
8.4	Further mutagenicity	IX	Level 1		7%	7%
8.5	Acute oral tox	VIII	Base set	17%	22%	22%
8.5.2	Acute inhalation tox	VIII	Base set	17%	22%	22%
8.5.3	Acute dermal tox	VIII	Base set	17%	22%	22%
8.6.1	Short-term repeated dose	VIII	Base set	17%	22%	22%
8.6.2	Sub-chronic tox	IX	Level 1		7%	7%
8.6.3	Long-term repeated tox	X	Level 2			5%
8.7.1	Developm. tox screening	VIII	Base set	17%	22%	22%
8.7.2	Developm. Tox study	IX	Level 1		7%	7%
8.7.3	Two-generation reprotox	IX	Level 1		7%	7%
8.9.1	Carcinogenicity	X	Level 2			5%
9.1.3	Short-term fish	VIII	Base set	17%	22%	22%
9.1.6	Long-term fish tox	IX	Level 1		7%	7%
9.3.2	BioAccumulation (fish)	IX	Level 1		7%	7%
9.6.1	Long-term bird	X	Level 2			No data



tion available to Industry for sharing (the so-called “promised data”). Pedersen et al. (2003) also consider the contribution of voluntary initiative on providing data, based on the information available in the US on the HPV Challenge Program, which covers base set tests for higher production volume substances. Regarding the low volume substances, there is an investigation performed on substances that are manufactured in Germany which are classified in IUCLID. Since the values shown in Tab. 10 are the results of a general survey, it is assumed that they also include data that are already present in IUCLID and in the US HPV Program.

The US HPV Challenge Program “challenges” companies to make publicly available data on chemicals produced or imported into the United States in quantities of one million pounds or more per year (about 500 tons). Companies have sponsored more than 2,200 HPV chemicals, with approximately 1,400 chemicals sponsored directly through the HPV Challenge Program and over 860 chemicals sponsored indirectly through international efforts. Only directly sponsored HPV Challenge Program chemicals make it to HPVIS (High Production Volume Information System). Currently, the HPVIS database contains over 340 submissions, representing almost 900 chemical substances (EPA, 2009a). Earlier (Bremer et al., 2007) we evaluated the most critical part with regard to animal numbers and costs, i.e. reproductive toxicity, and available test results. The HPV database contains eight one-generation and 10 two-generation studies as well as 43 developmental screening studies (for comparison, the EU New Chemicals Database includes 55 one- and 14 two-generation studies and no screening studies for 4,400 registered new chemicals). These figures for available data are ranging from 0.3-2%—substantially lower than assumed 7-22%. At the same time, it is highly unlikely that many further reproductive toxicity studies will be available for chemicals by individual companies and not used for registration purposes.

Another approach made by Allanou et al. (1999) counted the number of entries in the EU HPV list extracted from IUCLID management software. From 2,465 substances, Allanou et al. checked whether: i) the field of the toxicological endpoint was filled in the correspondent entry and ii) whether this string was a number. However, also assuming that all these 2,465 substances have been preregistered in the category of substances with a deadline of 2010, the percentage that came out from this query is less than 1% and it would have a negligible impact to the final results (Tab. 9).

3.2 Waiving

In Annexes VII through X, REACH regulation gives some standard rules for each endpoint about how to perform the test in the event that no data are available. REACH also provides rules indicating when a test may be waived: if there is no concern regarding exposure in a scientific demonstration, if performing the test is technically impossible, or when the result of a test is clearly derived from the information obtained for another endpoint. For example, a full developmental toxicity study (REACH § 8.7.2) shall only be performed for a substance under Annex IX provisions when there is a positive response from a developmental toxicity screening (REACH § 8.7.1). In

contrast, substances under Annex X provisions always require a full developmental study and the screening test is waived.

The possibility of a waiver is inherent to the physicochemical characteristic of the substance and therefore is independent of the tonnage that needs to be registered. The values proposed by Pedersen et al. (2003) are summarized in Tab. 10. Unfortunately, at the moment there are no data to confirm these numbers. There are other minor cases providing possibility for waiving. For example, a metabolite is covered by the testing of the starting substances. Since these opportunities have a minor impact on the final numbers, they are not taken into account in the final calculation. Moreover it should be noted that screening studies concerning safety factors for higher tonnage bands are not allowed, providing a substantial increase in testing demand for substances cover by Annexes IX and X provisions.

3.3 Final number of tests required for registration: current guidance and accepted methods

Tab. 11 is the result of the combination of both tables 9 and 10 applied to 53,048 and 47,858 substances to be registered according to the provisions in Annex IX and X respectively. The percentage of existing data is relative to the number of required tests, i.e. on the total number of substances subtracted by the percentage that can be waived. To estimate the total number of test animals needed for each endpoint, van der Jagt et al. (2004) have compiled information provided by some referenced organizations with extensive experience in this field. The final figure is the result of the application of official guidelines, as described in the Council Regulation 440/2008, which essentially implements the correspondent OECD guidelines as properly listed by

Tab. 10: Waiving possibility according to Pedersen et al. (2003)

	Information	Waiving
8.1.1	<i>In vivo</i> skin irritation	
8.2.1	<i>In vivo</i> eye irritation	
8.3	Skin sensitisation	
8.4	Further mutagenicity	70%
8.5	Acute oral tox	10%
8.5.2	Acute inhalation tox	50%
8.5.3	Acute dermal tox	60%
8.6.1	Short-term repeated dose	25%
8.6.2	Sub-chronic tox	75%
8.6.3	Long-term repeated tox	90%
8.7.1	Developm. tox screening	10%
8.7.2	Developm. Tox study	85%
8.7.3	Two-generation reprotox	85%
8.9.1	Carcinogenicity	90%
9.1.3	Short-term fish	
9.1.6	Long-term fish tox	
9.3.2	BioAccumulation (fish)	60%
9.6.1	Long-term bird	99%



Höfer et al. (2004). For the present study, the average number of animals per test as evaluated by van der Jagt has been considered for all endpoints except: REACH § 8.7.1. developmental toxicity screening, REACH § 8.7.2 developmental toxicity study (two species), and REACH § 8.7.3 two generation reprotoxicity. In fact, van der Jagt et al. (2004) did not take into account offspring, which are also test animals included in EU animal use statistics. For these endpoints, the number of animals per test as proposed by Höfer et al. (2004) was considered.

Tab. 12 lists the final result of this approach, showing that implementing REACH according to current guidance would require 141 million vertebrate animals! This value is clearly unacceptable, for both ethical and financial reasons (Bottini and Hartung, 2009). Feasibility should be taken into account as well, since Europe lacks the resources to run so many tests in such a short period of time (IEH Report, 2001). However, the introduction of Annexes VII through X clearly states that: “*Before new tests are carried out to determine the properties listed in this Annex, all available in vitro data, in vivo data, historical human data, data from valid (Q)SARs and data from structurally related substances (read-across approach) shall be assessed first.*” In this sense, REACH is one of the first legislative acts introducing a spirit of change in the field of toxicology, even though their full acceptance will require some time (Hartung, 2009).

4 Expected impact from alternative approaches: emerging validated/not accepted and methods under validation

In 2004, van der Jagt et al. published a report about how alternative approaches can reduce the use of test animals under REACH. The first step is focused on (Q)SAR (Quantitative Structure Activity Relationship) and read across. The basis of (Q)SAR approach is the hypothesis that a chosen property of a substance can be described in relation to the similarity of its chemical structure, as a whole or partially, with other chemicals for which the same property is known (Benfenati, 2007). The capability of (Q)SAR is evolving rapidly, aided by expanded computational technology and increased availability of databases providing more and more information on chemicals. Even though the capability of (Q)SAR modeling is clear, it is true that (Q)SAR tool can rarely be used as a standalone technique for the evaluation of complex toxicological endpoints. As described in the ECHA (2008a-c) Technical Guidance Documents, (Q)SAR is well placed in the ITS (Integrated Testing Strategy) for the assessment of substance toxicological properties, and its main role is to point out specific properties and to optimize the number of tests and animals used for each endpoint. According to van der Jagt et al. (2004) the extent to which (Q)SAR and read across have been used in the US HPV Challenge Program

Tab. 11: Total number of tests required for each endpoint based on actual preregistration considering both estimated existing data and possible waiving

This calculation of number of OECD guideline tests on individual chemicals is based on scenario 2 (table 7), i.e. the actually preregistered substances corrected for likely mistakes. It considers both existing data (tables 9) and possible waiving (table 10) but no alternative approaches.

	Information	Annex	53,048 substances ≥ 100 tonne/year Deadline 2013	47,858 substances ≥ 1000 tonne/year Deadline 2010	Total
8.2.1	<i>In vivo</i> eye irritation	VIII	41,377	37,329	78,707
8.3	Skin sensitisation	VII	41,377	37,329	78,707
8.4	Further mutagenicity	IX	14,800	13,352	28,153
8.5	Acute oral tox	VIII	37,240	33,596	70,836
8.5.2	Acute inhalation tox	VIII	20,689	18,665	39,353
8.5.3	Acute dermal tox	VIII	16,551	14,932	31,483
8.6.1	Short-term repeated dose	VIII	31,033	27,997	59,030
8.6.2	Sub-chronic tox	IX	12,334	11,127	23,461
8.6.3	Long-term repeated tox	X	0	4,547	4,547
8.7.1	Developm. tox screening	VIII	37,240	33,596	70,836
8.7.2	Developm. Tox study	IX	7,400	6,676	14,076
8.7.3	Two-generation reprotox	IX	7,400	6,676	14,076
8.9.1	Carcinogenicity	X	0	4,547	4,547
9.1.3	Short-term fish	VIII	41,377	37,329	78,707
9.1.6	Long-term fish tox	IX	49,335	44,508	93,843
9.3.2	BioAccumulation (fish)	IX	19,734	17,803	37,537
9.6.1	Long-term bird	X	0	479	479
	Total tests		377,887	350,488	728,378



is up to 44% for human health endpoints and 35% for environmental effects. Pedersen et al. (2003) consider the percentage of acceptability of (Q)SAR results for each endpoint. Tab. 13 lists the results obtained by applying RPA and Statistics approach to the extrapolated total number of tests. On average, the “good” (Q)SAR approach implies a reduction of 4.6%, the “fair” approach a reduction of 1.9%, and the “poor” approach a reduction of 0.1%. If this is true, the (Q)SAR benefit is practically negligible. The main hindrance in the applicability of (Q)SAR models is the limited applicability domain, which is generally well explained and measured for each model and based on the similarity of the structure of interest with the substances that have been used to build up the model.

Moreover, it should be further highlighted that (Q)SAR calculations are based on specific organic chemicals with a well defined structure. For this reason, any inorganic compounds, organometallic compounds, mixtures, and UVCBs are excluded by default. Barrat et al. (2007) tested 400 chemicals randomly selected from ESIS HPV and LPV. The output was that (Q)SAR could be taken into consideration for only half of them, just by excluding inorganic and ionic compounds, complex mixtures, and those chemicals with no unique chemical structure.

Notifiers can apply read across to categories of chemical structures, especially in the high tonnage area where there are a lot of refinery/production streams of broadly similar material.

The application of *in vitro* methods, which are also strongly supported by the REACH regulation, should help lower the number of animals required. Unfortunately, the development of such approaches for the complex and animal demanding endpoints is not even close to either validation or to regulatory acceptance.

In the following paragraphs all endpoints requiring *in vivo* testing are considered. In each case, some guesswork is used in an exercise to simulate the application of the different OECD TGs and to calculate the number of animals required in each step. Most of the assumptions are derived from personal experience. These assumptions were checked for plausibility by six REACH experts from industry and a regulatory authority. Even though there are no statistics supporting this conjecture, it may give a picture of the scenario required for REACH compliance.

The average number of animals per tested substance is derived according to the corresponding guideline. Lower numbers can result by testing several chemicals with one control; higher numbers might be necessary if the concentration response is not conclusive. Moreover, it should be considered that some of the most complex tests, such as 90 day repeated dose toxicity, carcinogenicity, etc., can be performed at the limit dose only instead of the three dose levels when there is no sign of toxicity for the chemical. Limit dose testing, where foreseen in the test guideline, can save up to 50% of the animals required for testing.

Tab. 12: Total number of animals required for each endpoint based on actual preregistration considering existing information and waiving but no alternative methods

This calculation of number of animals for OECD guideline tests on individual chemicals (table 12) is based on scenario 2 (table 7), i.e. the actually preregistered substances corrected for likely mistakes. It considers both existing data (tables 9) and possible waiving (table 10) but no alternative approaches. Animal numbers are from Höfer et al. 2004.

	Information	Annex	Total number of required tests	Animals per test on average	Total animals used	%
8.2.1	<i>In vivo</i> eye irritation	VIII	78,707	2	157,413	0.11%
8.3	Skin sensitisation	VII	78,707	23	1,810,254	1.28%
8.4	Further mutagenicity	IX	28,153	50	1,407,639	1.00%
8.5	Acute oral tox	VIII	70,836	8	566,688	0.40%
8.5.2	Acute inhalation tox	VIII	39,353	20	787,067	0.56%
8.5.3	Acute dermal tox	VIII	31,483	10	314,827	0.22%
8.6.1	Short-term repeated dose	VIII	59,030	50	2,951,501	2.09%
8.6.2	Sub-chronic tox	IX	23,461	32	750,741	0.53%
8.6.3	Long-term repeated tox	X	4,547	160	727,442	0.52%
8.7.1	Developm. tox screening	VIII	70,836	560	39,668,167	28.13%
8.7.2	Developm. Tox study	IX	14,076	150	2,111,458	1.50%
8.7.3	Two-generation reprotox	IX	14,076	3200	45,044,438	31.95%
8.9.1	Carcinogenicity	X	4,547	400	1,818,604	1.29%
9.1.3	Short-term fish	VIII	78,707	14	1,101,894	0.78%
9.1.6	Long-term fish tox	IX	93,843	400	37,537,032	26.62%
9.3.2	BioAccumulation (fish)	IX	37,537	108	4,053,999	2.88%
9.6.1	Long-term bird	X	479	70	33,501	0.02%
	Total				141,000,076	100.00%

Tab. 7 lists four different scenarios for the number of substances that will need some *in vivo* testing for registration. Scenario 4 represents roughly the Pedersen et al. (2003) predictions made about five years before the end of the preregistration phase and they are not further considered. Scenario 1 considers a full registration of all preregistered chemicals. This is by far the most pessimistic scenario and it hardly represents the real situation. Scenario 2 is probably quite near to the real situation in terms of total number of registered chemicals. However, the high number of chemicals preregistered at the upper tonnage level seems quite far from the present situation. Predictions in Scenario 3 of table 7 are quite optimistic, but they have a better spread over all 4 Annexes with their requirements and have been chosen for a more detailed assessment of *in vivo* testing requirements. This approach allows a more detailed distinction between different marketed tonnages that is not possible for the chemicals in the list of preregistered substances. This scenario foresees 44,632 chemicals marketed at level $\geq 1t/y$, 11,570 chemicals marketed at level $\geq 10t/y$, 5,721 chemicals marketed at level $\geq 100t/y$ and 6,286 chemicals marketed at level $\geq 1000t/y$. Noteworthy, this corresponds well with estimates given in recent presentations of ECHA representative as to the number of submission expected for end of 2010, which range between 4.000 and 10.000. It is evident that this starting point for the following assumption leads to a large underestimation of the final number of animals necessary for registration and of the related costs.

Costs are either taken from Fleischer (2007) survey or, when data were missing, from the personally communicated experience of a private contract research laboratory.

4.1 Skin/eye irritation and corrosion

This area has been largely covered by validated and EU/OECD accepted alternative methods, with the notable exception of eye irritation (OECD TG 405). Eye corrosion is covered, allowing screening for severe irritants first. Testing is required from Annex VIII onwards.

Estimates for no testing:

- already classified: 73% (ECB)
- human data: 1%
- skin corrosive: 3% (Hoffmann et al., 2005)
- pH <2 or >11.5: 2%
- flammable in contact with air or water: 0.1%
- peroxides: 0.1%
- acute dermal <400mg: 5%
- BfR rule base and (Q)SAR: 10% (Rorije and Hulzebos, 2005)
- *In vitro* eye corrosive: 5%
- Non-validated *in vitro*: 10%
- Read-across: 10%

This means that data are lacking for 26% of chemicals. Of these 45.2% can be gained without animal testing, i.e. 14.3% of chemicals will be tested according to OECD TG 405. Notably, ongoing validation activities promise to replace this test within few years. Given an average animal use of 1.3 animals per tested chemical, the most optimistic scenario results in the consumption of 10,229, 1,041, and 1,144 animals for chemicals

marketed at concentration higher than 10t/y, 100t/y, and 1000t/y respectively.

The estimated costs for running OECD TG 405 is about 1,100 euro per tested chemical.

4.2 Skin sensitization

The OECD TG 429 (Local Lymph Node Assay, LLNA) is the standard requirement from Annex VII onward. However, OECD TG 406 using 15-30 guinea pigs (maximization test vs. Buehler) can also be used according to the guidance (ECHA, 2008). ECB assumes 20% existing data. No testing is required for skin corrosives (3%). Since no validated alternative approaches exist and sensitization cannot be established by read-across, 77% of substances will require testing. Actually, some alternative approaches are under development. (Q)SAR activity in this field is quite interesting and several models are now publicly available. General perception is that (Q)SARs can be effectively employed as part of a battery of methods together with results from *in vitro* testing, as demonstrated by the results of two EU integrated projects, Caesar and Sens-it-iv (www.caesar-project.eu; www.sens-it-iv.eu). In the near future, they may provide tools for the reduction of the number of animals used for skin sensitisation assessment. At the moment, there is no general acceptance and/or knowledge and therefore no contribution from alternative methods is considered in the present study. No alternative method is considered suitable in the ECHA Technical Guidance (ECHA, 2008a).

Among the chemicals that will be tested for skin sensitisation *in vivo*, it is assumed that two-thirds will apply the LLNA as the clearly preferred test in the legislation and one-third will use guinea pig methods (23 animals assuming equal use of GPMT and Buehler). It is also assumed that in half of the cases, the validated reduced LLNA is used, consuming only eight animals instead of 16. Final counting assumes the application of each guideline for 25.7% of all registrations. Cost estimation is about 3,290 EUR for LLNA and 2,000 EUR for the reduced method. A study according to OECD TG 406 can cost up to 4,000 EUR.

4.3 Acute toxicity

Oral route OECD TG 420, OECD TG 423 or OECD TG 425 (accepted refinement and reduction methods) are required from Annex VII onward with a second route OECD TG 403 (inhalation) or OECD TG 402 (dermal) from Annex VIII onward. ECB assumes 56% oral and 34% of other routes available. It is assumed that dermal route is preferred to inhalation by 9:1.

3% of skin corrosive substances do not need to be tested. No validated and/or accepted alternatives are available. Notably,

Tab. 13: Estimates of (Q)SAR acceptance (%) as reported by Pedersen et al. (2003)

	Annex VIII	Annex IX	Annex X
Good	24	6	3
Fair	10	2.5	1.25
Poor	0.4	0.1	0.05



tiered testing variants for OECD TG 403 and OECD TG 402 are under discussion at OECD. These assumptions are summarized in Tab. 14, which also includes average costs.

Recently the national Coordinators of the OECD Environment, Health and Safety program have approved a new test guideline on Acute Inhalation Toxicity (Draft OECD TG 436), which uses a significantly reduced number of animals. The final approval of this new guideline is expected in late 2009 (ALTEX News, 2/2009).

4.4 Repeat dose toxicity

A 28-day study in one species is required from Annex VIII onward in the most relevant route. It is assumed that this is 90:9:1 for oral:dermal:inhalation. From Annex IX onward a 90-day study has to be added. ECB assumes availability of 34% and 7%, respectively.

Testing can be waived for no relevant exposure, assumed at 5%, thus resulting in test needs for 61% and 88%, respectively. It is not expected that long-term studies (12+ months, OECD TG 452) will be triggered. Notably, minimum animal numbers have been assumed with only three dose groups. Additional triggers of testing were not considered.

The use of OECD TG 452 is discouraged in the guideline (ECHA, 2008) and therefore it has been disregarded from this calculation. Contribution from OECD TG 453 and 422 is counted with carcinogenicity and reproductive toxicity assessment, respectively.

These assumptions are summarized in Tab. 15, which also includes average costs.

4.5 Mutagenicity

According to the guidance (ECHA, 2008a) Annex VII requires an Ames test, which in case of positive findings shall go to Annex VIII requirements for mutagenicity. Annex VIII foresees two more *in vitro* tests. According to Kirkland et al. (2005), the Ames test has a specificity for non-carcinogens of 73.9%, which means 26.1% false-positive results. Its sensitivity is about 90%. Thus, assuming about 10% real mutagens among chemicals, 23.5% of chemicals would be Ames-positive (9% real and 14.5% false-positive). In case of positive results, typically an *in vivo* test shall be considered; we assume that it will be carried

out in 80% of the cases, i.e. for 21.2% of Annex VII chemicals.

The false-positive rate for the combination of tests in Annex VIII ranges between 75-95% (Kirkland et al., 2005), which means it is fair to assume that together with the real positives about 90% of chemicals will be considered for *in vivo* and 80% of these, i.e. 72%, will be carried out.

For Annex IX and X, the *in vivo* test shall be considered independent of the *in vitro* results. The ECB assumed 7% data availability; and assuming again 80% execution of tests, 74.4% of substances will be tested.

The *in vivo* tests comprise different mutagenicity and the less frequently used germ-cell mutagenicity assays. The guidance document foresees 1-3 *in vivo* tests, to be chosen among OECD TG 474, 475, 483, 478 and 486. Requirements for each of these methods are quite different in terms of both animal consumption and costs. The average of 70 used in previous estimates (Höfer et al., 2004) seems realistic, as does the average cost of 10,800 euro per tested chemical.

4.6 Carcinogenicity

Animal testing for carcinogenicity is very much discouraged by the guidance: "A carcinogenicity study may, on occasion, be justified. If there are clear suspicions that the substance may be carcinogenic, and available information (from both testing and non-testing data) are not conclusive in this, both in terms of hazard and potency, then the need for a carcinogenicity study should be explored. In particular, such a study may be required for substances with a widespread, dispersive use or for substances producing frequent or long-term human exposures. However, it should be considered only as a last resort."

ECB assumes 4% available data, not relevant here.

It is assumed that 1% of the Annex X substances, and 0.1% of suspicious chemicals of the other Annexes will be tested in OECD TG 451. Application of OECD TG 453 can be considered as equivalent.

All carcinogenicity studies are quite long and complex, justifying the high cost per chemical of 780,400 euro upon the use of "only" 400 rats per study.

4.7 Reproductive Toxicity

Reproductive toxicity evaluation includes the developmental toxicity screening (8.7.1) required in Annex VIII plus developmental toxicity test (8.7.2) and two-generation reproductive toxicity study

Tab. 14: Assumptions for the requests of new *in vivo* Acute Toxicity studies

Cost and minimum animal number by OECD test guideline as well as estimated percentage of substances per tonnage level to be subjected to the different acute toxicity tests. See text chapter 4.3.

	OECD TG 420, 423, 425	OECD TG 403	OECD TG 402
Animals	8 – 15, average 12	40-50, average 45	25-30, limit test with 10, average 20
Cost (euro)	1,500 euro	11,700 euro	2,000 euro
VII	44% (-3%corr) = 42,7%		
VIII	42,7 %	10% of (66%-3%corr) = 6.4%	57.6%
IX	42,7 %	6.4%	57.6%
X	42,7 %	6.4%	57.6%



required in Annex IX (8.7.3). The developmental toxicity study is usually tested on one species but testing another non-rodent species is now recommended, basically changing the accepted current procedure for the assessment of reproductive toxicity. There is also a strong interconnection with other endpoints: a positive outcome on reproductive organs from the repeated dose toxicity study can trigger a two-generation toxicity study, while chemicals already classified as carcinogenic do not need reproductive toxicity testing as the risk measures must be adequate.

The following animal tests need to be considered:

- OECD TG 421, adopted on 27th July 1995: Reproduction/developmental toxicity screening test. Preferred species is the rat. The method requires at least 10 females and 10 males per dose, with three doses per chemical plus one control dose. Pups are counted. Höfer et al. (2004) calculate 560 rats and Fleischer (2007) consider an average price of 54,600 euro for the study on rats.
- OECD TG 422, adopted on 22nd March 1996: Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test. Preferred species is the rat. The method requires at least 10 females and 10 males per dose, with three doses per chemical plus one control dose. Pups are counted to give an average of 412 animals per chemical and a cost of 92,000 euro per study.
- OECD TG 414, adopted on 22nd January 2001: Prenatal developmental toxicity Study (EU Method B.31). Preferred rodent species is rat, preferred non-rodent species is rabbit. The method requires at least 20 pregnant females per dose, with three doses per chemical plus one control dose. Killing of the females is foreseen one day prior to the expected day of delivery, pups are counted as it is well known that foetuses perceive pain starting from approximately 60 percent of the gestation period (Close et al. 1997). Fleischer (2007) consider an average price of 63,100 euro for the study on rats and 92,500 euro for the study on rabbits. Rabbit consumption is estimated to be 560 per tests while rats are 784 per test.
- OECD TG 416, adopted on 22nd January 2001: Two-generation reproduction toxicity study (EU Method B.35) Preferred species is rat. The method requires at least 20 females and 20 males per dose, with three doses per chemical plus one control dose. Some of the F1 offspring are mated and F2 offspring are also included in the study. Höfer et al. (2004) calculate 3,200

rats and Fleischer (2007) consider an average price of 328,000 euro for the study on rats. We can assume 2,100 rabbits or 3,200 mice per chemical in case testing a second species is required with a cost of 328,000 euro per test (the lower number of 2,100 was used for the calculation). Testing on a second species is specifically recommended in the ECHA guideline.

- OECD TG 426, adopted on 16th October 2007: Developmental neurotoxicity. Preferred species is rat and the dosing procedure starts from pregnant females, without specifying how many male rats are used. The final request is for 20 litters, with no indication on the number of parents. We assume that at least 10 males and 10 females are selected as parents per dose group, with three doses per chemical plus one control dose. On average, the DNT study TG 426 requires 1,400 animals and costs 1.1 million euro.
- OECD TG 415, adopted on 26th May 1983: One-generation reproduction toxicity study (EU Method B.34). Preferred species is rat. The method requires at least 20 females and 20 males per dose, with three doses per chemical plus one control dose. This method is not further considered as it is assumed that the two-generation study (OECD TG 416) will be the preferred method of choice until agreement on TG 415 on OECVD level, even though some proposals for a revision of current strategy are under discussion (Spielmann and Vogel, 2007).

Average litter size of rats is 8.2 (Kidwell and Weeth 1959), while the average litter size for mice, the preferred second species for OECD TG 416, is 8.3 (Finn, 1963). For rabbits, average litter size is 5.5 (Blasco et al. 1993), resulting in about one-third fewer animals. Notably, while Höfer et al. (2004) calculate 3,200 rats for an OECD TG 416 per chemical, Cooper et al. (2006) estimate only 2,600. For consistency with the previous calculation, the Höfer et al. (2004) figures were used, but about 20% over-estimation in animal numbers for this important component might be possible.

The ECHA (2008a) has published endpoint-specific guidance to industry. In the following, this guidance is translated into an estimate of testing demands:

Standard requirements:

- Annex VIII (10-100t/y): OECD TG 421 or 422; since typically 28-day repeat dose study data will be available, we assume

Tab. 15: Assumptions for the requests of new *in vivo* Repeated Dose Toxicity Studies

Cost and minimum animal number by OECD test guideline as well as estimated percentage of substances per tonnage level to be subjected to the different repeated dose toxicity tests. See text chapter 4.4.

	28 days Studies			90 days Studies		
	OECD TG 407	OECD TG 410	OECD TG 412	OECD TG 408	OECD TG 411	OECD TG 413
Animals	40	40	40	80	80	80
Cost (euro)	49,400	49,600	105,500	115,700	135,000	250,000
≥ 10t/y	90% of 61%= 54.9% -33% repro= 21.9%	5.5%	0.6%			
≥100t/y	21.9%	5.5%	0.6%	90% of 88%= 79.2%	7.9%	0.9%
≥1000t/y	21.9%	5.5%	0.6%	79.2%	7.9%	0.9%



67% use of OECD TG 421 and 22% use of OECD TG 422, but “dependent on the nature of the alert(s)...it may be more appropriate to conduct a two-generation reproduction study ... or a prenatal developmental toxicity study” in addition; for this estimate it will be assumed that this is the case for 1% of the chemicals each for OECD TG 414 and OECD TG 416. Testing in a second species is normally not considered.

- Annex IX (100-1000 t/y): required method is OECD TG 414, occasionally including a non-rodent species (“a study in a second species will normally be required when the first study is negative,” for the purpose of this estimate we will assume that this is the case for 80%); in case of special concern, developmental neurotoxicity (DNT) testing should be taken into consideration as well.
- Annex X (≥ 1000 t/y): required method is OECD TG 416, occasionally including a non-rodent species; OECD TG 421/422 and OECD TG 414 are not required; in case of special concern, developmental neurotoxicity (DNT) testing should be taken into consideration as well.

Upgraded testing requirements: “Human exposures, particularly for consumers, are close to the levels at which toxicity might be expected,” we will assume that this is true, and in 1% of the cases the substance will move one level up.

Notably, “clear evidence of adverse effects” from OECD TG 421 or 422 will result in “no requirements for the conduct of a two-generation study at higher tonnage levels.” “A negative result ... will provide reassurance of the absence of this hazardous property.” For the purpose of our calculation, since reproductive hazard is rare and these screening tests typically do not give “clear evidence” we will assume 5% of such waiving for Annex IX and no effect for Annex X, since here no OECD TG 421/422 testing is foreseen. Noteworthy, in the European Commission regulation 134/2009 amending Annex XI of REACH (published in the Official Journal of the European Union on 16 February 2009) it is explicitly stated that derived no-effect levels “from screening test for reproductive/developmental toxicity shall not be considered appropriate to omit a prenatal developmental toxicity study or a two-generation reproductive toxicity study” prohibiting this major waiving opportunity.

Existing information allowing reproductive toxicity testing to be waived is rare (about 100-200 substances are currently recognized as reproductive toxicants).

An extensive survey (Bremer et al., 2007) has been developed over:

- Annex I of the EU Dangerous Substance Directive 67/548/EEC
- Proposition 65 list of the State of California Office of Environmental Health Hazard Assessment
- European Chemical Substances Information System and IUCLID Chemical Data Sheet Information System
- US National Toxicology Program (NTP) database of reproductive and developmental toxicity study abstracts
- US EPA’s Integrated Risk Information System database and peer-reviewed open literature
- Studies conducted according to recognized test guidelines

or which have at least examined a relatively broad range of reproductive or developmental parameters (US National Library of Medicine’s TOXNET-Developmental and Reproductive Toxicity study).

The output of the research was about information sufficient for only 71 substances positively classified. Even assuming that 2- to 20-fold more negative study results are available, for the purpose of REACH this number does not impact on overall test costs and animal use.

Human data are considered even more infrequently and are usually limited to drugs. The US EPA Integrated Risk Information System on high production volume chemicals, which is closest to the REACH dataset, contained information on reproductive toxicity tests for 17 of about 2,000 chemicals. We will assume 1% of available data for the purpose of this study.

Thus, existing data are negligible, and limit the opportunities to carry out read-across or modelling by (Q)SAR. To some extent, grouping of chemicals with testing prototypic members of a series of homologues might be considered, but experience from the drug area has taught that even enantiomers (thalidomide) or slight structural variants (retinoic acid) behave completely different with regard to reproductive hazard. We will assume a saving effect of 5% for the purpose of our study.

The guidance further suggests that substances “for which a mechanism of toxicity has been identified that is causally related to reproductive toxicity ... may be reasonably expected to exhibit the same pattern of reproductive toxicity.” However, only very few mechanisms of reproductive toxicity are known, which relate to many chemicals, with the possible exception of endocrine disruption. This is not a standard testing requirement in REACH. Thus, grouping cannot play a major role in a decision to waive testing, but we will assume another 5% of waived testing.

Testing can also be waived if the substance is “classified as a genotoxic carcinogen category 1 and mutagen category 3 or carcinogen category 2 and mutagen category 3, or a germ cell mutagen (Mutagen Cat. 1 or Cat. 2). For the purpose of our estimate we will assume 5% of such cases. Testing can further be waived if “all three criteria ... are met”: “(a) low toxicological activity (b) negligible systemic absorption and (c) no significant human exposure.” Meeting all three criteria is rare and will not exceed 5% of cases.

Data from other animal tests are seen as a critical substitute for reproductive toxicity testing, e.g. “sensitivity of repeated-dose toxicity studies for detecting effects on reproductive organs may be less than reproductive toxicity studies because of the lower number of animals per group” and “occur at lower doses during the development of fetuses and young animals than in adults.” On the contrary, further reproductive toxicity studies might be triggered “based in a Weight of Evidence assessment.”

Notably, “Repeated-dose toxicity studies may also provide indications to evaluate the need to investigate developmental neurotoxicity endpoints.” This is further explained as “Relevant triggers could be if the substance has been shown to:

- 1) Cause structural abnormalities of the central nervous system
- 2) Cause clear signs of behavioural or functional adverse effects



of nervous system involvement in adult studies e.g. repeated dose toxicity studies

- 3) Have a mode of action that has been closely linked to neurotoxic or developmental neurotoxicity effects

Evaluations of repeated-dose toxicity studies (Bitsch et al., 2006) suggest about 10% of chemicals cause neurotoxic effects, thus we assume that in half of the cases (5%) DNT studies will be considered and carried out in 2.5%, as suggested in the OECD TG 426. This is disputed by some practitioners, but optimistically we will assume that this is possible in half of the cases, resulting in a 50% cost reduction and no additional animals.

Furthermore, it is suggested that “Evidence of endocrine disruption seen in a repeated dose toxicity study provides a trigger for the conduct of a more comprehensive study, for example a two-generation study.” Evidence of endocrine disruption and pathology to endocrine glands is again seen for about 10% of chemicals (Bitsch et al., 2006), which might result in similar assumptions in 2.5% of two-generation studies triggered by repeat-dose studies in Annex VIII and IX.

The guidance does not identify any *in vitro* (“no officially adopted EU or OECD test guideline”) or (Q)SAR (“cannot be adequately covered by a battery of (Q)SAR models”) substitutes for testing. Reproductive toxicity is especially difficult for structure-based predictions, since sometime even enantiomers or slightest chemical modifications change the toxic profile, as is well known for hazardous substances such as thalidomide or vitamin A derivatives. In fact, a systematic assessment of the most promising commercial (Q)SAR programs led to disappointing results (Maslankiewicz et al., 2005) The software program DEREKfW has been challenged with the around 100 reproductive toxicants included in Annex I of Directive 67/548/EEC. About 90% of chemicals classified for “impaired fertility” and 81% of chemicals that cause harm to the unborn child were not detected. The TSCA (Toxic Substances Control Act) chemical category list of the US EPA’s new chemical program failed in 77% of cases to detect EU classified chemicals causing adverse effects to mammalian fertility, and 82% of developmental toxicants have not been correctly identified.

In vitro approaches are furthered by the EU ReProTect project (Hareng et al., 2005), but a replacement strategy is not foreseeable. Currently, the most important approach is the ongoing validation of replacing the two-generation test by an extended

one-generation reproductive toxicity test (Bremer et al., 2006, Spielmann and Vogel, 2007, ECETOC, 2008). However, *in vitro* positive results are considered a trigger for further testing (“a positive result in a validated *in vitro* test could provide a justification for further testing”).

Taking these considerations together, maximally 21% of animal tests can be waived based on 1% existing data, <5% grouping, <5% endocrine disrupters, <5% classified carcinogens and germ cell mutagens, and <5% negligible exposure. A contribution of *in vitro* or (Q)SAR alternatives is not foreseen. Noteworthy, there is some saving potential applying only a limit test with one dose >1000mg/kg and control group in all test guidelines, but the REACH guidance to industry does not even mention this opportunity, which was thus not further considered. All these considerations are summarized in Tab. 16, which also lists average costs.

4.8 Fish bioaccumulation

The official test (usually OECD TG 305) is required in Annex IX as well as for PBT substances.

The guidance is cautious (ECHA, 2008b): “In view of the importance of this endpoint in the assessment of a chemical, and the relatively small number of substances that have been properly tested, a cautious approach is needed.” ECB assumes data availability at 19%. The applicability of (Q)SAR models for elucidation of bioaccumulation is quite extensive for this endpoint (Weisbrod et al., 2007, Caesar Project 2009), providing further contribution to lower the demand of *in vivo* testing.

It is assumed that 10% of chemicals will actually be tested from Annex IX and X. Furthermore, it is assumed that about 1% of substances from Annex VII and VIII might qualify as PBT.

4.9 Short-term and long-term fish acute toxicity

Short-term toxicity testing on fish is required in Annex VIII, i.e., for substances marketed at a tonnage level higher than 10t/y. This test is waived if the substance is unlikely to cause aquatic toxicity, for example, if it is highly insoluble in water; we assume that this is the case for 10% of chemicals. ECB assumes that data exist for 49% of chemicals. The test is not necessary when a long-term toxicity study is available; ECB expects this for 7% of chemicals, but this should be typically on substances where the short-term test is also available. This means that the test will be carried out for 45.9% of chemicals. According to the

Tab. 16: Assumptions for the requests of new *in vivo* Reproductive Toxicity Study

Cost and minimum animal number by OECD test guideline as well as estimated percentage of substances per tonnage level to be subjected to the different acute toxicity tests. See text chapter 4.7.

	OECD TG 421	OECD TG 422	OECD TG 414	OECD TG 414 + 2 nd species	OECD TG 416	OECD TG 416 + 2 nd species	OECD TG 426
Animals	560	412	784	560	3,200	2,100	1,400
Cost (euro)	54,600	92,000	63,100	92,500	328,000	481,000	1,100,000
≥ 10t/y	67%	33%	1%		1% + 2.5% = 3.5%		1.25%
≥100t/y	67%	33%	95%	76%,	(80% of 95%)	3.5%	1.25%
≥1000t/y					100%	80%	1.25%



Tab. 17: Estimated number of animals required for registration dossiers based on scenario 3. Total number of animals is 54,4 million.

Endpoint	OECD TG	Average animals per test	≥ 1 t/y	≥ 10 t/y	≥ 100 t/y	≥ 1000 t/y	≥ 1 t/y	≥ 10 t/y	≥ 100 t/y	≥ 1000 t/y	Total	%
							44,632	11,570	5,721	6,286		
Eye Irritation	405	1.3	14%	14%	14%	14%	8,123	2,106	1,041	1,144	12,414	0.02%
Skin Sensitation	429	16	25.7%	25.7%	25.7%	25.7%	183,527	47,576	23,524	25,847	823,891	1.52%
	429 R	8	25.7%	25.7%	25.7%	25.7%	91,763	23,788	11,762	12,923		
	406	23	25.7%	25.7%	25.7%	25.7%	263,820	68,390	33,816	37,155		
Acute Toxicity	420, 423, 425	12	42.7%	42.7%	42.7%	42.7%	228,694	59,285	29,314	32,208	689,003	1.27%
	403	45		6.4%	6.4%	6.4%		33,322	16,476	18,103		
	402	20		57.6%	57.6%	57.6%		133,286	65,904	72,411		
Repeated Dose Toxicity	407	40		21.9%	21.9%	21.9%		101,353	50,115	55,063	1,187,122	2.19%
	410	40		5.5%	5.5%	5.5%		25,454	12,586	13,829		
	412	40		0.6%	0.6%	0.6%		2776.8	1,373	1,509		
	408	80			79.2%	79.2%			362,472	398,263		
	411	80			7.9%	7.9%			36,156	39,726		
	413	80			9.0%	9.0%			41,190	45,257		
Mutagenicity	see text	70	21.2%	72.0%	74.4%	74.4%	662,339	583,128	297,941	327,360	1,870,768	3.45%
Carcinogenicity	451	400	0.1%	0.1%	0.1%	1.0%	17,853	4,628	2,288	25,143	49,912	0.09%
Reproductive Toxicity	421	560		67%	67%			4,341,064	2,146,459		48,648,236	89.62%
	422	412		33%	33%			1,573,057	777,805			
	414	784		1%	95%			90,709	4,260,882			
	414 (2 nd species)	560			76%				2,434,790			
	416	3200		3.5%	3.5%	100%		1,295,840	640,734	20,114,299		
	416 (2 nd species)	2100				80%				10,560,007		
	426	1400		1.25%	1.25%	1.25%		202,475	100,115	110,000		
Bioaccumulation (fish)	305	16		1%	10%	10%		1,851	9,153	10,057	21,062	0.039%
Short Term Toxicity (fish)	203	42		45.9%				223,046			223,046	0.41%
Long Term Toxicity (fish)	210 (212, 215)	400		5%	10%	10%		231,400	228,834	251,429	711,662	1.31%
Avian Toxicity	205, 223	70				10%				44,000	44,000	0.08%

ECHA guideline on information requirements (ECHA, 2008b), OECD TG 203 is the accepted method for the assessment of short-term fish toxicity. The average cost for each test is 4,200 euro (Fleischer, 2007) with a consumption of 42 fish (van der Jagt et al., 2004). We further assume that in half of the cases the validated threshold-step-down approach will be used, which reduces animal use by 60%.

For substances covered by Annex IX, long-term toxicity study is always required and no additional test is added in Annex X. For long-term toxicity in fish, OECD TG 210, 212, and 215 are the preferred methods, as they consider different sensitive life stages of fish. The most used method is OECD TG 210, with an average cost of 26,300 euro and 400 fish per tested chemical.

It is assumed that long term toxicity on fish is required for 5% of substances produced in quantities <100t/y and 10% of substances produced in quantities ≥100t/y.

4.10 Avian toxicity

Experience with industrial chemicals for this assessment is minimal, but REACH requests this for Annex X. Thus there is little data or alternative methods available. Since waiving for substances above 1000 t/a is rather unlikely, about 10% testing is assumed. Two relevant OECD guidelines, i.e. OECD TG 223 and OECD TG 205, are available using 70 birds (ECHA, 2008c); it is assumed that both are used with equal frequency. The legislation asks for long-term toxicity, but no such test is


Tab. 18: Estimated costs required for registration dossiers based on scenario 3. Total cost is 9,5 billion euro

Endpoint	OECD TG	Average cost (thousand euro)	≥ 1 t/y	≥ 10 t/y	≥ 100 t/y	≥ 1000 t/y	≥ 1 t/y	≥ 10 t/y	≥ 100 t/y	≥ 1000 t/y	Total (thousand euro)	%
							44,632	11,570	5,721	6,286		
Eye Irritation	405	1.1	14%	14%	14%	14%	6,873	1,782	881	968	10,504	0.11%
Skin Sensitation	429	3.29	25.7%	25.7%	25.7%	25.7%	37,738	9,783	4,837	5,315	162,850	1.71%
	429 R	2	25.7%	25.7%	25.7%	25.7%	22,941	5,947	2,941	3,231		
	406	4	25.7%	25.7%	25.7%	25.7%	45,882	11,894	5,881	6,462		
Acute Toxicity	420, 423, 425	1.5	42.7%	42.7%	42.7%	42.7%	28,587	7,411	3,664	4,026	88,502	0.93%
	403	11.7		6.4%	6.4%	6.4%		8,664	4,284	4,707		
	402	2		57.6%	57.6%	57.6%		13,329	6,590	7,241		
Repeated Dose Toxicity (28d)	407	49.4		21.9%	21.9%	21.9%		125,171	61,891	68,003	1,832,718	19.22%
	410	49.6		5.5%	5.5%	5.5%		31,563	15,606	17,147		
	412	105.5		0.6%	0.6%	0.6%		7323.81	3,621	3,979		
	408	115.7			79.2%	79.2%			524,226	575,988		
	411	135			7.9%	7.9%			61,013	67,037		
	413	250			9.0%	9.0%			128,719	141,429		
Mutagenicity	see text	10.8	21.2%	72.0%	74.4%	74.4%	102,189	89,968	45,968	50,507	288,633	3.03%
Carcinogenicity	451	780.4	0.1%	0.1%	0.1%	1.0%	34,831	9,029	4,465	49,054	97,378	1.02%
Reproductive Toxicity	421	54.6		67%	67%			423,254	209,280		6,912,147	72.49%
	422	92		33%	33%			351,265	173,685			
	414	63.1		1%	95%			7,301	342,936			
	414 (2 nd species)	92.5			76%				402,175			
	416	328		3.5%	3.5%	100%		132,824	65,675	2,061,716		
	416 (2 nd species)	481				80%				2,417,860		
	426	1100		1.25%	1.25%	1.25%		159,088	78,662	86,429		
Bioaccumulation (fish)	305	10		1%	10%	10%		1,157	5,721	6,286	13,164	0.14%
Short Term Toxicity (fish)	203	4.2		45.9%				22,305			22,305	0.23%
Long Term Toxicity (fish)	210 (212, 215)	26.3		5%	10%	10%		15,215	15,046	16,531	46,792	0.49%
Avian Toxicity	205, 223	96.2				10%				60,469	60,469	0.63s%

currently available. Due to the small numbers involved, the high uncertainty connected to the present assumption does not affect the final results.

4.11 Summary of assumptions and results

Tabs. 17 and 18 summarize all the assumptions of the previous paragraphs. It is quite clear that the most demanding studies are in the area of reproductive toxicity testing, with about 90% of all animal use and 70% of the required costs for registration. This study used rather conservative estimates, i.e. adjusting the number of chemicals falling under REACH from 1994 due to the growth of chemical industry and the EU member

states, resulting in about 68,000 substances. It also used minimal animal numbers per test and neglected most triggering of additional tests by suspected CMR (carcinogen/mutagen/reproductive toxicant) and biopersistent chemicals as well as triggers from other *in vitro/in vivo/in silico* findings or yet not defined testing demands (endocrine disruption, respiratory irritation, respiratory sensitization, developmental neurotoxicity, etc.). A number of assumptions had to be made, and were reviewed by the practitioners named in the acknowledgement. The overall result suggests a demand of 54 million vertebrate animals and testing costs of 9.5 billion euro. Because of the nature of assumptions, this represents a best-case scenario. This number has to be compared to about 90,000 animals used for testing of new



chemicals per year in Europe. REACH aims to complete data collection by 2018. At that point, Annex VII and VIII chemicals, which do not require permission to carry out suggested animal tests, have to be registered. In parallel, for Annex IX chemicals registered by 2013, permission to carry out suggested animal tests shall be given within two years, and execution within the following years would be expected. Thus, most animal testing will start around 2012, i.e. two years after the registration of Annex X substances, and the majority of these tests should be completed in the relatively short period of 6 years. Theoretically, this means about 100-fold increase in animal use for chemical testing and about doubling of overall animal use in Europe. This clearly challenges the feasibility of the program. Without a major investment into high-throughput methodologies this program cannot be completed in a reasonable time frame. It is obvious that such methods will not be available by 2012.

Notably, in the areas of highest contribution to animal use and costs, i.e. reproductive toxicity, repeat dose toxicity, and long-term toxicity in fish, no relevant alternative approaches have emerged. At least no method is under validation, which means that no validation / peer-review / acceptance can be expected before 2018. The notable exception is the opportunity to change from the two-generation study to an extended one-generation study (Bremer et al., 2007, Spielmann and Vogel, 2007, Moore et al., 2009). This would reduce animal use for this test by 40-60%. This means about a 15% reduction in total animal numbers. This will only impact the cost of tests if the extensions of one-generation study, i.e. DNT and immunotoxicity, are not made mandatory for chemicals. Otherwise the costs of the two-generation-study and the extended-one-generation-study will not differ substantially.

5 Conclusions

The re-evaluation of animal numbers and costs of REACH demonstrates that estimates must be increased substantially. Preregistrations for REACH clearly exceeded expectations and several contributing factors, such as expansion of EU member states and growth of the chemical industry, were neglected in the earlier assessments. The guidance for industry published by ECHA does not offer the expected waiver opportunities and the availability of safety assessments from other sources was probably too optimistic. At the same time, despite an obvious stimulus, the development and validation of alternative methods has been minimal for the most complex endpoints. Successes in areas like topical toxicities, acute toxicity, acute fish toxicity and sensitization do not translate to major savings because of the dominance of reproductive toxicity testing requirements. This lack of progress is especially true in the most demanding areas of reproductive toxicity and long-term fish toxicity, with the notable exception of the extended one-generation test to replace the two-generation test. The feasibility of REACH on this basis has to be questioned. It seems to be inevitable to either prioritize testing requirements or reconsider testing approaches as currently suggested by EPA in the US (EPA, 2009b). It is beyond dispute that the primary aim of REACH is protecting hu-

man health and the environment from unwanted consequences of exposure to chemicals. The challenge will be to do it sensibly within the context of REACH while using all the information and experience we have and recognizing that most chemicals have been produced and used safely for many years without extensive testing on animals.

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References

- Allanou, R., Hansen, B. J. and Van der Bilt, Y. (1999). Public Availability of Data on EU High Production Volume Chemicals. JRC Report EUR 18966 EN (<http://ecb.jrc.ec.europa.eu/esis/>) (accessed on 17 July 2009).
- ALTEX News (2009). OECD: Experts approve new test guidelines for acute inhalation toxicity. <http://www.altex.ch/de/index.html?id=17&ncat=1&nid=114> (accessed on 17 July 2009).
- Angerer, G., Sartorius, C., Skulimowski, A. M. J., Nordbeck, R., Luo, Z., Wolf, O. and Delgado, L. (2007). Implementation of REACH in the New Member States. Part two: Business Case Studies in Selected New Member States. *JRC Scientific and Technical Reports*; ftp.jrc.es/EURdoc/22790_2-ExeSumm.pdf (accessed on 2 August 2009).
- Barrat, M., Hoffmann, S. and Hartung, T. (2007). The suitability of 400 REACH-relevant existing chemicals for evaluation by in silico methods. *Unpublished results*.
- Benfenati, E. (2007). Predicting toxicity through computers: a changing world. *Chemistry Central Journal* 32, 1-7.
- Bitsch, A., Jacobi, S., Melber, C., Wahnschaffe, U., Simetska, N. and Mangelsdorf, I. (2006). REPOSE: A database on repeated dose toxicity studies of commercial chemicals – A multifunctional tool. *Regulatory Toxicology and Pharmacology*, 46 (3), 202-210.

- Blasco, A., Santacreu, M. A., Thompson, R. and Haley, C. S. (1993). Estimates of genetic parameters for ovulation rate, prenatal survival and litter size in rabbits from an elliptical selection experiment. *Livestock Production Science*, 34 (1-2), 163-174.
- Bottini, A. and Hartung, T. (2009). Food for Thought ... on the Economics of Animal Testing. *ALTEX* 26, 1-9
- Bremer, S., Pellizzer, C., Hoffmann, S., Seidle, T. and Hartung, T. (2007). The development of new concepts for assessing reproductive toxicity applicable to large scale toxicological programs. *Curr. Pharm. Des.* 13, 3047-3058.
- Caesar Project (2009). Bioconcentration factor (BCF): The Endpoint. <http://www.caesar-project.eu/> (accessed on 17/07/2009).
- CEFIC (2009). Facts and Figures – The European Chemical Industry in a worldwide perspective, January 2009. http://www.cefic.be/factsandfigures/level02/growthindustry_index.html (accessed on 17 July 2009)
- Close, B., Banister, K., Baumans, V., Bernoth, E.M., Bromage, N., Bunyan, J., Erhardt, W., Flecknell, P., Gregory, N., Hackbarth, H., Morton, D. and Warwick C. (1997). Recommendation for euthanasia of experimental animals: Part 2. *Lab. Anim.* 31,14-15.
- Cooper, R. L., Lamb, J. C., Barlow, S. M., Bentley, K., Brady, A. M., Doerrer, N. G., Eisenbrandt, D. L., Fenner-Crisp, P. A., Hines, R. N., Irvine, L. F. H., Kimmel, C. A., Koeter, H., Li, A. A., Makris, S. L., Sheets, L. P., Speijers, G. J. A. and Whitby, K. E. (2006). *Critical Reviews in Toxicology*, 36, 69-98.
- Directive 67/548/EEC (1967). COUNCIL DIRECTIVE 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. *Official Journal of the European Communities*, P 196.
- Directive 92/32/EEC (1992). Council Directive 92/32/EEC of 30 April 1992 amending for the seventh time Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, *Official Journal of the European Communities*, L 154.
- ECHA (2008a). Guidance on information requirements and chemical safety assessment Chapter R.7a: Endpoint specific guidance. 428pp. http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r7a_en.pdf?vers=20_08_08 (accessed on 17 June 2009).
- ECHA (2008b). Guidance on information requirements and chemical safety assessment Chapter R.7b: Endpoint specific guidance. 234pp. http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r7b_en.pdf?vers=20_08_08 (accessed on 17 June 2009).
- ECHA (2008c). Guidance on information requirements and chemical safety assessment Chapter R.7c: Endpoint specific guidance. 235pp. http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r7c_en.pdf?vers=20_08_08 (accessed on 17 June 2009).
- ECHA Memo (2008). REACH Pre-registration & Registration – Questions and Answers. MEMO/08/240, Brussels, 11th April 2008.
- ECHA Press Release (2008). 19 December 2008 ECHA/PR/08/59. http://echa.europa.eu/doc/press/pr_08_59_publication_pre-registered_substances_list_20081219.pdf (accessed on 17 July 2009).
- ECHA Press Release (2009). 27 March 2009 ECHA/PR/09/03. http://echa.europa.eu/doc/press/pr_09_03_list_prereg_substances_20090327.pdf (accessed on 17 July 2009)
- ECETOC (2008). Workshop Report No. 12. Extended One-Generation Reproduction Toxicity Study, 14-15 April 2008, Barza d'Ispra, Italy, <http://www.ecetoc.org/>.
- EPA (2009a). High Production Volume Information System (HPVIS) <http://www.epa.gov/HPV/hpvis/index.html> (accessed on 17 July 2009).
- EPA (2009b). The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals. <http://www.epa.gov/osa/spc/toxicitytesting/> (accessed on 17 July 2009).
- Finn, C. A. (1963). Reproductive capacity and litter size in mice: effect of age and environment. *Journal of Reproduction and Fertility*, 6, 205-214.
- Fleischer, M. (2007). Testing Costs and Testing Capacity According to the REACH Requirements – Results of a Survey of Independent and Corporate GLP Laboratories in the EU and Switzerland. *Journal of Business Chemistry* 4, 96-114.
- Hareng, L., Pellizzer, C., Bremer, S., Schwarz, M. and Hartung, T. (2005). The Integrated Project ReProTect: a novel approach in reproductive toxicity hazard assessment. *Reproductive Toxicology*, 20, 441-452.
- Hartung, T. (2009). Toxicology for the twenty-first century. *Nature* 460, 208-212.
- Höfer, T., Gerner, I., Gundert-Remy, U., Liebsch, M., Schulte, A., Spielmann, H., Vogel, R. and Wettig, K. (2004). Animal testing and alternative approaches for the human health risk assessment under the proposed new European chemicals regulation. *Arch Toxicol.* 78, 549-564.
- Hoffmann, S., Cole, T. and Hartung T. (2005). Skin irritation: prevalence, variability, and regulatory classification of existing in vivo data from industrial chemicals. *Regul. Toxicol. Pharmacol*, 41, 159-166.
- IEH Report (2001). Testing requirements for proposals under the EC white paper 'strategy for a future chemicals policy'. Institute for Environment and Health, <http://www.cranfield.ac.uk/health/researchareas/environmenthealth/ieh/ieh%20publications/w6.pdf>, (accessed on 22 July 2009).
- Kidwell, J. F. and Weeth, H. J. (1959). Sex ratios and litter size in the laboratory rat. *J. Heredity* 49, 303-304.
- Kirkland, D., Aardema, M., Henderson, L. and Müller, L. (2005). Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens: I. Sensitivity, specificity and relative predictivity. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 584 (1-2), 1-256.
- Maslankiewicz, L., Hulzebos, E. M., Vermeire, T. G., Müller, J. J. A. and Piersma, A. H. (2005). Can chemical structure predict reproductive toxicity? RIVM report 601200005. <http://rivm.openrepository.com/rivm/bitstream/10029/7374/1/601200005.pdf>, (accessed on 17 July 2009).
- Moore, N., Bremer, S., Carmichael, N., Daston, G., Dent, M., Gaoua-Chapelle, W., Hallmark, N., Hartung, T., Holzum, B.,



- Hübel, U., Meisters, M.-L., Schneider, S., van Ravenzwaay, B. and Hennes, C. (2009). A modular approach to the extended one-generation reproduction toxicity study: Outcome of an ECETOC task force and International ECETOC/ECVAM workshop. *ATLA – Altern. Lab. Anim.* 37, 219-225.
- Pedersen, F., de Bruijn, J., Munn, S. and van Leeuwen, K. (2003). Assessment of additional testing needs under REACH – effects of (Q)SARs, risk based testing and voluntary industry initiatives. *JRC Report EUR 20863*. <http://home.hetnet.nl/~REACH/downloads/reachtesting-needsfinal.pdf> (accessed on 27 July 2009).
- REACH (2006). 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), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. *Official Journal of the European Union, L 396/1-849*.
- Regulation 134 (2009). COMMISSION REGULATION (EC) No 134/2009 of 16 February 2009 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annex XI. *Official Journal of the European Union. L 46, 3-5*.
- Regulation 440 (2008). COUNCIL REGULATION (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). *Official Journal of the European Union. L 142, 1-739*.
- Roe, D., Pease, D., Florini, K. and Silbergeld, E. (1997). Toxic Ignorance: The continuing absence of basic health testing for Top Selling Chemicals in the United States. Environmental Defense Fund, pp85 http://www.edf.org/documents/243_toxicignorance.pdf (accessed on 22 June 2009).
- Rorije, E. and Hulzebos, E. (2005). Evaluation of (Q)SARs for the Prediction of Skin Irritation/Corrosion Potential Physicochemical exclusion rules. ECB publication, pp45 http://ecb.jrc.ec.europa.eu/documents/QSAR/Evaluation_of_Skin_Irritation_QSARs.pdf (accessed on 18 June 2009).
- RPA and Statistics Sweden (2002). Assessment of the impact of new regulations in the chemicals sector. Final Report prepared for European Commission, DG ENTR. http://ec.europa.eu/enterprise/reach/docs/whitepaper/bia_report-2002_06_en.pdf (accessed on 24 July 2009)
- Spielmann, H. and Vogel, R. (2007). The extended 1-generation study (OECD 415), as a replacement of the mammalian 2-generation study (OECD 416). *AATEX, 14, 795-798*.
- van der Jagt, K., Munn, S., Tørsløv, J. and de Bruijn, J. (2004). Alternative approaches can reduce the use of test animals under reach. Addendum to the report: Assessment of additional testing needs under REACH Effects of (Q)SARs, risk based testing and voluntary industry initiatives. *JRC Report EUR 21405 EN*; http://ecb.jrc.ec.europa.eu/documents/REACH/PUBLICATIONS/Reducing_the_use_of_test_animals_under_REACH_IHCP_report.pdf (accessed 27 July 2009).
- Weisbrod, A. V., Burkhard, L. P., Arnot, J., Mekenyan, O., Howard, P. H., Russom, C., Boethling, R., Sakuratani, Y., Traas, T., Bridges, T., Lutz, C., Bonnell, M., Woodburn, K. and Parkerton, T. (2007). Workgroup Report: Review of Fish Bioaccumulation Databases Used to Identify Persistent, Bioaccumulative, *Toxic Substances. Environ Health Perspect. 115(2), 255-261*.
- White Paper (2001). Strategy for a future Chemicals Policy. Brussels, 27.2.2001 *COM(2001) 88 final*; www.sfc.fr/LivreBlancEU-REACH-01-2.pdf (accessed 2 August 2009).