Institute for Health
and Consumer Protection

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"

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Effects of (Q)SARS, risk based testing and voluntary industry initiatives

A great deal of additional information on the European Union is available on the Internet.
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SUMMARY

In September 2003 a report was published on the impact of the REACH system on the need for further testing taking into account already existing obligations and voluntary initiatives and applying certain assumptions regarding the use of estimation techniques such as grouping of chemicals, read-across, (Quantitative) Structure-Activity Relationships ((Q)SAR) and the outcome of screening tests and risk assessments (Pedersen et al. 2003). The report was based on the interim version of the REACH Consultation Document of May 2003 and did not address the number of test animals needed for the testing. The present document is an addendum to the previous report and specifically aims at identifying the potential savings of vertebrate test animals that can be obtained through application of these alternative approaches. It is based on the revised Commission proposal of 29 October 2003. Moreover an update of the cost estimates presented in Pedersen et al. (2003) is included.

The calculation of the test animals needed as a consequence of REACH has been carried out by using the same approach as employed by Pedersen et al. (2003). In this approach, the total amount of data required under REACH was established based on the information requirements as included in Annexes V – VIII of the legislative proposal. Then the amount of currently available data, as well as the data already promised under various programmes, was determined. The impact on the testing needs of so-called intelligent testing strategies applying methodologies such as (Q)SAR, grouping, read-across and possibilities for waiving according to Annex IX of REACH was assessed assuming three scenarios: 1) a standard scenario representing an average situation regarding acceptance of these methods, 2) a scenario based on minimum acceptance, and 3) a scenario with maximum acceptance. The currently available and expected data as well as the effect of the use of these methodologies were then 'subtracted' from the total quantity of data required under REACH, resulting in the estimated test requirements.

The number of vertebrate test animals needed for the individual endpoints was established by consulting test laboratories and this was multiplied by the estimated number of studies required under REACH, resulting in estimates of the total amount of test animals which will potentially be needed for the implementation of the REACH legislation.

The results of these calculations show that approximately 3.9 million additional test animals could potentially be used as a consequence of the introduction of REACH if the use of alternative methods is not accepted by regulatory authorities. However, a considerable reduction in animal use can be obtained if these techniques would be applied more intensively. The standard scenario based on average acceptance of these methods indicates potential savings of 1.3 million test animals. Maximum acceptance of these techniques would even enhance this saving potential to 1.9 million test animals. These savings can be obtained by introducing and accepting methods that are to a large extent available today.

Based on the average acceptance scenario, the estimated number of vertebrate test animals is 2.6 million animals (mammals, birds and fish) over a time period of 11 years (i.e. the time period for the full implementation of REACH), or 240,000 animals per year. This equates to 2-3% of the total number of vertebrate test animal used per year for experimental and other scientific purposes, including pharmaceutical testing, based on the 1999 figure of 9.8 million animals (EC, 2003b). After the implementation period of REACH, the number of test animals will most likely return to a base-line level corresponding to the notifications of new substances put on the market. By then, however, the huge knowledge gap we currently face for widely used existing chemicals will have been closed, enabling safer use of chemicals for the generations to come.

The overall direct testing costs based on the most likely scenario have been estimated at 1.5 billion EURO. This differs only slightly from the 1.6 billion EURO estimated in the 2003 Pedersen report. The average test costs per substance in the tonnage band from 1 – 10 tonnes per year is, however, reduced significantly compared to earlier estimates as a result of the reduced data requirements in Annex V (7,700 versus 12,100 Euro), whereas the costs per substance in the higher tonnage bands are only changed slightly. About 90% of the costs are attributed to human health related endpoints.

Applying intelligent testing strategies could reduce the need for tests by up to 70% for individual endpoints resulting in significant savings in testing costs and use of animals. Estimates considering the minimum and maximum use of (Q)SAR, grouping, read-across and possibilities for waiving show that the number of test animals required depends strongly (up to a factor of nearly 4 for various endpoints) on the success and acceptance of these methodologies. For some of the test animal intensive endpoints, no alternative testing is currently available, which effectively reduces the impact that these methods might have on animal testing. Therefore it is recommended that the current activities in the EU and the OECD as well as in industry and academia on the development, validation and adoption of both (Q)SAR methods, *in vitro* testing and read-across techniques (i.e. intelligent testing strategies) are intensified and focused on those endpoints that incur the highest benefits regarding reduction of test animal use and costs.

Increasing the efficiency, cost-effectiveness and focus of the risk assessment process, using new scientifically-sound techniques for creating and interpreting relevant data is essential. Such an approach can lead to a significant reduction in direct testing costs, the use of animals and speed-up the risk assessment process (Bradbury et al., 2004).

The Joint Research Centre (JRC) Activity on (Q)SARs, established under the JRC Work Programme for 2003-2006 and coordinated by the European Chemicals Bureau (ECB), is one of the actions by the Commission that is aimed at promoting the implementation of (Q)SARs and other estimation approaches. In addition, during the implementation of the Commission's interim strategy for REACH one of the REACH Implementation Projects (RIP 3.3) will focus on developing guidance on information requirements on intrinsic properties of substances, aiming in particular at developing intelligent testing strategies which should allow minimal use of test animals.

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1 INTRODUCTION

On October 29th, 2003 the European Commission published its proposal for a regulation concerning Registration, Evaluation and Authorisation of Chemicals (REACH) (EC 2003a). This proposal was preceded by an internet Consultation Document concerning REACH which was published by DG ENTR and DG ENV in May 2003. The draft REACH legislation addresses one of the key issues for chemicals - the lack of publicly available data on chemicals, a gap identified over 20 years ago.

Based on the text of the REACH consultation document the Joint Research Centre published in September 2003 a report entitled 'Assessment of additional testing needs under REACH' (Pedersen et al., 2003). This report evaluated the need for further testing under REACH taking into account already existing obligations and voluntary industry initiatives, and based on a number of assumptions regarding the use of estimation techniques such as (Q)SARs and the outcome of screening tests and risks assessments. The report by Pedersen et al. focussed on the testing costs for REACH but did not address the number of vertebrate test animals that could potentially be used as a consequence of implementing the new legislation. The present document is an addendum to the previous report and specifically aims at identifying the potential savings of vertebrate test animals that can be obtained through application of these alternative approaches. The method applied and the assumptions made are largely similar to the analysis carried out in the original report.

The assessment presented in this report is based on the final REACH proposal as published in October 2003 (EC 2003a). This means that the reduced Annex V- requirements, i.e. the exclusion of tests for cytogenicity for mammalian cells, growth inhibition in algae and the ready biodegradability from Annex V, are considered in this paper. These changes however, do not impact the number of vertebrate test animals, but do have an impact on the cost estimates (see section 4.2). Finally, it should be noted that Pedersen et al. did not consider the impact of increased use of *in vitro* methods as an alternative to whole animal testing, which is also the case with the present document

2 PROCEDURE

A stepwise procedure was used for estimating the total testing needs as a consequence of REACH (EC, 2003a). Only testing needs directly incurred by REACH in addition to testing already required under current legislation and testing already conducted or promised to be conducted by industry as a result of voluntary initiatives have been taken into account. The following stepwise approach has been followed:

1. Identification/estimation of number of substances within the volume bands for testing requirements
2. Identification of existing data coverage for High Production Volume Chemicals (HPVCs)
3. Estimation of likely data coverage for non-HPVCs
4. Identification of data to be provided by voluntary initiatives
5. Identification of possibilities for use of intelligent testing strategies, e.g. (Quantitative) Structure-Activity Relationships ((Q)SAR), grouping, read-across and possibilities for waiving.
6. Assessment of likely acceptance of waiving and requests for further testing
7. Estimation of number of tests needed for each endpoint for each tonnage band
8. Estimation of the number of test animals required for the different endpoints

For each of the endpoints for which data are required according to Annexes V to VIII of the REACH Document and for each of the four tonnage bands, the data coverage resulting from the following four elements was established:

- Amount of available data
- Data promised under various programmes
- Impact of (Q)SAR, grouping and read-across methods
- Waiving (for other reasons as, e.g., unlikely exposure)

The resulting test needs for each endpoint and tonnage band were then estimated as total testing requirements minus the estimated data already covered by the four elements mentioned above. The REACH legislation strongly promotes and in the case of vertebrate animal testing actually requires sharing of test data between companies, and it will provide the tools needed. For this reason duplicate testing is assumed not to take place and if it were to take place, it is considered to be the result of a voluntary initiative from industry, and not as a direct consequence of the REACH legislation. Therefore, data sharing was assumed for each substance resulting in one test package per substance.

For a discussion of the various considerations regarding data coverage for each endpoint, the reader is referred to the paragraphs 2.1 to 2.7 in Pedersen et al. (2003). It is noteworthy, however, that for the developmental toxicity study (endpoint 6.7.2 in REACH) it is assumed that the test is carried out in two species. This has been taken into account in both the estimates of test animals needed as well as the cost estimates. However, it is likely to be an overestimation as a second study in a different species will often be waived and only required in case of a negative result in the first test.

The information on testing needs for the individual endpoints, combined with an estimate of the numbers of substances provided the matrix on the basis of which the need for vertebrate test animals was calculated. For the test animal estimate the following studies were considered (numbers indicate the REACH reference number):

6.2.1 *In vivo* eye irritation
6.3 Skin sensitisation
6.4.4 Further mutagenicity studies
6.5.1 Acute oral toxicity
6.5.2 Acute inhalation toxicity
6.5.3 Acute dermal toxicity
6.6.1 Short-term repeated dose toxicity
6.6.2 Sub-chronic toxicity
6.6.3 Long-term repeated dose toxicity
6.7.1 Developmental toxicity screening study
6.7.2 Developmental toxicity study
6.7.3 Two-generation reproductive toxicity study
6.9 Carcinogenicity
7.1.3 Short-term fish toxicity
7.1.6 Long-term fish toxicity
7.3.2 Accumulation in aquatic species
7.6 Long-term bird toxicity

NB: for Toxicokinetics (endpoint 6.8.1) the legislative text assumes 'assessment of the toxicokinetic behaviour of the substance to the extent that can be derived from the relevant available information'.

To estimate the total amount of test animals anticipated as a consequence of REACH, the number of test animals needed for each endpoint study had to be established. For the environmental studies, information was provided by a Danish consultancy firm (DHI). For all other studies, a Dutch institute (TNO) has been consulted on how many animals are normally (average) used for the different toxicity tests, including the minimum and maximum range. Both firms are considered to have extensive experience with carrying out (eco) toxicology studies and/or experience in preparing dossiers for submission to regulatory authorities. The estimates on the mammalian and non-mammalian test animals (birds, fish and fish eggs) required per study type/endpoint are provided in Appendix I.

To clarify how the average number and the range (min – max) of test animals anticipated to be used has been calculated the following should be noted. As the testing guidelines are not always explicit on how a toxicity study should be conducted, the numbers of test animals required for a certain endpoint study might vary. Most figures in Appendix I are based on the content of the test guidelines. Where an expert judgement was employed for interpretation or providing a typical animal use estimate, this is indicated. In addition, where more than one test was available for the same endpoint, the figure representing the most commonly used endpoint study was applied in the calculation (light blue background). Finally, in some cases the 'average' number of test animals used equals the 'maximum' number of test animals used. This is where a limit or minimal test (with fewer animals) cannot provide the requested / intended information and a full test would be used. More details are available in the footnotes of the Table in Appendix I.

Regarding the calculated numbers of test animals per endpoint the following should be noted. In the case of *in vivo* skin and eye irritation it has not been taken into account that the test animals can be re-used for new studies. Also, the amount of animals anticipated for range finding has not been taken into account, as it is assumed that for filling in the currently existing data gaps, the studies have to be carried out for substances for which some information on the toxicity of the substance is already available. Therefore, range finding studies would be needed in only a limited number of cases. It should also be noted that for endpoint 6.7.2, the developmental toxicity study, it has been assumed that this study will be carried out in two species. This we

consider a conservative approach, especially taking into account the large contribution of this endpoint to the total amount of test animals required, as the second study is required only when the outcome of the first test is negative. It should be noted that for the two-generation reproductive toxicity study and the developmental toxicity study, offspring have not been taken into account.

The extent to which (Q)SAR and read-across can be used as an alternative to animals test has been demonstrated in the US Challenge Program for HPV substances (Bradbury et al. 2004) (**Table 2.1**). Of the data needed on human health endpoints, 50% was covered by previously unpublished studies submitted by the industry whereas 44% of the data was estimated by use (Q)SARs and read-across methods (in fact 88% of the remaining data gap). Only 6% therefore needed to be obtained by testing. For environmental data, 58% of the missing data was available as unpublished studies, 35% were estimated by use of (Q)SARs and read across methods (in fact 83% of the remaining data gaps) and 7% were obtained from tests.

Table 2.1 Experience from the US HPV Challenge Program (Auer, 2004)

	Human health	Environmental effects
Adequate studies	50 %	58 %
Estimation	44 %	35 %
Testing	6 %	7 %

Three different scenarios were applied for estimating the impact of alternatives to animals testing: 1) a standard scenario reflecting the current practices on acceptance of (Q)SAR, grouping, read-across and possibilities for waiving, but not taking into account possible new techniques, 2) a scenario assuming minimal acceptance, and 3) a scenario assuming maximum acceptance of these methods (including techniques to be developed in coming years). The scenarios are described in detail in Pedersen et al. (2003) and the main assumptions regarding use of these alternatives methods are summarised below.

In estimating the number of test animals assuming the 'standard' scenario of (Q)SAR, grouping, read-across and possibilities for waiving, it was assumed that for 70% of the substances produced in < 1,000 tonnes/year (non-HPVCs), (Q)SARs and read-across will be accepted where no data are available. This is about 10 – 20% lower than the actual use of these techniques in the U.S. HPV Challenge Program (Bradbury et al., 2004). Furthermore for the endpoints not covered by the U.S. HPV Challenge Program, but where estimation techniques have already been developed, an assessment was made by the Danish EPA on the quality of the (Q)SARs for the different endpoints. Depending on the scores granted by the EPA, ranging from good, fair to poor, it was assumed that respectively 60%, 30% and 0% of the test needs could be covered by (Q)SARs.

In order to calculate the total number of test animals in the scenario assuming minimal use of (Q)SAR, grouping, read-across and possibilities for waiving, similar levels of acceptance of (Q)SARs were used as in the business impact study which was carried out by RPA (2003). Further details can be found in Pedersen et al. (2003).

For calculating the scenario assuming maximum acceptance of (Q)SAR, grouping, read-across and possibilities for waiving it was assumed that the approach already used today under the U.S. HPV Challenge Program will also be accepted in the EU. Hence, the same acceptance probability for the endpoints[1] covered was used for non-HPVCs as for the HPVCs (substances

[1] The applied acceptance of (Q)SAR and read-across (70%) was lower than in the US-EPA HPV Challenge Program

produced in > 1,000 tonnes/year). For the endpoints not covered by the U.S. HPV Challenge program, but where estimation techniques have already been developed, scores were assigned based on the assessment by the Danish EPA. These ranged from good, fair to poor, leading to the assumption that 80%, 40% and 10%, respectively, of the test needs can be covered by (Q)SARs.

The cost estimate is based on the approach used by Pedersen et al. (2003) and applies the estimated number of tests required under REACH under the assumptions regarding alternatives to animal tests discussed above. The costs of the individual tests are taken from the Business Impact Study carried out by RPA (2003) unless noted otherwise in the spreadsheets (available from the ECB website).

where (Q)SAR/read-across acceptance was about 80-90%.

3 RESULTS

The estimated number of tests needed for the different data endpoints, expressed as the percentage of the total number of phase-in substances, is presented in **Figure 1**. The estimate takes into account the basic information requirements for each tonnage band, the already available tests, the tests industry has already committed to, the possible use of (Q)SARs, grouping and read-across and the possibilities for waiving.

Based on these assumptions it is estimated that the skin sensitisation test is the test that needs to be conducted for most of the phase-in substances (35%) followed by further mutagenicity tests (22%) and the eye irritation test (18%). Other endpoints involving vertebrate test animals are required for 10% or less of the total number of phase-in substances.

Table 3.1 provides an overview of the estimated test animal needs over an implementation period of 11 years for the three scenarios described in Section 2. The min-max range indicates the corresponding calculations using 'minimum test animals' per study and 'maximum test animals' respectively for the different end point studies (see Table in Appendix I).

Table 3.1 Test animal needs under REACH (in millions) over an implementation period of 11 years.

Scenario	Average no. animals	min-max[3]
Mammalian		
Standard use of (Q)SAR etc.[1]	2.4	2.0 - 2.8
Min. use of (Q)SAR etc.[1]	3.5	2.8 - 4.1
Max. use of (Q)SAR etc.[1]	1.9	1.6 - 2.2
Non-mammalian[2]		
Standard use of (Q)SAR etc.[1]	0.23	0.19 - 0.27
Min. use of (Q)SAR etc.[1]	0.40	0.35 - 0.51
Max. use of (Q)SAR etc.[1]	0.16	0.13 - 0.18
Total test animals		
Standard use of (Q)SAR etc.[1]	2.6	2.2 - 3.1
Min. use of (Q)SAR etc.[1]	3.9	3.2 - 4.6
Max. use of (Q)SAR etc.[1]	2.1	1.7 - 2.4

1. (Q)SAR, grouping, read-across and possibilities for waiving
2. Birds, fish and fish eggs
3. Based on estimated minimum and maximum animal use per study

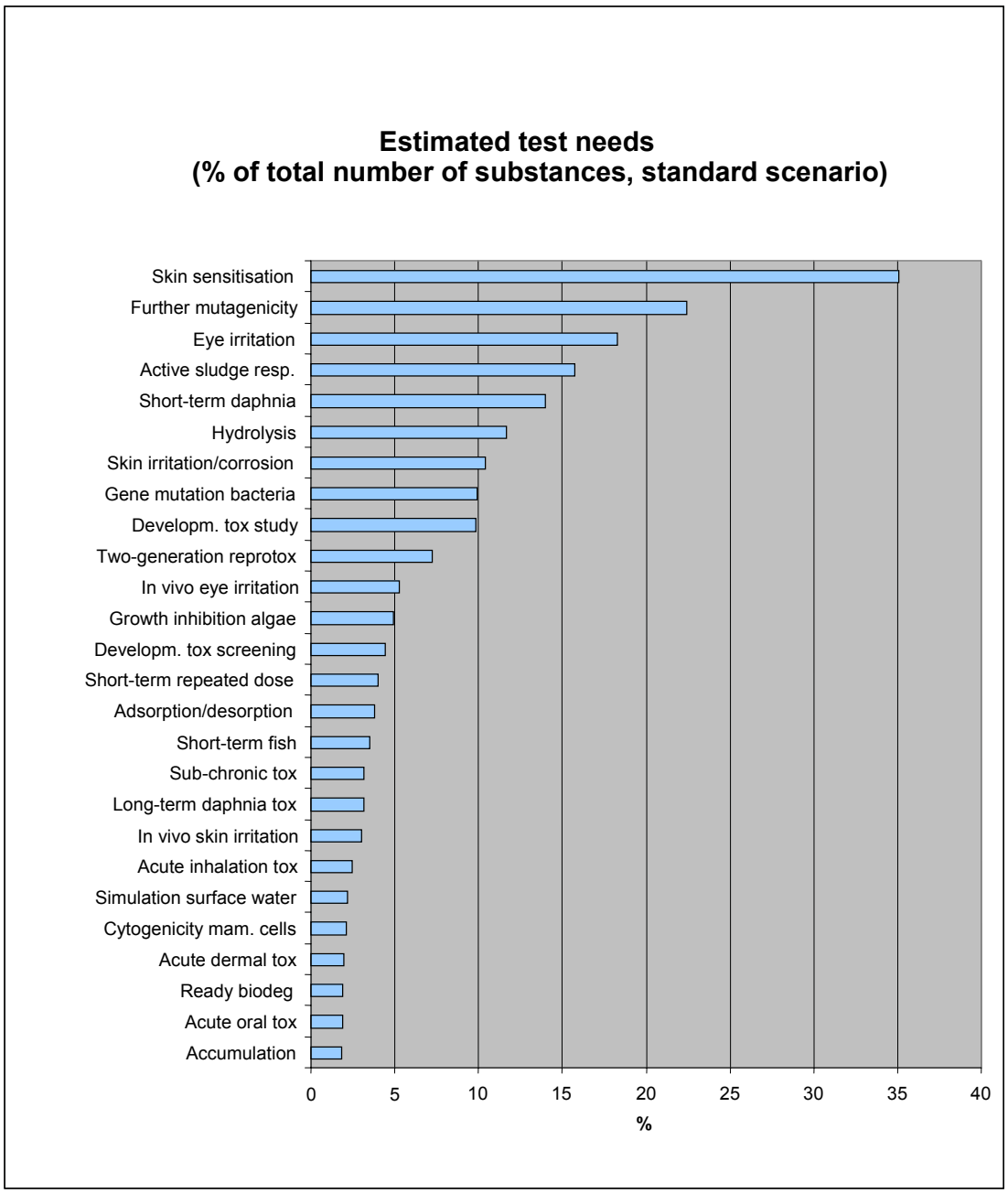


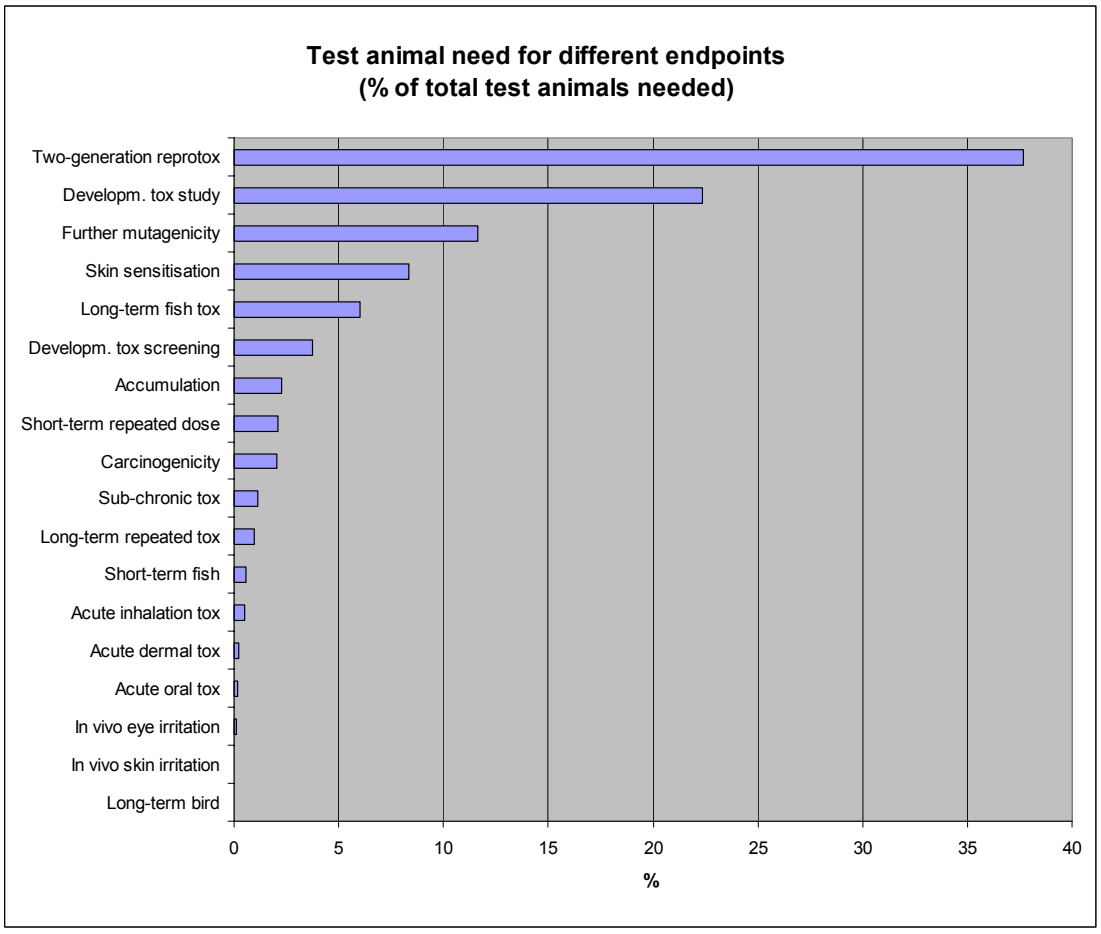
Figure 1 Estimated percentage of the total number of phase-in substances that will need to be tested for the different endpoints.

The results in **Table 3.1** show that when assuming the use of alternatives to animal testing corresponding to the 'standard' scenario described in the previous section, the total sum of mammalian and non-mammalian animals needed under REACH is estimated at 2.6 million (2.2-3.1). Of this total, 2.4 million animals will be mammalian and 0.23 million non-mammalian. As mentioned earlier, this calculation uses the assumption that the developmental toxicity study is carried out in two species. If only one developmental toxicity study is carried out the number of mammalian test animals would amount to 2.1 million (1.7 – 2.4).

Table 3.1 also indicates the effect of a best-case scenario (assuming maximum use of (Q)SAR, grouping, read-across and possibilities for waiving) versus a worst-case scenario (assuming minimum use of these methods). If the use of alternative methods is applied and accepted more extensively by regulatory authorities the number of mammalian test animals could be reduced from 4.1 to 1.6 million (a difference of about a factor 2.6) and the number of non-mammalian test animals from 0.51 to 0.13 million (a difference of a factor of almost 4). Hence the potential impact of using intelligent testing strategies on the total number of test animals required under REACH is significant. The impact, however, is reduced due the lack of alternative methods for certain endpoints for which existing *in vivo* studies require a high number of test animals (e.g. reproductive toxicity testing).

Figure 2 provides an overview of the estimated test animal needs for the different endpoint studies in the implementation phase of REACH. This figure indicates a major reason for the limitation of alternative testing on the estimated use of test animals. Currently no alternatives to animal testing are available for the three main contributors to the overall test animal use (two-generation reprotoxicity, further mutagenicity (*in vivo*) studies, and developmental toxicity studies), which contribute with 72% to the total test animal need. For the next three main contributors (skin sensitisation, long-term fish studies, and accumulation) the acceptance of the (Q)SAR is considered to vary from 10 – 37%. This suggests that further exploration of alternative testing and promotion of the acceptance of existing alternative testing methods is strongly needed.

Figure 2 Estimated test animal need for the different endpoints.



4 DISCUSSION

4.1 TEST ANIMALS

The results presented in this report show that approximately 3.9 million additional test animals could potentially be used as a consequence of the introduction of REACH if the use of alternative methods is not accepted by regulatory authorities. However, a considerable reduction in animal use could be obtained if these techniques are applied more intensively. The impact of (Q)SAR, grouping, read-across and possibilities for waiving in place of test animals, is notable but limited if compared to the total number of test animals required under REACH, due to the lack of suitable alternatives to animal testing for the highest contributors (which accounts for 72% of the test animals used as a consequence of REACH).

The estimated total amount of animals used for experimental and other scientific purposes in 1999 was 9.8 million (COM(2003)). About 10% of these are used for toxicological and other safety evaluations. The remaining 90% are used in:

- biological studies of a fundamental nature;
- research and development of human medicine, dentistry and veterinary medicine
- production and quality control of human medicine, dentistry and veterinary medicine
- education and training
- diagnosis of disease
- and other purposes

The 10% of animals used for toxicological and other safety evaluations include those for safety evaluation of products and devices for human medicine and dentistry and for veterinary medicine. This includes about 150,000 animals used for testing of '*products/substances falling under the scrutiny of authorities concerned with safety of health and of the environment by chemical products'*, such as industrial chemicals and pesticides corresponding to about 1.5% of the test animals used in 1999.

Using the standard scenario, the estimated number of vertebrate test animals potentially needed for the implementation of REACH is 2.6 (2.2-3.1) million over a time period of 11 years, i.e. the time period for full implementation of REACH. This corresponds to about 240,000 animals per year, or 2 - 3 % of the test animals used in 1999.

The anticipated increase of overall test animal use as a consequence of REACH will last for a time period of 11 years, while knowledge on currently widely used chemicals that has been lacking for years will be acquired, enabling a safer use of chemicals for generations to come. After the implementation period, the testing requirements will return to a base level corresponding to the number of new registrations.

It should be noted that the use of test animals for new chemicals under REACH will actually be lower than under the existing legislation due to the reduced data requirements at the tonnage band under 10 tonnes per year. To illustrate this, the base set for new chemicals under the current legislation include *in vivo* acute toxicity, skin irritation, eye irritation, skin sensitisation, a 28-day repeated-dose toxicity study, and an acute toxicity test in fish. This amounts to about 109 test animals per chemical, according to Appendix I. The base set is required for all chemicals registered from 1 tonne and higher in the current New Chemicals legislation. In REACH the requirements are considerably reduced for the tonnage band 1– 10 tonne, where the only test requiring vertebrates is the skin sensitisation test (23 animals, according to Appendix I).

Currently, about 120 new chemicals are registered in this tonnage band each year. Under the new legislation, this would therefore reduce the animals required over an 11 year time period by about 144,000 animals, compared to the current chemical legislation. Moreover, at present most test animals are used for acute toxicity studies, while REACH focuses on long-term endpoints that are more relevant for the assessment of human health, i.e. reproductive toxicity and mutagenicity.

It should be emphasised that the estimates could be further reduced by including the further development and acceptance of (Q)SAR, grouping, read-across and possibilities for waiving as well as other approaches like thresholds for toxicological concern (Kroes et al. 2004), provided that this development is focused on the endpoints where the benefit in terms of reduction of animal use and costs is highest. Increasing the efficiency, cost-effectiveness and focus of the risk assessment process, using new scientifically-sound techniques for creating and interpreting relevant data is essential. Such an approach can lead to a significant reduction in direct testing costs and the use of animals and could speed-up the risk assessment process considerably (Bradbury et al., 2004).

4.2 TEST COSTS

The cost for the testing requirements in the implementation period of the final REACH proposal from October 2003 is estimated at 1476 Million EURO (range: 1,143-2,274 Million EURO, representing the different scenarios). This is slightly lower that estimated earlier based on Consultation Document from May 2003: 1561 Million EURO (range: 1180-2423 Million EURO) (Pedersen et al. 2003). The difference is mainly due to the reduced test requirements at the tonnage band from 1-10 tonnes per year, where the estimated test cost per substance is reduced from 12,100 EURO to 7,700 EURO. In both cases the calculations are made under the assumptions described in Section 3. The cost per substance in the higher tonnage bands remains more or less the same.

Of the estimated costs over 11 years at about 1.5 Billion EURO, 32% is attributed to the developmental toxicity studies, 25% to the two-generation reproductive toxicity studies, 9% to the *in vivo* mutagenicity studies (further mutagenicity), and 8% to the sub-chronic toxicity studies. Other endpoints account for less than 5% of the total costs each.

The testing requirements and the costs depend on the produced quantity of a substance. In the standard scenario, the total testing costs are distributed over the different tonnage bands as follows: 10% (1-10 tonnes/year), 25% (10-100 tonnes/year), 27% (100-1,000 tonnes/year), and 38 % (> 1,000 tonnes/year). Consequently the main financial burden will be on the substances with the highest tonnage levels.

As already indicated in Pedersen et al. (2003), the testing cost per produced tonne is much higher in the lowest tonnage bands, if the test costs are considered for an average substance. The test cost is 255 EURO/tonne for a substance produced with a volume of 3 tonnes/year and 7 EURO/tonne for a substance produced in 3,000 tonnes/year.

5 CONCLUSION

The current document describes the impact of implementing the REACH proposal of October 2003 on animals used for testing purposes as well as on the direct testing costs. Only the effect of REACH in addition to existing obligations of the current legislation or voluntary initiatives regarding submission of data on substances has been considered.

The estimates are based on the ECB's current understanding of how the testing strategy and adaptation rules will be implemented. The background and method as well as the estimated number of phase-in substances are the same as used by Pedersen et al. (2003). The information on number of test animals used in different tests was not, however included in the Pederson report. This means the estimates are based on the information available to the ECB regarding number of substances, availability of data, ongoing initiatives on providing data by industry and other sources, possibilities for use of (Q)SAR, grouping, read-across and waiving, or requiring tests based on current risk assessment considerations.

According to the estimates, the highest numbers of test are required for the endpoints skin sensitisation (35%), further (*in vivo*) mutagenicity studies (22%), and eye irritation (18%). Other endpoints involving vertebrate test animals are required for 10% or less of the phase-in substances.

The results presented in this report show that approximately 3.9 million additional test animals could potentially be used as a consequence of the introduction of REACH if the use of alternative methods is not accepted by regulatory authorities. However, a considerable reduction in animal use could be obtained if these techniques are applied more extensively. The standard scenario based on average acceptance of these methodologies indicates potential savings of 1.3 million test animals. Maximum acceptance of these techniques would even enhance this saving potential to 1.9 million test animals.

Of the total amount of test animals that could potentially be used under REACH, about 72% will be required for carrying out two-generation reproductive toxicity studies, developmental toxicity studies and further mutagenicity (*in vivo*) studies.

The estimated number of test animals required for the implementation of REACH is about 240,000 animals per year, which equals to 2 - 3 % of the test animals used in 1999 for experimental and other scientific purposes.

The anticipated increase of the overall test animal use will last for a limited time period of 11 years (the implementation period for REACH), enabling knowledge about currently widely used chemicals that has been lacking for years to be acquired, and providing better basis for safe use of chemicals for generations to come. Moreover, compared to the existing legislation, the need for test animals per registered substance is much lower in the tonnage band from 1 – 10 tonne per year, as specified in Section 4.1.

It is emphasised that the expected further development of (Q)SARs and improved acceptance of (Q)SAR, grouping, read-across and possibilities for waiving, will most likely reduce the presented estimates, e.g. by focusing the development of alternative methods on endpoints where the benefit will be highest in terms of reduced use of test animals as well as costs.

The direct testing costs resulting from the REACH proposal of October 2003 have been estimated at about 1.5 Billion EURO assuming use of alternatives to animal testing corresponding to the described standard scenario. The main difference from the estimates published earlier (Pedersen et al. 2003) is the lower price for tests per substance in the 1-10

tonnage band, where the costs is reduced from 12,100 EURO to 7,700 EURO, due to the reduced test requirements in Annex V. Almost 90% of the total direct testing costs are attributed to the human health studies. Furthermore, it is estimated that of these costs, about 32% is attributed to the developmental toxicity studies, 25% to the two-generation reproductive toxicity studies, 9% to the *in vivo* mutagenicity studies and 8% to the sub-chronic toxicity studies.

The testing requirements and the costs depend on the produced quantity of a substance: 10% (1-10 tonnes/year), 24% (10 -100 tonnes/year), 27% (100-1,000 tonnes/year), and 38 % (> 1,000 tonnes/year).

It should be noted for some of the most test animal intensive endpoints, no alternative tests are currently available. Therefore it is recommended that the current activities in the EU and the OECD as well as in industry and academia on the development, validation and adoption of both (Q)SAR methods and in-vitro tests are intensified and focused on endpoints that incur the most benefits from both a test animal and cost perspective.

The Joint Research Centre (JRC) Activity on (Q)SARs, established under the JRC Work Programme for 2003-2006 and coordinated by the European Chemicals Bureau (ECB) is one of the actions by the Commission that is aimed at promoting the implementation of (Q)SARs and other estimation approaches. In addition, during the implementation of the Commission's interim strategy for REACH, one of the REACH Implementation Projects (RIP 3.3) will focus on developing guidance on information requirements on intrinsic properties of substances, aiming in particular at developing intelligent testing strategies which should allow minimal use of test animals.

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Appendix 1 Estimation of the number of test animals (vertebrates) used per test

1. Note to the table: The testing guidelines are not always explicit on how a toxicity study should be set up and therefore some of the numbers might vary depending on the study set up and/or study laboratory. Most figures in the table are based on the content of the test guidelines. Where an expert/expert judgement is employed for interpretation or providing a typical animal use estimate, this is indicated.

2. Note to the table: In case of the possibility of a selection of one test out of several tests for the same endpoint, the figures presented in the row with the light blue background were used in the calculations.

3. Note to the table: In some cases the 'normal' number of test animals used equals the 'maximum' number of test animals used. In cases where a limit test can not provide the requested/ intended information a full test will be used.

		Method (Annex V and OECD)	Title	Min (Limit[2]) Control/treated	Max	Full Test Control/treated	Normally	Comments
6.1.1	*In vivo* skin irritation	B.4	Acute toxicity (skin irritation)	1	3	1 - 3	2[3]	
6.2.1	*In vivo* eye irritation	B.5	Acute toxicity (eye irritation)	1	3	1 - 3	2[3]	
	Skin sensitisation			16	25	25	23[3]	LLNA is the preferred test for REACH[4]
	Classical	B.6	Skin sensitisation	5/10	30	10/20	15[3]	

[2] limit test = usually this means one high (2000 mg/kg normally) dose group and one control group. Limit test ≠ Min, but normally they imply the same number of test animals. When such a test is available this is indicated between brackets '(limit)'.

[3] estimate after consultation with a Dutch test laboratory, TNO.

[4] The 'base set' required for Annex V and Annex VI (1 – 10 tonnes and up) under REACH is equal to the skin sensitization test (23 animals). The base set for New Chemicals (NC) required for all chemicals registered from 1 tonne and up consist of an *in vivo* acute toxicity test (two routes, oral 8 and dermal 10), skin irritation (2), eye irritation (2), skin sensitization (23), a 28-day repeated dose toxicity (50) and an acute toxicity test in fish (14), totaling 109 test animals.

	Method (Annex V and OECD)	Title	Min (Limit[2]) Control/treated	Max	Full Test Control/treated	Normally	Comments
LLNA	B.42	LLNA	4(+4)/12	25	5(+5)/15	23[3]	Currently carried out with controls (+ n), over time omitted
6.4 Mutagenicity							
6.4.4 *In vivo* mutagenicity studies (**one** of these tests is carried out)			25	50	50	50[3]	Min based on waiver for one sex
	B.11	Mutagenicity - In vivo mammalian bone-marrow chromosome aberration test	10/(10+10) (limit)	50	10+10/30+interim[5]	50	In some cases there is a waiver for one sex (- 25)
	B.12	Mutagenicity - In vivo mammalian erythrocyte micronucleus test	10/(10+10) (limit)	50	10+10/30+interim	30	Often the limit test is carried out
6.5 Acute toxicity							
6.5.1 By oral route (**one** of these tests is carried out)			3	9	6 - 9	8[3]	Usually B.1 tris is carried out
	B.1 bis	Acute toxicity (oral) fixed dose method	3/3	9	6 - 9	8[3]	
	B.1 tris	Acute toxicity (oral) – Acute	3	9	6 - 9	8[3]	

[5] interim = the additional animals added to the test group in case of possible interim sacrifices

	Method (Annex V and OECD)	Title	Min (Limit[2]) Control/treated	Max	Full Test Control/treated	Normally	Comments
		toxic class method					
6.5.2 By inhalation	B.2	Acute toxicity (inhalation)	10/10 (limit)	40	10/30	20[3]	Usually limit test is carried out
6.5.3 By dermal route	B.3	Acute toxicity (dermal)	5/5 (limit)	20	5/15	10[3]	Usually limit test is carried out
6.6 Repeated dose toxicity							
6.6.1 Short-term repeated dose toxicity study (28 days) (the most relevant test (exposure route) is carried out)			20	60	40 - 60	50[3]	Usually no limit test is used for sub acute
Oral	B.7	Repeated dose (28 days) toxicity (oral)	10/10 (limit)	60	10/30 + interim (=20)	50[3]	
Inhalation	B.8	Repeated dose (28 days) toxicity (inhalation)	NA	60	10/30 + interim (=20)	50[3]	
Dermal	B.9	Repeated dose (28 days) toxicity (dermal)	10/10 (limit)	60	10/30 + interim (=20)	50[3]	
6.6.2 Sub-chronic toxicity study (90-day) (only one of the following tests is carried out)			16	32	32	32[3]	In general B.27 is carried out
	B.26	Sub-chronic oral toxicity test.	20/20 (limit)	120	20(+20)/60 + interim (=20)	80[6]	

[6] IEH (2001): *Assessment of the Feasibility of Replacing Current Regulatory In Vivo Toxicity Tests with In Vitro Tests within the Framework Specified in the EU White Paper 'Strategy for an EU Chemicals Policy'* (Web Report W10), Leicester, UK, MRC Institute for Environment and Health (at http://www.le.ac.uk/ieh/ posted December 2001)

	Method (Annex V and OECD)	Title	Min (Limit[2]) Control/treated	Max	Full Test Control/treated	Normally	Comments
		Repeated dose 90 - day oral toxicity study in rodents					
	B.27	Sub-chronic oral toxicity test. Repeated dose 90 - day oral toxicity study in non-rodents	8/8 (limit)	32	8/24	8/24[3]	
	B.28	Sub-chronic dermal toxicity test: 90-day repeated dermal dose study using rodent species	20/20 (limit)	120	20(+20)/60 + interim (=20)	-	
	B.29	Sub-chronic inhalation toxicity test: 90-day repeated inhalation dose study using rodent species	20/20	120	20(+20)/60 + interim (=20)	-	
6.6.3 A long- term repeated toxicity study	B.30	Chronic toxicity test	8/24	160	Rodent 8/24 Non Rodent 40/120	160[6]	
6.7 Reproductive toxicity							
6.7.1 Screening for reproductive/-developmental toxicity	OECD TG 421	Reproductive/ developmental toxicity screening test	20/20	80	20/60	80[3]	

	Method (Annex V and OECD)	Title	Min (Limit[2]) Control/treated	Max	Full Test Control/treated	Normally	Comments
6.7.2 Developmental toxicity study	B.31	Teratogenicity test – rodent and non-rodent	never used	144	Non rodent : 24/72 females + 24/24 males Rodent: 16/32	100[3]	Pups not counted, (females arrive pregnant, no males)
6.7.3 and 6.7.4 Two-generation reproductive toxicity study	B.35	Two generation reproduction toxicity test	never used	448	2 x 28 (male + female)/ 2 x 28 x 3 x 2 generations	448[3]	Pups not counted
6.8 Toxicokinetics							
6.8.1 Assessment of the toxicokinetic behaviour of the substance to the extent that can be derived from the relevant available information	B.36	Toxicokinetics	Depends on study design and target, but single dose rat gives test animal use of 22	198[3]	22 - 198	60[3]	Min test not available. Animal use depends on test design, (single dose/-repeated dose/carcinogenicity/reprotoxic).
6.9 A carcinogenicity study	B.32	Carcinogenicity test	300	400	100/300	400[6]	
7.1 Aquatic toxicity							
7.1.3 Short-term toxicity testing on fish	C.1	Acute Toxicity for Fish	7/7 (limit)	42	7/35	14	
7.1.6 Long-term toxicity testing on fish							
7.1.6.1 Fish early-life stage (FELS)	OECD TG 210	Fish early-life	300	420[6]	60 (+60)/300	400[7]	Preferred test,

[7] estimate after consultation with a Danish test laboratory (DHI)

	Method (Annex V and OECD)	Title	Min (Limit[2]) Control/treated	Max	Full Test Control/treated	Normally	Comments
toxicity test (OECD 210)		stage (FELS) toxicity test					others tests not normally carried out
7.3.2 Bioconcentration in (one) aquatic species, preferably fish	C.13	Bioconcentration: Flow-through fish test	never used	108	108	108	
7.6 Reproductive toxicity to birds	OECD TG 206	Avian reproduction test	never used	70	70	70	

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