



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

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assessment of chemical substances pose
legal barriers for the use of alternatives
to animal testing?**

RIVM Letter report 2014-0148
M.B. Heringa et al.



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Colophon

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Publiekssamenvatting

Voor de beoordeling van risico's van chemische stoffen voor mens en milieu worden veelal dierproeven gebruikt. Alternatieven daarvoor mogen alleen worden ingezet als de wettelijke beoordelingskaders daar expliciet de mogelijkheid voor bieden. Het RIVM heeft bij tien van deze Europese beoordelingskaders onderzocht of dat het geval is. Bij negen van de tien kaders wordt naar alternatieve mogelijkheden voor dierproeven verwezen en doen zich dus geen wettelijke belemmeringen voor. Bij het tiende kader, voor de toelating van diergeneesmiddelen, is het onduidelijk: de wet vermeldt de mogelijkheid niet, maar in de onderliggende richtlijn (*guideline*), die volgens de wet moet worden nageleefd, worden wel alternatieven aangereikt. Dit maakt de juridische status van de mogelijkheid om ze in te zetten onduidelijk.

Uit het onderzoek blijkt ook dat het vooral technisch wetenschappelijke barrières zijn die de inzet van alternatieven voor dierproeven belemmeren, en niet zozeer wettelijke. Er bestaan bijvoorbeeld geen alternatieven voor bepaalde dierproeven, of ze zijn nog onvoldoende geschikt of gevalideerd. Aanbevolen wordt eraan te werken om deze praktische belemmeringen weg te nemen.

De studie signaleert daarnaast nog twee aandachtspunten: als eerste gaat het om het gebruik van resultaten van alternatieve methoden voor dierproeven bij de risicobeoordeling bij calamiteiten en bij de vaststelling van industriële locaties waar gevaarlijke stoffen aanwezig zijn. Specifieke dierproefresultaten nemen hier vaak een prominente plaats in. De resultaten van alternatieve methoden passen niet zonder meer in methodieken die in een aantal landen voor deze risicobeoordelingen worden gebruikt.

Daarnaast verdient de gevaarsclassificatie, etikettering en verpakking (CLP) van schadelijke stoffen aandacht. Het kader REACH, dat leidend is en waarvoor data voor de CLP worden gegeneerd, schrijft voor dat alternatieven mogelijk zijn mits deze geschikt zijn voor de CLP. Voor de CLP zijn echter voor sommige classificaties geen alternatieve methoden beschikbaar en de classificatie criteria beperken de mogelijkheid om alternatieve methoden te ontwikkelen.

De studie is in opdracht van het ministerie van Economische Zaken uitgevoerd, naar aanleiding van een motie in de Tweede Kamer bij de recente aanpassing in de Wet op dierproeven. Mogelijke wettelijke belemmeringen voor geneesmiddelen worden momenteel in een aparte RIVM-studie onderzocht.

Trefwoorden: alternatieven voor dierproeven, wettelijke belemmeringen, EU beoordelingskaders, risicobeoordeling

Abstract

For the assessment of risks of chemical substances for man and environment, animal studies are commonly performed. Alternatives for these studies may only be applied if the legal frameworks for the assessments explicitly offer a possibility for them. RIVM has analyzed ten of such European frameworks for assessment of chemical substances whether such a possibility is present. In nine of the ten frameworks, reference is made to the possibility to use alternative methods for animal tests and thus pose no barriers for them. In the tenth framework, for the acceptance of veterinary medicinal products, it is not clear: the Directive does not mention this possibility, but in the underlying, mandatory (but not legally binding) guideline alternative methods are suggested. This makes the legal status of the possibility to use alternative methods unclear in this framework.

The investigation also shows that it's mostly practical barriers that obstruct the use of alternatives for animal tests, and not so much legal barriers. There is, for example, a lack of alternatives for some animal tests, or they are not sufficiently suitable or validated. It is recommended to direct the attention to the removal of these practical barriers.

The study notices two other points of attention. The first concerns the use of results from alternative methods in the risk assessments for calamities and for the determination of industrial locations with hazardous substances. Specific animal test results are often of high importance there. The results of alternative methods do not directly fit into the calculation methodologies applied by some countries for these risk assessments.

Secondly, the classification, labelling and packaging (CLP) of chemical substances needs attention. The framework REACH, which is leading and for which the data used for CLP are generated, states that alternatives are possible, on the condition that the results of alternative methods are suitable for CLP. For some classifications, however, no alternative test methods are available and the classification criteria limit the use/development of alternative methods.

The study was performed under commission of the Dutch Ministry of Economic Affairs, because of questions from the Dutch Parliament when the new Law on animal testing was adjusted. Possible legal barriers for human pharmaceuticals are currently investigated in a separate RIVM study.

Keywords: alternatives for animal testing, legal barriers, EU frameworks, risk assessment

Contents

Contents – 7

Summary – 9

1 Introduction – 11

2 Method – 13

3 Legal barriers per regulation – 17

3.1 REACH – 17

3.2 Biocides – 18

3.3 Plant protection products – 19

3.4 Novel foods – 20

3.5 Food improvement agents – 21

3.5.1 Food enzymes – 22

3.5.2 Food additives – 22

3.5.3 Food flavourings – 22

3.6 Food contact materials – 23

3.7 Feed additives – 24

3.8 Veterinary medicinal products – 25

3.9 Cosmetics – 27

3.10 Detergents – 28

3.11 Dependent framework: CLP – 29

3.12 Dependent utility: derivation of OELs – 30

3.13 Dependent utility: emergency response planning and land use planning – 30

4 Discussion and conclusions – 31

5 Acknowledgements – 35

6 References – 37

Summary

The Dutch Parliament wants to be informed on the presence of legal barriers to apply alternatives to animal testing. To this end, the Ministry of Economic Affairs has commissioned RIVM to investigate whether such legal barriers exist in the regulatory frameworks for the toxicity and risk assessment of substances, excluding pharmaceutical substances for humans.

Ten regulatory frameworks were identified in which (eco)toxicological data of the substance need to be submitted. These ten frameworks in the area of industrial chemicals and food and product safety were analyzed for legal barriers to apply alternatives to animal testing for the required toxicological data. A distinction was made between legally binding texts in the EU (regulations, directives and decisions) and non-binding texts such as guidelines and commission recommendations. Any barriers found in the latter were considered to be practical barriers, not legal barriers.

Additionally, several "dependent" frameworks were considered, i.e. frameworks where (eco)toxicological data of a substance are not demanded, but are necessary for deriving limits and classifications in the framework. These frameworks are therefore dependent on the data collected through other frameworks.

Legal barriers for the use of alternatives to animal tests were identified only in the framework for classification, labelling and packaging (CLP; one of the dependent frameworks), and possibly in that for veterinary medicinal products (where the regulation requires animal tests, but one of the mandatory guidelines allows for alternatives). For other frameworks, the use of alternatives is solely hampered by practical barriers, such as availability, limited scientific robustness of the method, lack of predictivity for more complex systemic endpoints, lack of scientific validation, and a (resulting) lack of options for alternatives in guidance documents, which are all outside the scope of this study. These barriers, as well as costs aspects, have an impact on the regulatory acceptance. To stimulate the use of alternatives, resources and future studies would therefore be best focused on these practical barriers.

Attention is also needed for the necessary amendment of dependent frameworks and utilities such as for occupation exposure limits, emergency response planning and land use planning, in order to maintain sufficient protection of human and environmental health when alternatives come into play.

1 Introduction

On 22 September 2010, Directive 2010/63/EU of the European Union (EC, 2010) was published, which obliged the Netherlands to adapt its “Wet op de Dierproeven (WoD)” accordingly. The European Directive is based on the principle that animals have an intrinsic value which must be respected and that the use of animals for scientific and educational purposes should only be considered in case no alternative for the animal test is available. It aims to enhance the protection of animals used for scientific and educational purposes (e.g., by the 3Rs – refinement, reduction and replacement) and to enhance the competitiveness of research and industry in the European Union. A few requirements from the European Directive lead to changes in the Dutch situation and oblige the Dutch government to adapt the Dutch law “Wet op de Dierproeven”, such as:

- Projects for animal tests must be approved beforehand by an authorized body, resulting in a license for the project.
- Member states must install a national committee for the protection of animals that are used for scientific purposes.
- Every breeder, supplier and user must have sufficient employees that are competent and skilled in their tasks.
- Every breeder, supplier and user must install a body for animal welfare.
- Reports should include non-technical summaries.

The proposal for the adapted Dutch law was accepted by the Dutch Parliament on December 10, 2013, with several amendments. The discussion in the Parliament on the adapted law triggered the motion Heerema/Ouwehand (TK 2013-2014, 33 692nr. 34) from the Parliament requesting the Dutch government to investigate which legal barriers exist for the application of alternatives to animal tests and to adapt laws and regulations to facilitate the use of alternatives by the industry. The action plan, that was offered to the Parliament on February 28, 2014, by the Minister for Agriculture (Ministry of Economic Affairs), indicates that in 2014, it will be assessed which legal frameworks contain barriers for the use of alternatives for animal tests in the relevant domains of the Ministries of Economic Affairs, of Health, Welfare and Sport and of Infrastructure and the Environment. The Ministry of Economic Affairs subsequently asked RIVM to carry out this assessment for the toxicity testing of chemical substances, which is described in this report.

2 Method

More specifically, this report addresses the question “Which legal barriers exist for the use of alternatives for animal tests in the relevant safety assessment frameworks for chemical substances (in the area of industrial chemicals, food and consumer products), excluding human medicines?” The legal barriers in frameworks for human medicines are assessed separately in another study. To answer this question, all legal frameworks that require toxicological data for evaluation (and admission) of chemical substances were analyzed for possibilities and barriers for the use of alternatives (table 1).

Table 1. List of legal frameworks evaluated for legal barriers for the use of alternative methods for animal tests.

	Legal frameworks	Regulation or Directive
1	REACH	Regulation (EC) No 1907/2006
2	Biocides	Regulation (EC) No 528/2012
3	Plant protection products	Regulation (EC) No 1107/2009 Regulation (EC) No 283/2013 and Regulation (EC) No 284/2013)
4	Novel foods	Regulation (EC) No 258/97, under revision
5	Food improvement agents: a. Enzymes b. Additives c. Flavourings	Regulation (EC) No 1331/2008 Regulation (EC) No 1332/2008 Regulation (EC) No 1333/2008 Regulation (EC) No 1334/2008
6	Food contact materials	Regulation (EC) No 1935/2004
7	Feed additives	Regulation (EC) No 1831/2003 Regulation (EC) No 767/2009
8	Veterinary medicinal products	Directive 2001/82/EC, supplemented with changes in Directive 2004/28/EC and Directive 2009/9/EC
9	Cosmetics	Regulation (EC) No 1223/2009
10	Detergents	Regulation (EC) No 648/2004

This study is comparable to a previous analysis for legal barriers that hamper the use of the benchmark dose (BMD) approach (Brandon et al., 2013). The BMD approach can lead to a refinement and reduction of animal use and ten legal frameworks were analyzed for legal barriers to use this approach. The conclusion was that no barriers were found, but it was recommended that legislation, as well as concomitant guidelines, at any future revision should be adjusted to recommend explicitly the use of the best available approach for deriving a POD in general, currently being the BMD approach. The current assessment also searches for legal barriers, in approximately the same legal frameworks, but then for the use of any alternative to animal tests.

“Alternatives to animal tests” are defined here as methods in a broad sense that lead to refinement, reduction or replacement (3Rs) of animal tests. For a definition of animal tests, for which alternatives are needed, Directive 2010/63/EU (EC, 2010) on “the protection of animals used for scientific purposes” gives a description:

- any use, of an animal for experimental or other scientific purposes, or educational purposes, “which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.” “The elimination of pain, suffering, distress or lasting harm by the successful use of anaesthesia, analgesia or other methods” does not exclude such a procedure from being an animal test. The killing of animals solely for the use of their organs or tissues is excluded.
- with the following animals:
 - a) live non-human vertebrate animals, including:
 - (i) independently feeding larval forms; and
 - (ii) foetal forms of mammals as from the last third of their normal development;
 - b) live cephalopods (“inkfish”).
where a non-human vertebrate animal at an earlier developmental stage than described above, which is “allowed to live beyond that stage of development and, as a result of the procedures performed, is likely to experience pain, suffering, distress or lasting harm after it has reached that stage of development” is included as well.
- excluding normal veterinary practices, agriculture and animal husbandry. Under this definition from the Directive, the use of zebrafish embryo’s in a toxicity test up to 120 h after fertilization, for example, is not considered an animal test for which alternatives are needed, but can actually be an alternative, as the larval form of this fish is seen as not independently feeding yet in this time period (Strahle et al., 2012).

The current study was limited to the legal texts of the listed frameworks; possible jurisprudence from evaluations of individual substances was not included. There are three basic types of EU legislation: regulations, directives and decisions¹. A regulation is similar to a national law with the difference that it is applicable in all EU countries and/or was applicable in all EEC and/or EC countries. Directives set out general rules to be transposed into national law by each country as they deem appropriate.

A decision only deals with a particular issue and specifically mentioned persons or organizations. A distinction was made between the legally binding texts of regulations, directives, and decisions, and the non-binding texts in guidance documents, guidelines, and commission recommendations: barriers stated in the latter were not considered as legal barriers as they are not binding, but were considered to be practical barriers.

The study did not include possible barriers other than legal barriers in the use of alternatives, if they are legally allowed, such as practical barriers like availability of or access to alternative methods. The question whether alternative methods have a possible higher predictive value for humans is also beyond the scope of the present evaluation. Also, the question if certain information requirements can be waived if other information (e.g. from alternatives) is available, is not addressed. These are in-depth scientific questions in the field of “intelligent/integrated testing strategies” and these are beyond the scope of the current study. However, the legal texts are screened for barriers to apply waiving a.o., as waiving can reduce the number of animals used and is therefore seen as an alternative method.

Besides the listed frameworks, there are also safety assessment frameworks that require quantitative toxicological data, but derive this information from

¹ http://europa.eu/eu-law/decision-making/legal-acts/index_en.htm

other regulations or public databases. Hence, these regulations do not ask for specific animal tests. Examples are:

- the derivation of occupational exposure limits (OELs),
- the Regulation for Classification, Labeling and Packaging (CLP, 1272/2008/EC), and
- the derivation of Acute Exposure Reference Values (AERVs) such as the Dutch Intervention Values for dangerous substances.

Possible consequences of the use of alternative methods for the quality of the safety assessment in such frameworks are shortly addressed.

3 Legal barriers per regulation

3.1 REACH

Regulation (EC) No 1907/2006 (EC, 2006) describes the Registration, Evaluation, Authorization and restriction of CHemicals (REACH) and according to article 1 of the Regulation: is to ensure a high level of protection of human health and the environment, including the promotion of alternative methods for assessment of hazards of substances, as well as the free circulation of substances on the internal market while enhancing competitiveness and innovation. REACH registration covers the manufacture and use of all industrial chemicals when not already covered by other safety assessment regulations (e.g. Biocides Regulation).

Annex VII-X list the required information for the various tonnage bands, among which *in vivo* tests are specifically listed for some endpoints, e.g. for sensitization (requirement 8.3 in Annex VII, thus applying for all chemicals produced at > 1 tonne/year). Options to waive this test are given in column 2 of these requirement tables. In the example of the *in vivo* test for skin sensitization, the test can be waived if there is already sufficient information to classify for skin sensitization or corrosion, if the substance is a strong acid or base, or if the substance is flammable in air at room temperature. In addition to the options in column 2 in Annex VII-X, Annex XI provides waiving options, as described further below.

The use and promotion of alternatives to animal tests is an important aspect within REACH, exemplified by the aim given in recital 1: "This Regulation should also promote the development of alternative methods for the assessment of hazards of substances." The possibility to use alternatives is legally provided by Article 13.1: "Information on intrinsic properties of substances may be generated by means other than tests, provided that the conditions set out in Annex XI are met. In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example, *in vitro* methods or qualitative or quantitative structure-activity relationship models or from information from structurally related substances (grouping or read-across)." Furthermore, title III of REACH is named "Data sharing and avoidance of unnecessary testing" and provides explicit rules to avoid unnecessary testing of animals.

Annex XI states that a registrant may adapt the standard testing regime (given in Annex VII to X) if the results of alternative methods show that animal testing does not appear scientifically necessary. Listed alternative methods are: grouping and read-across, (Q)SARs, biokinetic models, *in vitro* tests, existing data, substance-driven, exposure driven testing, optimized *in vivo* tests and Weight-of-Evidence (WoE) based on several independent sources of information. Conditions for each alternative method are given, generally requiring that results are adequate for the purpose of classification and labelling and/or risk assessment, that the method is scientifically valid, and that adequate and reliable documentation of the applied method is provided. For example: "results are derived from a (Q)SAR model whose scientific validity has been established". Annex VI on information requirements even contains statements promoting the use of alternatives: "The registrant should also collect all other available and relevant information on the substance regardless whether testing for a given

endpoint is required or not at the specific tonnage level. This should include information from alternative sources (e.g. from (Q)SARs, read-across from other substances, *in vivo* and *in vitro* testing, epidemiological data) which may assist in identifying the presence or absence of hazardous properties of the substance and which can in certain cases replace the results of animal tests." And: "New tests on vertebrates shall only be conducted or proposed as a last resort when all other data sources have been exhausted."

Furthermore, according to article 117(3) of REACH: "Every three years the Agency, in accordance with the objective of promoting non-animal testing methods, shall submit to the Commission a report on the status of implementation and use of non-animal test methods and testing strategies used to generate information on intrinsic properties and for risk assessment to meet the requirements of this Regulation." The report of 2014 (ECHA, 2014) states that

a 'read-across' or category approach, by example, was used in up to 75% of analyzed dossiers for at least one endpoint" in the registrations held by ECHA on October 1, 2013, and shows that read-across has been applied for a certain percentage of dossiers for all human health and environmental endpoints. Regardless of whether these adaptations are ultimately accepted by ECHA in compliance checks (not covered by this report), this shows industry is readily applying this alternative method.

In conclusion, although specific *in vivo* tests are explicitly mentioned under the information requirements in the REACH Annexes for some endpoints, the Regulation provides the possibility to use alternatives when proven to be scientifically valid, and their results are adequate for the purpose of classification and labelling and/or risk assessment, and adequate and reliable documentation of the applied method is provided. Thus, there are no legal barriers to use alternatives to produce toxicological data.

3.2 Biocides

The registration of biocides is regulated in Regulation (EC) No 528/2012 (EC, 2012), the Biocidal Product Regulation (BPR). It concerns the placing on the market and use of biocidal products intended to protect humans, animals, materials or articles against harmful organisms by the action of active substances contained in the biocidal product. The purpose of the BPR is to improve the free movement of biocidal products while ensuring a high level of protection of both human and animal health and the environment.

Annex II and Annex III contain the information requirements for the preparation of the dossier for active substances and for the biocidal product accompanying an application for the approval of an active substance, respectively. *In vivo* tests are specifically demanded for several toxicological endpoints, for example sensitization, genotoxicity or reproductive toxicity. However, it is stated that "new tests involving vertebrates shall be conducted as the last available option to comply with the data requirements set out in this Annex when all the other data sources have been exhausted." Therefore, in light of the importance of reducing testing on vertebrates, column 3 of the tables in the Annexes provides specific rules for the adaptation of some of the data elements which might require testing in vertebrates. This does not apply to all data elements requiring *in vivo* information.

Furthermore, for biocidal products *in vivo* testing of the product is not required if there are valid data available on each of the components in the mixture

sufficient to allow classification of the product according to the rules laid down in Directive 1999/45/EC (EC, 1999) and Regulation (EC) No 1272/2008 (CLP; EC, 2008a), and synergistic effects between the components are not expected. Annex IV furthermore contains general rules for the adaptation of the data requirements. This Annex states that existing data based on weight of evidence, (Q)SARs, *in vitro* methods, and grouping of chemicals and read-across may be used instead of testing when they show that testing does not appear scientifically necessary and specific conditions are met. These conditions include a.o. that “the results are derived from a scientific validated model/method, the results must be adequate for the purpose of classification and labelling and risk assessment, the results have adequate and reliable coverage of the key parameters addressed in the corresponding test method, and the results cover an exposure duration comparable to or longer than the corresponding test method if exposure duration is a relevant parameter.” Also the applied method must be adequately and reliably documented.

In conclusion, although *in vivo* animal testing is explicitly mentioned under the information requirements for several toxicological endpoints, the Regulation provides the possibility to use alternatives to animal testing when these alternatives have been proven to be adequate and reliable. Thus, there are no legal barriers to use alternatives to produce toxicological data.

3.3 Plant protection products

The registration of plant protection products (PPP) is controlled in Regulation (EC) No 1107/2009 (EC, 2009a). It concerns the placing of PPPs on the market, which forms one of the most important ways of protecting plants and plant products against harmful organisms, including weeds, and of improving agricultural production. The purpose of this Regulation is “to ensure a high level of protection of both human and animal health and the environment and at the same time to safeguard the competitiveness of Community agriculture.” A foundation for the use of alternative methods to animal testing is provided in this Regulation. Recital 40 states that “the use of non-animal test methods and other risk assessment strategies should be promoted. Animal testing for the purposes of this Regulation should be minimised and tests on vertebrates should be undertaken as a last resort. Therefore, rules should be laid down to avoid duplicative testing and duplication of tests and studies on vertebrates should be prohibited. For the purpose of developing new plant protection products, there should be an obligation to allow access to studies on vertebrates on reasonable terms and the results and the costs of tests and studies on animals should be shared.” Articles 61 and 62 elaborate further on rules on avoidance of duplicative testing and sharing of tests and studies involving vertebrate animals, respectively.

Regulations (EU) 283/2013 (EU, 2013a) and 284/2013 (EU, 2013b) set out the data requirements for active substances and plant protection products, respectively, but are subordinate to Regulation (EC) No 1107/2009. Paragraph 5 of the Annex, in both Regulations, discusses tests on vertebrate animals and states that these tests may only be used when no other validated methods are available. *In vitro* methods and *in silico* methods are specifically mentioned to be considered when designing experiments. In addition, reduction and refinement

methods are encouraged. Tests involving human and non-human primates are prohibited according to paragraph 5.3 in the Annex. Specific data requirements on mammalian toxicological and metabolism studies are described in part A, section 5 of the Annex, ecotoxicological studies in section 8, and residue studies in section 6. In these sections, *in vivo* animal testing is requested for several toxicological endpoints and for determination of residues in food of animal origin. For example, it is stated that “the short-term oral toxicity of the active substance to rodents (90-day), usually the rat, a different rodent species shall be justified, and non-rodents (90-day toxicity study in dogs), shall always be reported.” For other endpoints, such as for example skin or eye irritation, a tiered approach is prescribed starting with *in vitro* methods before testing *in vivo*. Another example of applying refinement and reduction is found for generational studies. A reproduction study in rats over at least two generations shall be reported, however the OECD extended one-generation reproductive toxicity study may be considered as alternative approach. For products the relevant calculation methods used for the classification of mixtures as laid down in Regulation (EC) No 1272/2008 (EC, 2008a) can be applied as an alternative approach. For tests to determine residues in animals fed with plants on which the plant protection product has been applied and from which animal products are meant for consumption (section 6), no alternative methods are mentioned. However, paragraph 5 provides an option to use alternative methods when available. Paragraph 6 of the Annex indicates that the specific test methods to be used are given in a separate document (Commission Communication 2013/C 95/01 and 2013/C 95/02 (EC, 2013c and d)), which are meant to be updated regularly when new (alternative) methods become available.

In conclusion, although *in vivo* animal testing is explicitly mentioned under the information requirements for several toxicological endpoints, the Regulations provide the possibility to use alternatives to animal testing. Some scientifically accepted alternatives are already given as possibilities in the annex of the two more detailed Regulations, and by regular updates of Commission Communication 2013/C 95/01 and 2013/C 95/02 (EC, 2013c and d), new alternatives that have been proven to be adequate and reliable are supposed to become accepted, too. Thus, there are no legal barriers to use alternatives to produce toxicological data.

3.4 Novel foods

Regulation (EC) No 258/97 (EC, 1997a) concerns the placing on the market of novel foods (NF) and novel food ingredients. The Regulation itself does not address animal testing. Article 4.4 lays down that the Commission must write down recommendations on the information necessary to support an application and the presentation of such information. The current Commission Recommendation 97/618/EC on scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients (EC 1997b) contains a section (3.6) on animal testing. In this section, scheme XIII describes the set of toxicological information which is needed to assess the NF. Here it makes a distinction between food for which substantial equivalence can be established and foods for which this is not possible. With substantial equivalence is meant that “a new food or food component is found to be substantially equivalent to an existing

food or food component". In that case, "it can be treated in the same manner with respect to safety" and if the NF does not contain new toxicants or changed levels of existing toxicants and is not expected to be allergenic, no additional tests are needed. If substantial equivalence cannot be established, "the safety assessment based on a case-by-case evaluation must consider following elements: consideration of the possible toxicity of analytically identified individual chemical components; toxicity studies *in vitro* and *in vivo* including mutagenicity studies, reproduction and teratogenicity, studies as well as long term feeding studies, following a tiered approach on a case-by-case basis; studies on potential allergenicity".

The text of the Commission Recommendation indicates that animal studies may be needed as part of the toxicological assessment of NF depending on the characteristics of the NF. However, a recommendation is not legally binding, a case-by-case approach is mentioned, and the Recommendation also mentions *in vitro* studies and a tiered approach.

EFSA's Scientific Committee on Food (SCF) has also published a guidance: the "Guidance on submissions for safety evaluation of sources of nutrients or of other ingredients proposed for use in the manufacture of foods" from 2001 (SCF, 2001). This guidance does not provide further details. Regarding toxicological data it says ".... The available data should be submitted in the first instance. The extent of the data needed will depend on safety considerations in relation to the fate of the source in the body. Any deviations from requirements already established for food additives ... should be justified."

In conclusion, there are no legal requirements for animal tests and therefore also no legal barriers to use alternatives to animal testing. A new proposal for an updated Novel Food Regulation is currently under discussion in the EC, but has not been accepted yet.

3.5 Food improvement agents

On December 16, 2008, several Regulations related to food enzymes, food additives and food flavourings, together called the "Food Improvement Agent Package" were adopted. Regulation (EC) No 1331/2008 lays down a common procedure for the authorisation of food additives, food enzymes and food flavourings (EC, 2008b). Also three more specific Regulations were adopted, i.e. Regulation (EC) No 1332/2008 on food enzymes, Regulation (EC) No 1333/2008 on food additives and Regulation (EC) No 1334/2008 on flavourings and food ingredients with flavouring properties (EC, 2008c, d, and e). None of these Regulations contain statements as to the principles and methodologies of hazard and risk assessment.

Regulation (EC) No 234/2011 (EC, 2011) lays down the implementation of Regulation (EC) No 1331/2008. Article 5 of this Regulation describes the general provisions on data required for risk assessment. According to this article, the applicant should take into account the latest guidance documents adopted or endorsed by the Authority available at the time of the submission of the application. The safety evaluation strategy and the corresponding testing strategy shall be described and justified with rationales for inclusion and exclusion of specific study and/or information.

Below the specific aspects for the risk assessment of food enzymes, food additives, and food flavourings are described.

3.5.1 *Food enzymes*

Article 8 of Regulation (EC) No 234/2011 elucidates on the specific data requirements for the risk assessment of food enzymes (EC, 2011). It states (in paragraph 1.1) that information shall be provided on biological and toxicological data with the core areas subchronic toxicity and genotoxicity covered (paragraph 2). This Regulation is amended by Commission Implementing Regulation (EU) 562/2012 which applies to food enzymes only and lays down derogation from submitting toxicological data in some specific cases and the possibility of grouping food enzymes under one application under certain conditions (EU, 2012). In case of 'low risk' food enzymes, the submission of toxicological data is not required.

EFSA has provided guidance on the submission of an application dossier for enzymes (EFSA, 2009a). Chapter 4 defines the toxicological data that is needed. As said, the core data set exists of subchronic data and genotoxicity data. For the assessment of genotoxicity, a tiered approach is recommended starting with in vitro testing followed by in vivo testing only when a positive result is obtained in vitro and it cannot be ruled out that the in vitro finding is relevant for the in vivo situation. For the assessment of systemic toxicity, a "subchronic oral toxicity study as described in OECD guideline 408 should be performed." Hence, animal testing seems mandatory. However, it is also stated that "test methods described by the OECD and other provisions adopted under European legislation are recommended" and "there may be circumstances under which it may be appropriate to deviate from the above mentioned core set. Such deviations include exemption from certain tests, or use of alternative protocols or use of alternative assays or tests. In such cases a scientific justification should be provided and additional types of considerations or mechanistic studies may be needed". These statements provide opportunities to use alternatives to animal testing when a robust scientific justification is given.

3.5.2 *Food additives*

Article 6 of Regulation (EC) No 234/2011 (EC, 2011) and a scientific statement of the ANS panel of EFSA (2009b) describe the specific data requirements for risk assessment of food additives. Biological and toxicological data should cover the following core areas: toxicokinetics, subchronic toxicity, genotoxicity, chronic toxicity/carcinogenicity and reproductive and developmental toxicity. Details on the testing strategies are described in the guidance for submission for food additive evaluations (EFSA, 2012). In this document, reference is made to the 3 R's: "In contrast to the Scientific Committee for Food (SCF) guidance document published in 2001, which describes core and supplementary toxicological studies, this guidance describes a tiered approach which balances data requirements against other considerations such as use and animal welfare. The Panel recommends that an integrated testing strategy, which may include alternative approaches, should be used to further support the risk assessment."

3.5.3 *Food flavourings*

Article 10 of Regulation (EC) No 234/2011 describes the specific data requirements for risk assessment of flavourings (EC, 2011). Biological and toxicological data should cover the following core areas: examination for structural/metabolic similarity to flavouring substances in an existing flavouring group evaluation (FGE), genotoxicity and – where applicable – subchronic toxicity, developmental toxicity and chronic toxicity and carcinogenicity. The

EFSA guidance (EFSA, 2010) provides details on the data requirements. In vivo genotoxicity data and data on subchronic toxicity, developmental toxicity and chronic toxicity are not needed for all flavouring agents. This depends on structural, metabolic similarity to flavouring agents in an existing flavouring group evaluation, the results of in vitro genotoxicity data, whether the flavouring agent is metabolized to innocuous products and whether the intake of the flavouring agent exceeds the threshold of toxicological concern.

It is however stated that “deviations from the requirements ... are acceptable if adequate scientific justifications are provided. Such deviations may include different testing strategies and/or approaches”. This statement might provide opportunity to use alternatives to animal testing when a robust scientific justification is given.

In conclusion, the Regulations concerning food improvement agents lay down that toxicological data is needed for authorization but does not elucidate if alternative methods may be used for that purpose. Specific data requirements are laid down in guidance documents, which include in most cases in vivo toxicity studies. Nevertheless, the use of a tiered approach and integrated testing strategies is promoted reducing animal testing. Also, the EFSA guidances seem to leave some room for alternatives to animal testing by stating that deviations to the guidance are acceptable on the condition that adequate scientific justifications are given.

3.6 Food contact materials

Food contact materials are regulated in Regulation (EC) No 1935/2004, and amended by Regulation (EC) No 596/2009 (EC, 2004a, consolidated version). This Regulation aims “to ensure the effective functioning of the internal market in relation to the placing on the market in the Community of materials and articles intended to come into contact directly or indirectly with food, whilst providing the basis for securing a high level of protection of human health and the interests of consumers.” It applies to materials and articles in their finished state, which are already, are intended or can reasonably be expected to be brought into contact with food.

Article 9 describes the application requirements for authorizing a new substance. An application dossier must be submitted containing amongst others a technical dossier “containing the information specified in the guidelines for the safety assessment of a substance to be published by the Authority”. When such guidelines are not yet published by the Competent Authority, applicants can use the ‘Guidelines of the Scientific Committee on Food for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation’ (EFSA, 2008).

In this guidance document, data requirements are set. The requirements for the extent of the toxicological dataset depend on the level of migration of the substance. If the substance will not migrate into food at a higher level than 0.05 mg/kg food, it is not required to perform studies in animals; only three genotoxicity studies *in vitro* will suffice (OECD 471, 473 and 476) if, at least, all three tests are negative. In case of a positive *in vitro* genotoxicity study, further mutagenicity tests, including *in vivo* assays, may be required to elucidate the genotoxic potential of the substance. If the substance migrates in more than 0.05 mg/kg food, additionally a 90-day repeated dose study (OECD 408, study

in rodents) is required. If the migration is higher than 5 mg/kg food, data requirements are even extended with a second 90-day repeated dose study in another species, studies on reproduction in one species, and developmental toxicity, normally in two species, and studies on long-term toxicity/carcinogenicity, normally in two species. These studies should be carried out according to prevailing EU or OECD guidelines, including "Good Laboratory Practice". No possibilities to use alternative testing are mentioned in the guideline. The current Dutch law applying to food contact materials (i.e. non-plastics) refers also to the EFSA Note for Guidance for data requirements.

In conclusion, the legislation on food contact materials does not prescribe which toxicity tests are required for authorisation, but requires that the guidance is followed. The guidance document prescribes animal tests and does not mention the possibility to use alternatives. Therefore, there is no true legal requirement for animal tests, but indirectly there is no way to avoid them. Indirectly, this poses a barrier to apply alternatives, which could be removed by adapting the guidance document of EFSA.

3.7 Feed additives

Feed additives is a collective name for additives to feed and feed with special purposes (diet feed). As diet food for humans is also not considered in this analysis, diet feed was not further considered either. The placement of additives to feed on the market is laid down in Regulation (EC) No1831/2003 (EC, 2003). The purpose of this Regulation is "to establish a Community procedure for authorising the placing on the market and use of feed additives and to lay down rules for the supervision and labelling of feed additives and premixtures in order to provide the basis for the assurance of a high level of protection of human health, animal health and welfare, environment and users' and consumers' interests in relation to feed additives, whilst ensuring the effective functioning of the internal market." When a new additive or a different use of an additive is sought to be authorised, an application dossier must be submitted. There are no direct statements on how the information must be gathered, but it is stated that "the feed additive shall not have an adverse effect on animal health, human health or the environment" (Article 5(2)) and that "a copy of the studies which have been carried out and any other material which is available to demonstrate that the feed additive satisfies the criteria laid down in Article 5(2) and (3)" shall be submitted.

Regulation (EC) No 429/2008 contains the detailed rules for implementation of Regulation No 1831/2003 (EC, 2008f). The contents of an application dossier should fulfil the general requirements as set out in Annex II. For some feed additives the specific requirements in Annex III are to be satisfied (article 3(1)). Annex II states that "the determination of physico-chemical, toxicological and eco-toxicological properties must be performed in accordance with the methods established by Council Directive 67/548/EEC, as last amended by Commission Directive 2004/73/EC, or with updated methods recognised by international scientific bodies. The use of methods other than these must be justified". Specifically on alternatives to animal testing, it says "the use of in vitro methods or of methods refining or replacing the usual tests using laboratory animals or reducing the number of animals used in these test shall be encouraged. Such

methods shall be of the same quality and provide the same level of assurance as the method they aim to replace." On the other hand, further on in Annex II and III, specific *in vivo* toxicity tests are mentioned for the safety assessment and referred to as "shall be carried out" or "shall be performed" and the application dossier should be compliant with these requirements as is stated in article 3. In addition, it is stated that a toxicological NOAEL must be established. Furthermore, if necessary, ADIs should be established and MRLs should be set in accordance with procedures for residues of veterinary medicinal products.

In conclusion, for feed additives, specific animal tests are demanded for several toxicological endpoints, but the use of the 3Rs principle is encouraged as long as the alternatives are of the same quality and provide the same level of assurance as the test they replace. Thus, there are no legal barriers for the use of alternatives to animal testing in the regulations within the authorisation process of feed additives.

3.8 Veterinary medicinal products

Directive 2001/82/EC, as amended by Directive 2004/28/EC and Directive 2009/9/EC, and currently under revision, regulates the marketing of veterinary medicinal products in the EU (EC, 2001, consolidated version). Veterinary medicinal products (VMP) must be authorised by a competent authority in a member state before they can be marketed in that member state. Simultaneous authorisation in more member states can be applied for by mutual recognition, decentral or central procedures. According to Article 12, "the application for marketing authorisation shall include all the administrative information and scientific documentation necessary for demonstrating the quality, safety and efficacy of the veterinary medicinal product in question." It is further stated that the application shall contain "results of pharmaceutical (physico-chemical, biological or microbiological) tests; safety tests and residue tests; pre-clinical and clinical trials; tests assessing the potential risks posed by the medicinal product for the environment."

Annex I of Directive 2001/82/EC, as amended, sets further information requirements for these tests, subdivided in requirements for immunological VMPs and VMPs other than immunological. For VMPs other than immunological, tests are required for assessing:

- 1) the pharmacology and toxicology (repeated-dose, reproduction, developmental, genotoxicity etc.) of the active substance
- 2) efficacy of the product in target animal
- 3) the target animal safety (i.e. tolerance) of the product
- 4) residue levels tests in target animal species in the case the VMP is intended for food producing animals, to determine to what extent residues may persist in foodstuffs produced from these animals, including the determination of a withdrawal period.

The precise tests that should be performed are not mentioned, but phrases like "A developmental toxicity test in the rat is required." (3.4.2. in chapter I of Part 3 of Annex I) and "In the case of substances or veterinary medicinal products intended for use in food-producing animals, repeat-dose (90 day) toxicity testing shall be performed in a rodent and a non-rodent species in order ..." (3.2. in chapter I of Part 3 of Annex I) indicate *in vivo* testing is mandatory.

For immunological VMPs, safety and efficacy tests in the target species are required to show the potential risks under the proposed conditions of use in animals and to demonstrate its efficacy. User safety is discussed based on these tests; no toxicity tests in laboratory animals are required for immunological VMPs. Initially, batch safety tests in the most sensitive target species are required, to ensure the efficacy and safety of a batch. Residue studies are commonly not required, only in case of possible residues in foodstuffs.

As already stated, Directive 2001/82/EC, as amended, does not state the precise tests that should be performed to fulfil the information requirements. It is stated that "...the scientific guidelines relating to the quality, safety and efficacy of veterinary medicinal products published by the European Medicines Agency (Agency) and the other pharmaceutical Community guidelines published by the Commission in different volumes of "The rules governing medicinal products in the European Union" " should be taken into account when assembling a dossier. These guidelines can be found on the EMA website². The data requirements and thus the guidelines for the efficacy and pharmacology were not part of the scope of this study and were thus not analysed.

To evaluate the safety of residues of veterinary drugs in human food and for target animals, the European Medicines Agency (EMA) has made numerous guidance documents, many of which are in harmony with the Veterinary International Conference on Harmonization (VICH). One important example is the guideline on a general approach to testing for safety of residues in human food (EMA 2009). The 3R's principle is an important issue for the VICH, which is made clear by the statement in the objective of this guideline that "VICH seeks to minimize animal testing by supporting the 3R's principle. One of the expressed goals of VICH is to strive to eliminate repetitious and unnecessary testing through harmonization of regulatory requirements for the registration of veterinary products, a goal that undoubtedly leads to a reduction in the number of animals used for product development and registration. Whenever possible, flexibility, minimum number of animals, as well as alternative in vivo and in vitro tests have been recommended". In addition, VICH reviews the guidelines periodically to investigate whether the guidelines can be amended "to assure they conform to the most recent alternative testing developments". A foundation for applying alternatives to in vivo testing is made by the remark that "the guidelines do not preclude the possibility of alternative approaches that may offer an equivalent assurance of safety, including scientifically based reasons as to why such data may not need to be provided". This shows that attention is paid by EMA to the worldwide developments in alternatives to animal testing in the veterinary medicinal products evaluation and authorization processes.

In conclusion, the Directive seems to require specific laboratory animal tests and does not mention the possibility to use alternative methods, but does state that the guidelines should be taken into account. In the guideline (Safety: general approach), it is specified that minimal animal testing is strived for and that, whenever possible, alternative tests have been recommended and will not be precluded when offering an equivalent assurance of safety.

The mentioning of specific laboratory animals to be used may suggest that there is a legal barrier for the use of alternatives, but indirectly this barrier may be uplifted by the mandatory guidelines. Currently, the legal status of cross referencing to guidelines is unclear.

²

http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000173.jsp&mid=W00b01ac058002d89a

3.9 Cosmetics

In Europe, the safety of cosmetic products is regulated by Regulation (EC) No 1223/2009 on cosmetic products, which succeeds the Cosmetic Products Directive (76/768/EEC) since July 2013 (EC, 1976 and 2009c). The Cosmetic Products Regulation aims at ensuring a high level of protection of human health and requires manufacturers or importers to take responsibility for the safety of their cosmetic products under normal or reasonably foreseeable conditions of use. Environmental concerns related to cosmetic products or its ingredients and risks for workers in the manufacture of the cosmetic ingredients and products are not covered by the Cosmetic Products Regulation but should be considered through the application of REACH.

The safety of cosmetic products is supported by prohibitions and restrictions of some cosmetic ingredients, as listed in Annex II and III respectively. Specific attention is given to substances for which concerns exist with respect to human health, including (hair/skin) colorants, preservatives and UV-filters. Such substances must be listed in in the Annexes IV, V and VI, respectively, in order to be allowed in cosmetic products.

With respect to animal testing, the Cosmetic Products Regulation has made a firm statement.

Since 11 March 2009, the Cosmetic Products Regulation prohibits the placing on the market of cosmetic products where the final formulation, the ingredients or combination of ingredients have been tested on animals after this date (art 18.1, Cosmetic Products Regulation). The performance of animal testing of finished cosmetic products or (combination of) ingredients within the EU is also prohibited by the Cosmetic Products Regulation. Derogation from this ban was granted until 11 March 2013 in order to test repeated-dose toxicity, reproductive toxicity and toxicokinetics (art 18.2, Cosmetic Products Regulation).

The Regulation states that animal testing *must* be replaced by alternative methods that are accepted and validated by the OECD. Only in exceptional circumstances, Member States can request the Commission to grant a derogation, but only after the Scientific Committee (SCCS) is consulted and only if there is potential for a serious health concern and the specific ingredient is widely used and cannot be replaced.

It is noted that as of yet, to the knowledge of the authors of this report, no cosmetics have been approved on the basis of non-animal tests only.

The chemical ingredients applied in cosmetics may, however, also need to be registered under REACH. This has created some uncertainty about whether testing on animals can take place in order to comply with REACH, or whether it should not, in order to comply with the Cosmetics Regulation. A news article of 27 October, 2014, on the ECHA website³ provides an explanation: "The European Commission, in cooperation with ECHA, has now clarified the relationship between the marketing ban and the REACH information requirements as follows:

- Registrants of substances that are exclusively used in cosmetics may not perform animal testing to meet the information requirements of the REACH human health endpoints, with the exception of tests that are done to assess the risks to workers exposed to the substance. Workers

³ http://echa.europa.eu/view-article/-/journal_content/title/clarity-on-interface-between-reach-and-the-cosmetics-regulation (accessed 27 November 2014)

in this context, refers to those involved in the production or handling of chemicals on an industrial site, not professional users using cosmetic products as part of their business (e.g. hairdressers).

- Registrants of substances that are used for a number of purposes, and not solely in cosmetics, are permitted to perform animal testing, as a last resort, for all human health endpoints.
- Registrants are permitted to perform animal testing, as a last resort, for all environmental endpoints.

Therefore, the testing and marketing bans in the Cosmetics Regulation do not apply to testing required for environmental endpoints, exposure of workers and non-cosmetic uses of substances under REACH. Registrants of substances registered exclusively for cosmetic use will still have to provide the required information under REACH wherever possible, by using alternatives to animal testing (such as computer modelling, read-across, weight of evidence etc.)."

In conclusion, as the Cosmetic Products Regulation prohibits the use of animal testing on cosmetic products or its ingredients for human health endpoints, alternative test methods *must* be used to produce toxicological safety data. Thus, the Cosmetic Products Regulation contains no legal barriers, but rather a legal obligation to use alternatives for animal testing, which need to be validated and accepted nonetheless. There where animal testing is permitted for simultaneous compliance with REACH, REACH offers opportunities for use of alternatives. Thus, also in these cases, no legal barriers exist.

3.10 Detergents

Regulation (EC) No 648/2004 (EC, 2004b) on detergents aims "to achieve the free movement of detergents and surfactants for detergents in the internal market while, at the same time, ensuring a high degree of protection of the environment and human health." For this purpose, it considers mainly the biodegradability of ingredients, the presence of fragrance allergens and the content of phosphates in detergents. Animal tests are required in case the obligatory biodegradability tests show that a substance is not sufficiently biodegradable. Article 4 states that surfactants and detergents can only be placed on the market if they meet the ultimate aerobic biodegradability criterion given in Annex III, which is 60 or 70% mineralization in 28 days depending on which of the listed methods is used. If a substance does not meet this criterion, articles 5 and 9 describe that test results on the primary biodegradation (Annex II) must be submitted in an application for derogation, as well as the information described in Annex IV. Annex IV states that at least information on identity, use and potential recalcitrant metabolites shall be provided. Toxicity tests (point 4.2.2 in Annex IV) on fish, daphnia, algae and bacteria "should be requested only where such information is necessary and proportionate", in order "to avoid unnecessary animal testing". According to article 5.2, such tests shall be carried out on the basis of a tiered approach defined in a guidance document. This guidance document can be found in the Commission Recommendation C (2005) 5677 (EU, 2005), which requires PNEC values of the parent substance, based on "typically acute fish, daphnia and algae toxicity as a minimum". For metabolites, it prescribes the use of non-test or non-fish test methods first, with the fish test only necessary in case the alternative methods cannot show

sufficiently low ecotoxicological risks. Only the tests on fish are regarded as animal tests for which alternatives are desired. According to this guidance, alternative methods can thus be used for the metabolites, but not so clearly for the parent substance. However, due to the text in Annex IV and the word “typically” for the performance of, among others, the acute fish test, in the Commission Recommendation, it does appear possible to use alternative methods also for the parent substance. A more clear text on this in the Commission Recommendation would be helpful to show this possibility.

In conclusion, this legal framework requires an animal test for not sufficiently biodegradable substances and only where such information is necessary and proportionate. This is not further defined in the Regulation, but in the mandatory Commission Recommendation. Although this guideline does suggest an acute fish test needs to be performed for the parent substance, the Regulation offers a possibility to use alternatives in Annex IV. Thus, there are no legal barriers for the use of alternative methods in the framework, but some clarification in the Commission Recommendation would be helpful.

3.11 Dependent framework: CLP

Regulation (EC) No 1272/2008 (based on the United Nations Globally Harmonized System (UN-GHS)) on classification, labelling and packaging (CLP) of substances and mixtures (EC, 2008a) aims to ensure a high level of protection of human health and the environment as well as the free movement of substances, mixtures and articles by obligating manufacturers, importers and downstream users to classify their substances and mixtures and label and package them in a specified way. The Regulation does not require toxicological data to be submitted, but describes how available toxicological data can lead to certain classifications, which in turn lead to labelling and packaging requirements. The classification therefore depends on availability of toxicological data, e.g. produced for other frameworks, such as REACH. The description of which toxicological test results lead to which classification, however, is highly focused on animal tests. For example, for classification on acute toxicity, LD₅₀ values are indicated to be necessary. However, article 5.1c requires the collection of any other information generated in accordance with section 1 of Annex XI of REACH, which includes *in vitro* methods and (Q)SARs, and article 9.3 allows the use of other information that were not derived with accepted or validated test methods in a weight of evidence evaluation in line with section 1.2 of Annex XI of REACH. The weight of the evidence should be sufficient. For skin corrosion or irritancy, for which OECD test guidelines for alternative, *in vitro* tests are available, CLP Annex I, paragraph 3.2.2.1 allows the use of *in vitro* tests to help make classification decisions for skin irritation / corrosion. Consequently, if alternative methods are used for authorization of substances under other frameworks, this information can be adequate for classification of the substances, according to the current CLP Regulation. However, for some hazard classes, specific requirements are included in the classification criteria that limit the use of alternative approaches such as (Q)SARs and read-across. For example, chapter 3.4.2.2.4.3 of Annex I on the classification of substances for skin sensitization requires additional information besides positive results from close structural analogs, thereby limiting the use of read-across. Also, table 3.5.1 of chapter 3.5.2.2 on the classification criteria for germ cell mutagenicity for category 2 states that substances which show a chemical structure relationship to known germ cell mutagens shall be considered for classification as Category 2 mutagens. This limits the use of read-across and may require

additional testing for a classification as a Category 1B mutagen. These substances would then not be properly labelled and packaged, when the classification is based on read-across only, and workers, consumers, and environment would remain insufficiently protected without additional testing.

In conclusion, although animal test data are focused on in this framework, there are possibilities, and thus no legal barrier to use alternative methods for most hazard classes. However, the classification criteria for some hazard classes limit the use of alternative methods, while REACH requires that alternative methods are adequate for the purpose of classification and labelling and (REACH Annex XI part 1). In this way, the CLP framework poses a barrier for the use of some alternative methods within REACH.

3.12 Dependent utility: derivation of OELs

Inter)national frameworks for OEL derivation, such as performed by national committees or the EC Scientific Committee on Occupational Exposure Limits (SCOEL), heavily rely on information in the public literature and publicly available reports. Often the publicly available information has been generated to fulfill data requirements within other frameworks. If in the latter frameworks alternatives for animal testing are considered sufficiently valid to replace animal testing and the alternative is applied, it may have as a consequence that important information for frameworks such as the derivation of OELs no longer becomes available. If the alternatives comply with the requirements of the framework concerned, but does not meet the information need for OEL setting, it may hamper an adequate control of exposures at the workplace. It is acknowledged that also within REACH workplace exposures are addressed and DNELs for occupational exposures are derived. Discussions are ongoing about the role of DNELs derived for occupational exposures in risk management at the workplace.

3.13 Dependent utility: emergency response planning and land use planning

Adequate risk management in scenarios of emergency response planning (ERP) and for the purpose of land use planning (LUP; as required *e.g.*, within the SEVESO II Directive 96/82/EC), requires data on potential risks following acute exposures. Examples are Acute Exposure Reference Values (AERVs) for ERP and so-called probit relations for LUP.

AERVs are thresholds for predefined levels of toxicity or severity of toxicity and are used in support of the public health management of chemical incidents, which requires that AERVs should enable comparison of the public health impacts of the chemical exposure and of the possible emergency response measures such as shelter-in-place or evacuation. For this purpose, quantitative information is needed about health effects following acute exposures in case of a chemical incident. In most frameworks, acute toxicity data are only used for the purpose of classification and labelling for which purpose qualitative or semi quantitative information obtained by non-animal testing may be sufficient. As with OELs, there is no legal framework with data requirements for the derivation of AERVs or probits. It should then be realized that if acute exposure tests, such as the acute inhalation toxicity test (*i.e.*, the adjusted LC₅₀ test (OECD TG403), are replaced by alternatives that do not generate quantitative data that meets the data needs for AERV or probit derivation, this would hamper risk management for the purpose of ERP and LUP.

4 Discussion and conclusions

The purpose of our analysis was to determine whether there are any legal barriers for the use of alternatives to animal tests, in order to pinpoint where action is needed by politicians and the European Commission (as writers of EU regulations) to remove such barriers to clear the way for these alternatives. The ten European regulatory frameworks requiring toxicity data, analyzed in this study, show different constructions in the requirement of animal tests and the possibility to use alternatives, especially in the placement of requirements in the legally binding regulation or directive or in a non-binding guidance document, guideline, or recommendation referred to in the regulation or directive:

1. One framework prohibits the use of animal tests and obligates the use of validated and accepted alternatives for assessing consumer health (Cosmetics)
2. Five frameworks require specific animal tests in the regulation or directive, but also give the opportunity in the regulation or directive to use alternatives if they are proven to be scientifically valid and reliable (REACH, Biocides, Plant protection products, Feed additives, Detergents).
3. One framework seems to require specific animal tests in the directive and does not mention the possibility of using alternatives there, but states that the guidance or commission recommendation must be followed. One example guideline document subsequently gives a possibility to use alternatives when scientifically valid and justified (Veterinary medicinal products).
4. Three frameworks do not mention any requirement for animal testing in the regulation or directive, but in most cases refer to a guidance document or recommendation for the necessary information.
 - a. For two of these frameworks, this guidance or recommendation does require animal tests, but offers the possibility to use alternatives (if scientifically justified; Novel foods, Food improvement agents).
 - b. For one of these frameworks, this guidance requires animal tests and does not give full opportunity to use alternative methods (Food contact materials).
 - c. For one case, no guidance was mentioned or found (Diet feed).

Upon closer analysis, the framework for feed additives appeared not to give specific requirements for toxicological data, and is therefore left out of this grouping and further discussion.

The frameworks mentioned under 1 and 2 clearly do not pose a legal barrier for the use of alternatives, no amendments would be necessary there to enable the use of alternatives. It must be remarked that the REACH Regulation gives the clearest possibilities to use alternatives, with adaptations indicated in column two of the tables listing required *in vivo* tests and a reference to Annex XI which describes the general conditions under which an alternative method is acceptable. In the biocides framework, adaptation possibilities are given in column 3 of the tables with required *in vivo* tests, but no general conditions for an alternative method are given. In the plant protection regulation, the user of an alternative method can only refer to a general paragraph stating that the tests on vertebrates may only be used when no other validated methods are available. In the detergents framework, the situation around the acute fish test for the parent substance is not very clear, thus clarification in the Commission

Recommendation would help in showing alternative methods are indeed possible.

For the framework under 3 (Veterinary medicinal products), there seems to be a legal barrier to use alternative methods, as these are not given as possibility in the regulation or directive. It is not clear what the precise legal status of the guideline is, when the directive states this must be followed. Officially, such documents are non-binding, but there seems no alternative when such a guideline is referred to in the binding legal text. In any case, it would be most transparent when the possibility to use alternatives is embedded in the regulation or directive, i.e. the binding legal text, by an amendment of Directive 2001/82/EC (Veterinary medicinal products). This would be the responsibility of the European Medicines Agency (EMA). The duration of an amendment procedure cannot be estimated, as it depends heavily on how debated a proposed amendment is and how much obstruction it will face.

The frameworks under 4 provide no legal requirements for animal tests and correspondingly, no legal barriers to use alternatives. Barriers are only present in the guidance, and thus, to enable the use of alternatives, the guidance documents of these frameworks (Novel foods, Food improvement agents, Food contact materials and Diet feed) need to be adapted. No legal actions are necessary. EFSA is the responsible body for the updating of the guidance documents for these frameworks under point 4, the duration of which again cannot be estimated as it depends on the amount of debate it faces.

Besides these identified amendments in some of the frameworks requiring toxicity data, an amendment of the CLP Regulation (Regulation (EC) No 1272/2008) is necessary to accommodate the use of test results of alternative methods for CLP for all hazard classes. This would simultaneously remove the barrier the CLP Regulation now poses for using alternatives under REACH, as the REACH Regulation requires that an alternative method is suitable for CLP. Further, the scientific and legal consequences of an increased use of alternatives to animal tests on risk assessment or standard setting within frameworks depending on data requirements of other frameworks need consideration. Examples of such frameworks are the derivation of OELs for workplace exposure, AERVs for emergency response planning, and probit relationships for land use planning.

The most frequent barrier for the use of alternative methods, found in almost all frameworks, is the condition that an alternative method must be shown to be scientifically valid, justified and accepted. This is not a legal barrier, but a practical one, which has been identified previously (e.g. Vandebriel & Opperhuizen, 2011; Adler et al., 2011). It therefore falls outside the scope of this study, but it needs to be stressed nevertheless that for many toxicological endpoints (e.g. for repeated dose toxicity and reproductive toxicity), no validated, accepted alternative methods are currently available yet for full replacement of the *in vivo* tests, as is currently necessary for cosmetics. Furthermore, toxicokinetic tools, necessary to make *in vitro* data more meaningful for the effects on a whole human body, are lacking as well (Adler et al., 2011). The absence of legal barriers in most frameworks therefore does not mean the road is free to apply the alternatives yet, especially for cosmetics. Moreover, it is noted that as of yet, to the knowledge of the authors of this report, no cosmetics have been approved on the basis of non-animal tests only, probably because of the lack of adequate methods to predict endpoints such as repeated dose toxicity, reproductive toxicity, etc. Further development and validation of alternative methods and complementary tools needs to be stimulated to give a clear opportunity for alternatives to animal tests.

This lack of robust and scientifically sound alternative methods also hampers the regulatory acceptance of alternative methods, which is often mentioned as a barrier for the use of these alternatives (e.g. Ball et al., 2014; Piersma et al., 2014; Vandebriel & Opperhuizen, 2011). A lack of regulatory acceptance, therefore, does not so much relate to legal barriers, as the current study confirms, but rather to practical barriers. Specifically, a lack of regulatory acceptance can be due to the fact that a method is not yet scientifically robust, does not predict the relevant endpoint, is not within the chemical domain and/or is not sufficiently validated.

Vandebriel & Opperhuizen (2011) concluded that the chain of development, validation and implementation of an alternative method would run smoother and faster if the developers of a method and regulators and industry would exchange views at the beginning of this chain. Regulators can then indicate what type of methods are needed for the purpose of risk assessment and the criteria they should meet (e.g. relevant endpoints), industry can provide input on how costs are compared, and developers can show what is scientifically possible.

Through expert enquiries, Schiffelers et al. (2014) concluded that the main barriers for regulatory acceptance of alternatives are "the uncertainty about the value of the new 3R models (micro level), the lack of harmonization of regulatory requirements and acceptance criteria (meso level) and the high levels of risk aversion (macro level)". Additionally, Piersma et al. (2014) identified the limitations and uncertainties in the applicability domain and unsuitability to cover several, especially complex, systemic endpoints as a bottleneck, and for industry, sometimes costs are an issue. The major importance of the costs has been stated by DG Enterprise and Industry in the discussion on the implementation of the extended one generation reproductive toxicity study (EOGRTS) in the REACH Committee. These type of practical barriers therefore also need to be addressed to pave a "free road" for the use of alternative methods.

To conclude, barriers for the use of alternatives to animal tests have been identified in the dependent framework for CLP, and possibly in that for veterinary medicinal products. For other frameworks, the use of alternatives is solely hampered by practical barriers, such as availability, limited scientific robustness of the method, lack of predictivity for more complex systemic endpoints, lack of scientific validation, and a (resulting) lack of options for alternatives in guidance documents, which are all outside the scope of this study. These barriers, as well as costs aspects, have an impact on the regulatory acceptance. To stimulate the use of alternatives, resources and future studies would therefore be best focused on these practical barriers.

Attention is also needed for the necessary amendment of dependent frameworks and utilities such as for occupation exposure limits, emergency response planning and land use planning, in order to maintain sufficient protection of human and environmental health when alternatives come into use.

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