Regulatory Toxicology and Pharmacology 117 (2020) 104786

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

# Analysis and reflection on the role of the 90-day oral toxicity study in European chemical risk assessment

Misha Vrolijk [a], Hubert Deluyker [b], Aalt Bast [a], Alie de Boer [c,*]

[a] University College Venlo, Campus Venlo, Maastricht University, the Netherlands
[b] Former Scientific Advisor of the European Food Safety Authority (EFSA), the Netherlands
[c] Food Claims Centre Venlo, Campus Venlo, Maastricht University, the Netherlands

ARTICLE INFO

*Keywords:*
Risk assessment
Regulatory science
Chemical safety
Toxicology
Sub-chronic toxicity
EFSA
EMA
ECHA

ABSTRACT

The 90-day toxicity study is one of the studies used in the safety assessment of food ingredients, medicines or other chemical substances. This paper reviews the current role of the 90-day oral toxicity study in European regulatory dossiers of chemicals by reviewing EU legislation and EU and OECD guidance documents. Regulatory provisions with regard to necessity, objectives and design of such 90-day toxicity studies vary between the different sectors addressed in this review. Most often the 90-day study is expected to be part of the standard test battery used for chemical risk assessment, without necessarily being a legal requirement and its objectives may vary between regulatory domains. Exceptions, when a 90-day study is not required are spelled out in the chemicals legislation and for food contact materials. The sectorial study design requirements of the 90-day toxicity study are very often embedded in the OECD TG 408 protocol. Differences in study objectives are not necessarily reflected in specific study designs. Considering the call for the reduction of using experimental animals for scientific purposes and the fact that a 90-day study may serve different purposes, consistency between the necessity to conduct such a study, its objectives and the study design to achieve these objectives may improve judicious use of laboratory animals. Thus there may be an opportunity to reflect and further optimise the design of *in vivo* toxicology studies, such as the 90-day study. This should be based on a systematic analysis of past studies and risk assessments.

## 1. Introduction

Consumers, workers and other members of the public are exposed to chemicals that have been introduced on the market either deliberately or are present as contaminants. In the former case, European Union legislative frameworks that regulate chemicals, medicines and food products share the aim of ensuring the highest level of consumer protection upon exposure to such chemicals ( and the Council, 2002a; 2004a, 2006a). To allow the introduction of chemicals, medicines and food and feed ingredients, the European Union considers the available scientific evidence about the potential risks of such products. For the evaluation of these, the risk manager (the Commission and the Member States competent authorities) receives scientific support from the concerned national and EU scientific assessment agencies, including ECHA (the European Chemicals Agency, dealing with chemicals in general), EMA (European Medicines Agency, assessing medicinal products) and EFSA (European Food Safety Authority, evaluating chemicals in food and feed) (Commission of the European Communities, 2008).

Scientific evidence submitted by a company seeking marketing authorisation through a regulatory dossier must contain the studies necessary to be able to assess the safety of a chemical substance. Taken together, such studies need to provide an overview of the potential toxicological effects of the compound, taking into consideration their intended use. The regulations describing the required scientific evidence differ by sector. In general, however, a pre-market safety dataset is expected to include safety and kinetic information generated through *in silico* and *in vitro* studies, *in vivo* experimental animal studies, and, in the case of e.g. medicines or novel foods in human and pharmaceuticals, and feed additives in animals also human or target animal safety data, respectively. The study details, such as the methods to be used, that are necessary to generate the relevant safety data may either be laid down in the applicable legislations or are described in guidance documents

* Corresponding author.Food Claims Centre Venlo Maastricht University, Campus Venlo Faculty of Science and Engineering, Maastricht University, P.O. Box 8 5900, AA Venlo, the Netherlands.
*E-mail address:* a.deboer@maastrichtuniversity.nl (A. de Boer).

https://doi.org/10.1016/j.yrtph.2020.104786
Received 8 May 2020; Received in revised form 12 September 2020; Accepted 18 September 2020
Available online 22 September 2020

issued by the concerned scientific agencies.

The safety data are typically generated through a series of successive studies (Chhabra et al., 1990). Within this framework of gradual accumulation of data on the safety characterisation of a chemical, one of the studies that is frequently conducted is the repeated-dose 90-day oral toxicity study in rodents, often referred to as the 90-day toxicity study (Parasuraman, 2011). The duration of the 90-day toxicity study has been suggested to be originating from the length that US Navy personnel spent on submarines, where they were exposed to many newly, man-made chemicals (National Research Council, 1994). In general, this study aims to characterise the health hazards resulting from sub-chronic daily oral exposure for at least 90 days to different levels of a test substance, when compared to an otherwise similar control group (OECD, 2018).

The relevance of this type of study within a scientific assessment has however been questioned for low toxicity chemicals by Taylor and colleagues (Taylor et al., 2014; Taylor and Andrew, 2017), who concluded that for this type of chemicals a 90-day toxicity study did not add value in characterising the risk, beyond what was already known from a 28-day study. Concurrently, the concern regarding needless use of animals for experimental studies has been translated into a European Directive (Directive, 2010/63/EU) that calls for the replacement, reduction and refinement (known as the 3R principle) of using experimental animals for scientific purpose (European Parliament and the Council, 2010). Taken together, there is a growing need to justify the conduct of a 90-day study.

The aim of this paper is to contribute to this debate by reviewing, in the different regulatory domains, the requirement to include a 90-day oral toxicity study in regulatory dossiers; what its objectives and design expectations are. Based on this, the authors reflect on aspects that may merit further consideration and how to possibly achieve these.

## 2. Materials and methods

The scope of the paper is limited to the assessment of safety following oral exposure in rodents. Hence, the scientific assessment of e.g. the dermal route of exposure studied with cosmetics is outside the scope of this paper.

The materials used in this study concern the European requirements that affect the conduct of 90-day studies for the safety assessment of chemicals, medicines, and food and feed products. This includes cross-cutting documents, i.e. the EU's Good Laboratory Practices (GLP) Directives (Directive 2004/9/EC and Directive 2004/10/EC (European Parliament and the Council, 2004b; 2004c), as well as the Directive on animal testing for scientific purposes (Directive, 2010/63/EU). Also cross-cutting test guidelines from the Organisation for Economic Co-operation and Development (OECD) were considered, focusing on the guidance on the conduct of studies to assess sub-chronic oral safety in rodent species, OECD test guidelines (TG) 408 (OECD, 2018). The historic developments of TG 408 are briefly described in Annex 1.

Also, sectoral EU-level regulatory requirements and guidance documents were reviewed. Guidance documents provide recommendations on the interpretation of the legally binding requirements and are thus, in principle, not legally binding in nature. Nevertheless, they provide further insights as to when a 90-day study is expected, what purpose the study serves, as well as reflections on the design of the study.

According to Regulation (EU) No 1272/2008, specific safety information must be provided on chemicals marketed within the EU, to classify, label and package (CLP) these substances and mixtures (European Parliament and the Council, 2008a). Also, substances that are used for the production of food, feed or medicines and that do not present a product in its finished state fall under this CLP Regulation. CLP aims to determine the hazardous properties of chemicals or mixtures that need specific classification, which is the starting point of communicating potential hazards. However, CLP cannot be seen as completely separate from other main regulations dealing with chemicals in the EU (including Regulation (EC) No 1907/2006 on Regulation, Evaluation, Authorisation and Restriction of Chemicals (REACH) (European Parliament and the Council, 2006a) and Regulation (EU) No 528/2012, the Biocidal Products Regulation (BPR) (European Parliament and the Council, 2012)), it rather can be regarded as a parallel classification assessment based on the existing information in the dossier of the chemical. Hence, classification of substances is directly influenced by the data requirements of these other domains. The CLP regulation is therefore not addressed separately in this paper.

Only consolidated versions of legal acts and guidance documents in which amendments are integrated (i.e. the most recently updated versions) were used. These documents were reviewed to identify and characterise the safety study requirements, and determine whether and how they vary between sectors with regard to the obligatory nature to conduct a 90-day toxicity study (Table 1), the study objectives (Table 2), and the study design requirements (Table 3). For the characterisation of the study design (Table 3) the following aspects were assessed: (i) whether and, if so, which prior information is considered in the design of the study; (ii) whether a test hypothesis is to be stated explicitly; (iii) whether and which test material specifications are to be described; (iv) what the test species and the treatment groups are to be, including the number of animals per treatment group; (v) what outcomes are to be measured (vi) whether guidance is provided regarding statistical analysis; and (vii) whether adherence to study quality standards, in particular principles of good laboratory practice, is required.

Instead of a 90-day study, a repeated-dose study of somewhat shorter

**Table 1**
Is a 90-day study expected?

| Domain | Subchronic study[a] | 90-day study[a] |
|---|---|---|
| Food and feed | | |
| GM food | Regulation (EU) No 503/2013 | Regulation (EU) No 503/2013 |
| Feed additive | Regulation (EC) No 429/2008 | Regulation (EC) No 429/2008 |
| Pesticide | Regulation (EU) No 283/2013 | Regulation (EU) No 283/2013 |
| Food improvement agent[b] | Regulation (EU) No 234/2011 | Guidance, with exceptions (EFSA ANS Panel, 2012; EFSA CEF Panel, 2010, 2009) |
| Novel food[c] | Regulation (EU) No 2017/2469 | Guidance (EFSA NDA Panel et al., 2016) |
| Food contact material | Not mentioned | Guidance, with exceptions (EFSA CEF Panel et al., 2008) |
| New nutrition source[d] | Not mentioned | Guidance (EFSA ANS Panel et al., 2018) |
| ***Medicinal product*** | | |
| Human | Directive 2001/83/EC | Not mentioned |
| Veterinary | Directive 2001/82/EC | Not mentioned |
| ***Chemicals*** | | |
| REACH | Regulation (EC) No 440/2008, with exceptions | Regulation (EC) No 440/2008, with exceptions |
| Biocides | Regulation (EU) No 528/2012, with exceptions | Regulation (EU) No 528/2012, with exceptions |

[a] Legislation = requirement explicitly described in legislation; Legislation, with exceptions = requirement explicitly described in legislation, as well as exceptions to this requirement Guidance = requirement described in guidance documents; Guidance, with exceptions = requirement described in guidance documents, as well as exceptions to this requirement Not mentioned = need to conduct this type of study not described in legislation or guidance documents.

[b] Food improvement agents: grouped overview of food enzymes, food additives, food flavourings, as defined in Regulation (EC) No 1331/2008.

[c] Novel foods: focusing on new (and not traditional) foods in the definition of Regulation (EU) No 2283/2015.

[d] New nutrition sources: grouped overview of new nutrition sources as used in food supplements (Directive 46/2002/EC), to be added in foods *i.a.* for fortification purposes (Regulation (EC) No 1925/2006), or used in foods for special groups (Regulation (EU) No 609/2013).

**Table 2**
Study objectives of 90-day study

| Domain | Hazard identification | Hazard characterisation | | Basis for designing further longer duration tox studies | | Source |
|---|---|---|---|---|---|---|
| *OECD TG 408* | ***Identify toxicological profile*** | ***Relationship dose and effect*** | ***Point of departure*** | ***Basis for design chronic toxicity study*** | ***Identify need for additional studies*** | (OECD, 2018) |
| GM food | L/G[a] | L/G | n.m.[b] | L/G | L/G | (EFSA GMO Panel, 2011; EFSA GMO Unit, 2014; EFSA Scientific Committee et al., 2011; European Commission, 2013a) |
| Feed additive | L/G | n.m. | n.m. | L/G | n.m. | (EFSA FEEDAP Panel et al., 2017; European Commission, 2008) |
| Pesticide | L/G | L/G | L/G | L/G | n.m. | (Directorate E Directorate-General Health & Consumer Protection, 2001; European Commission, 2013b) |
| Food contact material | O [c] | O | O | O | O | (EFSA CEF Panel et al., 2008; European Commission, 2011) |
| Food improvement agent | L/G | L/G | L/G | L/G | L/G | (EFSA ANS Panel, 2012; EFSA CEF Panel, 2010, 2009) |
| Novel food | L/G | n.m. | L/G | n.m. | L/G | (EFSA NDA Panel et al., 2016) |
| New nutrition source | O | O | O | O | O | (EFSA ANS Panel et al., 2018) |
| Human medicine | L/G | L/G | n.m. | n.m. | n.m. | (EMA, 2010) |
| Veterinary medicine | L/G | L/G | L/G | L/G | n.m. | (EMA - VICH, 2003) |
| REACH | O | O | O | O | O | (ECHA, 2011; European Council, 2008) |
| Biocides | L/G | L/G | L/G | L/G | L/G | (ECHA, 2017; European Council, 2008) |

[a] L/G = study objective is explicitly described in legislation or guidance documents;
[b] n.m.(not mentioned) = study objective is not explicitly described in legislation nor guidance documents;
[c] O = explicit reference is made to OECD TG 408 (as highlighted by the matching shaded areas) regarding this study objective in legislation or guidance documents.

or longer duration (ranging from 28 days to 9 months) may be required or recommended. Even though this paper focusses on 90-day studies, insights into repeated dose toxicity studies in the range of 4 weeks–9 months that may be carried out in lieu of a 90-day study are therefore also considered.

## 3. Results

The results of the three studied aspects of 90-day toxicity studies are shown in three separate tables: whether there is an obligation to conduct a 90-day toxicity study (Table 1), the study objectives (Table 2), and the study design requirements (Table 3). These results are reviewed sector-by-sector below, starting with the findings from cross-cutting regulations and guidances.

### 3.1. Cross-cutting guidances and regulations

#### 3.1.1. Good laboratory practice

The OECD has adopted GLP principles for non-clinical safety assessment of chemicals in the various domains covered in this paper (OECD, 1999). Adherence to GLP principles concerns *i.a.* the test facility, test materials, quality assurance programmes and study reporting. It aims to ensure that the data collected are of sufficient quality and rigour so as to be reproducible, thus facilitating the exchange of data between different jurisdictions. In the EU, the GLP principles have been laid down in two Directives. Directive 2004/9/EC[6] states that adherence to the OECD GLP guidelines needs to be verified during inspections and audits of laboratories and studies within EU Member States. Directive 2004/10/EC requires EU Member States to harmonise legislation and procedures in both the application and verification of adherence to the OECD GLP principles (European Parliament and the Council, 2004c). All EU sectorial legislation mention the need to adhere to the OECD guideline or more generally refers to the need to adhere to GLP standards.

#### 3.1.2. OECD TG 408

The 90-day toxicity study in rodents (TG 408) can be summarised to serve five objectives (Table 2). These can be grouped as follows. It can be used to identify hazards, by studying the toxicological profile of a substance. The study can also be used for hazard characterisation purposes, analysing dose and effect relationships or to determine of the point of departure (including the establishment of a no-effect level). Thirdly, the results from the 90-day toxicity study can be used as basis for designing chronic toxicity studies or to identify the need to conduct of further toxicity studies (OECD, 2018). TG 408 describes aspects of the design of the 90-day oral toxicity study (Table 3). While the study may, in principle, serve multiple purposes, there is no mention of an expectation to explicitly state which objectives are to be addressed that will determine the design of the study.

The preferred animal species for the 90-day toxicity study is the rat. The test substance is to be administered to at least three dose groups and a control group, consisting of a minimum of 20 animals per group. TG 408 does not include any explicit suggestions regarding power calculations to determine minimum sizes of the dose groups to meet the study objectives. The dose levels are suggested to be based on results obtained by repeated dose or range finding studies, together with any existing toxicological information available for the test compound. Also, the highest dose level chosen should aim to induce toxicity. During exposure, the animals should be closely observed for toxicity by making daily general clinical observations. The 90-day study furthermore needs to include other general weekly measurements (e.g. weight and food and water consumption) and detailed final observations (e.g. ophthalmological examination, haematology, clinical biochemistry, urinalysis, gross necropsy and histopathology) (OECD, 2018).

Regarding statistical analysis, TG 408 remarks that appropriate and generally acceptable statistical methods should be used for evaluation of the collected data. No mention is made whether to describe if the study was sufficiently powerful to detect the stated differences of biological relevance. The protocol does not mention whether GLP principles are to be adhered to in conducting of a 90-day toxicity study, as this is the subject of another OECD guideline as described in section 3.1.1 (OECD, 1999).

**Table 3**
Study design guidance.

| Domain | Req. prior studies[a] | Hypothesis stated[a] | Test material specified[a,b] | Test species[a,c] | Dose groups[a,d] | | | Observations[a,e] | Statistical analyses[a,f] | Source |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | #[i] | # animals per group[j] | power calculation | | | |
| *OECD TG408* | *Yes* | *n.m.* | *n.m.* | *Rodent, pref. rat* | *≥4* | *≥20* | *n.m.* | *Yes* | *Yes* | (OECD, 2018) |
| GM food | Suggested | Yes | Yes: FP | Rodent | ≥3 | Power-based | Yes | | | (EFSA GMO Panel, 2011; European Commission, 2013a) |
| Feed additive | n.m. | | Yes: AS | Pref. rodent* | | | | | | (EFSA FEEDAP Panel et al., 2017; European Commission, 2008) |
| Pesticide | n.m. | | Yes: AS | Rat & dog | | | | | | (Directorate E Directorate-General Health & Consumer Protection, 2001; European Commission, 2013b) |
| Food contact material | n.m. | | Yes: AS | | | | | | | (EFSA CEF Panel et al., 2008; European Commission, 2011) |
| Food improvement agent | n.m. | | Yes: AS | | | | | | | (EFSA ANS Panel, 2012; EFSA CEF Panel, 2010, 2009) |
| Novel food | n.m. | | Yes[+] | | | | | | | (EFSA NDA Panel et al., 2016) |
| New nutrition source | n.m. | | Yes: FP or AS[++] | | | | | | | (EFSA ANS Panel et al., 2018) |
| Human medicine | | Suggested | Yes: AS | Rodent and non-rodent | | Power-based | Yes | | | (EMA, 2010) |
| Veterinary medicine | | | Yes: AS | Rodent and non-rodent | | | | OECD TG408 & TG409 | OECD TG408 & TG409 | (EMA - VICH, 2003) |
| REACH[h] | | | Yes: AS | | | | | | | (ECHA, 2011; European Council, 2008) |
| Biocides | | | Yes: AS | | | | | | | (ECHA, 2017; European Council, 2008) |

= Grey shaded areas highlight similar study design requirements by the respective legislation or guidance with the OECD TG408 requirements.

= Grey shaded areas highlight similar study design requirements by the respective legislation or guidance with the OECD TG408 requirements.
[a]Yes = Design element is explicitly described in legislation or guidance documents; Suggested = design element is not explicitly mentioned, but seems to be suggested in legislation or guidance documents n.m. (not mentioned) = study design element is not explicitly described in legislation or guidance documents; OECD TG408/409 = explicit reference is made to OECD TG 408 or TG 409 regarding the study design element in legislation or guidance documents.
[b]Specification of test material. FP = formulated product; AS = active substance; No- = not mentioned+"for 'whole foods', the testing requirements should be determined using a case-by-case approach, as special considerations are required with regard to dose selection and the avoidance of possible nutritional imbalances"[++] test to be conducted "on the source" as it will be used in products (FP or AS).
[c]Test species specifications.
[d]Dose groups specifications: (i) number of groups, (ii) number of animals per group, (iii) use of power calculation to determine group size [i] = including untreated (control) group [j] = half of which are males.
[e]Specifications regarding type of observations (which concern clinical, biochemical and postmortem observations).
[f]Specifications regarding statistical analyses and reporting.
[g]Yes = general references are made to GLP standards; OECD GLP = a general reference is made to OECD standards or OECD GLP standards specifically.
[h]Method B26 and B27 replicate the OECD TG 408 and OECD TG 409 of 1998 completely.

### 3.2. Food and feed safety

The General Food Law (GFL), Regulation (EC) No 178/2002, serves as the framework regulation for foods and aims to provide the legal basis for a high level of protection of human health, as well as ensuring effective functioning of the European market (European Parliament and the Council, 2002a). The GFL does not provide details on how to study the safety of a food product. When it concerns new nutrients however, the need to provide scientific evidence on its safety is referred to Article 29 (1) of the GFL, which describes in general that EFSA can be requested by the European Commission to issue a scientific opinion, or can do so on its own initiative (European Parliament and the Council, 2002a). Particular requirements for placing on the market of different types of food or feed products are found in more specific regulations. Separate regulations deal with genetically modified (GM) foods (European Parliament and the Council, 2003a), feed additives (European Parliament and the Council, 2003b), pesticides (European Parliament and the Council, 2009), food contact materials (European Parliament and the Council, 2004d) (and specifically plastic materials and articles intended to come into contact with food (European Commission, 2011a)), food improvement agents (European Parliament and the Council, 2008b), and novel foods (European Parliament and ) and new nutrition sources (European Parliament and the Council, 2013; 2006b, 2002b).

Food improvement agents include food enzymes, food additives and food flavourings. While they are dealt with separately in Regulations (EC) No 1332/2008, 1333/2008 and 1334/2008, respectively (European Parliament and the Council, 2008c; 2008d, 2008e); they do however share a common authorisation procedure which is described in Regulation (EC) 1331/2008 and further specified in Commission Implementing Regulation (EU) 234/2011 (European Commission, 2011b). Before food improvement agents are added to the Union lists of approved food improvement agents, the Commission can request the scientific opinion of EFSA regarding their safety. Such applications are to be (i) added to food as a nutrient source (such as for fortification purposes), for example vitamins and minerals (European Parliament and the Council, 2006b); (ii) food supplements (European Parliament and the Council, 2002b); or (iii) components to be added to the positive list of ingredients used in foods for specific groups (European Parliament and the Council, 2006b). In all these cases safety needs to be proven before they are to be incorporated in the positive lists as found in the Annexes to these regulations.

The Novel Food Regulation defines two types of novel foods: (i) new ingredients or products made by novel production techniques, that fall within one of ten predefined categories; or (ii) 'traditional foods' that are part of the common diet in countries outside the EU (European Parliament and the Council, 2015). For both types of novel foods, the regulation requires that scientific evidence substantiates safety before these products can be placed on the EU market. According to its Article 11, EFSA should consider whether a novel food is safe, its composition and conditions of use will not pose a safety risk to human health, and ensure that its usage (when replacing other foods) will not become nutritionally disadvantageous for consumers. For traditional foods, Implementing Regulation (EU) 2017/2468 and the corresponding EFSA guidance document specify that safety can be established by providing data on

historical use outside the EU (EFSA NDA Parasuraman, 2011; European Commission, 2017a). For newly produced foods however, a complete scientific dossier is required for the authorisation procedure, as described in Commission Implementing Regulation (EU) 2017/2469 and EFSA's guidance on novel foods (EFSA NDA Parasuraman, 2011; European Commission, 2017b).

#### 3.2.1. Is a 90-day study expected?

As summarised in Table 1, the sector specific legislation varies as to whether subchronic safety effects are to be studied and whether this needs to include a 90-day study. For GM foods, feed additives and pesticides a 90-day toxicity study is explicitly required (European Commission, 2013a, 2013b, 2008). For food improvement agents and novel foods, legislation prescribes that details about subchronic effects need to be included in the dossier (European Commission, 2011b; European Parliament and the Council, 2015, 2008b). Their technical guidance documents specify that these subchronic effects can be studied with the 90-day toxicity test, which implies that the 90-day toxicity study is part of the standard test battery (EFSA ANS Panel, 2012; EFSA CEF Panel, 2010; 2009; EFSA NDA Parasuraman, 2011). Even though also the guidance document on food additives suggests the 90-day toxicity test for subchronic toxicity testing, specific exceptions to the use of the 90-day toxicity study are foreseen in this guidance document (EFSA ANS Panel, 2012).

In the legislation dealing with food contact plastics and new nutrition sources, the need to conduct a subchronic study is not specified (European Commission, 2011a; European Parliament and the Council, 2013, 2006b, 2002b). Still, guidance documents for preparing scientific dossiers for these authorisation requests suggest the 90-day toxicity study is expected to be part of the standard test battery or minimum dataset (EFSA ANS Parasuraman, 2011; EFSA CEF Parasuraman, 2011), for food plastics depending on the level of migration. The note on plastic food contact materials specifies potential exceptions to this requirement (EFSA CEF Parasuraman, 2011).

#### 3.2.2. Study objectives of the 90-day toxicity study

The purpose of the 90-day toxicity study in different food authorisation procedures does not always include each of the five study objectives from TG 408 (OECD, 2018). In fact, only guidance documents for new nutrition sources and plastic food contact materials refer explicitly to TG 408 to describe the study objectives of the 90-day toxicity study (EFSA ANS Parasuraman, 2011; EFSA CEF Parasuraman, 2011). Guidance documents for food improvement agents foresee three main objectives of i.e. hazard identification & characterisation (including to establish a NOAEL or BMDL), as well as providing the basis for the design of further longer duration toxicity studies (EFSA ANS Panel, 2012; EFSA CEF Panel, 2010; 2009). These correspond with those in TG 408, even if these guidance documents do not explicitly mention the TG 408.

Identifying the toxicological profile of a compound is described as a study objective for feed additives (EFSA FEEDAP Parasuraman, 2011; European Commission, 2008), pesticides (Directorate E Directorate-General Health and Consumer Parasuraman, 2011; European Commission, 2013b), GM foods (EFSA GMO Panel, 2011) and novel foods (EFSA NDA Parasuraman, 2011), whereas only for GM foods and pesticides the establishment of a dose-effect relationship is defined (including a NOAEL for pesticides) and for novel foods the point of departure (including a NOAEL or BMDL) is explicitly mentioned (EFSA GMO Unit, 2014; EFSA Scientific Committee et al., 2011). The 90-day toxicity study can be used as a basis for designing chronic toxicity studies for GM foods, pesticides, and feed additives (EFSA FEEDAP Parasuraman, 2011; EFSA GMO Panel, 2011; EFSA GMO Unit, 2014; EFSA Scientific Committee et al., 2011; European Commission, 2013a; 2013b; European Parliament and the Council, 2003b). For novel foods, it can also serve the purpose of identifying the need for additional studies (EFSA NDA Parasuraman, 2011).

#### 3.2.3. Study design, data analysis and quality standards

Whereas most of the sub-domains specify the required test material to be the active substance in the 90-day toxicity study, in the case of GM foods the full product needs to be tested. For new nutrition sources it is not quite clear what material needs to be tested as the guidance document requires "the test to be conducted on the source" (EFSA ANS Parasuraman, 2011).

All legal and guidance documents require the use of rodent species (preferably rats) as test species in the 90-day toxicity, either indirectly by referring to TG 408 or by mentioning this explicitly. For pesticides, the use of two animal species (rats and dogs) is required (Directorate E Directorate-General Health and Consumer Parasuraman, 2011; European Commission, 2013b). Most food and feed domains require minimally 4 dose groups and 20 animals per dose group, consistent with TG 408. None of these legal or guidance documents mention anything about power calculations. Only GM foods deviate from TG 408 in specifying the requirement of performing power analyses for calculating the number of animals per dose groups (EFSA GMO Panel, 2011; EFSA GMO Unit, 2014; EFSA Scientific Committee et al., 2011; European Commission, 2013a). Regarding the requirements for clinical, biochemical and post-mortem observations, statistical analyses and GLP adherence, no noticeable differences were found among the different food and feed domains, as they largely refer to TG 408 and the OECD GLP guidance (OECD, 1999).

### 3.3. Medicinal products

For medicinal products, three legislative acts are of importance. The authorisation and supervision procedures for medicinal products as well as EMA's responsibilities are laid down in Regulation (EC) No 726/2004 (European Parliament and the Council, 2004a). Directive 2001/83/EC lays down rules related to human medicinal products (European Parliament and the Council, 2001a), whereas Directive 2001/82/EC regulates veterinary medicines (European Parliament and the Council, 2001b). Directive 2001/83/EC describes the requirements for the scientific dossier that must be submitted for the authorisation of the medicinal product. The analytical, pharmaco-toxicological and clinical requirements with respect to testing the product are found in its Annex I. Annex I of Directive (2001)/82/EC describes the requirements for veterinary medicines other than immunological medicines.

#### 3.3.1. Is a 90-day study expected?

Both for human and veterinary medicinal products, single dose toxicity and repeated dose toxicity studies are expected in scientific dossiers to provide toxicological information (Table 1) (European Parliament and the Council, 2001a; 2001b). In the case of human medicines, repeated dose toxicity studies are required when the medicine is intended to be used in a multiple dosing regime in subjects, and the mode and scheme of administration of these studies shall closely reflect the clinical dosing plan (European Parliament and the Council, 2001a).

Repeated dose toxicity studies are however not necessarily conducted in the form of 90-day toxicity studies as the duration depends on the actual exposure (European Parliament and the Council, 2001a; 2001b). For human medicines, two repeated dose studies generally will be conducted: one short term and one longer term. The short-term study lasts between two to four weeks, while the duration of the longer-term study depends on the conditions of clinical use (European Parliament and the Council, 2001a). The duration of repeated dose toxicity studies for medicinal products intended for use in non-food-producing animals mostly depends on the duration of the clinical use and should be consistent with the relevant guidelines (EMA - ICH, 2009; 2008, 1999). In case of medicinal products intended for use in food-producing animals, the duration of the repeated dose toxicity test shall be 90 days (EMA - VICH, 2003).

#### 3.3.2. Study objectives of the 90-day toxicity study

Repeated dose toxicity studies are intended to reveal and determine any physiological, anatomical or pathological changes by the repeated administration of the active substance(s) of the medicine under examination, and to identify the dose-response relationships (European Parliament and European Parliament and the Council, 2001; 2001b). For human medicinal products, this specifically is described to include the identification of potential toxic effects in particular target organs (European Parliament and European Parliament and the Council, 2001). The EMA guidance on repeated dose toxicity testing for veterinary medicine residuals furthermore specifies that results from this study can also be used to perform additional testing in case of specific toxicological concerns (EMA - VICH, 2003).

#### 3.3.3. Study design, data analysis and quality standards

When conducting non-clinical trials for human medicines, EMA's general guideline on repeated dose toxicity assumes that GLP quality standards are adhered to (EMA, 2010). This guideline further describes various recommendations and requirements related to the quality of the tested substance, the treatment and handling of experimental animals, dose and administration, observations, as well as the data analysis, presentation of results and the conclusions that can be drawn. Conducting a repeated dose toxicity study in one species of mammals is only acceptable when clearly justified, with the guidelines recommending carrying out the studies in two species of animals (one of which non-rodent). In the guideline, specific emphasis is put on selecting the dose regimen and route of administration that are based on the intended clinical use of the product, to ensure that the animals are sufficiently exposed to the active substance and its metabolites. The guideline document further defines that the size of the treatment groups used in the repeated dose toxicity study should be sufficient to 'allow [for] meaningful scientific interpretation of the data generated', indicating that the number of animals should depend on the experiment, the planned analyses and estimated effect sizes (EMA, 2010). For human medicinal products, previous studies should also reveal qualitative and quantitative analyses of toxic reactions, without referring to whether and how these results should be used in repeated dose toxicity studies (European Parliament and the Council, 2001a).

In the case when the active substance(s) or veterinary medicinal products are intended for use in food-producing animals and veterinary drug residues can end up in human foods, 90-day toxicity tests shall be performed in one rodent and one non-rodent species (EMA, 2010). When the active substances or veterinary medicinal products are intended to be used in non-food-producing animals only, a repeated-dose toxicity study in one animal species is sufficient. All toxicological studies need to be conducted with the active substance(s) instead of the formulated product. Before studying repeated dose toxicity of veterinary medicinal products that are used in food-producing animals, single dose studies should reveal *i.a.* the dose to be used in the repeated dose toxicity study (European Parliament and the Council, 2001b). For further design specifications (related to the number of animals per group and the observations), reference is made to OECD TG 408.

For both veterinary and human medicine safety testing, the design of any animal safety study is expected to reflect the clinical use of the product (EMA - ICH, 2009; 2008; EMA, 2010). The duration of a sub-chronic toxicity study may therefore need to be adjusted, based on the duration of use and the type of medicinal product, to either a study of 6 months duration for rodent animals or 9 months for non-rodents.

### 3.4. Chemicals

The two main regulations dealing with chemical safety in the EU are REACH (European Parliament and the Council, 2006a) and BPR (European Parliament and the Council, 2012). The aim of REACH is to ensure a high level of human health and environmental protection (European Parliament and the Council, 2006a). Regulation (EC) No 440/2008, implementing REACH, describes the test methods for the identification of the physico-chemical and toxicological properties of chemicals, which are required for the authorisation of these substances (European Council, 2008). This list of endpoints differs based on the amount of the chemical produced: the higher the quantities manufactured and marketed, the more demanding the list is in terms of both the type and number of the endpoints to be examined and the experiments to be conducted.

Biocidal products are used to protect humans, animals, materials or articles against harmful organisms. They are regulated under the BPR, which aims to harmonise regulations related to biocidal products, while ensuring protection for human health and the environment, by requiring pre-market authorisation of biocidal products and their active components (European Parliament and the Council, 2012). Regarding safety, the approval application for an active chemical should contain a scientific dossier (as described in Annex II) on the *active substance* of the biocidal product and a dossier (Annex III) on at least one representative biocidal product *containing* this active substance.

#### 3.4.1. Is a 90-day study expected?

The study of subchronic toxicity and the conduct of a 90-day toxicity are required. There are however exceptions: as described in REACH's Annexes VII to X (European Parliament and the Council, 2006a), the frequency and duration of human exposure to chemicals defines whether a sub-chronic toxicity study is required in the authorisation procedure. For chemicals sold in quantities <10 tonnes/year, no repeated dose toxicity testing is required, whereas chemicals imported or manufactured in quantities from 10 to 100 tonnes/year only require a 90-day toxicity study, next to a 28-day repeated dose toxicity test, when specific considerations are met (related to *i.a.* potential serious toxic effects, accumulation, no NOAEL identified yet). Chemicals in quantities of 100–1000 tonnes/year (Annex IX) always require a 90-day toxicity study, in addition to the short-term 28-day toxicity study. Only when (i) chemicals undergo immediate disintegration; (ii) relevant human exposure is excluded; (iii) an available reliable short-term study shows severe toxicity; (iv) a reliable chronic study is available; or (v) a chemical is insoluble, unreactive, not inhalable and no evidence of absorption and toxicity is found in the 28-day study, this requirement can be disregarded. Chemicals that are manufactured or imported in the highest quantity (≥1000 tonnes/year) should always be studied in a 90-day toxicity test, and under specific circumstances also long-term repeated toxicity studies (≥12 months) may be required.

To establish repeated-dose safety, 28-day, 90-day and long-term studies of at least 12 months are required for testing the potential toxicity of the bioactive substance (as described by BPR's Annex II) (European Parliament and the Council, 2012). Only when the active substance undergoes immediate disintegration and sufficient data is presented upon the cleavage products for potential effects or relevant exposure can be excluded, such subchronic toxicity studies do not need to be performed. Unreactive, insoluble and non-inhalable chemicals do not require 90-day toxicity studies and the 90-day toxicity study is not required when a reliable 28-day study shows severe toxicity and the NOAEL allows extrapolation to 90-days toxicity studies.

#### 3.4.2. Study objectives of the 90-day toxicity study

The objectives of the 90-day toxicity study in the authorisation procedures for chemical compounds and biocidal products include each of the five study objectives in TG 408 (OECD, 2018). For REACH, explicit reference is made to OECD TG 408 concerning the objectives (European Parliament and the Council, 2006a). In the guidance document for biocides (ECHA, 2017), the objectives of the 90-day toxicity study are clearly defined, including hazard identification & characterisation purposes (including to establish a NOAEL or BMDL), as well as providing the basis for the design of further longer duration toxicity studies. These correspond with those objectives defined in TG 408, even if the guidance documents do not explicitly mention the TG 408.

#### 3.4.3. Study design, data analysis and quality standards

The criteria for the design of the 90-day toxicity study are defined in the Methods B26 and B27 of REACH and BPR (ECHA, 2017), which replicate the OECD TG 408 and TG 409 of 1998. Even though these TGs have been updated after 1998 (most recently, TG 408 was updated in 2018 (OECD, 2018)), methods B26 and B27 do not seem to be updated to reflect these adaptations. The different requirements regarding study design, data analysis and quality standards are therefore mostly consistent with TG 408. In particular, methods B26 and B27 do not specify requirements for prior studies and the need to state the objectives or conduct a power calculation. They do specify that the tests should be conducted with the active substance, which test species should be used, what the number of dose groups and animals per groups should be, which observations should be reported, that appropriate statistical analyses should be performed and that the conduct of the study should be done in compliance with GLP.

## 4. Discussion

The results confirm that, while a 90-day oral toxicity study is often part of the safety assessment of chemical substances, there is variation in the regulatory provisions with regard to the regulatory obligation to conduct such a study, its objectives, and design. The below discussion considers each of these areas across the various regulatory domains.

### 4.1. Is a 90-day study expected?

In the EU legislation and guidance covered in this paper, a study of subchronic toxicity is generally expected. (The OECD TG408 does not address this point explicitly.) This may, but does not necessarily always include a 90-day toxicity study. There are two types of notable exceptions to this principle. On the one hand, for GMOs, pesticides, and feed additives the regulator has decided that a 90-day study should always be conducted as part of the safety assessment (European Commission, 2013a, 2013b, 2008). What scientific validity there is for making this a requirement in those areas (while not in others) is not clear. For example on GMOs, Knudsen and Poulsen, 2007 reported that meaningful 90-day studies could be done to detect biological/nutritional/toxicological effects of a novel gene insert. However, Bartholomaeus et al. (2013) concluded that such whole food animal toxicity studies are unnecessary and scientifically unjustifiable. On the other hand, REACH legislation states that whether a 90-day study is mandatory or not depends on the tonnage of the product on the market (European Parliament and the Council, 2006a). It also considers scientific reasons for such exceptions, such as when relevant human exposure can be excluded.

There are advantages for not making a 90-day study systematically mandatory. The safety assessment procedure of substances is regarded as a process consisting of successive but distinct, phases, whereby information from each phase is used to determine the set-up of experimental studies of the following phase (Chhabra et al., 1990). Given that a 90-day study is part of this series of studies, it may be that the prior information already shows that such a study will add little to no information (Taylor et al., 2014; Taylor and Andrew, 2017). This is consistent with Directive 2010/63/EU on the protection of animals used for scientific purposes, which aims to minimise use experimental animals for scientific purposes, unless there is a clear need for it (European Parliament and the Council, 2010).

The results also show that even when a 90-day study is not legally mandatory, guidance may create the expectation to provide this type of study either systematically or as part of tiered approach to assess subchronic toxicity. This is particularly the case where the legislation has mandated the regulatory agency to address this, as is the case in the common authorisation procedure for food improvement agents (described in Article 5 (3), Regulation 234/2011). This raises a question as to the role of the legislation versus the guidance in defining requirements. These roles appear to vary between sectors. It would seem that, as a rule, the legal requirements should spell out, and also be limited to, what safety aspects need to be covered in general; whereas the role of guidance is well suited to spell out how these requirements are to be met (Deluyker, 2017). For example, legislation may require that subchronic toxicity always be considered when sub-chronic or longer duration of exposure can be expected, but that exceptions may be granted where scientifically justified.

### 4.2. Study objectives

OECD TG 408 specifies that the study can be initiated for different purposes, describing objectives that can be grouped as hazard identification and hazard characterisation; but the 90-day study can also form the basis for further studies (*i.a.* chronic toxicity studies) (OECD, 2018). The objectives of a 90-day toxicity study or any other form of subchronic toxicity study, are discussed explicitly in legislation on GMOs, feed additives and pesticides (European Commission, 2013a, 2013b, 2008). For the other areas, they are addressed in guidance documents. Some domains explicitly list the potential study objectives whereas other guidance documents refer to TG 408 without providing further specifications. The reasons for and impact of these differences may merit further exploration: the identified differences within and between domains may not result in differences in practice, but this cannot be excluded. It would seem preferable to not restrict the possible range of objectives, but at the same time expect that the main study objective(s) be stated explicitly.

### 4.3. Study design

#### 4.3.1. Role of study objectives

One would expect that the study objectives will largely determine the study design. For example, the design of a study which aims to identify adverse effects at pharmacologically active concentrations is expected to be quite different from the design of a study which aims to identify a no-effect level. For human and veterinary medicines this differentiation is reflected in the directives (European Parliament and the Council, 2001b; 2001a) and their associated guidance documents (EMA - ICH, 2009; 2008, 1999; EMA - VICH, 2003; EMA, 2010).

In other domains, little information seems to be provided on how the objectives of a 90-day toxicity study will affect the study design. Hence, it is not surprising that (with the sole exception of GM food assessment, where a hypothesis is required (EFSA GMO Panel, 2011; EFSA GMO Unit, 2014; EFSA Scientific Committee et al., 2011; European Commission, 2013a), next to the suggestions for human medicines to use a hypothesis-based approach when assessing toxicity) none of the studied documents (including TG 408), specify that a hypothesis is required for conducting a 90-day toxicity study. This suggests that a 90-day toxicity study is not hypothesis-based, but rather hypothesis-generating. Also, the establishment of a dose-reponse curve does not require a 'hypothesis' in the strictest sense, i.e. a 'null-hypothesis' that is tested and can possibly be rejected.

#### 4.3.2. Role of prior studies

The need for careful consideration of prior studies, including on toxicokinetics (Rozman and Doull, 2000), in the design of sub-chronic and chronic toxicity studies is well known (Rozman, 1993). OECD TG 408 foresees that the study is conducted after initial information has been obtained from an acute or a repeated-dose 28-day toxicity tests. While it would seem unlikely that any 90-day study would be initiated without prior information on acute and subacute toxicity, it is not clarified what type of information should be used from these prior studies to the conduct of a subsequent 90-day sub-chronic oral toxicity study.

The studied legislation and guidance documents vary in their description of the need for prior studies as input for the 90-day toxicity study. Only for human and veterinary medicines, is such data explicitly

expected (EMA, 2010). The studied documents of GM food, feed additives and novel food suggest that prior studies are needed when designing the 90-day toxicity study, although it is not clearly indicated to that this is expected (EFSA FEEDAP Parasuraman, 2011; EFSA GMO Panel, 2011; EFSA NDA Parasuraman, 2011; European Commission, 2013a; 2008). In various other domains however, no specific requirements are made regarding whether or what type of prior information is needed and how such prior findings affect the key objectives and subsequently, the design of the 90-day study.

#### 4.3.3. Specific design aspects

Already in 1982 Gale and Sheppard (1982) advocated that the 90-day rat study should not be considered as routine. Rather, they argued, *"[…] there should be a basic framework around which a study protocol is built which is specific for the type of chemical to be investigated"* (Gale and Sheppard, 1982). TG 408 provides recommendations on the choice of the test species, the dose groups, the types of observations to be made, and statistical analyses to be conducted (OECD, 2018). For example, TG 408 states that a minimum of 20 animals per dose group is needed. However, it does not clarify what the basis for the number to be used per dose group; the choice of the dose groups nor the purpose of the statistical analyses.

The various domain-specific documents that were considered show (Table 3) that also in these areas key elements in the design of a study are very broad and not described so as to be aligned with the specific objectives of the study. This may be interpreted to suggest that the same study set-up is expected to be adequate to achieve differing objectives.

In contrast with TG 408 and with other domains, the requirements for subchronic toxicity testing in GM foods and human medicines authorisation procedures state that the number of animals to be tested depends on the parameters measured and the effect sizes of these parameters (EFSA GMO Panel, 2011; EMA - ICH, 2009; 1999; EMA - VICH, 2003). This is critical, as the power calculation forms the scientific basis for justifying that the design of the study is expected to be able to detect suspected effects of substances at a level that is biologically meaningful.

Hence, it is important that, along with a statistical analysis, the power of the study be documented *post-hoc* to assess whether the study was able to detect statistically significant differences at a level that is considered to be of biological relevance (EFSA Scientific Committee et al., 2017a). It also serves as input in the design of subsequent studies, thereby potentially avoiding e.g. that an effect may show up in several studies as biologically significant but no study ever demonstrated it was statistically significant or vice versa.

Challenges in interpreting results both within and between studies from a biological and statistical perspective are well known (Lewis et al., 2002). For example, weaknesses in the statistical analyses on small group sizes have been reported by Na et al. (2014); and Schmidt et al. (2016) have studied how modern statistical methods may help address concerns regarding statistical significance and biological relevance. Statistical analysis approaches of 90-day studies share challenges common with other repeated-dose toxicity studies (Kavlock et al., 1996; Schmidt et al., 2016). Hence they are not expected to be an explicit part of a 90-day study guidance but rather are the subject of separate guidance (EFSA Scientific Committee et al., 2017a; 2017b). This generally concerns guidance on data analysis after the data has been collected. What can be beneficial is that the data analysis that is envisioned be justified for the intended purpose i.e. to achieve the study objectives. For example, for the conduct of a benchmark dose (BMD) analysis, Kavlock et al. (1996) studied the importance effects of key design aspects, i.e. number of dose groups, dose spacing, dose placement, and sample size per dose group on the calculation of the BMD for developmental toxicity.

### 4.4. Relevance of a 90-day study

The relevance and added value of a 90-day toxicity study with the objective of setting the NOAEL for compounds with low toxicity in a 28-day study has been discussed by Taylor and colleagues (Taylor et al., 2014; Taylor and Andrew, 2017). They used a limited dataset and (implicitly) assumed that the key variation in the design of these studies was the duration of treatment. They concluded that conducting such a 90-day oral toxicity test, in addition to a 28-day study does not necessarily provide new insights for low toxicity industrial chemicals. Further research conducted by Luechtefeld et al (2016) on a larger number of chemicals with a NOAEL $\geq$ 1000 mg/kg bw/day in a 90-day toxicity study, (only) some 70% had a 90-day NOAEL at that level i.e. the rest were lower.

After analysing a dataset containing studies from both the ECHA database and studies that were used by the Agency for Toxic Substances and Disease Registry regarding drinking water assessment, Lampe et al. (2018) suggested that an extrapolation factor of 10 would allow for the extrapolation of NOAELs and BMDs identified from a 28-day study to a 90-day study (Lampe et al., 2018). This safety factor is then applied, along with others, as appropriate (EFSA Scientific Committee et al., 2012). They explored, through a sensitivity analysis, whether some design characteristics affected the relationship between the 28-day and 90-day NOAEL and found little difference, except when a small number of animals per dose group was used in the 28-day study.

On the other hand, Wang and Gray (2015) studied the effect of duration along with other study design aspects i.e. test species (mouse, rat) and sex on outcome variables (lesion site). They found that the value of subchronic toxicity studies (12–14 weeks) for chronic toxicity (2 years) and for sex/species (rats versus mice) comparisons in predicting the site of lesion by the same chemical was subject to considerable uncertainty (Wang and Gray, 2015). This suggests that several elements of the study design, rather than just duration of exposure, may need to be considered in an analysis of the predictive value of shorter term studies for long term toxicity (Roberts et al., 2015).

## 5. Conclusions & future perspectives

The analysis presented in this paper shows that the 90-day toxicity study is still considered essential in different regulatory domains. Also in other jurisdictions, such as the USA, no alternatives to the use of the 90-day oral toxicity study have been validated or accepted (Hartung, 2018). What is less clear from the reviewed documents, is what study designs are expected to achieve the main chosen objective(s) and minimise the subsequent difficulties in the interpretation of the results.

For this study design the EU, with the important exception of human pharmaceuticals, seems to most often rely (directly or indirectly) on the OECD guidance TG 408 which offers numerous technical specifications. In contrast, in human medicines there are much fewer such specifications and the emphasis is on justification of key study design decision. These approaches can be viewed as complementary. One puts the emphasis on describing necessary conditions (similar to e.g. complying with GLP requirements) and the other takes the perspective of what are sufficient conditions.

The interpretation of the results of an individual subchronic toxicity study, let alone a set of toxicity studies, may be quite challenging (Lewis et al., 2002). Also, many authors (e.g. Bokkers and Slob, 2005; Chapman et al., 2013; Merone et al., 2014; Nelms et al., 2018) have questioned current laboratory-animal based approaches and suggested use of potential alternative methods to improve the predictive value of the experimental studies in the safety assessment thereby simultaneously contributing to the reduction of animal use and improving the efficiency of, for instance, drug development.

Much attention and resources are indeed currently devoted to the development and validation of non-animal tests or how animal-based studies could be made obsolete by extrapolation from shorter-duration studies. In parallel, there may also be an opportunity to reflect on possibilities to optimise the design of *in vivo* toxicology studies, such as the 90-day study. It is now 40 years after the OECD 408 study design guidance was issued; it may be worthwhile to look again at the design

guidance for these studies so as to facilitate the subsequent interpretation of the results.

Our review of the legislation and related guidance documents merely provides a theoretical perspective on the use of 90-day toxicity studies to substantiate authorisation requests. Along with simulation studies (Kavlock et al., 1996), large-scale systematic analysis of past studies (and risk assessments) could help to identify best practices. The existence of differing practices in different regulatory domains is in that regard not necessarily a disadvantage. The advent of big data and AI along with modern statistical methods lend themselves to such applied research on design optimization.

Results may lead to updating previous guidance on study design optimisation either through scientific literature (Chhabra et al., 1990), and/or the updating of EU topical guidance, such as EMA's; the development of EU cross-cutting guidance (similar to the NTP guidance in the USA (National Toxicology Program, 2011)) and/or the updating of guidance at international level (ICH or OECD) to ensure Mutual Acceptance of data (MAD) principles are met (OECD, 2020). This is not to say that guidance documents need to prescribe in utmost detail how to conduct every type of study and not remain open to serendipitous findings. Providing both flexibility, along with the necessity to justify key design choices, may save resources, improve the quality of studies, while providing transparent justification for the use of animals and thus meet the EU's stated objectives in this domain.

## Declaration of competing interest

The authors declare that they have no known financial competing interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors thank Dr. George Kass for providing his critical perspective on the paper. This research has been made possible with the support of the Dutch Province of Limburg.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yrtph.2020.104786.

## References

Bartholomaeus, A., Parrott, W., Bondy, G., Walker, K., 2013. The use of whole food animal studies in the safety assessment of genetically modified crops: Limitations and recommendations. Crit. Rev. Toxicol. Rev Toxicol. https://doi.org/10.3109/10408444.2013.842955.

Bokkers, B.G.H., Slob, W., 2005. A comparison of Ratio Distributions Based on the NOAEL and the benchmark approach for Subchronic-to-Chronic extrapolation | Toxicological Sciences | Oxford Academic. Toxicol. Sci. Sci 85, 1033–1040.

Chapman, K.L., Holzgrefe, H., Black, L.E., Brown, M., Chellman, G., Copeman, C., Couch, J., Creton, S., Gehen, S., Hoberman, A., Kinter, L.B., Madden, S., Mattis, C., Stemple, H.A., Wilson, S., 2013. Pharmaceutical toxicology: Designing studies to reduce animal use, while maximizing human translation. Regul. Toxicol. Pharmacol. Toxicol Pharmacol 66, 88–103. https://doi.org/10.1016/j.yrtph.2013.03.001.

Chhabra, R.S., Huff, J.E., Schwetz, B.S., Selkirk, J., 1990. An overview of Prechronic and Chronic toxicity/Carcinogenicity Experimental study Designs and Criteria used by the National toxicology Program. Environ. Health Perspect. Health Perspect 86, 321. https://doi.org/10.2307/3430970.

Commission of the European Communities, 2008. European Agencies - the Way Forward {SEC(2008) 323} (Brussels).

Deluyker, H., 2017. Is scientific assessment a scientific discipline? EFSA J 15.

Directorate E Directorate-General Health & Consumer Protection, 2001. Draft Guidance Document GUIDANCE for the SETTING of an ACUTE REFERENCE DOSE (ARfD).

ECHA, 2017. Guidance on the Biocidal Products Regulation Volume III Human Health-Assessment & Evaluation (Parts B+C). https://doi.org/10.2823/143042. Helsinki, Finland.

EFSA ANS Panel, 2012. Guidance for submission for food additive evaluations. EFSA J 10, 2760.

EFSA ANS Panel, Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Dusemund, B., Filipičč, M., Frutos, M.J., Galtier, P., Gundert-Remy, U., Kuhnle, G.G., Lambré, C., Leblanc, J., Lillegaard, I.T., Moldeus, P., Mortensen, A., Oskarsson, A., Stankovic, I., Waalkens-Berendsen, I., Woutersen, R.A., Wright, M., Di Domenico, A., Fairweather-Tait, S., McArdle, H., Smeraldi, C., Gott, D., 2018. Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources. EFSA J 16, 5294. https://doi.org/10.2903/j.efsa.2018.5294.

EFSA CEF Panel, 2010. Guidance on the data required for the risk assessment of flavourings to be used in or on foods. EFSA J 8, 1623. https://doi.org/10.2903/j.efsa.2010.1623.

EFSA CEF Panel, 2009. Guidance of the scientific Panel of food contact material, enzymes, flavourings and Processing Aids (CEF) on the submission of a Dossier on food enzymes for safety evaluation. EFSA J 7, 1305. https://doi.org/10.2903/j.efsa.2009.1305.

EFSA CEF Panel, Silano, V., Bolognesi, C., Castle, L., Cravedi, J., Engel, K., Fowler, P., Franz, R., Grob, K., Gürtler, R., Husøy, T., Kärenlampi, S., Mennes, W., Milana, M.R., Penninks, A., de Fátima Tavares Poças, M., Smith, A., Tlustos, C., Wölfle, D., Zorn, H., Zugravu, C., 2008. Note for guidance for the preparation of an application for the safety assessment of a Substance to be used in plastic food contact materials. EFSA J 6, 21r. https://doi.org/10.2903/j.efsa.2008.21r.

EFSA FEEDAP Panel, Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. de L., Bories, G., Chesson, A., Cocconcelli, P.S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., Villa, R.E., Wallace, R.J., Wester, P., Anguita, M., Dujardin, B., Galobart, J., Innocenti, M.L., 2017. Guidance on the assessment of the safety of feed additives for the consumer. EFSA J 15, 5022. https://doi.org/10.2903/j.efsa.2017.5022.

EFSA GMO Panel, 2011. Guidance for risk assessment of food and feed from genetically modified plants. EFSA J 9, 2150. https://doi.org/10.2903/j.efsa.2011.2150.

EFSA GMO Unit, 2014. Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment. EFSA J 12, 3871. https://doi.org/10.2903/j.efsa.2014.3871.

EFSA NDA Panel, Turck, D., Bresson, J., Burlingame, B., Dean, T., Fairweather-Tait, S., Heinonen, M., Hirsch-Ernst, K.I., Mangelsdorf, I., McArdle, H., Naska, A., Neuhäuser-Berthold, M., Nowicka, G., Pentieva, K., Sanz, Y., Siani, A., Sjödin, A., Stern, M., Tomé, D., Vinceti, M., Willatts, P., Engel, K., Marchelli, R., Pöting, A., Poulsen, M., Salminen, S., Schlatter, J., Arcella, D., Gelbmann, W., Sesmaisons-Lecarré, A., Verhagen, H., Loveren, H., 2016a. Guidance on the Preparation and Presentation of an Application for Authorisation of a Novel Food in the Context of Regulation (EU) 2015/2283. EFSA J 14.

EFSA NDA Panel, Turck, D., Bresson, J., Burlingame, B., Dean, T., Fairweather-Tait, S., Heinonen, M., Hirsch-Ernst, K.I., Mangelsdorf, I., McArdle, H., Naska, A., Neuhäuser-Berthold, M., Nowicka, G., Pentieva, K., Sanz, Y., Siani, A., Sjödin, A., Stern, M., Tomé, D., Vinceti, M., Willatts, P., Engel, K., Marchelli, R., Pöting, A., Poulsen, M., Schlatter, J., Gelbmann, W., de Sesmaisons-Lecarré, A., Verhagen, H., Loveren, H., 2016b. Guidance on the Preparation and Presentation of the Notification and Application for Authorisation of Traditional Foods from Third Countries in the Context of Regulation (EU) 2015/2283. https://doi.org/10.2903/J.EFSA.2016.4590. EFSA J 14.

EFSA Scientific Committee, Antunović, B., Barlow, S., Chesson, A., Flynn, A., Hardy, A., Jeger, M., Knaap, A., Kuiper, H., Lovell, D., Nørrung, B., Pratt, I., Rietjens, I., Schlatter, J., Silano, V., Smulders, F., Vannier, P., 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA J 10, 2579. https://doi.org/10.2903/j.efsa.2012.2579.

EFSA Scientific Committee, Antunović, B., Barlow, S., Chesson, Andrew, Flynn, A., Hardy, A., Jeger, M., Knaap, A., Kuiper, H., Lovell, D., Nørrung, B., Pratt, I., Rietjens, I., Schlatter, J., Silano, V., Smulder, F., Vannier, P., 2011. Guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA J 9, 2438. https://doi.org/10.2903/j.efsa.2011.2438.

EFSA Scientific Committee, Hardy, A., Benford, D., Halldorsson, T., Jeger, M.J., Knutsen, H.K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J.R., Silano, V., Solecki, R., Turck, D., Younes, M., Bresson, J., Griffin, J., Hougaard Benekou, S., van Loveren, H., Luttik, R., Messean, A., Penninks, A., Ru, G., Stegeman, J.A., van der Werf, W., Westendorf, J., Woutersen, R. A., Barizzone, F., Bottex, B., Lanzoni, A., Georgiadis, N., Alexander, J., 2017a. Guidance on the assessment of the biological relevance of data in scientific assessments. EFSA J 15. https://doi.org/10.2903/j.efsa.2017.4970.

EFSA Scientific Committee, Hardy, A., Benford, D., Halldorsson, T., Jeger, M.J., Knutsen, K.H., More, S., Mortensen, A., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Silano, V., Solecki, R., Turck, D., Aerts, M., Bodin, L., Davis, A., Edler, L., Gundert-Remy, U., Sand, S., Slob, W., Bottex, B., Abrahantes, J.C., Marques, D.C., Kass, G., Schlatter, J.R., 2017b. Update: use of the benchmark dose approach in risk assessment. EFSA J 15, 4658. https://doi.org/10.2903/j.efsa.2017.4658.

E, 2010. Repeated Dose Toxicity. CPMP/SWP/1042/99 Rev. 1 Corr.

EMA - ICH, 2009. ICH Guideline M3(R2) on Non-clinical Safety Studies Fort He Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals (CPMP/ICH/286/1995) step. 5.

EMA - ICH, 2008. Note for Guidance on Non-clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (CPMP/ICH/286/95) step. 3.

EMA - ICH, 1999. Note for Guidance on Duration of Chronic Toxicity Testing in Animals (Roden Tand Non Rodent Toxicity Testing). CPMP/ICH/300/95.

EMA - VICH, 2003. Topic GL31 Safety Studies for Veterinary Drug Residues in Human Food: Repeat-Dose (90) Toxicity Testing.

European Commission, 2017a. Commission Implementing Regulation (EU) 2017/2468 of 20 December 2017 laying down administrative and scientific requirements concerning traditional foods from third countries in accordance with Regulation (EU) 2015/2283 of the European Parliament and of t. OrkesterjournalenJ L 351, 55, 63.
European Commission, 2017b. Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foo. OrkesterjournalenJ L 351, 64–71.
European Commission, 2013a. Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council. OrkesterjournalenJ L 157, 1–48.
European Commission, 2013b. Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection produc. OrkesterjournalenJ L 93, 1–84.
European Commission, 2011a. Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. OrkesterjournalenJ L 12, 1–89.
European Commission, 2011b. Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OrkesterjournalenJ L 64, 15–24.
European Commission, 2008. Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessme. OrkesterjournalenJ L 133, 1–65.
European Council, 2008. Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, evaluation, authorisation and Restriction of chemicals (REACH. OrkesterjournalenJ L 142, 1–793.
European Parliament and the Council, 2015. Regulation (EU) 2015/2283 on novel foods. OrkesterjournalenJ L 327, 1–22.
European Parliament and the Council, 2013. Regulation (EU) No 609/2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control. OrkesterjournalenJ L 181, 35–56.
European Parliament and the Council, 2012. Regulation (EU) No 528/2012 on the market and use of biocidal products. OrkesterjournalenJ L 167, 1–123.
European Parliament and the Council, 2010. Directive 2010/63/EU on the protection of animals used for scientific purposes. OrkesterjournalenJ L 276, 33–79.
European Parliament and the Council, 2009. Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market. OrkesterjournalenJ L 309, 1–50.
European Parliament and the Council, 2008a. Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures. OrkesterjournalenJ L 353, 1–1355.
European Parliament and the Council, 2008b. Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OrkesterjournalenJ L 354, 1–6.
European Parliament and the Council, 2008c. Regulation (EC) No 1332/2008 on food enzymes. OrkesterjournalenJ L 354, 7–15.
European Parliament and the Council, 2008d. Regulation (EC) No 1333/2008 on food additives. OrkesterjournalenJ L 354, 16–33.
European Parliament and the Council, 2008e. Regulation (EC) No 1334/2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. OrkesterjournalenJ L 354, 34–50.
European Parliament and the Council, 2006a. Regulation (EC) No 1907/2006 concerning the Registration, evaluation, authorisation and Restriction of chemicals (REACH), establishing a European chemicals agency. OrkesterjournalenJ L 396, 1–849.
European Parliament and the Council, 2006b. Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods. OrkesterjournalenJ L 404, 26–38.
European Parliament and the Council, 2004a. Regulation (EC) No 726/2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency. OJL 136, 1–33.
European Parliament and the Council, 2004b. Directive 2004/9/EC on the inspection and verification of good laboratory practice (GLP). OrkesterjournalenJ L 50, 28–43.
European Parliament and the Council, 2004c. Directive 2004/10/EC on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances. OrkesterjournalenJ L 50, 44–59.
European Parliament and the Council, 2004d. Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food. OrkesterjournalenJ L 338, 4–17.
European Parliament and the Council, 2003a. Regulation (EC) No 1829/2003 on genetically modified food and feed. OrkesterjournalenJ L 268, 1–23.
European Parliament and the Council, 2003b. Regulation (EC) No 1831/2003 on additives for use in animal nutrition. OrkesterjournalenJ L 268, 29–43.
European Parliament and the Council, 2002a. Regulation (EC) No 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OrkesterjournalenJ L 31, 1–24.
European Parliament and the Council, 2002b. Directive 2002/46/EC on the approximation of the laws of the Member States relating to food supplements. OrkesterjournalenJ L 183, 51–57.
European Parliament and the Council, 2001a. Directive 2001/83/EC on the Community code relating to medicinal products for human use. OrkesterjournalenJ L 311, 67–128.
European Parliament and the Council, 2001b. Directive 2001/82/EC on the Community code relating to veterinary medicinal products. OrkesterjournalenJ L 311, 1–66.
Gale, E., Sheppard, D., 1982. Can the Design of the 'Routine' 90 Day Rat Study Be Improved? Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-68511-8_11, 68-68.
Hartung, T., 2018. Rebooting the generally recognized as safe (GRAS) approach for food additive safety in the US. ALTEX 35, 3–25. https://doi.org/10.14573/altex.1712181.
Kavlock, R.J., Schmid, J.E., Setzer, R.W., 1996. A Simulation study of the Influence of study design on the Estimation of benchmark Doses for Developmental toxicity. Risk Anal. 16, 399–410. https://doi.org/10.1111/j.1539-6924.1996.tb01474.x.
Knudsen, I.B., Poulsen, M., 2007. Comparative safety testing of genetically modified foods in a 90-day rat feeding study design allowing the distinction between primary and secondary effects of the new genetic event. Regul. Toxicol. Pharmacol. Toxicol Pharmacol 49, 53–62. https://doi.org/10.1016/j.yrtph.2007.07.003.
Lampe, B.J., Fuller, E., Kuppusamy, S.P., 2018. A quantitative comparison of points of departure between 28-day and 90-day repeated dose studies with a proposed extrapolation factor. Regul. Toxicol. Pharmacol. Toxicol Pharmacol 92, 189–200. https://doi.org/10.1016/j.yrtph.2017.12.007.
Lewis, R.W., Billington, R., Debryune, E., Gamer, A., Lang, B., Carpanini, F., 2002. Recognition of Adverse and Nonadverse effects in toxicity studies. Toxicol. Pathol. Pathol 30, 66–74. https://doi.org/10.1080/01926230252824725.
National Research Council, 1994. Review of the U.S. Naval Medical Research Institute's Toxicology Program. The National Academies Press, Washington, DC. https://doi.org/10.17226/9140.
National Toxicology Program, 2011. Specifications for the Conduct of Studies to Evaluate the Toxic and Carcinogenic Potential of Chemical, Biological and Physical Agents in Laboratory Animals for the National Toxicology Program (NTP).
Nelms, M.D., Simmons, J.E., Edwards, S.W., 2018. Adverse Outcome Pathways to Support the assessment of chemical mixtures. In: Chemical Mixtures and Combined Chemical and Nonchemical Stressors. Springer International Publishing, Cham, pp. 177–201. https://doi.org/10.1007/978-3-319-56234-6_7.
OECD, 2020. Mutual Acceptance of Data (MAD) [WWW Document]. https://www.oecd.org/env/ehs/mutualacceptanceofdatamad.htm. (Accessed 9 November 2020).
OECD, 2018. OECD Guidelines for the Testing of Chemicals, Section 4 - Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents. Paris.
OECD, 1999. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring - Quality Assurance and GLP. Paris.
Parasuraman, S., 2011. Toxicological screening. J. Pharmacol. Pharmacother. Pharmacol Pharmacother 2, 74. https://doi.org/10.4103/0976-500X.81895.
Roberts, R., Callander, R., Duffy, P., Jacobsen, M., Knight, R., Boobis, A., 2015. Target organ profiles in toxicity studies supporting human dosing: Does severity progress with longer duration of exposure? Regul. Toxicol. Pharmacol. Toxicol Pharmacol 73, 737–746. https://doi.org/10.1016/j.yrtph.2015.10.021.
Rozman, K.K., 1993. Use of acute toxicity data in the design and interpretation of Subchronic and Chronic toxicity studies. In: Yacobi, A., Skelly, J.P., Shah, V.P., Benet, L.Z. (Eds.), Integration of Pharmacokinetics, Pharmacodynamics, and Toxicokinetics in Rational Drug Development. Springer Science+Business Media, New York, pp. 39–48. https://doi.org/10.1007/978-1-4757-1520-0_6.
Rozman, K.K., Doull, J., 2000. Dose and time as variables of toxicity. Toxicology 144, 169–178. https://doi.org/10.1016/S0300-483X(99)00204-8.
Schmidt, K., Schmidtke, J., Kohl, C., Wilhelm, R., Schiemann, J., Van Der Voet, H., Steinberg, P., 2016. Enhancing the interpretation of statistical P values in toxicology studies: implementation of linear mixed models (LMMs) and standardized effect sizes (SESs). Arch. Toxicol. Toxicol 90, 731–751. https://doi.org/10.1007/s00204-015-1487-8.
Taylor, K., Andrew, D.J., 2017. The added value of the 90-day repeated dose oral toxicity test for industrial chemicals with a low (sub)acute toxicity profile in a high quality dataset: an update. Regul. Toxicol. Pharmacol. Toxicol Pharmacol 90, 258–261.
Taylor, K., Andrew, D.J., Rego, L., 2014. The added value of the 90-day repeated dose oral toxicity test for industrial chemicals with a low (sub)acute toxicity profile in a high quality dataset. Regul. Toxicol. Pharmacol. Toxicol Pharmacol 69, 320–332.
Wang, B., Gray, G., 2015. Concordance of Noncarcinogenic Endpoints in Rodent chemical Bioassays. Risk Anal. 35, 1154–1166. https://doi.org/10.1111/risa.12314.