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The last resort requirement under REACH: From principle to practice

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ABSTRACT

REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) is a European Union regulation that aims to protect human health and the environment from the risks posed by chemicals. Article 25 clearly states that: "*[i]n order to avoid animal testing, testing on vertebrate animals for the purposes of this Regulation shall be undertaken only as a last resort.*" In practice, however, the standard information requirements under REACH are still primarily filled using animal studies.

This paper presents examples illustrating that animal testing is not always undertaken only as a last resort. Six over-arching issues have been identified which contribute to this: (1) non-acceptance of existing animal or non-animal data, (2) non-acceptance of read-across, (3) inflexible administrative processes, (4) redundancy of testing, (5) testing despite animal welfare concerns and (6) testing for cosmetic-only ingredients.

We, members of the Animal-Free Safety Assessment (AFSA) Collaboration, who work together to accelerate the global adoption of non-animal approaches for chemical safety assessment, herein propose several recommendations intended to aid the European Commission, the European Chemicals Agency and registrants to protect human health and the environment while avoiding unnecessary animal tests - truly upholding the last resort requirement in REACH.

1. Introduction

1.1. The Animal-Free Safety Assessment collaboration

The Animal-Free Safety Assessment (AFSA) Collaboration is a multi-stakeholder initiative developed to accelerate the global adoption of modern chemical safety assessment using non-animal approaches (AFSA, 2018). The AFSA Collaboration brings together leading industry and not-for-profit organisations with a shared goal to better protect people and our planet, by replacing animal testing with more predictive and relevant approaches. It has several key areas of activity, including:

- Increasing the understanding, uptake and acceptance of non-animal approaches in jurisdictions with or without cosmetic animal-testing bans through educational resources and targeted publications

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Abbreviations

(Q)SAR	(Quantitative) Structure-Activity Relationship
AFSA	Animal-Free Safety Assessment
BoA	ECHA Board of Appeal
CLP	Classification, Labelling and Packaging
DNEL	Derived No Effect Limit
EC	European Commission
ECHA	European Chemicals Agency
ECJ	European Court of Justice
eMSCA	evaluating Member State Competent Authority
EOGRTS	Extended One-Generation Reproductive Toxicity Study
FELS	Fish, early-life stage toxicity test
HPVIS	High Production Volume Information System
IUCLID	International Uniform Chemical Information Database
MSC	Member State Committee
MSCA	Member State Competent Authorities
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PNDT	Prenatal Developmental Test
RAAF	Read-Across Assessment Framework
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SIR	Standard Information Requirements
STOT RE	Specific Target Organ Toxicity - Repeat Exposure
TG	Test Guideline
US EPA	United States Environmental Protection Agency

- Accelerating global regulatory alignment in chemicals and medicines sectors
- Identifying barriers to regulatory acceptance
- Developing strategies to overcome regulatory barriers through sharing experiences and case examples.

One significant area of interest to the AFSA Collaboration is how chemical safety assessments are carried out in practice, especially when legislation mandates a paradigm shift from animal testing towards animal-free approaches - as is the case for the last resort requirement in the European Union's regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (European Union, 2006).

1.2. The last resort requirement in REACH

REACH aims to protect human health and the environment from the risks posed by chemicals (European Union, 2006). Companies that manufacture or import chemicals in the EU must register their substances and provide standardised information on their properties and hazard according to the intended production or import volume. This tonnage level serves as a proxy of exposure, each level with its own Annex. Each Annex lists a 'tick-box' list of cumulative standard information requirements (SIRs) consisting of intrinsic chemical properties, toxicological and ecotoxicological hazards which must be fulfilled. The '3Rs' principle of Replacement, Reduction, and Refinement of use of animals in scientific research is a key element of the REACH legislative text, exemplified by Article 1 plainly stating that "*[t]he purpose of this 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*" [emphasis added], and reinforced by Article 13 "*Information on intrinsic properties of substances may be generated by means other than tests … In particular for human toxicity,* ***information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods***" and Article 25(1) "*[i]n order to avoid animal testing, testing on vertebrate animals for the purposes of this Regulation shall be undertaken* ***only as a last resort***" [emphasis added]. Since REACH was launched in 2006, there has been much progress. Science has evolved - there are now even more non-animal approaches available, such as *in silico* tools, *in vitro* assays, 'omics, defined approaches, next-generation risk assessment, as well as initiatives dedicated to the development of non-animal approaches for every endpoint in REACH, including complex endpoints (ONTOX, 2021; PrecisionTox, 2021; RISK-HUNT3R, 2021). Furthermore, there has been a testing and marketing ban on the use of animal testing for cosmetics in the EU since 2013 (European Union, 2009). Despite this commitment to the 3Rs and the last resort requirement, due to the structure of REACH it is challenging for registrants to argue that an *in vivo* SIR is not required, even if the test is unlikely to make a difference to the overall safety assessment. As a result, the SIRs under REACH are still primarily filled using animal studies - with overall animal numbers going far beyond the original estimates for REACH (Knight et al., 2023) and set to increase further (Rovida et al., 2023).

1.3. A brief summary of evaluation under REACH

To understand why acceptance of non-animal approaches is low, it is important to understand the process of REACH dossier evaluation. In brief, REACH registration begins with the submission of a registration dossier, by a registrant (or group of registrants), containing information on their substance according to the specific use and manufacture/import volume, to the European Chemicals Agency (ECHA). Evaluation under REACH consists of three areas: a compliance check, examination of testing proposals and substance evaluation. Within each evaluation, the registrant can comment during the decision process and/or appeal the decision, however, there is a time limit enforced and after this passes the evaluator is unable to accept any additional information from the registrant. However, the registrant is permitted to adapt a requested study, even after a final decision has been given.

Compliance check is carried out on 20% of the registrations (European Chemicals Agency, 2019b). The outcomes may be that no information is required at present or that additional testing, or further information, is required for SIRs in the relevant tonnage band e.g. an additional animal test. ECHA communicates this by sending a draft decision letter to the registrant, who can accept or contest the draft decision. If contested, the registrant has 30 days to provide comments to ECHA, who review the registrant's comment and may or may not amend the draft decision. Next, the Member State Committee (MSC) is notified about the draft final decision by ECHA. If the MSC proposes no amendments, then the draft final decision is adopted. Upon receiving this final decision, the registrant has another opportunity to appeal, by filing to the independent Board of Appeal (BoA), who consider the scientific reasoning and arguments from the registrant and ECHA/MSC and who may uphold or amend the decision.

Another area under evaluation is examination of testing proposals. A testing proposal must be submitted by the registrant if a new Annex IX or Annex X (animal) test is intended to be performed to fulfil SIR. The testing proposal is published by ECHA, allowing third parties 45 days to submit "*scientifically valid information and studies that address the relevant substance and hazard endpoint, relating to the testing proposal*" (European Union, 2006). This process is intended to ensure that the best use has been made of existing information, particularly information on existing vertebrate tests. The possible outcomes are acceptance, acceptance with modifications, acceptance or rejection but additional tests are required, or rejection of the testing proposal.

Substance evaluation is the third type of evaluation, intended to

clarify whether a substance may harm human health or the environment. Different from the other two evaluations, substance evaluation is carried out by an evaluating Member State Competent Authority (eMSCA). The draft decision may request further information from the registrants, which can be tests not included in SIR if it fits the criteria to address a concern, and could lead to change in risk management measures.

This is only a brief summary of evaluation under REACH - a more detailed description is outside the scope of this manuscript and is described elsewhere (European Chemicals Agency, 2022c, 2022e).

1.4. The low acceptance of non-animal approaches in REACH

REACH has several mechanisms embedded within the regulation to allow the use of non-animal approaches, including:

- The inclusion of the Articles described above which promote the use of non-animal approaches and stipulate that animal testing should only be carried out as a last resort
- The SIRs being largely drafted in a manner that requires information to inform a specific outcome versus specifying a(n) (OECD Test Guideline) study
- The presence of Annex XI which permits the use of adaptations (omission of a SIR test) if the registrant can illustrate that testing does not appear scientifically necessary, testing is technically not possible, or where exposure scenarios mean testing can be omitted (a substance-tailored exposure-driven adaptation)
- The speedy adoption of updated test methods (including many non-animal approaches) via the amendment to Regulation (EC) No 440/2008 laying down test methods pursuant to the REACH regulation (European Union, 2008).

However, SIRs are still achieved predominantly with animal tests and the regulatory acceptance of non-animal approaches remains low (European Chemicals Agency, 2020b, 2023c; Westmoreland et al., 2022). Whilst acknowledging the importance of relying on validated methods to demonstrate safety, there is too often an automatic predisposition that SIRs can only be met though adherence to an *in vivo* OECD Test Guideline. This imposes a barrier to the inclusion of knowledge generated by non-animal approaches, prior guideline studies, non-guideline studies, or guideline studies with limitations on the public availability of detailed data. In spite of the mechanisms in REACH designed to achieve the aim of animal testing as a last resort, journal articles providing clear frameworks and proposals to better integrate non-animal methods in regulatory frameworks (Ball et al., 2022; Health Canada, 2021; Pereira et al., 2022) and pressure from the European Parliament to accelerate the phasing out of animal use in regulatory testing (European Union, 2021), evidence suggests that often, unless a fully validated non-animal study exists, which provides identical information to the animal test, non-animal data are systematically questioned and rejected by ECHA and/or Member States during compliance check and/or substance evaluations, ultimately leading to a request for the animal study to be performed (Fentem et al., 2021). It also appears that there is limited consideration of comments to support non-animal approaches during the commenting period for testing proposals (Taylor et al., 2014).

Furthermore, the use/acceptance of the oft-cited read-across and *in silico* (quantitative) structure activity relationship ((Q)SAR) models to fulfil SIR has reduced (read-across) or remained fairly static (*in silico*) as reported in The Use of Alternatives to Testing on Animals for the REACH Regulation (Table 1), published as per Article 117(3) in REACH, hereafter referred to as Article 117(3) report(s) (European Chemicals Agency, 2020b, 2023c). This is surprising, given the publication of guidance documents such as the ECHA Read-Across Assessment Framework (RAAF) (European Chemicals Agency, 2017c) and the Practical Guide on how to use and report (Q)SARs (European Chemicals Agency, 2016b) developed to address the variable quality in read-across and (Q)SAR submissions. This may suggest that registrants are having such approaches rejected or pre-emptively use experimental tests as the path of least resistance.

Table 1
Options used to fulfil SIR in REACH in 2016, 2019 and 2022, given as percentages. Compiled from ECHA's 2020; 2023 Article 117(3) reports (European Chemicals Agency, 2020b, 2023c).

Option used	2016 (%)	2019 (%)	2022 (%)
Experimental	27.6	27.1	30.9
Read-across/category	27.7	25.1	22.8
QSAR	3.0	2.6	2.8
Weight of evidence	3.7	3.7	4.1
Other	5.6	4.8	3.3
Data waiver	10.8	7.7	7.1
Testing proposal	0.3	0.2	0.2
No information	21.2	28.7	28.8

Moreover, even with Annex XI, which expressly permits the adaptation of SIRs using a variety of non-animal and non-testing information, and contains clear language around so-called "*suitable*" non-animal methods, defined as "*sufficiently well developed according to internationally agreed test development criteria … for the entry of a test into the prevalidation process*", personal communications to the authors indicates that there is a fear, or assumption, that non-animal methods will be rejected by regulators, borne out of experience that they must provide information directly equivalent to that of animal tests.

Shifting the focus onto animal studies, the limitations of animal studies for predicting effects in humans is something that is seldom discussed. For example, a systematic review reported that the results from 18%, 37%, and 45% of animal studies were contradicted, replicated, and not tested in human randomised trials, respectively (Hackam and Redelmeier, 2006). Another systematic review on the use of corticosteroids for six different medical interventions (traumatic head injury, haemorrhage, thrombolysis in acute ischaemic stroke, general acute ischaemic stroke, neonatal respiratory distress syndrome and osteoporosis), showed that concordance between animal studies and clinical trials were observed for only 50% (3/6) of the interventions (Perel et al., 2007). There are also several examples in the literature in which extensive animal testing failed to predict critically severe toxicity in humans (Olson et al., 2000; Van Norman, 2019), and studies show that comparing toxicity between species that are phylogenetically more similar to each other than a rodent species to humans still provides a considerable measure of uncertainty, even when using the same substance under the same conditions (Wang and Gray, 2015). The results from this study were considered in agreement with previously conducted studies (Gold et al., 1989; Haseman and Lockhart, 1993). Overall, it is clear that there are limitations and uncertainties associated with all tests, animal and non-animal (Paul Friedman et al., 2023), however these should not be unduly focused on - the benchmark of both should be their capacity to characterise human-relevant toxicity (for human health endpoints) and species-extrapolated toxicity (for environmental endpoints).

In this paper, we provide our perspective on how the last resort requirement is being applied in practice, using a selection of real-world examples. Recommendations to improve adherence and implementation are proposed, and are intended to aid the European Commission, ECHA and registrants in protecting human health and the environment in full compliance with the legal requirement to ensure that animal testing is carried out only as a last resort.

2. Real-world experience of animal testing as a last resort

It is challenging to obtain specific documentation on the non-animal approaches, adaptations and waivers deemed suitable to fulfil SIR in specific dossiers by ECHA. Even though the Article 117(3) report is

published every three years and provides information on the options used to fulfil information requirements (e.g. experimental data, data waivers, read-across etc, see Table 1) it does not publish specific instances where an adaptation was submitted and the data considered acceptable, or the data rejected. However, there is an exception following a compliance check dossier update, where ECHA provides a final letter to indicate that the registrant met/didn't meet the initial data request. Consequently, there is only a single example illustrating adherence to the last resort requirement, disodium 4,4′-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2′-disulphonate and no examples of a solely *in vitro* alternative being submitted, and then rejected. Furthermore, in several of the case studies, multiple endpoints were covered in the ECHA decision. Single endpoints/test requirements have been selected for this paper, most of which involve a weight of evidence utilising several lines of evidence, often incorporating existing animal data as well as non-animal approaches, rather than an approach based solely on a non-animal approach. This is not intended to provide an unbalanced view, it is intended to highlight registrants real-world experience of trying to stand by animal testing as a last resort.

2.1. Disodium 4,4′-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl) amino]stilbene-2,2′-disulphonate

Substance name (CAS): Disodium 4,4′-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2′-disulphonate (CAS 16090-02-1)

Type of check: Compliance check.

Test requested: OECD 408 - repeated dose 90-day oral toxicity study in rodents.

Adaptation used: Annex XI, Section 1.2 (weight of evidence).

Final ECHA decision: Adaptation accepted.

Description: During a compliance check of disodium 4,4′-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2′-disulphonate, a fluorescent whitening agent in paper, textile and household detergents, it was considered by ECHA that the substance may attach to constituents of standard diets used in animal studies (European Chemicals Agency, 2020a). Therefore, the final decision letter from ECHA requested additional information on several endpoints, including a repeated dose 90-day oral toxicity study in rodents (OECD 408) with food restriction 2 h before and 2 h after oral gavage dosing. After receiving the final decision letter from ECHA, the registrant updated their International Uniform Chemical Information Database (IUCLID) registration dossier with the following two studies (which were inadvertently missed during the first literature search): (1) a repeated dose 28-day oral toxicity study in rodents (OECD 407) with a test material containing 82.5% of the substance in which a NOAEL was established at 1000 mg/kg bw/day (corresponding to 825 mg/kg bw/day of the substance). The study was performed according to GLP and was available as secondary literature and (2) a two-year chronic toxicity study with a test material containing 91.7% of the substance in which a NOAEL was established at 10000 ppm (corresponding to 481 mg/kg/day in males of the substance and 725 mg/kg/day of the substance in females). The study pre-dated GLP and was available as secondary literature. Since both studies were considered "Valid without restriction" by the US Environmental Protection Agency (EPA) and available from the US EPA's High Production Volume Information System (HPVIS) database, the studies were considered to be Klimisch 2 rated studies, despite only being available as secondary literature, where Klimisch is a scoring system encouraged by ECHA to be used in order to evaluate the reliability of experimental studies (European Chemicals Agency, 2023b; Klimisch et al., 1997).

In the dossier, the registrant discussed the adequacy and reliability of the two studies, provided a weight of evidence assessment, and self-classified the substance as Not Classified for Specific Target Organ Toxicity - Repeat Exposure (STOT RE). The registrant invoked the adaptation in Annex XI, Section 1.2 of the REACH regulation. After having updated the IUCLID dossier, the registrant received a follow-up to dossier evaluation decision from ECHA in which it was indicated that the SIRs subject to the second deadline in the final decision letter (which included the SIR of an OECD 408) had been met (these documents are confidential so this was a personal communication). Consequently, ECHA appears to have accepted the data provided and the invoked adaptation by the registrant.

2.2. Alkenes, C6-11 (branched), hydroformylation products, distn. residues, heavy cracked fraction

Substance name (CAS): Alkenes, C6-11 (branched), hydroformylation products, distn. residues, heavy cracked fraction substance (CAS 98072-31-2)

Type of check: Compliance check.

Test requested: OECD 414 - Pre-natal developmental toxicity study by oral route, in a second species (rabbit).

Adaptation used: Annex XI, Section 3 (substance-tailored exposure-driven waiving).

Final ECHA decision: Request for test removed, following European Court of Justice ruling.

Description: During a compliance check for the alkenes, C6-11 (branched), hydroformylation products, distn. residues, heavy cracked fraction substance, ECHA requested an OECD 414 prenatal developmental toxicity study in a second species (rabbit) (European Chemicals Agency, 2021a; European Chemicals Agency, 2021b; OECD, 2018a). The registrant submitted an exposure-based adaptation in accordance with Annex XI, Section 3 of REACH, and supported this with a dose-range finding study in rabbits. Furthermore, the registrant provided information that included a derived no-effect level (DNEL) based on a dose-range finding study that demonstrated significant animal welfare concerns in the typical non-rodent species (i.e., the study showed that rabbits did not tolerate exposures to an oily substance) as well as a DNEL derived from a quantitative structure activity relationship (QSAR) model that predicts rabbit developmental effect levels from rat data (the typical first species). A conclusion was made by the registrant that the exposure would be significantly below the DNELs. In its contested decision, ECHA stated that the registrant's lack of exposure scenarios meant the argumentation could not be accepted. In comments to the draft decision, the registrant corrected the absence of exposure scenarios, providing an updated attachment as part of the comments, and additionally specified the sections in the registration dossier which supported the substance-tailored exposure-based adaptation. Nonetheless, the final decision letter maintained that the exposure scenarios were not provided and that the exposures were not below the DNELs. The registrant appealed this rejection and argued that the exposure scenarios were provided in response to the draft decision, and that the QSAR model should be used to predict a rabbit DNEL, as the evidence suggested the substance is not well-tolerated by rabbits. Furthermore, the registrant's detailed exposure scenarios included higher-tier exposure estimation models, and demonstrated that even in a worst-case scenario, exposures would be well below the conservatively-derived DNELs.

Following the appeal, ECHA re-examined the decision-making process leading to the adoption of the contested decision. In the course of the re-examination, it was concluded that certain information was not taken into account and the request for the rabbit prenatal developmental toxicity study was removed, supported by a European Court of Justice (ECJ) ruling in favour of the registrant (European Court of Justice, 2021).

2.3. Benzoic acid, C12-15 alkyl esters

Substance name (CAS): Benzoic acid, C12-15 alkyl esters (CAS 68411-27-8)

Type of check: Compliance check.

Test requested: OECD 210 - Fish, early-life stage (FELS) toxicity

test.

Adaptation used: Annex XI, Section 1.5 (read-across).

Final ECHA decision: Adaptation rejected.

Description: In November 2017, ECHA issued a final decision dossier compliance check on the exclusive use cosmetic ingredient benzoic acid, C12-15 alkyl esters registered at Annex X under REACH. Within the decision letter, ECHA challenged the read-across approach used to satisfy multiple (eco)toxicological SIR (European Chemicals Agency, 2017a). One of the endpoints challenged by ECHA was chronic fish toxicity, which had been fulfilled using a specific adaptation to waive based on lack of acute toxicity, poor water solubility and rapid biodegradability to avoid the need for vertebrate testing. ECHA thus requested an OECD 210 Fish, early-life stage (FELS) toxicity test (OECD, 2013). Upon receipt of the compliance check the lead registrant agreed that the previous waiver was scientifically weak and sought to develop a more robust strategy. The proposal included a detailed assessment of all available aquatic toxicity data for all structurally similar alkyl benzoate ester substances registered under REACH. These data indicated a clear structure-activity relationship with an apparent "cut-off" for chronic toxicity to aquatic organisms (including fish) for higher C-chain length alkyl benzoate esters, which can be explained using water solubility as a surrogate for bioavailability. High quality, reliable and relevant data for a shorter C-chain alkyl benzoate ester analogue source substance (isodecyl benzoate) indicated it was not chronically toxic to fish or invertebrates at the limit of solubility (LoS) test media. Since the water solubility of the target (benzoic acid, C12-15-alkyl esters) is measurably lower than the water solubility of the source (isodecyl benzoate), an extrapolated read-across justification led to the conclusion that the target will be non-toxic at the LoS. This enhanced read-across strategy and hypothesis was proposed by the lead registrant to the consortium members co-registrants. However, the consortium members disagreed (with the main concerns being further rejection of the read-across approach by ECHA and possible enforcement action) and instead commissioned a new OECD 210 test, using a water accommodated fraction limit test design. As a result, one registrant exercised its legal right under REACH to opt-out of the new chronic OECD 210 test in the registration dossier in accordance with Article 11(3)(c) and the updated REACH co-registration dossier with the required opt-out justification was successfully submitted to ECHA on Dec 2, 2022. The result of the new OECD 210 test carried out in parallel by the other registrants confirmed that benzoic acid, C12-15-alkyl esters was not chronically toxic to fish at the LoS, as already predicted by the alternative read-across approach. Therefore, the new OECD 210 test was an unnecessary use of vertebrate animals (estimated ~150 fish in the limit test design) and the information requirement could have been adequately covered by read-across, given it led to the same conclusion. At the current time of writing, the registrant of the opt-out dossier has not received any feedback from ECHA on the read-across justification used to satisfy the chronic fish information requirement.

2.4. Pyrrolo[3,4-c]pyrrole-1,4-dione, 3,6-bis[4-(1,1-dimethylethyl) phenyl]-2,5-dihydro

Substance name (CAS): Pyrrolo[3,4-c]pyrrole-1,4-dione, 3,6-bis[4-(1,1-dimethylethyl)phenyl]-2,5-dihydro (CAS 84632-59-7)

Type of check: Testing proposal.

Test requested: OECD 443 - extended one-generation reproductive toxicity study (EOGRTS).

Adaptation used: n/a.

Final decision: Board of Appeal found in favour of registrant.

Description: A testing proposal was put forward by the registrant to conduct an OECD 443 extended one-generation reproductive toxicity study (EOGRTS) on pyrrolo[3,4-c]pyrrole-1,4-dione, 3,6-bis[4-(1,1-dimethylethyl)phenyl]-2,5-dihydro (also known as 3,6-bis(4-tert-butylphenyl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione), being registered at the 10–100 tonnes per annum tonnage band, because an OECD 421 reproduction/developmental toxicity screening test study showed some pup mortality (European Chemicals Agency, 2015a; OECD, 2016a; OECD, 2018b). However, between the time the draft decision was sent to the Member State Committee and the adoption of the contested decision, the registrant became aware of another dossier containing another OECD 421 test for the same substance using a different sub-strain of rodent and a different vehicle. In this study, no parental, reproductive, or developmental toxicity was observed up to the limit dose (1000 mg/kg/day). However, ECHA refused to take these study findings into account before the adoption of the contested decision as this study was submitted after an administrative cut-off point, even though ECHA acknowledged that the information within could impact the decision whether or not to perform an OECD 443 study. An appeal was filed by the registrant to the BoA who found that ECHA breached its Article 25 (1) requirement by requesting animal testing without consideration of available information in other dossiers. The BoA stated that the other registrant's screening test was considered as substantial new information which could impact the need to carry out a new OECD 443. The BoA also found that ECHA's procedures were too rigid and upheld the registrant's appeal, annulling the contested decision and compelling ECHA to begin the decision-making process again, taking both screening studies into account before making a conclusion on whether an OECD 443 is needed.

2.5. Aziridine

Substance name (CAS): Aziridine (CAS 151-56-4)

Type of check: Testing proposal.

Test requested: OECD 210 - Fish, early-life stage (FELS) toxicity test.

Adaptation used: Annex I.

Final decision: Unknown.

Description: The registrant submitted a test proposal for an OECD 212 fish short term toxicity study on embryo and sac-fry stages (OECD, 1998) for aziridine, which was rejected in favour of an OECD 210 FELS test (OECD, 2013) in accordance with Article 40(3) in REACH (European Chemicals Agency, 2012b). The registrant maintained that an OECD 210 test was unnecessary as there was no need to investigate further on the effects to aquatic organisms, as per Annex I of REACH. The updated registration dossier, including the justification on the waiving of this test, was submitted and confirmed as received. However, ECHA still requested the long-term OECD 210 toxicity test based on the original test proposal and did not take the update into account. ECHA stated that any updates of a registration dossier, even those containing the waiving statements, received after a draft decision has been sent to the MSCA for their comments cannot be taken into account for the purposes of that decision. As a result, the registrant appealed this through the BoA, but at the same time the Executive Director of ECHA rectified the contested decision, after which the appeal was withdrawn (European Chemicals Agency, 2012a).

2.6. Aluminium chloride, aluminium chloride basic and aluminium sulphate

Substance name (CAS): Aluminium chloride (CAS 7446-70-0), aluminium chloride basic (CAS 1327-41-9) and aluminium sulphate (CAS 10043-01-3)

Type of check: Substance evaluation.

Test requested: OECD 474 - combined *in vivo* mammalian erythrocyte micronucleus test in bone marrow and an OECD 489 - modified *in vivo* mammalian comet assay.

Adaptation used: Annex XI, Section 1.5 (read-across).

Final decision: Request for test removed, following Board of Appeal finding in favour of registrant.

Description: Following the substance evaluations of aluminium chloride, aluminium chloride basic and aluminium sulphate, the eMSCA

requested an OECD 474 combined *in vivo* mammalian erythrocyte micronucleus test in bone marrow (OECD, 2016b) and an OECD 489 modified *in vivo* mammalian comet assay (OECD, 2016c) with additional specific investigations on oxidative DNA damage on liver, kidney, glandular stomach and duodenum tissues due to genotoxicity concerns. The registrants contested that the *in vitro* and *in vivo* studies used by the eMSCA to suggest concern for genotoxicity were unreliable, in contrast to the *in vitro* studies submitted by the registrant which gave no indication of genotoxicity, supported by a read-across from dialuminium chloride pentahydroxide. ECHA rejected the read-across on the basis that it was insufficient to exclude the concern for genotoxicity as it lacked "*confirmation of the bioavailability/specific toxicokinetic data*". The registrant lodged an appeal and the BoA found that ECHA failed to demonstrate the existence of a potential risk, failed to demonstrate that the additional *in vivo* studies would lead to improved risk management measures, and failed to demonstrate that all available evidence had been considered when rejecting the read-across. The BoA annulled the contested decisions and the requirement for the *in vivo* tests were removed (European Chemicals Agency, 2019a).

2.7. Carbon tetrachloride

Substance name (CAS): Carbon tetrachloride (CAS 56-23-5)

Type of check: Substance Evaluation.

Test requested: OECD 416 - two-generation reproductive toxicity study later amended to OECD 443 EOGRTS via the inhalation route.

Adaptation used: n/a.

Final decision: Request for test removed, following Board of Appeal finding in favour of registrant.

Description: Following an opinion from the ECHA Member State Committee (MSC) concerning reproductive/developmental toxicity and worker safety, carbon tetrachloride underwent substance evaluation (European Chemicals Agency, 2015b). An OECD 416 two-generation reproductive toxicity study (OECD, 2001) was originally requested by the eMSCA, which was amended to a request for an OECD 443 EOGRTS via the inhalation route (OECD, 2018b) after taking proposals for amendment and the appellants' comments into account. This request was appealed by the registrants for several reasons, including that risk management measures were already adequate and protective at doses to which workers could reasonably be expected to be exposed. This was based on the fact that existing data established that reproductive toxicity only occurred at high levels of exposure, and the existing NOAELs ensured sufficient protection of human health. The registrants argued that performing an OECD 443 was unlikely to lead to a lower NOAEL and further risk management, and thus breached the principle of proportionality. Finally, the registrants also argued that the request for an OECD 443 breached Article 25(1) of REACH and failed to ensure that the minimum number of animals were used as per the EU Directive on the protection of animals used for scientific purposes (European Union, 2010). The BoA found in favour of the registrants and stated "*that, under substance evaluation, in order to request additional information the Agency must be able to … demonstrate that the information requested has a realistic possibility of leading to improved risk management measures.*"

2.8. Summary of issues identified

The examples above describe several occasions where animal testing has been requested by an eMSCA/ECHA in what appears to be a default option and not as a last resort. They also show how the Board of Appeal functions as a gatekeeper, upholding Article 25 where necessary. However, by considering these examples, several broad and recurring issues have been identified, and merit further reflection (Fig. 1).

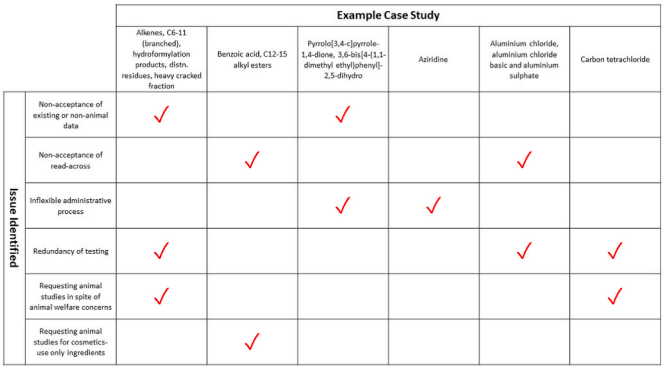

Fig. 1. Summary of issues identified in selected case studies demonstrating non-adherence to the last resort requirement.

2.8.1. Non-acceptance of existing or non-animal data

Several of the examples demonstrate how existing data or non-animal data are not accepted to fill SIR under REACH, illustrating the high burden of proof placed on the registrants when using a weight of evidence approach: alkenes, C6-11 (branched), pyrrolo[3,4-c]pyrrole-1,4-dione, 3,6-bis[4-(1,1-dimethylethyl)phenyl]-2,5-dihydro. Furthermore, data from studies published in peer-reviewed journals have been rejected, limiting the prospects of using toxicological studies published in peer-reviewed journals to bolster a weight of evidence under Annex XI, Section 1.2. With respect to other existing data being rejected, other models or assays may be more appropriate than the default OECD guideline method, as they better predict the chemical safety for human and/or the environment. Following the purpose of REACH as stated in Article 1, the scientific adequacy of the test for the purpose of the regulation should prevail.

2.8.2. Non-acceptance of read-across

Read-across is one of the most used non-animal approaches for data gap filling in registrations submitted under REACH, for which ECHA has developed the RAAF, a systematic approach to fulfilling SIR by means of read-across (European Chemicals Agency, 2017c). Experience has taught that meeting the requirements of the RAAF is not always relevant to its acceptance (personal communication]), and can often only be done only with hindsight – after the testing has been performed. Several examples, illustrate that even when a robust read-across argument is posed, this can still be deemed as insufficient by ECHA (aluminium chloride) or animal testing may be performed by registrants to avoid potential rejection (benzoic acid, C12-15 alkyl esters), and later shown to provide an identical outcome to a read-across prediction.

2.8.3. Inflexible administrative process(es)

The examples outline several occasions where an inflexible administrative process has meant alternative ways of addressing SIRs that would not result in unnecessary animal experimentation were not taken into account e.g. pyrrolo[3,4-c]pyrrole-1,4-dione, 3,6-bis[4-(1,1-dimethylethyl)phenyl]-2,5-dihydro and aziridine, where timing of new information availability vs. aspects of process were in conflict; aziridine where rigid adherence to process was observed even when new information became available.

2.8.4. Redundancy of testing

Several case examples (alkenes, c6-11 (branched), aluminium chloride, aluminium chloride basic and aluminium sulphate, carbon tetrachloride) describe good faith attempts to use non-animal approaches expressly provided for in Annex XI, which were later rejected. The alkenes example shows that by using several lines of evidence (including the use of exposure-based adaptation in accordance with Annex XI combined with a novel QSAR), it was unnecessary to conduct an experiment in rabbits which was already associated with severe animal welfare concerns. Similarly, scientific understanding of existing genotoxicity data on aluminium salts was essential to understand the genetic toxicity hazards posed, rather than defaulting to animal testing. For

carbon tetrachloride, existing data indicated that toxicity only occurred at high levels of exposure and risk management measures were protective, meaning any additional (animal) testing would be redundant.

2.8.5. Requesting animal studies despite animal welfare concerns

There are several examples of animal studies being requested despite substantial animal welfare concerns. The alkenes example suggested there were tolerability issues around dosing the substance to rabbits, resulting in concerns for animal welfare as well as the scientific utility and relevance of the study for the purpose of human and environmental safety assessment. The carbon tetrachloride example showed that ECHA failed to ensure that the minimum number of animals were used as per the EU Directive (European Union, 2010).

2.8.6. Requesting animal studies for cosmetics-use only ingredients

Finally, there is an example of an animal test being requested for a substance, benzoic acid C12-15 alkyl esters, used exclusively for cosmetics. Although ECHA are permitted to request (*in vivo*) data for cosmetic-only ingredients, we recommend that ECHA allow the fulfilment of REACH SIRs only through non-animal approaches for cosmetic-only ingredients to avoid undermining the intentions of the Cosmetic Products Regulation (European Union, 2009)

3. Recommendations for governance and enforcement of the last resort requirement

The examples described have been used to identify issues which could be addressed through several recommendations, proposed below, for governance and enforcement of the last resort requirement. These recommendations are separated into specific actions for the European Commission, ECHA and the registrants - to improve the efficiency of REACH by maximally utilising existing animal data to avoid animal testing where knowledge and reason warrants doing so. Some of these recommendations, such as those for ECHA, may also be applicable to other regulatory agencies in the EU.

3.1. European Commission

3.1.1. Uphold the integrity of the Cosmetic Products Regulation (EC) No 1223/2009

The Cosmetic Products Regulation (EC) No 1223/2009 (European Union, 2009), which came fully into force in 2013, prohibits the animal testing of cosmetics ingredients placed on the EU market. However, this regulation can clash with REACH, if a new (cosmetic) ingredient is registered (European Chemicals Agency, 2014). In order to uphold the integrity of this regulation, the EC should amend one (or both) regulations to ensure that new animal data are not required, requested, or generated for purposes of REACH compliance for ingredients used exclusively in cosmetics, as was done for benzoic acid, C12-15 alkyl esters, described above. However, the EC have stated in their response to the European Citizen's Initiative 'Save Cruelty-free Cosmetics - Commit to a Europe without Animal Testing' that no legislative changes will take place before the outcome of two court cases which are underway, related to interface between these two regulations (European Commission, 2023). Furthermore, (non-animal) data submitted under the regulation should be encouraged to be repurposed for use under REACH, especially if relevant routes/levels of occupational exposure are taken into consideration.

3.1.2. Ensure revisions to regulations increase the uptake of non-animal approaches

The European Commission (EC) must demonstrate a strong commitment to Article 1 of REACH **"***The purpose of this 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 …***"** This could be achieved in several ways, including by making the language in regulations more flexible and by not adding any new or expanded animal testing requirements, as described previously (Pereira et al., 2022). This would have a direct impact on the last resort requirement, by making it easier for registrants to adhere to it as well as reducing the totality of animals used to inform safety in use for a given registration.

3.1.3. Ensure revisions to regulations continue to uphold the principles enshrined in Directive 2010/63/EU

Directive 2010/63/EU is one of the most stringent regulations to protect animals in the world and its principles, focused on the 3Rs of replacement, reduction and refinement, must continue to be upheld by the EC (European Union, 2010). For example, Article 11 and Article 12 state "*When choosing methods, the principles of replacement, reduction and refinement should be implemented through a strict hierarchy of the requirement to use alternative methods. Where no alternative method is recognised by the legislation of the Union, the numbers of animals used may be reduced by … implementing testing strategies, such as the use of in vitro and other methods that would reduce and refine the use of animals*." and "*The use of animals for scientific or educational purposes should therefore only be considered where a non-animal alternative is unavailable.*" This must be kept at the forefront of the mind when chemical regulations are revised, especially when new animal tests are being proposed. Furthermore, many toxicity tests are carried out at considerably higher doses than typical exposure scenarios *"to ensure that any test results can reliably show whether a chemical causes severe health effects or not"* (European Chemicals Agency, 2022a, 2022b, 2022d). This advice is aimed as a means of avoiding repeated animal tests but the legal obligation to apply all 3Rs, or replacement, reduction and refinement, must remain the principal objective.

3.2. ECHA

3.2.1. Reduce the financial risk associated with obtaining existing animal data for read-across and publish successful read-across case studies

The purchase of animal data from a third party for use in read-across by registrants can be a significant financial investment - associated with a substantial risk considering that read-across data are often rejected when reviewed by ECHA during compliance checks, as exemplified by the examples highlighted above (European Chemicals Agency, 2017a, 2019a). In order to reduce this financial risk, legal certainty of regulatory acceptance of read-across is needed. Developing a successful read-across case can be difficult and the (perceived) high frequency of rejection by ECHA only serves to dampen the enthusiasm of registrants to pursue read-across as an way to fulfil the last resort requirement. The RAAF (European Chemicals Agency, 2017c), or a specific detailed guidance document, should be updated/developed to include additional case studies of acceptable and unacceptable read-across examples. The RAAF should also highlight new technology used to identify appropriate analogues, such as matched molecular pairs (MMF) or quantitative similarity (qSIM) scores (Lester et al., 2023; Lester and Yan, 2021) and new ways to approach read-across, for example by using read-across in combination with non-animal approaches (Alexander-White et al., 2022; Ouedraogo et al., 2022). By doing these in tandem, the acceptance of fit-for-purpose and relevant read-across would improve, thus lowering the cost to registrants from obtaining existing animal data and incentivising the use of this technique. With respect to using existing data, the EC's Joint Research Centre is working hard to make better use of academic data in regulatory assessments, as they are co-leading an OECD-coordinated project on how to better utilise data contained in peer-reviewed publications, therefore ECHA could use its outcomes as another case study for read-across.

3.2.2. Create an open communication channel for registrants

To date, in final decisions notified to registrants after a compliance check, it is specified that the registrant can seek content clarification by

using the ECHA helpdesk. At this helpdesk, it is possible to select a topic, post the question, include attachments, and give contact details. However, this type of communication is makes it more difficult to have an open and two-way dialogue. We recommend that ECHA creates an improved and formal expert scientific committee which could serve as an advice channel in which it is possible for registrants to initiate open communication with the agency, as described elsewhere (Pereira et al., 2022), and it is promising that the EC have committed to investigating the feasibility of such a committee in their response to the European Citizen's Initiative 'Save Cruelty-free Cosmetics - Commit to a Europe without Animal Testing' (European Commission, 2023). This would allow both parties to discuss freely how best to use the available data to minimise the use of animal tests and this communication channel would also help to build trust between the involved entities. As ECHA stated in its 2020 report under Article 117(3) that it is concerning how many registration dossiers are still non-compliant (European Chemicals Agency, 2020b) and a study has shown that the introduction of new governmental channels of communication serves to achieve more efficient results, which could lead to better compliance (Sanina et al., 2017) and significantly minimise the number of animals used to establish human health and environmental safety.

3.2.3. Revise administrative processes

ECHA should build upon their strong commitment to the use of non-animal approaches by increasing flexibility in their administrative processes. Inflexible administrative process such as strict adherence to deadlines had an impact on four of the case examples described above, pyrrolo[3,4-c]pyrrole-1,4-dione, 3,6-bis[4-(1,1-dimethyl ethyl)phenyl]-2,5-dihydro, aluminium chloride, aluminium chloride basic and aluminium sulphate, aziridine and carbon tetrachloride. ECHA of course need deadlines in order to assess chemicals effectively but requesting an animal test just to fulfil a(n) (arbitrary) deadline does not align with the last resort requirement. Furthermore, compliance check decision letters should be amended to remind registrants of their obligation to generate information by non-animal means whenever possible and to only test on animals as a last resort and that Annex XI adaptations should be considered, even after a final decision. We suggest that the administrative processes are reviewed to take these into account.

3.2.4. Build confidence in the accurate prediction of human health and environmental effects by non-animal approaches

A report from ECHA on non-animal methods, relating to the current status of regulatory applicability under REACH, CLP and Biocidal Products regulations was released in 2017 (European Chemicals Agency, 2017b). ECHA encouraged registrants to use reliable non-animal methods and made it clear that there are various sources of information from ECHA that can be used to understand how non-animal methods can be used to meet legal requirements. Limitations of non-animal approaches in the report were covered in brief for read-across approaches and in-depth for computer modelling and *in vitro* methods. For animal studies, it has been recognised that there is an uncertainty in extrapolating the obtained results to humans. However, as there are decades of experience with animal studies, there is sufficient confidence that uncertainty can be addressed by using an overall interspecies uncertainty factor. Where non-animal approaches have the benefit that they can be built on human data or human-derived cell--lines, these methods are lacking the extensive experience that the scientific community feels they have with animal tests. Only when non-animal approaches are used more widely, with more experience on what they can do well and less well, will confidence increase in their use. Any test, whether an animal model or a non-animal approach, will have its limitations and the key to success is increased use and experience to develop insights in these limitations and applicability domains. Then the confidence that is needed to replace one testing model with an improved one will be built. The benchmark for this should be adequate prediction of the effects of the test substance on human and environment, in a robust, repeatable way. To ensure confidence continues to grow with the use of these non-animal approaches, we recommend that ECHA hold a workshop on a yearly basis, similar to those held in 2016 and 2023 (European Chemicals Agency, 2016a, 2023a) and the one arranged by the US National Academy of Sciences in 2019 workshop (National Academies of Sciences, 2022) and participate or hold training events to build confidence in the use of these approaches.

3.3. Registrants

3.3.1. Ensure registrants are preparing robust and complete dossiers

There are several examples (e.g. disodium 4,4′-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2′-disulphonate, alkenes, C6-11 (branched), hydroformylation products, distn. residues, heavy cracked fraction substance) where a dossier has been submitted to ECHA with data missing or incomplete. Registrants could undergo thorough training on dossier preparation and submission to reduce this happening. This could be incorporated into the recommendation for ECHA to create an open communication channel for ECHA-registrant dialogue. Improved communication and training would lead to better quality dossiers.

4. Conclusions

The AFSA Collaboration have set out the current reality of the last resort requirement, as described in Article 25 of REACH, as not being adequately upheld, illustrated through single endpoint case studies. It is important to note that there are over 2500 ECHA decisions available online, and this paper focuses on personal experiences of the co-authors, who feel that even with mechanisms within REACH to encourage the use of non-animal approaches e.g. flexibility in the studies used to fulfil SIRs, as well as adaptations being permitted under Annex XI, the paradigm shift away from animal testing still seems a long way away.

Overall, these examples described within demonstrate that if a scientific approach to understanding the actual safety of the substance is taken, it should be possible to make a robust decision without the need to resort to animal testing. At present however, this science appears to be employed solely to argue why the default 'expected' animal test should not be done, rather than an upfront acceptance that these approaches will, and should, be used as a matter of routine to demonstrate substance safety - and resorting to animal testing should increasingly be the exception.

The main challenges experienced are not insurmountable but do require a willingness from all actors to ensure that all alternative avenues are explored prior to an animal study being conducted. Several solutions are proposed in this paper which require ownership as well as collaboration between by key stakeholders (the European Commission, ECHA and registrants). The recommendations should be seriously considered during the forthcoming, as well as any future revision(s) of the REACH regulation to create the conditions needed to ensure animal testing as a last resort becomes a reality.

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James Dawick: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Benjamin Carrick:** Writing – original draft, Formal analysis, Conceptualization. **Graham Ellis:** Writing – original draft, Formal analysis, Conceptualization. **Roberto Ferro:** Writing – original draft, Formal analysis, Conceptualization. **Katy Goyak:** Writing – original draft, Formal analysis, Conceptualization. **Chantal Smulders:** Writing – original draft, Formal analysis, Conceptualization. **Ricky A. Stackhouse:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Espe Troyano:** Writing – original draft, Formal analysis, Conceptualization. **Carl Westmoreland:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Blanca Serrano Ramón:** Writing – original draft, Formal analysis, Conceptualization. **Vanessa Rocha:** Writing – original draft, Formal analysis, Conceptualization. **Xiaoling Zhang:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization.

Declaration of competing interest

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

Data availability

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