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Non-Animal Methods of Chemical Testing: Replacement, Reduction and Refinement under European Union Law

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Abstract

In regulatory toxicology, animal tests are habitually applied in hazard assessments to identify the potential toxic endpoints of chemicals to human health and the environment. This paper identifies major challenges associated with the perceptions of the need to rely on animal studies and argues that regulatory culture, resting upon familiarity and confidence in animal studies, hinders the transition to non-animal methodologies (NAMs). These challenges are further compounded by a deferential judicial approach that often fails to uphold the 'last resort' principle prescribed by EU chemical legislation. Drawing upon novel empirical data from 32 stakeholder interviews conducted as part of the PrecisionTox project, the study explores the social, technical, and legal barriers to NAM uptake. The findings suggest that progress requires a shift in mindset, improved validation protocols, and proactive legal engagement to replace 20th-century testing methods with 21st-century science.

Keywords: NAMs, 3Rs, REACH, chemical risk assessment, animal testing, judicial oversight

Introductory remarks

In regulatory toxicology, animal tests are habitually applied in hazard assessments to identify the potential toxic endpoints of chemicals to human health and the environment. This is particularly true with regards to industrial chemicals, which are subject to strict requirements on their registration, authorisation, and, in some cases, placing on the market. Testing here is deployed for the assessment and evaluation of intrinsic properties of chemical substances, but for products such as biocides and plant protection products there is a second stage of approvals for final products in which the chemicals are ingredients. Further animal testing may then follow. This is true across many legal systems governing chemical risk assessment around the world including the European Union (EU), which is the main focal point of this study. However, under social, ethical, and economic pressures, there has been a gradual consideration of other testing methods, which aim for the replacement, reduction, or refinement of animal testing (3Rs, as explained below) (Russell & Burch, 1959).

These non-animal (or new approach) methodologies ('NAMs') bring together a variety of different methods, including *in vitro* cellular and tissue models, *in silico* computational approaches, *in chemico* reactivity, high-throughput technologies, and the use of model invertebrate species, such as fruit flies, nematodes, water fleas, and embryos of zebrafish or clawed frog. These 3Rs approaches to animal testing are frequently endorsed by policymakers around the world, yet despite the diversity of methods available under this umbrella term of NAMs, their deployment in regulatory risk assessment remains limited. To some extent this is due to complexities surrounding NAMs, which, rather than comprising a uniform and discrete technology, encompass quite different disciplines and technological forms. As we will see, however, many of the barriers associated with the understanding and uptake of NAMs

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extend beyond the technical and into social realms (Bearth et al. 2025; Čavoški et al. 2025).

This paper identifies major challenges associated with the perceptions of the need to rely on animal studies and their value for regulatory application. The paper argues that, far from being a question of scientific advance, it is regulatory culture, which rests upon familiarity and confidence in animal studies, that also hinders the transition to NAMs and better compliance with 3Rs. The paper contends that these challenges are further compounded by courts' deferential approach to scientific decision-making. This leads to courts not upholding a 'last resort' principle as prescribed by EU chemical legislation but endorsing a recourse to animal testing in the face of scientific uncertainty. These challenges are identified from novel empirical data that the authors generated as part of the PrecisionTox project (PrecisionTox; https://precisiontox.org). The PrecisionTox project reviews the safety assessment of chemicals without resort to traditional mammalian testing. The overarching goal of PrecisionTox is to draw upon evolutionary principles and their practical application to establish cost-effective chemical testing paradigms that are '3Rs' compliant.

The empirical study involved 32 interviews with different groups of stakeholders involved in the risk assessment and management of chemicals, namely industry, regulators, and policymakers, across Europe and in non-European common law jurisdictions. The 32 semi-structured interviews were conducted by using Zoom software, including one-to-one and small group interviews from January 2023 to August 2023. The study included 12 interviewees from the industry, 10 regulators and 10 policymakers. The industry respondents were largely from transnational corporations. The regulators and policymakers were predominantly from EU, but the study included interviewees from other representative jurisdictions, since chemical testing and the NAMs debate affects global trade. The study identified that barriers to NAMs are not purely technical but predominantly involve a range of social factors. This empirical study differentiates the research from plentiful but narrower scientific studies, which tend to limit the scope of the problem to the technological maturity of the next generation of chemical risk assessment methods (Čavoški et al., 2024). Furthermore, the paper focuses on EU courts' oversight of scientific decision-making with regards to chemicals governed by REACH, which has received a limited attention in legal scholarship.

This paper is structured as follows. Section 2 introduces the role of animal testing and alternative methods and discusses the existing scholarship that documents the slow uptake of non-animal approaches. Section 3 goes on to describe the legislative landscape in the EU related to research and testing on animals as applied across a range of chemical sectors. Following an overview of animal use data and reaction in the EU, section 4 then compares the animal testing situation in the USA, as a major trading partner of the EU. An analysis of social and technical insights surrounding animal testing and its alternatives, based on empirical data, is provided in Section 5. Section 6 then analyses the judicial oversight of animal-based testing in Europe prior to a concluding Section 7.

Animal testing and NAMs

As early as 1831, the work of Marshal Hall posited that studies should be conducted with the 'least possible infliction of suffering' (Hubrecht & Carter, 2019). The term '3Rs' (as the replacement, reduction, and refinement of animal testing) was introduced by Russell and Burch in 1959 and is now regarded as reflecting internationally established principles (Council Directive 2010/63/EU). The EU, for example, has developed legislation regarding the use of animals in science, which we shall discuss later, and intergovernmental activities also seek to limit the harm caused by testing (Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM); https://ntp.niehs.nih.gov/whatwestudy/niceatm/iccvam). Through harmonisation, the OECD's agreement on the Mutual Acceptance of Data (MAD) contributes to reducing animal testing because data generated by a member country in accordance with the OECD's Test Guidelines (TGs) and Principles of Good Laboratory Practice (GLP) is to be accepted by other member countries and 'adhering non-member countries', thus limiting the duplication of tests. Now often synonymous with national testing requirements, the Test Guidelines of the OECD (TG) are standard methods covering, for example, the testing for toxic effects from repeated dermal applications over a 21- or 28-day study of at least 10 animals (OECD, 1981); the study of reproductive performance and offspring growth and development in rats (OECD, 2001); and the study of at

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least 20 rats to understand the potential for neurotoxic effects from a chemical exposure (OECD, 1997). Animal studies typically involve measurements of animal weight or water and food consumption; the microscopic examination of tissues and cells; organ weights; and signs of toxicity and abnormalities. However, the OECD Guidance Document on Experimental Animals also recognises the 3Rs and the evidence that animals experience pain, distress, and suffering, and therefore sets the principles that this should not be severe, with animals to be killed humanely (OECD, 2002).

As part of a wider shift to reduce animal testing, many alternative approaches to chemical testing have been developed over time. The label of 'new approach methodologies' (NAMs) is often used as an umbrella term that bring together different forms of methods. As used in this paper, and as explained further below, NAMs encompass methods used as alternatives to animal testing that seek to fulfil the 3Rs ambition. Table 1 provides key approaches that are deployed for the purposes of chemical risk assessment, though some of these methods can be used in combination (Singh et al., 2018; Schneider et al., 2021; Punt et al., 2020; Hartung & Hoffman, 2009; Cronin et al., 2009; Attene-Ramos et al., 2005; Palsson 2002; US EPA, 2025; Čavoški et al., 2024).

Table 1: Key Approaches to NAMs

NAM Approach	Brief Summary
In vitro	A broad term of any non-whole animal study encompassing tests using organs, tissues, cell cultures, cell lines, and / or sub-cellular aspects such as mitochondria. Other microphysiological systems seek to replicate functionality as small-scale reproductions of aspects of human physiology, such as 'organ-on-chip' (OoC) and organoid methods.
In silico	Computational or 'non-testing' methods including machine learning and artificial intelligence and which can also be used in the planning and analysis of other tests, or as prediction tools. This also includes quantitative structure-activity relationship (QSAR) models.
In chemico	Tools for taking physicochemical measurements of the reaction of a chemical on biology to understand the changes to, for example, covalent bonds and effects on electrons.
High throughput technologies	Uses automated equipment to rapidly test large numbers of samples for biological activity, often associated with providing transcriptomics, metabolomics, and other 'omics' data.
Alternative model organisms	In addition to providing ecotoxicological data on the species used, such organisms are used where toxicity pathways are comparable to humans, such as invertebrates of fruit flies, nematodes, and zooplankton, and early life stages of other organisms.

In the EU, an animal is defined under Council Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes Art. 1 as:

(a) live non-human vertebrate animals, including:

(i) independently feeding larval forms; and

(ii) foetal forms of mammals as from the last third of their normal development;

(b) live cephalopods.

This therefore allows the use of whole, living organisms that fall outside the definition (see 'Alternative Model Species' in Table 1, above) in science and toxicology testing in the EU. However, this definition does not sit easily with public perception of what an animal is, and such distinctions of types of animals are not made in the USA. This differing of expectations has broader implications on industry: for example, the internationally recognised corporate standard of the Leaping Bunny Logo asserts that cosmetics and household products and their ingredients should not be tested on:

> 'non-human animals in the kingdom "Animalia"... Note this definition differs from that of EU Directive 2010/63 [above].'

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(Leaping Bunny Program; www.leapingbunny.org)

The definition of the term 'NAMs' is therefore also not without debate, as we can question what it is that NAMs are setting out to replace, and the quest for clarity continues (Colbourne et al., 2025).

Despite some complexities associated with these alternative methods that will be discussed further in the paper, there are some important advantages of NAMs that are worth pointing out. It is well known that traditional toxicology is expensive and time consuming (Schmeisser et al., 2023; Zaunbrecher et al., 2017). This is coupled with the fact that there remain a significant number of chemicals on global markets that are not yet tested (European Environment Agency, 2023). If NAMs allow for the greater generation and processing of data on chemical hazard, the number of untested chemicals may be reduced. A good illustration is the deployment of 3R-compliant model organisms such as fruit and water flies, which, with their ease of breeding, rapid development, and well-characterized genetics, allow a greater testing throughput (National Research Council, 2000).

Significant scholarship has been undertaken so far on different aspects related to animal testing and the shift to NAMs. An important part of that research is devoted to the human relevance of animal testing by questioning the accuracy and effectiveness of this testing to inform on human harm and harm to the environment (Dinkar, 2018; Rishell, 2018; Akhtar 2015; Balls, 2021; Swaters et al., 2022; Archibald, 2018, p. 1; Johnson & Smajdor, 2019; Van Norman, 2019). This scholarship doubts the applicability of animal testing as a predictor of human health outcomes (whether that be for the presence or the absence of a toxic response) and one study even suggests that the dog is a better predictive model than rodents, despite rodents being the primary animals used for toxicity safety assessments of industrial chemicals as a proxy for humans (Clark & Steger-Hartmann, 2018). Groff emphasises the high numbers of animals used in research and toxicity testing, including in drug, medical device, chemical, cosmetic, personal care, household, and other product sectors (Groff et al., 2014). Animal use and disposal, coupled with the throughput of chemicals and supplies, contributes to pollution as well as adverse impacts on biodiversity and public health.

Besides the predictability and accuracy of animal testing, scholars are concerned with issues surrounding the conduct of animal testing. Ethical issues, including, especially, an emphasis on unnecessary pain inflicted to animals during the testing, are frequently debated in scientific literature (Rollin, 2007; Hansen & Kosberg, 2019; Kolar, 2015; Festing & Wilkinson, 2007; Weber et al., 2021). In wider literature, this leads to further discussion about whether animals should carry improved legal protection, which could be enforced through laws promoting animal welfare or more fundamentally by animals being accorded rights (Gross & Tolba, 2015; Dinkar, 2018; Ogden et al., 2017). Even where some degree of mammalian testing is accepted, scientists are concerned about the minimum expected standards for conducting animal testing, while considering future avenues to improve animal experiments (Liguori et al., 2017). Taylor and Weber raise the issue of transparency in publishing information on animal-based research projects, which may conceal some deficiencies, particularly in describing the harms occasioned to animals (Taylor et al., 2024; Lilley et al., 2014).

As NAMs developed over time, scholarship also expanded to include wide-ranging discussions about the many forms of NAMs applied both in academic and industrial research and development (Scholz, et al., 2013). Recent pharmaceutical results have suggested in vitro and in silico technologies may be 'more promising than animal models to predict human responses,' and a JRC report from 2021 listed many in vitro methods that can inform on specific human health endpoints (Pistollato, et al., 2021). Conversely, there are concerns about the ability of NAMs to characterise chronic toxicity, and the importance of standardised reporting frameworks to assist the assessment of data-rich approaches, such as omics, is often stressed (Schmeisser et al., 2023). Equally, there are concerns of the ability of NAMs to evaluate systemic toxicity, which is attributed to the difficulty in replicating the large amount of information for assessing the many hazard classes and sub-classes that animal tests provide (Čavoški et al., 2024). This is coupled with the claims of the ability of mammalian testing to provide a holistic

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picture of toxicity that more closely represents human biology, and which NAMs currently struggle to recapitulate. There are reservations that a transition to NAMs will risk 'off target' effects being missed, although this debate is further complicated by assertions that animal studies poorly represent some harmful outcomes, such as developmental neurotoxicity (Smirnova et al., 2014). Additionally, the need to combine methods so that human biology can be represented is a shift in approach that regulatory toxicologists must face. It is unlikely that a single new method will simply replace a programme of animal testing (Pistollato, et al., 2021), so regulators may require a change in mindset to adopt alternative methods and assess the weight of evidence from various tests.

Discussions also included several important lines of inquiry on the wider examination of the '3Rs concept' (Rodriguez Perez et al., 2023), and the examination of different jurisdictions and their commitment to the application of that concept. To that end, significant research has been undertaken with regards to EU policy to facilitate the uptake of NAMs (Knight et al., 2023). Such work has examined the extent to which animals are used to ensure compliance under various EU laws governing chemicals (Ibrahim, 2006). One of the major criticisms emphasised is that, despite repeated commitments to the contrary, animal testing persists (Fentem et al., 2021; Macmillan et al., 2024), with limited progress in the regulatory acceptance of NAMs (Taylor et al., 2014). Equally, there is debate about the validation of methods and comparisons between validation of animal methods, which put NAM validation on an unequal footing (Piersma et al., 2018; Locke et al., 2021). As NAMs have experienced only limited uptake, research has been undertaken to discuss ways forward.[1] Lilienblum and others consider that the 'strategy of the development of alternative methods should be more directed towards the refinement or reduction of in vivo tests' in the short and medium term (Ashton et al., 2014).

In summary, complexities surrounding NAMs occupy a noteworthy part of the existing scholarship. Some of the debates are predominantly focused on scientific barriers to uptake of novel technologies (Prior et al., 2019; Zaunbrecher et al., 2017; Bearth et al., 2024), such as the capacity of the science to fully replicate human biology and elucidate on systemic toxicities. Moreover, there is limited validation of alternative methods, which inevitably limits confidence in their use. Elsewhere academic commentary focuses on associated legal, policy and regulatory issues (Schiffelers et al., 2012). There is a plethora of issues arising out of the regulatory structure that might inhibit the take-up of NAMs, including the familiarity of regulators with data from animal studies and the lack of trust in data provided by industry to support hazard assessment of chemicals in Europe, as well as the influence of access to global markets (Čavoški et al., 2024).

Animal Testing Commitments in the EU

The European Union has a long lineage of legislation governing the use of animals for testing. National laws on protecting animals in science experimentation were harmonised in the European Union in 1986 by the adoption of Council Directive 86/609/EEC. In large part, this Directive reflects the agreement in the Council of Europe, also in 1986, of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, even though the gradual rate of ratifications to this Treaty meant that it was 1991 before it entered into force. The Convention and the Directive contain similar provisions for animal welfare and protection, but in practice it is the Directive that provided the main point of protection for animals in science across the European Union.

In 2002, a European Parliament resolution called for the European Union to revise and strengthen Council Directive 86/609/EEC of 1986, to reflect improved understanding of animals' sense of pain, harm, and suffering (Council Directive 2010/63/EU, Recital 6). It took until 2010 and the adoption of Directive 2010/63 on the Protection of Animals used for Scientific Purposes to pass these more detailed and transparent measures, which remain in force today. The scope of the Directive was also extended to include cephalopods (e.g., octopus and cuttlefish) and some foetal forms of mammals. The increasing awareness and protection of animal welfare is considered an important contributor towards phasing

[1]

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out animal testing altogether. Directive 2010/63 therefore also promotes the progression of alternative methods, and it implements the 3Rs hierarchy.

The 2010/63 Directive contends that only where an alternative method is not recognised in legislation should there be resort to animal testing by employing methods carefully selected for relevance. Animal use numbers are to be reduced (such as through reuse and sharing of animal tissues and organs) and minimal pain and distress is to be caused. This Directive introduces classifications of procedures, with those resulting in severe, long-lasting pain, suffering, or distress being prohibited. The species used are expected to be those with the lowest capacity for pain, and the use of endangered species should be kept to a 'strict minimum.' Further restrictions are in place for the use of non-human primates, reflecting particular concern on the part of the public and added ethical and practical issues due to their advanced behavioural, environmental, and social needs.

In addition, the 2010/63 Directive acknowledges that certain EU legislation requires safety testing as part of marketing requirements, so that risks to humans, animals, and the environment can be managed. Some such instruments require animal testing as part of the regulatory regime but seek to avoid repetition of animal tests by sharing data produced from recognised methods across the Union. By way of examples, in the key legislation for the safety assessment of industrial chemicals, Article 13 of the Regulation (EC) 1907/2006 Concerning the Evaluation, Authorisation, and restriction of Chemicals (REACH) requires information on the intrinsic properties of substances to be generated 'by means other than vertebrate animal tests' by using alternative methods 'whenever possible.' Council Regulation (EC) No 440/2008 (TMR) lays down test methods, including the tests and testing methods for chemicals under the REACH Regulation. REACH Art. 13(2) commits to the regular revision of the TMR in an attempt to reduce the number of animals used in testing by including new and updated alternative test methods and there have been six such amendments to date. Additionally, testing on vertebrate animals is only to be undertaken 'as a last resort,' according to Art. 25 of REACH, which proposes data sharing and the joint submission of information. Annex XI then provides the rules allowing standard testing (typically animal testing, such as a 28-day repeated-dose toxicity study) to be adapted, such as by the use of a weight of evidence justification; valid qualitative or quantitative structure-activity relationships models (QSARs); validated in vitro methods; and the grouping of chemicals with read-across between data rich to data poor chemicals (REACH Annex XI s1.2-1.5). Regulation (EU) 528/2012 Concerning the Making Available on the Market and Use of Biocidal Products (the 'Biocidal Products Regulation') also requires animal testing on vertebrates only to be undertaken 'as a last resort', and testing should not be repeated. Instead, enquiries are expected to be made into existing animal data (Biocidal Products Regulation 2012, Art. 62(2)). This serves as an example of the relative homogeneity of requirements in relation to the 3Rs across chemical regulations in the EU.

In a more radical departure, with the help of public pressure, from 2013 the EU banned the marketing of cosmetics that had been tested on animals, and a European Parliament resolution in 2018 called for a world-wide ban (Pistollato et al., 2021). However, the 2013 ban continues to stand alone within European chemical regulation, despite it being considered 'an important signal on the value that Europe attaches to animal welfare' (European Commission; https://ec.europa.eu/commission/presscorner/detail/en/IP_13_210). Additionally, the ban does not guarantee animal-free testing for cosmetics because certain core ingredients may have been tested under REACH for registration purposes. Such ingredients may be used in a variety of settings beyond cosmetics and animal tests may have been conducted to provide information about worker safety or ecotoxicological endpoints, prior to any question of cosmetics regulation (Culliney, 2021).

Table 2 provides a summary of the EU legislative instruments in force and the relevant articles, introduced above:

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Table 2: Relevant EU Legislative Instruments and Articles	
EU Legislative Instrument	**Article**
Council Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes, 2010 O.J. (L 276) 33	Article 4: Principle of Replacement, Reduction and Refinement Article 6: Methods of Killing (minimum pain, suffering, and distress) Article 7 & 8: Endangered Species; Non-human Primates Article 13: Choice of Methods (minimum number of animals; reduce the duration and intensity of suffering to the animal to the minimum possible; involve animals with the lowest capacity to experience pain, suffering, distress, or lasting harm) Article 16: Reuse Article 18: Sharing Organs and Tissues Article 54: Reporting (Member States shall collect and make publicly available, on an annual basis, statistical information on the use of animals in procedures)
Regulation (EC) 1907/2006 Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals, 2006 O.J. (L 396) 1 (REACH)	Article 13: General Requirements for Generation of Information on Intrinsic Properties of Substances (Information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods; [TMR] to replace, reduce, or refine animals testing) Article 25: Data Sharing and Avoidance of Unnecessary Testing, Objectives and General Rules (Testing on vertebrate animals for the purposes of this Regulation shall be undertaken only as a last resort) Annex VII to X: Standard Information Requirements Annex XI: General Rules for Adaptation of the Standard Testing Regime Set Out in Annexes VII to X
Council Regulation (EC) No 440/2008 Laying Down Test Methods, 2008 O.J. (L 142) 1 (the Test Method Regulation)	(Specifies tests)
Regulation (EU) 528/2012 Concerning the Making Available on the Market and Use of Biocidal Products, 2012 O.J. (L 167) 1 ('Biocidal Products Regulation').	Article 62: Data Sharing (Testing on vertebrates for the purposes of this Regulation shall be undertaken only as a last resort. Testing on vertebrates shall not be repeated for the purposes of this Regulation)

Despite the legislative wording depicting animal testing as a last resort, the 3R principles are not uniformly applied in the application of EU chemical regulations (European Parliament Resolution, 2021). While the use of the grouping and read-across within the standard information requirements of REACH constitutes the most frequently used adaptation, it is also commonly rejected due to a lack of thoroughness and rigour (Schmeisser et al., 2023). It is worth noting that adaptations are a very useful concept under REACH, which allows applicants to deviate from 'standard testing regime' prescribed by Annexes VII to X and rely on some other methods, such as the use of historical human data; a weight of evidence approach; or in vitro methods. Additionally, the responsibility of utilising NAMs does not rest solely

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with industry, as regulators are considered to be sceptical of accepting data from NAMs due to concerns about the lack of complexity in comparison with whole organisms; the lack of toxicodynamic information; and the unclear association with exposure duration (Schmeisser et al., 2023). Examples of the mistrust in NAMs includes a request for an animal test that resulted in the same outcome the original read-across had given, and a Board of Appeal decision found that requests by ECHA for animal tests were not proportionate as the anticipated results were unlikely to lead to an improvement in risk management measures (Macmillan et al., 2024).

A further requirement under Council Directive 2010/63/EU is that animal use data is to be submitted to the European Commission from Member States (Art. 54). The most recent data shows a fluctuating yet stubborn picture. The data of 2020 from Member States and Norway shows a small decrease in the total number of animals that have been used for the first time in research and testing from 8.8 million in 2018, to 8.5 million in 2019, and 7.9 million in 2020 (European Commission, 2020).[1] Of course, external factors always make comparisons difficult, and the Covid-19 pandemic provides an excellent example; 11 of the 16 Member States whose animal use numbers decreased in 2020 attributed this, in part, to, for example, lockdown measures, and four of the responding countries attributed their increase to the very same reason. Yet, in 2021 the total numbers of animals used increased to 9.4 million, then in 2022 (the most recent data) the figure decreased to 8.4 million animal uses (European Union, 2024). The report describes the 2021 increase only as being of an 'exceptional nature.'

Table 3 categorises the type of use of the total 8.48 million animal uses in research and testing in 2022. The largest proportion of animal uses is for basic research (for example, about the nervous and immune systems), and while only 13% of animal uses relates to regulatory use, this still accounts for over one million animal uses in the year. Translational and applied research refers to areas such as animal welfare, human cancer and animal diseases, and the 'other' grouping includes, *inter alia*, research on prevention of species loss.

Table 3: Purpose of Use as a Percentage of 8.48 million Uses of Animals for Scientific Purposes	
Basic Research	37%
Translational and Applied Research	35%
Regulatory Use	13%
Other	9%
Routine Production	6%

The greatest regulatory animal use is for batch potency testing,[2] followed by developmental toxicity, ecotoxicity, batch safety testing, repeated dose toxicity, and reproductive toxicity.

In 2022, just under half (47.8%) of animals used were mice, with use also of, for example, thousands of other rodents, rabbits, dogs, pigs, monkeys, domestic fowl, reptiles, amphibians, fish, and cephalopods. Just under half a million animal uses (n=499,931) related to toxicity testing (including pharmacology), with almost 3000 animal uses to test skin irritation / corrosion, which is particularly concerning, as this occurs despite updates to the REACH legislation in 2016 making non-animal methods the default for this health endpoint (ECHA, 2023). Overall, there appears to be a general inertia in moving away from animal testing.

As a signal of the present public view on animal testing, over one million European citizens signed a petition that was submitted to the European Commission (EC) in January 2023, calling for: a strengthening of the cosmetics animal testing ban; an end to new forms of animal testing within chemicals' regulations; and a commitment to a legislative proposal to phase out animal testing before the end of that legislative term (European Union, 2021). In response, the EC committed to develop a roadmap with stakeholders, and to initiate other (unspecified) actions to accelerate the reduction in animal use (European Commission, 2023).

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How does the EU compare with the USA?

Under the Toxic Substances Control Act (TSCA), chemicals in the USA are evaluated depending on their status as a 'new' or 'existing' substance, whereas no such differentiation is made in the EU (US EPA, OCSPP, n.d.). While new chemicals that are not on the TSCA inventory of existing chemicals are assessed under a 'gatekeeper' programme to identify whether measures are needed before the chemical is placed on the market (US EPA, n.d.), the EU approach of 'no data no market' allows substances to be marketed as long as a registration dossier has been submitted (REACH, Art. 5), which contains registrant-generated toxicological and ecotoxicological information about the substance in accordance with the quantity imported or manufactured per year, starting with a one tonne limit (REACH, Art. 10). This is a lower general requirement quantity than the USA, although some substances are subject to data reporting at a lower tonnage where there is thought to be an unreasonable risk (US EPA reporting, n.d.; US EPA Section 4, n.d.). The European Chemicals Agency (ECHA) undertakes a check on only a percentage of the submitted dossiers, where they ensure compliance with the standard information requirements (REACH, Art. 41). Evaluations of substances, prioritised according to risk, are undertaken by the competent authorities of Member States under a 'Community rolling action plan' (REACH, Art. 44-45).

As in the EU, the updated TSCA of 2016 includes the requirement to reduce and replace the use of vertebrates in testing, and to promote the development of alternative methods (US EPA, 2024; US EPA 2025b). Akin to the test methods in the EU, the EPA Strategic Plan includes a list of new approach methodologies (NAMs), although this is updated annually (US EPA, 2024; US EPA 2021). Test methods required by the EPA are typically OECD test guidelines or those accepted by the Office of Chemical Safety and Pollution Prevention (OCSPP), which also includes NAMs (US EPA, 2022), with the OCSPP maintaining a 'Master List' of test guidelines that can be used to generate data (US EPA, 2019). This appears clearer compared to the EU, where the use of validated methods appears to be preferred over, say, a weight of evidence approach using non-validated methods. This discretion over what is considered acceptable by regulators in the EU leads to uncertainty that is not entirely overcome by a large amount of soft law guidance in disparate locations, which is not always readily coherent.

As for the impacts of these different approaches on the level of animal use, any comparison is difficult to draw. The United States Department of Agriculture (USDA) compiles statistics annually for some of the animals used in experiments, however, under the Animal Welfare Act those 'purpose bred' for experiments, such as rats and mice, are not required to be included (Humane World for Animals; https://www.humaneworld.org/en/issues/animals-used-experiments-faq), and these are predominantly the animals used in traditional chemical toxicity testing.

The EU may lag a little behind the USA in terms of policy signals for non-animal testing, with the formation of a roadmap only now being developed by the European Commission, whereas the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) published 'A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States' in 2018 (National Toxicology Program, 2018). Also, in applying the 3Rs principles, the USA can be considered as having a more flexible system that has resulted in the EPA and FDA accepting numerous Defined Approaches (DAs) that utilise NAMs (OECD, 2016), whereas only two are accepted in the EU (Schmeisser et al., 2023).[3] The USA is not without criticism though. In addition to the lack of animal data, the EPA had quietly dropped their 'hard deadline' of 2035 (and an interim 2025 goal) to phase out the use of mammals in chemical safety testing (Grimm, 2024; Inside EPA 2021). Additionally, data up to 2015 indicates that thousands of grants over the preceding seven years had been steadily awarded from the National Institutes of Health (NIH) for research involving mice (Lauer, 2016; Singer & Lochke 2023), and the non-profit organisation PETA (People for the Ethical Treatment of Animals) had made moves to sue the NIH for awarding grants for research into the sepsis disease that involves animal testing, when over a

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dozen peer-reviewed studies have questioned the adequacy of mice as a model for this disease (*Peta v Tabak*, 2023). However, more recently both the EPA and FDA have reaffirmed their intentions to phase out animal testing (US FDA, 2025; Dinan, 2025). Both jurisdictions, therefore, appear to struggle in demonstrating a fixed trajectory away from animal testing.

A Last Resort? Empirical Insights into Animal Testing in the European Union

As discussed earlier, despite different policy approaches on 3Rs across jurisdictions, the uptake of NAMs as alternative approaches to animal testing has been limited. Different barriers have been identified in the existing scholarship encompassing scientific, regulatory, social, and economic barriers. This section of the paper draws upon our empirical data from stakeholder interviews to analyse in greater depth the barrier presented by the current understanding of, familiarity with, and confidence in animal studies among key stakeholders in the chemical risk assessment process. This barrier brings together different lenses and perceptions in grasping the value proposition of both animal studies and NAMs. Awareness of this issue is valuable in addressing some of the concerns associated with NAMs.

Despite the REACH stipulation of the use of animals as a last resort, an overwhelming majority of participants in the empirical study voiced their concerns about the inadequate application of this principle in practice. As one interviewee pointed out that:

> *"The animal testing is the last resort, as is already mentioned in Article 25 that it should be. But seeing that last resort principle actually put into practice, it's very rare"(Interviewee I2).*[4]

This has been coupled with dismay at the continuing resort to an old technology "*invented in 1950s despite new ways of doing testing such as computational approaches, bioinformatics*" (Interviewee I2). Interviewees failed to find similar examples in other sectors where novel technologies struggled to gain a foothold. These general observations open a wider discussion of how society is being locked into a technology track from which it is difficult to escape and why this should be the case (Čavoški et al., 2025).

Different reasons have been put forward during the empirical study that may provide insight into the limited traction that NAMs have gained, with many of these associated with perceptions of animal testing. The legislative barrier has been seen as a key obstacle in the EU when it comes to the replacement of animal testing. It is worth noting that findings of the study indicate several facets that fall under the 'legal barrier' to NAMs. It may include provisions in the legal text that acts as a barrier followed by a legal interpretation of that legal text that may impede the uptake of NAMs. It may also include legal and regulatory culture entrenched among regulators that become difficult to shift.

With regard to the legal text acting as barrier, the following remark made by one of the interviewees illustrates the point:

> *"The biggest barrier is the legal one. The legal framework is so difficult to change that that is actually suppressing what the institutions feel they can do and should do. And then the whole regulatory machinery is kind of linked to the legal basis, and that also becomes highly inflexible. Number one is the law. It is like an immovable system. Legislation tends to get more diverse and more complicated."(Interviewee P5)*

This quote raises an important point of the significance of a legal basis in making judgements about the deployment of NAMs. As pointed out earlier, REACH promotes the use of alternative testing by requiring the use of animal testing as a last resort. In addition, there are other legal bases that could provide for a better use of NAMs. Annex XI is particularly key in this respect. Under Annex XI adaptations of REACH, NAMs derived from in vitro and QSAR methods are specified, and grouping and weight of evidence allow some potential use of NAMs. Despite this flexibility offered by Annex XI, regulators often do not consider these alternatives, such as applying weight of evidence from a variety of sources, as a valid means of providing alternative information. This is explained by a narrow interpretation of this legal basis coupled with limited expertise interpreting

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data sets resulting from the deployment of different forms of NAMs. There remain some good examples of engagement of the regulators with scientific community in exploring better deployment of alternatives that fall within the remit of Annex XI. A recent initiative involves grouping and read-across, a common alternative approaches to animal testing in chemical risk assessment in which chemicals are grouped typically based on chemical structure, and data is interpolated from well-tested, data rich substances and read across to data poor substances in the same group.. Recent research has suggested that by grouping according not to structure but to shared molecular effects across source and target substances, a more reliable and regulatory acceptable grouping may emerge, leading to greater opportunities for read-across from historic in vivo data to negate the need for yet more animal testing (Viant et al., 2024).

There is also a perception that EU chemical legislation tends to be prescriptive as to method and thus restricts the use of NAMs. This brings to the fore the wording of the legal texts which may pre-date recent developments in NAMs. The close association of the EU Regulation (EC) 1272/2008 on Classification, Labelling and Packaging of Substances and Mixtures (CLP) with REACH is considered a further limitation on the use of NAMs to assess safety in place of animal tests. For example, the criteria for the classification of a substance as acutely toxic includes the wording:

> *"The preferred test species for evaluation of acute toxicity by the oral and inhalation route is the rat, while the rat or rabbit are preferred for the evaluation of acute dermal toxicity."(CLP Annex I, s3.1.2.2.1)*

Such specific wording and references elsewhere in CLP of producing acute toxicity estimate values by converting LD50 or LC50 values (CLP, Annex I, Table 3.1.1) also indicate not just an expectation, but what could be further interpreted as being a requirement to generate data from animal studies.

NAM data is not absent from CLP, however, with mentions of in vitro methods and 'weight of evidence' for skin corrosion and irritation endpoints, albeit 'validated and accepted' in vitro (CLP, Annex I, s3.2.2.2.4). However, a confused and confusing message for the application of NAM data does appear, as sub-categories for skin corrosion relates directly to animal exposures only:

> "Category 1: Destruction of the skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least one tested animal after exposure" (CLP, Annex I, Table 3.2.1)

Alongside the prescriptive nature of EU legislation coupled with its potentially narrow interpretation, is a wider issue related to the deeply embedded view of animal testing as a 'gold standard'. This is closely related to the entrenched regulatory culture that may slow down the uptake of NAMs. The participants in the empirical study emphasised two main interconnected issues that are key for this discussion – reliability of animal testing and their ability to accurately predict harm as illustrated by the two quotes below:

> *"There's a whole group of toxicologists that believe that the animal study is never a good predictor for the human" (Interviewee R5).*

"I think the biggest barrier is the idea that the animal test is a good test". (Interviewee I4)

The reliability of animal testing is often perceived as coming from the longevity of their use and the level of confidence regulators draw from this. Animal studies are still perceived as the backbone of hazard and risk by regulators (Interviewee R2). This was often described as "familiarity" with the animal testing in decision-making, which provides an easier route than attempting to understand the science between NAMs testing. Additionally, a 'higher bar' is applied frequently for the acceptance of NAMs in terms of their reliability, so that the variability of responses from individual animals in an animal study is considered acceptable (and even expected), whereas variations of results from a NAM test may lead to the rejection of that method or result. Regulators are accustomed to dealing with whole organism data and their departure from this type of testing may bring unease and discomfort to well established practices.

> *"Some of the regulators have always accessed material with one way of doing it. And therefore, they have the comfortable situation of 'We have always done this like this, and therefore it's relevant, and therefore we would like to continue doing it like this.' I could have sympathy. For this we*

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need the same level of confidence." (Interviewee I6)

One of the key challenges is the interpretation of NAMs testing, which is different from the interpretation of a legal text, coupled with the validation of such tests. The regulator is faced with a battery of tests and a complex task in extrapolating results. In performing this extrapolation, it becomes challenging to convert the results in such a way that there is a correlation with the type of results that animal studies provide. A good illustration is a 90-day rat study, which involves the assessment of 32 organs and several methods to assess the toxicity of each organ. Thus, to validate NAMs against each of these specific parameters is problematic where NAMs take a more comprehensive approach (for example in monitoring bioactivity to assess a range of adverse outcome pathways). In our data, validation was also recorded as a crucial issue among different representatives of civil society. Across civil society organisations propagating different agendas such as animal welfare or green agenda, there is a common understanding that validation is key to address the mistrust that exist between the industry and civil society.

As one interviewee also pointed out, the acceptance of NAMs is particularly challenging when NAMs testing indicates a less conservative view of hazard than that suggested by animal testing; there is then a tendency to dismiss the value of the NAM test rather than apply a different interpretation of the results (Interviewee I4). A criticism is also that NAMs tend to be validated against the outcome from the existing animal tests (due to the 'gold standard' assumption). Yet a NAM may have been developed to represent a human response, and if one accepts the argument that animals are a poor proxy for human health, the continuing comparison with animal data is constructing a barrier to the take up of NAMs.

It is also worth noting that some animal tests were never validated and were accepted only as a result of long use and familiarity with these testing methods. Related to this is also the bigger question of the ability of animal testing to accurately predict harm. Some interviewees pointed out that the choice of animals we use is not necessarily due to similarity of their traits to humans but due to the fact that these types of animals 'thrive' under laboratory conditions (Interviewee R9). In order to improve validation of NAMs, we need "*clear protocols based on performance standards for NAMs coupled with the reproducibility across multiple sites*" (Interviewee I10). The solution may lay in having a more flexible approach whereby validation can be reformed to reflect different levels of certainty that could be acceptable for different purposes. In practice this will open up room for NAMs and their role in screening and prioritisation of chemicals, which could be validated against less rigorous standards than those used in quantitative assessments. This new approach is well articulated in the following quote:

> *"So, the alternative then needs to be validated. Because there are so many NAMs we have to prioritise which ones go through the process of validation. And maybe we need to think about validation in a different way. Clearly there should be different tiers of validation, maybe at the highest tier of validation where you require all sorts of inter-laboratory things, and meeting all sorts of criteria should be left for some kind of study that is really important for the decision making. And then maybe a lower level of validation for things that maybe are only useful for screening purpose or prioritisation, and then maybe one even lower level of validation that's only for when you want to find new compounds, at the R&D development stage of a new chemical. And maybe even validation principle should change. It should be more about, you know, test being fit for purpose.*" (Interviewee R3)

Besides creating these new protocols and guidance on what human relevance entails, there was a general agreement that there is a need for a mind-set change regarding NAMs validation and a reconsideration of alternative ways of validation, by moving, to some extent, from OECD test guidelines as a cornerstone of validation. This again points to a need to change the regulatory perceptions of the roles and significance of validation.

There are some essential questions concerning the suitability of animal testing to assess the effects of chemicals on the environment and biodiversity. Some participants highlighted the genetic diversity that we

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are limited to by using the same laboratory animals (Interviewee I4). The continuous use of selected species in laboratory testing produces skewed results, which fail to capture the diversity that is present in the wider environment. Issues of capacity and resources have been identified as factors that still perpetuate the use of animal testing. There was a general agreement that there is a wide choice of labs performing animal studies, which is certainly not the case with regards to deployment of different forms of NAMs. This is coupled with limits of who is employed by the industry, especially in smaller companies, which lack the facilities and resources of multinational industry. Consultants are well connected to labs, which gives confidence to clients when employing them, and they tend to suggest animal studies to their clients as they know this will be more likely to be accepted by the regulators than would NAMs testing. This may also be challenged if costly testing is suggested and compounded by the fear of costs from additional testing later required using animal studies. Some of the interviewees mentioned that there are financial incentives for consultants to prioritise animal studies, as this would result in secured financial gain for them upon completion of the work. As pointed to by one of the interviewees:

> *"It's easy money for a consultant, and if you are purely looking at the financials, you've got the choice of, let's say, performing a 90-day study for £250,000 or performing a NAM based approach for £300,000."(Interviewee I3)*

This emphasis on animal studies as a secure way to ensure dossier approval, taken together with other barriers, has an economic knock-on effect. As with any new technology, such as with renewable energy technology, the expectation is that over time this new technology will become cheaper. However, the lack of resources both in terms of human resources and NAM-based facilities, leads to a vicious circle of continuous reliance on animal testing. This situation was further exacerbated by the supply chain crisis following the Covid-19 pandemic. Some of the interviewees also reflected on wider perceptions of NAMs being cheaper, which may be the case if you are undertaking single receptor screening compared to an animal study, but the likelihood is that multiple assays will be needed (Interviewee R2). For complex endpoints, the costs are anticipated to be more expensive, possibly using a combination of methods, thereby maintaining reliance on animal testing.

Societal concerns about the shift from animal testing to NAMs occupy an important place within the wider discussion. There is a wider understanding of the negative considerations associated with animal testing such as ethical, cultural, and economics, which raises consumers' awareness about the need for change. However, despite this growing awareness, citizens remain wary of a shift to non-animal testing happening too quickly. It was well documented in the study that citizens and citizens' associations are active in raising their concerns with policymakers by urging them not to move too rapidly (Interviewee P3). This influences policymakers who want to make sure that a new robust system is put in place, followed by an appropriate socialisation of this new technology. As one interviewee pointed out "the public wants to be reassured that whatever is there on the market is safe" (Interviewee R3). This is certainly further intensified by debate in the civil society between those non-governmental organisations that are pushing an animal welfare agenda, as opposed to those pursuing a public health agenda. Some interviewees pointed out that progress to NAMs is very slow and that many concerns are impressionistic, rather than a reflection of the actual status of NAMs for purposes of chemical regulation. This is coupled with limited political commitment to NAMs sending out a very feeble signal to citizens about the need to embrace non-animal alternative testing methods. One way of highlighting to citizens a shift to non-animal testing would be to adopt the reduction in the number of animals used for testing as a performance indicator for regulators both in the EU and other jurisdictions, accompanied by forms of actively managed reduction. Finally, the empirical study also brought to the fore the needs for societal education as a key factor in enabling a shift from animal testing. Without this, the radical shift to NAMs will not happen. A parallel with Covid was provided to illustrate how societal change is possible and it could happen quickly when required:

> *"You know, we all went from knowing very little about vaccinations to kind of getting a daily update and our knowledge as a population kind of grew exponentially around what a vaccine is, and compliance as well. So, you know people*

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that wouldn't have gone to get a vaccination, and so there was a whole kind of societal education that drove change. I guess that's what I'm getting at, and I just wonder what's the role of society in this whole NAM debate?" (Interviewee I1)

The Last Resort Principle: Appeals and Judicial Oversight in Europe

This section analyses the role of the ECHA Board of Appeals, the Court of Justice of the European Union (CJEU), and the EU General Court in interpreting and ruling upon the requirement that animal testing should be employed only as a last resort.[5] Before doing so, it may be helpful to explain some features of challenges to decisions made by ECHA. There is a Board of Appeal within the European Chemicals Agency (ECHA) which, while part of the administrative fabric of the Agency, is considered as discharging functional independence in overseeing appeal proceedings (*GE Betz v OHIM – Atofina Chemicals (BIOMATE)*, 2004).[6] The task of deciding appeals against ECHA decisions is allocated to the Board of Appeals under Article 76 of REACH but at the same time is carefully circumscribed to particular types of decisions listed in Art. 91 of REACH or by Art. 62 of the Biocidal Products Regulation. This means that there are a group of challenges not within the remit of the Board, including those relating to the interpretation and scope of REACH itself, which will proceed to the European Court of Justice without appeal to the Board of Appeals.

There is an important distinction in relation to competence of the Board of Appeals when compared to the Court. Any review by the latter is restricted to issues of legality of the decision reached, including procedural correctness. This stems from Article 263(1) of the Treaty for the Functioning of the European Union (TFEU) which states that:

> "The Court of Justice of the European Union shall review the legality of legislative acts, of ... the Commission ... intended to produce legal effects vis-à-vis third parties. It shall also review the legality of acts of bodies, offices or agencies of the Union intended to produce legal effects vis-à-vis third parties."

Note that the subject of review is the European Commission in making decisions on risk management, rather than to the risk assessment decision of ECHA, though, of course, the former may very much rest on the latter. Again, this is different to Board of Appeal hearings in which ECHA is the respondent to any appeal coming before the Board.

In terms of the grounds for review, it has been convincingly argued by Navin-Jones that the Board of Appeals faces no restrictions confining the review 'legality' as laid out in Article 263(1), so that review can extend beyond the lawful nature of the decision to include other grounds including the technical and scientific basis of the decision (Navin-Jones, 2015). Navin-Jones illustrates this by reference to the decision in *Lanxess Deutschland GmbH v. ECHA* in which the Appellant sought to amend a registration dossier to include results from a 13-week, mouse-based, sub-chronic toxicity study generated by the US National Toxicology Program, rather than conducting a similar, 90-day sub-chronic animal test. ECHA argued that this was not as such a challenge to the lawful nature of the testing requested. The Board of Appeal did uphold the challenge on the basis that there was no legal certainty that the US test results would be forthcoming, but even if there was no challenge to the legality of the ECHA decision, the Board of Appeal certainly felt itself free to explore the background factual and technical issues. This accords with the status of the Board as part of ECHA itself in that, given ECHA is charged with certain tasks, including the use of animal testing as a last resort, it should be open to the Board to review technical progress in meeting such obligations.

The context of decisions regarding chemical hazard is often a desire to protect human health and the priority given to this in case law regarding the application and interpretation of the EU chemicals law can override other ethical misgivings. The weight of evidence applied by regulators in assessing applications by industry reveals a cautious approach to assessing the scientific evidence and its reliability, which is then supported by the General Court and the CJEU on appeal. When it comes to alternatives to animal testing, according to the General Court, in exercising the weight of evidence, ECHA is bound by the principle of scientific excellence in analysing the intrinsic properties of a substance

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(*PlasticsEurope v. ECHA*, 2019, para. 93-94). This tends to generate discussions as to which studies can be regarded as 'reliable scientific studies' to demonstrate the hazardous properties of the chemicals involved as well as the weight of evidence to be applied, and as indicated above, such studies rely typically on established animal testing.

This approach of prioritising human health considerations is often supported by reasoning that involves resort to the precautionary principle. This is summed up by the General Court as follows:

> "the process for assessing substances, which encompasses the adoption of a decision requesting further information in the evaluation of a substance, is intended to achieve a more detailed evaluation of substances which are considered to be priority in the light of the risks they are likely to pose for human health and for the environment. Such an evaluation, which is conducted by the designated authority taking into account the precautionary principle … must be entrusted to scientific experts." (*BASF Grenzach GmbH v. ECHA, 2019*)

Note that the precautionary principle may be invoked not only to support the generation of data through further animal testing but also to support the testing regime as proportionate. For example, in the Board of Appeal decision in *Goldmann GmbH v ECHA (2020),* the Appellant argued that requiring a 90-day toxicity study was disproportionate, especially since it involved animal testing that might not yield significant results, but ECHA justified the study to the satisfaction of the Board by invoking the precautionary principle, arguing that the risks warranted comprehensive testing. As for arguments by the appellant that ethical concerns had been overlooked, although the Board stated that animal testing is only permissible where no other suitable options exist, it found that ECHA had adequately considered alternative approaches, and that the extent of animal testing required was justified when weighed against potential risks of cardiotoxicity.

Because challenges in the Court, in contrast, are confined to issues of legality, it is not for the Court to substitute its own view for that of the expert, and instead the task is to ensure that ECHA has acted throughout in accordance with its legal authority, both procedurally and substantively, and has not gone beyond the scope of the legal powers assigned to it. This involves a degree of deference accorded by the Court to the expert judgement of the agency. Given the complexity of scientific decision-making, some degree of discretion ought to be allowed in reaching an expert determination. This is not to forgo review, as this is demanded by Article 263(1) of the Treaty (above) in relation to the legality of the decision made. In a case challenging the withdrawal of plant protection products by the European Commission, the judicial task was described as follows:

> "If the Commission is to be able to pursue effectively the objective assigned to it, account being taken of the complex technical assessments which it must undertake, it must be recognised as enjoying a broad discretion. However, the exercise of that discretion is not excluded from review by the Court. The Court has consistently held that in the context of such a review the Community judicature must verify whether the relevant procedural rules have been complied with, whether the facts admitted by the Commission have been accurately stated and whether there has been a manifest error of appraisal or a misuse of powers." (*P. Industrias Quimicas del Valles SA v. Commission of the European Communities*, i, para. 75-76)

However, as best illustrated by the case of *French Republic and European Commission v CWS Powder Coating GmbH and others (2025)*, the courts are ready to review hazard classifications to ensure that these align with clear scientific proof of the hazard cited. The challenge in this case was to Delegated Regulation (EU) 2020/217, which classified titanium dioxide (TiO_2) as a carcinogen by inhalation. The Court ruled that the studies relied upon by ECHA's Risk Assessment Committee (RAC) did not demonstrate that TiO_2 in powder form posed a carcinogenic risk under normal conditions of use and followed the General Court in annulling the Delegated Regulation. The first ground of review in this case focused on whether there was a manifest error in relation to the particle density value. In addressing this issue, the General Court readily embarked on a highly technical to demonstrate how by using the density value of unagglomerated primary particles, instead of agglomerated particles of

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titanium dioxide, the RAC failed to take into account all factors of significance to this case (para 100; Čavoški et al., 2023). This raised concerns about the Court's knowledge and expertise to make a judgment on highly scientific issue as well as whether this falls within the remit of the Court's judicial review role. Broadly the CJEU determined that the General Court had exceeded its powers of judicial review but were still correct to hold that the RAC had failed to take account of all relevant factors in its assessment of the study on which the decision to classify was based. [7]

Although not a case concerning animal testing, the *Titanium Dioxide* case is noteworthy for several reasons. First, it exemplifies a European system of chemical safety assessment based on hazard, to the point that the CJEU emphasised that hazard classification should focus exclusively on the intrinsic properties of the substance under assessment. Questions of particle size and even exposure scenarios were thought not relevant to the consideration of hazard classification. Secondly, while it is critical of the approach of the General Court for overreaching its powers of judicial review, it proceeds nonetheless to annul the Delegated Regulation. More specifically, the General Court should not have engaged in an enquiry to determine the question of the appropriateness of the value of the density of titanium dioxide particles attributed by the RAC in considering the phenomenon of agglomeration of those particles. This was a question requiring scientific assessment such that the General Court could not substitute its findings for those of the competent authorities. Yet despite this, the CJEU was not prepared to set aside the decision on the basis that the decision was well founded on other legal grounds. This leads to a final issue to note which concerns deference. The decision of the General Court in embarking on its own enquiry is hardly deferential but was allowed to stand. As cases come before the courts where assessment made on the evidence from NAMs is perhaps rejected by regulators and where the last resort principle is invoked to challenge the dismissal of evidence provided by new non-animal methods, the *Titanium Dioxide* case suggests that European courts may go further than one might have envisaged in its oversight of the science relied on by competent authorities. The deference given to the expert judgement of an agency remains appropriate, but perhaps with less certainty as to how, when and on what basis the courts may so defer. As it stands, however, regulators in the EU remain an important group governing NAM use, because of continuing preference for animal studies accompanied by court deferral to the scientific methods underpinning assessment.

In the USA, deference to agencies is a hot topic given the recent demise of *Chevron* deference following the ruling of the Supreme Court in *Loper Bright Enterprises v. Raimondo* (2024). It should be noted, however, that the model of deference as laid down in *Chevron U.S.A. Inc. v. Natural Resources Defense Council, Inc* (1984) extends much more widely than the equivalent doctrine in EU or UK law. This is because, in both legal systems, there is little experience of delegation of rule-making power to regulatory agencies. In the European context a body like ECHA is an independent agency with a Management Board containing representatives of the Member States of the EU. It has the power to register and evaluate chemical substances and to make decisions authorising or restricting their marketing and sale within the EU. As we see from the *Titanium Dioxide* case, such decisions can be challenged before an internal Board of Appeals and ultimately by the Court of Justice of the EU. The Court will act as the final authority on matters of construction or interpretation of ECHA's powers under (say) REACH and there would be no question of the court deferring to the Agency on its interpretation of its own powers, no matter what the ambiguity in the framing of those powers. Resolving such ambiguity is very much the function of the CJEU, which, for example, regularly takes cases on deference from the courts of EU Member States for a definitive ruling to resolve ambiguity in EU law.

To this extent, the position in Europe, was already much more closely aligned to that now existing in the USA post *Loper Bright* (Willis, 2025). In the USA, as pointed out in one commentary on the Lopez Bright decisions, with Chevron deference gone in terms of interpretation of the statutory remit, an "extremely deferential standard of review remains with respect to agency fact finding." (Bamberger et al., 2024). The idea of deference, when in play in the EU system, relates precisely in this way to the scientific or technical judgement exercised by agencies such as ECHA in processes such as hazard assessment. As

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explained above, the Court will review the powers of the agency in line with ordinary principles of statutory interpretation and if such powers have been applied in a procedurally correct manner, and if the decision is free from manifest error, then the court will defer to and not seek to displace the decision reached. This leaves court review focussed on legality of agency action and not in a role in which they are second guessing the science. Nonetheless, it remains part of that review of legality to police the last resort principle and to make adequate enquiry into alternative non-animal methods where a party claims that these provide an adequate evidential basis for a decision to be made.

In the EU, the combined effect of review of legality in a precautionary setting that demands a decision in the face of scientific uncertainty together with a deferential approach to scientific decision-making is that often regulatory requirements for further animal tests are upheld by the Court. At times this is simply by acknowledging the last resort principle but then accepting that this must have been fed into the scientific call for further testing. This is well illustrated by the case of *Polynt SPA v ECHA* in which the appellants argued that the conduct of a study required by ECHA would lead to the sacrifice of approximately 600 animals, causing the applicant company to breach its own policies on animal welfare and to lose the benefits of selling a substance that can be considered by the market as cruelty free. In upholding the request for such testing, the General Court stated that:

> "It is true that the objective of ensuring animal protection is also pursued by the REACH Regulation, in particular by Article 13(1) and Article 25(1) thereof. According to that latter provision, testing on vertebrate animals for the purposes of that regulation is to be undertaken only as a last resort. However, the information requirements set out in Annexes VII to X to the REACH Regulation confirm that animal testing cannot be avoided in every case. In some cases, only testing on vertebrate animals will provide sufficient scientific information to enable measures to be taken to protect human health and the environment." (*Polynt SPA v. ECHA,* 2021, para. 34-35)

This points to a central dilemma in REACH, which is that the espousal of the 'last resort' principle is compromised by the technical annexes to the Regulation, which directly advocate vertebrate animal testing. Under deference, this leads to the court upholding not a last resort principle, but a first recourse to animal testing in the face of scientific uncertainty. It is true that the task of ECHA is to minimise such uncertainty in the interests of chemical safety but the push for certainty is invoked in a manner that sweeps away ethical considerations. The very limited progress in halting animal testing in chemicals regulation surely attests to this. We see something of this in the aforementioned *Polynt* case. Polynt applied for interim measures to suspend the operation of the decision, claiming it would suffer serious and irreparable harm, including the need to conduct animal testing. It was necessary for Polynt to apply an application for interim protection, because otherwise a decision of the Board of Appeals would take effect and animal testing would commence even though an appeal to the General Court had been lodged. As Orzan points out, this implicitly ranks considerations of the protection of public health above any consideration of animal welfare (Orzan, 2024). To support the application for interim measures suspending the testing, Polynt are then required to prove that the contested decision will lead to direct damage to its corporate interests, whether financial or reputational. As summed up in a similar case, "consequently, while their argument that the implementation of the contested decision would harm animal welfare does indeed have an ethical dimension, it cannot establish the urgency of the suspension sought for the applicant" (*Nouryon Industrial Chemicals and Others v Commission*, 2020, para. 25).

On the issue of the REACH annexes and their dependence on methodologies involving animal testing, this indicates the significant entrenchment of toxicology built on test methods with over seventy-five years of application. New approach methodologies are developing rapidly whereas REACH, passed in 2006 with a phased implementation, began life before the turn of the Millennium prior to the publication of a White Paper in 2001 (European Commission, 2001). This timescale meant that REACH coincided but failed to allow for developments in fields such as big data and

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AI and huge advances in omics. Once in place, reliance on animal testing methods built into the Annexes of REACH began to inhibit the take up of NAMs into regulatory testing.

There are doubts regularly expressed about the reliability of animal testing as a predictor of adverse outcomes in humans. Such doubts stem from the challenges of extrapolating testing results from animals to humans due to interspecies differences in, for example, anatomy, physiology and metabolism. There are questions about the reproducibility of findings even across testing on the same species and challenges in the precise estimation of risk in the human population on the evidence of animal test results. It is not generally open to successfully raise such doubts in the courtroom, given the focus on legality and the endorsement of animal test methods in the Regulation itself. In any case, the long history and well-established tradition of animal testing make it difficult to dislodge and we see from many reported cases that where uncertainty prevails, as it often does in questions of chemical safety, the resort is to yet more animal testing.

Conclusion

There is no doubt that the dominance of animal testing is maintained in part by the wide range of new chemicals and chemically derived products requiring testing to gain market access. In Europe, animal testing provides the primary tool to assess the hazards associated with such chemicals. However, with the further development of science and societal understanding of impacts of testing on animals, there is a policy shift to a new agenda that called for the replacement, reduction and refinement of animal testing (the 3Rs). Many different forms of NAMs have been developed following scientific advances, particularly in biosciences and bioinformatics, raising an aspiration, perhaps an expectation, that gradually they will be key in upholding the 3Rs principle. Thus far, however, there has been no substantial reduction of animal testing over the lifetime of the REACH regulation, which is the key EU legislation, and which, on the face of it, with its 'last resort' principle, promotes a 3Rs policy commitment.

There are various barriers that explain the slow uptake of NAMs including scientific, regulatory, societal, technical barriers. This paper focused on identifying perceptions of animal testing, which prove to be key components of various types of barriers. For example, both the familiarity with animal testing and the inertia embedded into the governance structure form constituent elements of the regulatory barriers to NAMs. Equally, perceptions among the wider public that NAM testing might dilute what is seen as a rigorous level of safety generate societal challenges for NAMs as novel technology. Through the lens of primary empirical data gathered in engaging with the industry, policy makers and regulators, we have located barriers standing in the way of the elimination of animal testing by resort to NAMs.

We have begun work on how such barriers might be torn down and are actively pursuing three types of initiatives. The first is wide public engagement, which might help to develop a wider understanding the value proposition of NAMs which can then be tailored to multiple audiences. There is a challenging task involved in engaging in public debate on complex scientific method, but the ethical and moral issues addressed here might drive the deliberations. The second initiative involves the scientific community within public and private research teams, regulatory agencies, and policy makers. For example, the paper demonstrates that not only do we need to invest in developing NAMs, but we must cultivate quicker and better tailored routes to the validation of NAMs as they come forward.

Finally, there is ample room for legal research initiatives. Driving forward legislative change is demanding especially in a regional bloc made up of 27 EU member states. This leads to questions of constitutionality and what types of change require legislative reform. The whole area of animal testing is replete with soft law provisions such as protocols, practice guidelines and laboratory procedures that might be capable of moulding a more 3R compliant approach. At the level of court supervision, this paper has shown that the role of courts in paving a way for novel technologies is not always well-considered in legal scholarship, notwithstanding the key role that the courts play in delivering the stated commitment of the EU to end animal testing. Our analysis demonstrated a cautious approach by courts in assessing the scientific evidence and its reliability, which leads to deference to scientific decision-making

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by the courts. It pointed to the CJEU's reliance on the precautionary principle not only to support the generation of data through further animal testing but also to support the testing regime as proportionate. This ultimately erodes the application of the principle of last resort as stipulated by REACH. These findings expose the need not only to engage with industry, civil society, policy makers and regulators as often contended, but also to work with judges and other legal professionals to understand complexities in interpretation and application of EU chemicals law. Only when these public, scientific and legal initiatives coalesce might we begin to make progress in replacing the cruel testing methods of the 20th century with a more humane approach led by 21st century science.

Notes

1. The data does not include UK information from any of the dates covered, to allow comparisons to be made.
2. The greatest regulatory use by legislation type is for human medicinal products (46.7%); followed by veterinary medicinal products (27.7%) and then industrial chemicals (13.8%).
3. Predictions from Defined Approaches (DA) are rule based, consisting of a fixed data interpretation procedure (DIP) from a defined set of information source.
4. References to interviewee stakeholder groups are I=industry, R=regulatory, P=policy respondent. Each respondent is assigned a numerical figure within their stakeholder group.
5. The General Court has the responsibility to hear cases brought up individual and legal persons against acts passed by the EU institution and thus receives, at first instance, all cases related to implementation of REACH. The Court of Justice of the EU (CJEU) acts as the appellate court and its decisions are final. This is not to say that questions about implementation of REACH cannot come through preliminary reference procedure in which case the preliminary questions of national courts would be heard before the CJEU.
6. This is a case involving another EU Board of Appeal, in the form of the Office for Harmonization in the Internal Market (OHIM).
7. It is worth noting that the applicants brought the appeal before the Court of Justice of the EU as a second instance court in this case. The CJEU in its judgment of 1st August 2025 dismissed the appeals and upheld the General Court's annulment of the EC's classification as a suspected human carcinogen. In doing so, the CJEU diverged from the Advocate General's opinion. With regards to the argument that the Court acted ultra vires in evaluating the particle density, the CJEU conclude that 'the General Court erred in finding that, in the present case, it was for it to assess the appropriateness of the choice of the standard density value of titanium dioxide particles used by the RAC for the purposes of applying the Morrow calculation'; however, it did not err in law in holding that the RAC had failed to take into account all the relevant factors in order to calculate the lung overload for the purposes of the assessment of the Heinrich study by means of that calculation' (para. 125).

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