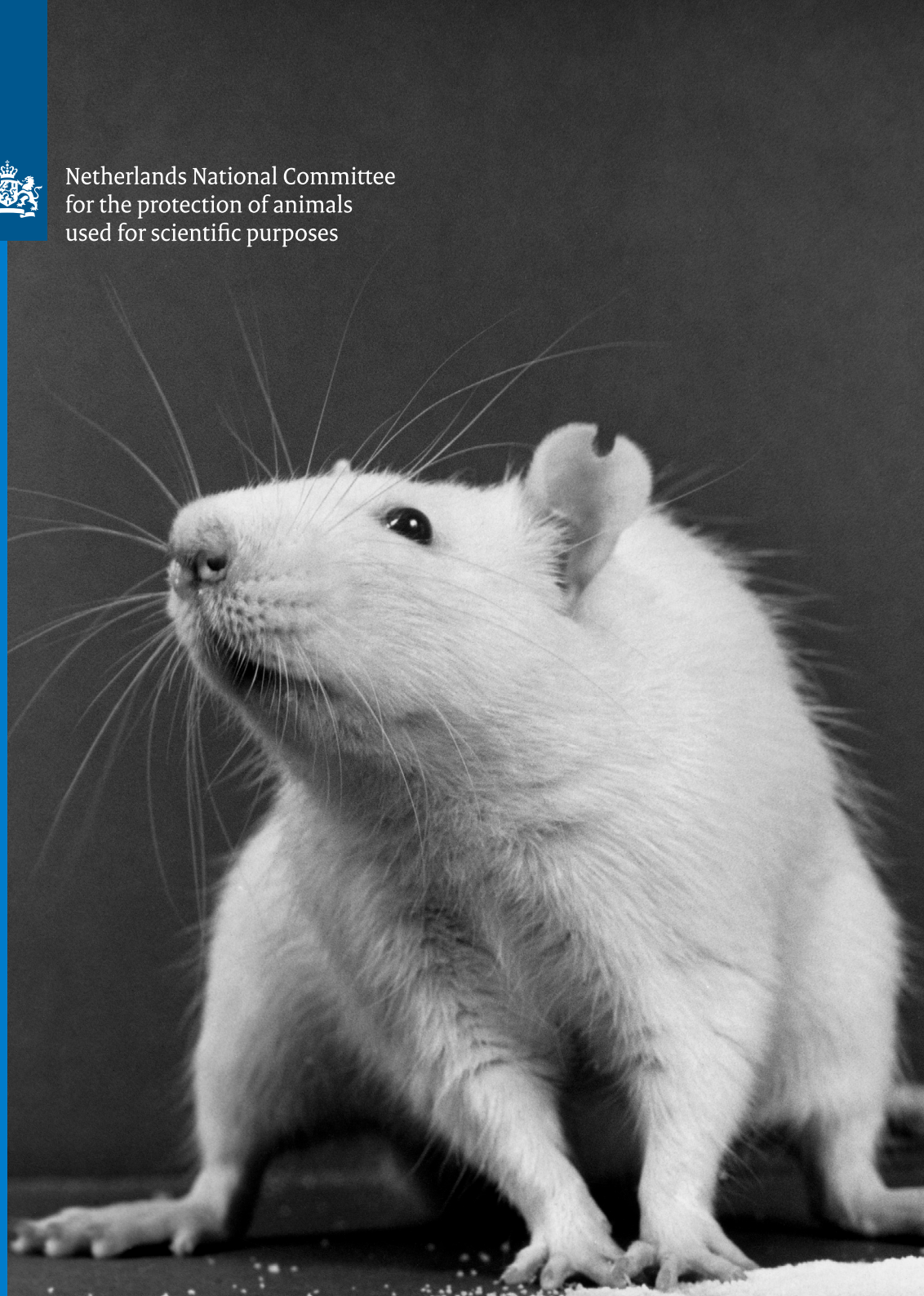
Netherlands National Committee
for the protection of animals
used for scientific purposes

Severe suffering in regulatory toxicity testing



Signal report from the Netherlands National Committee for the protection of animals used for scientific purposes (NCad)

For today's experimental animals and tomorrow's innovations

Netherlands National Committee for the protection of animals used for scientific purposes

The NCad

The Netherlands National Committee for the protection of animals used for scientific purposes (NCad) is an independent advisory body that protects the welfare of animals used for scientific purposes. The statutory duties of the NCad, established in 2014 pursuant to Council Directive 2010/63/EU, include providing solicited and unsolicited advice to the Minister of Agriculture, Fisheries, Food Security and Nature (LVVN), the Central Authority for Scientific Procedures on Animals (CCD) and the animal welfare bodies on the acquisition, breeding, housing, care and use of animals in procedures and on alternatives to animal testing. The NCad draws up Codes of Practices in addition to advisory reports. It organises meetings and workshops, gives presentations, and consults with its stakeholders. In this way, the NCad realises visible improvements for the Replacement, Reduction and Refinement (3Rs) of animal testing and animal-free innovation.

Composition NCad
From left to right: Roger Adan, Coenraad Hendriksen, Wim de Leeuw, Henk Smid (chair), Jan-Bas Prins, Reineke Hameleers, Katja ten Cate

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Introduction

The NCad is concerned about signals from the field that have reached us regarding an increase in suffering in the regulatory toxicity testing of chemicals. This increase is related to a scientific discussion about determining dose levels, which eventually led the European Chemicals Agency (ECHA) to issue two advisory reports for the field to state its view on the necessity to select the highest possible dose levels. The signalling of an increased impairment of animal welfare in experimental animals made the NCad decide to issue a signal report to the Minister of LVVN on its own initiative. Based on the analysis conducted through relevant literature and interviews with various stakeholders (see Annex 1), the NCad concluded that there is an increase in the severity of suffering within the moderate suffering category, and considers an increase in severe suffering as sufficiently plausible too.

These developments contradict the policy of the Dutch government to reduce, refine and replace animal testing (3R policy), under which, if no animal-free testing method is yet available, the method that causes the least suffering has to be chosen. With this signal report, in line with the role the government has assumed as international frontrunner for protection of animals in research, we request an adequate response from the Minister of LVVN aimed to prevent severe suffering. Hereby the NCad also draws attention to the aforementioned consequences of the ECHA advice and makes recommendations to minimise the increased suffering in regulatory toxicity testing. Some recommendations relate to the more intensive use of supplementary preliminary studies. These should be expected to lead to an improvement of the scientific quality and validity of research conducted for the protection of humans from hazardous substances.

As we believe that our recommendations can only be given optimal substantiation with the involvement of all relevant departments, we request the response of the Minister of LVVN to be part of government-wide policy, including the policy areas of Infrastructure and Water Management, Health, Welfare and Sport and Social Affairs and Employment.

In this signal report we first describe the problem definition with a brief description of the background, followed by the NCad's working method, assessment and recommendations. Finally, the explanatory note to this signal report includes a more detailed description of the background and an analysis of the findings that led to the assessment and recommendations.

Problem statement

Background in brief

One of the application areas of animal research is regulatory toxicity of chemicals. These studies must be conducted in accordance with specific guidelines to comply with the REACH regulation for the determination of intrinsic hazard properties[1] of chemicals (see Annex 2 for a description of the relevant regulatory frameworks and guidelines). In 2022, two advisory documents were published by the European Chemicals Agency (ECHA) relating to the design of the required studies. These documents, titled 'Advice on dose-level selection for the conduct of reproductive toxicity studies (OECD TGs 414, 421/422 and 443) under REACH' and 'Advice on dose-level selection for the conduct of sub-acute and sub-chronic assays under REACH', aim to clarify the selection of doses in toxicological studies according to existing test guidelines, in order to make the results of the studies more useful for the safety evaluation of a substance. About the highest dose level, these documents state that *'it should be demonstrated that the aim is the it highest possible dose level without severe suffering or death to the animal'*, unless no toxic effects of the substance are observed at the limit dose of 1000 mg/kg bw per day, in which case it is sufficient to only test the limit dose. However, now that the advice is implemented in research, it is leading to signals about an increased impairment of animal welfare from institutions where this research is being conducted. These signals are likewise recognised and shared by representatives from academia and by organisations such as NC3R,[2] ECETOX[3] and CEFIC,[4] including through publications in peer-reviewed journals (see attached list of consulted sources) and by representatives of consulted institutions.

[1] Intrinsic hazard properties, also known as 'hazard', indicate the adverse health effects that a substance has the potential to cause. The ultimate risk of a substance is determined by the combination of intrinsic hazard properties and the degree and route of exposure.

[2] NC3Rs is a scientific organisation, based in England, that works nationally and internationally with the research community to replace, refine and reduce the use of animals in research and testing.

[3] ECETOC is a centre for ecology and toxicology of the European chemical industry. ECETOC works with leading scientists from academia, governments and industry to develop and promote reliable and practical scientific solutions for a safe, sustainable and healthy world.

[4] CEFIC is the European Chemical Industry Association: the forum of large, medium and small chemical industries that together account for about 14% of global chemical production.

Harm-benefit analysis

First it should be noted that, for the NCad, animal-free research should be the premise for ethical arguments (intrinsic value of animals) and for scientific arguments (translational value of animal models, see, e.g. Schmeisser et al., 2023). However, the reality is that complete replacement is not yet possible for legal and scientific reasons. This makes a harm-benefit analysis, coupled with attention to the aspects of reduction and refinement the foci for the NCad. The ethical consideration involves the following question:

> *Does adjusting the highest dose to the borderline of severe discomfort, as proposed by ECHA, improve safety evaluation? And if so, does this outweigh the increase in the severity of animal suffering reported by various stakeholders?*

To answer this question, an inventory was made of the reasons and consequences of the ECHA advice in terms of animal use and suffering as well as in terms of optimisation of toxicity testing. This analysis is described in the explanatory note.

Method

To compose this signal report, the NCad consulted relevant publications and public sources. A list of consulted sources is enclosed. It should be emphasised that the ECHA advice, issued in 2022, relates to ongoing and future long-term studies for which study reports are not yet available. Consequently, concrete numbers on the effects of the ECHA advice, such as on severity categories, cannot yet be provided, but field experiences and findings are consistent in terms of observations. The NCad's research therefore focused on these experiences and findings. To this end, in-depth interviews were held with stakeholders and independent experts from academia, industry, contract research organisations (CROs) and public authorities (see Annex 1 for an overview of interviewees).

Assessment and recommendations

As indicated in the 'Problem definition' section, the focus of this signal report is a harm-benefit analysis. To answer this question, the NCad made an inventory of the underlying reasons and consequences of the ECHA opinions. Below is the NCad's assessment and subsequent statement on the harm-benefit analysis. The NCad makes a number of recommendations aimed at prioritising animal welfare without compromising the quality of toxicity testing.

Assessment

The NCad observed that the subtle change introduced by the ECHA advice in the description of the highest dose compared to the existing OECD test guidelines for toxicological studies, namely as 'the highest **possible** dose level without severe suffering or death', has led to several studies in which overdosing actually occurs. This is confirmed by the institutions where this research is being conducted, who observe an increase in severe suffering.

Toxicity testing of substances for human, animal and environmental safety has broad political and public support, as evidenced, by the results of the recent EU barometer 'Attitudes of Europeans towards the Environment' published in May 2024,[5] and was legally formalised in the REACH Regulation after approval by the European Parliament in 2006. The protection of experimental animals also has broad public and political support, as evidenced by a recent European citizens' initiative aimed at phasing out animal testing[6] and the European Commission's response committing to developing a roadmap to ultimately phase out animal testing for chemical safety assessments.[7] The protection of animals used for scientific purposes is a priority for the European Commission (Council Directive 2010/63/EU). The EU directive requests that non-animal methods should be used when available. For the more complex research questions, which are the subject of the ECHA advice, non-animal methods are not yet available and animal testing must be conducted to comply with the REACH regulation (see Annex 3 for an overview of animal tests specified in the REACH regulation). However, the impairment of the animal welfare should be balanced against the benefit of the study, and the study should be designed to cause the least suffering to the animals and require the least number of animals. The underlying reason for ECHA to issue its advice was its finding, based on a dossier analysis, that some of the reproductive toxicity studies were conducted at doses that were too low ('underdosing'), which, according to ECHA, resulted in studies from which no conclusions on the safety of a substance could be drawn.

[5] Attitudes of Europeans towards the Environment - Eurobarometer Report https://europa.eu/eurobarometer/surveys/detail/3173

[6] European citizens' initiative ' Save Cruelty Free Cosmetics - Commit to a Europe Without Animal Testing' https://citizens-initiative.europa.eu/save-cruelty-free-cosmetics-commit-europe-without-animal-testing_en

[7] Press release 25 July 2023: Commission acts to accelerate phasing out of animal testing in response to a European Citizens' Initiative https://ec.europa.eu/commission/presscorner/detail/en/IP_23_3993

Both 'underdosing' and 'overdosing' are factors in the ethical evaluation that make the acceptance of an animal study problematic. Suffering of the individual animal is a significant determinant here, and the NCad holds the view, in line with the OECD test guidelines to which the ECHA advice refers, that severe suffering in animal studies for regulatory toxicity testing should not be admissible. Advice on the highest dose level in regulatory toxicity testing concerns policy from the European Commission.

It becomes clear from the interviews and the scientific literature that, through input from Bureau REACH,[8] the Netherlands has made an important contribution to the realisation of the ECHA advice. From its responsibility as delegated authority on implementation of European laws and regulations on chemicals, the values of risk minimisation and safety perception have been the leading considerations. This is evident, for instance, from the scientific publications published by Bureau REACH in support of the ECHA advice and annual reports that mention the activities on this subject. The NCad observes that only minimal space has been created both nationally and internationally to look for solutions from an animal welfare perspective and innovative methods to prevent underdosing in studies. This is not in line with the 3R policy from the Dutch government and the European Commission.

Recommendations

With respect to the above assessment, the NCad gives the following recommendations to the minister of LVVN:

Recommendation 1. Take immediate action on opportunities to prevent serious suffering in regulatory toxicity testing.
With the implicit 'acceptance' of severe suffering, the Dutch 3R policy and the European Council Directive 2010/63/EU on the protection of animals used for scientific purposes is insufficiently translated in the ECHA opinions. The NCad advises the minister to ensure that, in accordance with the REACH regulation, regulatory toxicity testing will be conducted in line with the policy to protect experimental animals.

- Advocate that the ECHA advisory reports on dose-level selection explicitly state that dose levels do not give rise to severe suffering or death. To this end, the phrase of a '*highest possible dose level without severe suffering or death*' in these reports should be changed to a description more in line with the OECD test guidelines of a '*highest dose level with some toxic effects but not death or severe suffering*'.
- Call for the establishment of clear and detailed guidelines for the execution and reporting of dose range-finding studies with the aim of preventing both underdosing and overdosing in regulatory toxicity testing. Explicit attention should be paid to improving the scientific usefulness and quality of the toxicity studies as well as to striving for minimal suffering and reducing the number of animals.
- Ask independent experts to draw up recommendations on intensifying and optimising complementary preliminary studies, in addition to dose range-finding studies. Complementary preliminary studies offer opportunities to address uncertainties in dose-level selection, such as through toxicokinetic studies, read-across, qualitative structure-activity relationships (QSAR) and artificial intelligence (AI).
- Promote communication between regulators and implementers by initiating a working group between concerned parties, if possible in collaboration with the European Partnership for Alternative Approaches to Animal Testing (EPAA).
  The importance of communication was brought to the fore, as on the sidelines of the interviews a picture emerged of a fundamental difference in vision between regulators and implementers (industry, CROs, academia): the regulators, formalistic and bound by mandates, and the implementers, pragmatic with an important element of expert assessment. While differences in views are not part of the problem definition, they do have an impact on animal welfare. Hence a notable finding in this study is that the implications for animal welfare appeared to be unknown to regulatory bodies. In this context, the NCad recommends that policymakers take a neutral position in relation to stakeholders and from that position interpret developments objectively and independently.

[8] Bureau REACH is delegated by the Dutch' government to deal with REACH-related issues.

Recommendation 2. Anticipate for future impact of the ECHA advice.
Given that the ECHA advice is currently being implemented, it is relevant to anticipate consequences such as overdosing in studies. In addition, the revision of the REACH regulation is under preparation. There are indications that the upcoming revision will lead to an increase the number of the animal tests that are the subject of the ECHA advice. The NCad advises the minister to ensure that an expansion in the identified problem of severe suffering is avoided. Specifically, this means:

- Seeking independent expert advice on the consequences of overdosing on the validity of study results. An interpretation of the validity of studies in which overdosing occurred is not the scope of this NCad signal report. However, a widely supported view emerges from interviews with experts that overdosing can lead to indirect effects that are not relevant for the hazard assessment.
- Continuing to engage with industry to monitor developments surrounding the ECHA advice and its implications. Overdosing may have as the consequence that studies are considered invalid for market authorisation by bodies other than ECHA, for example outside Europe. Having to re-conduct these studies should be avoided.
- During the revision of the REACH regulation, pushing for the inclusion of innovative methods, such as toxicokinetic studies, read-across, QSARs and AI, to ensure maximum implementation of the 3R policy. The NCad deems this to be a *joint* responsibility of the ministries to ensure that the contribution of experts on these innovative methods is explicitly included in the input from the Netherlands for the purpose of revising the REACH regulation.

Explanatory note on the signal report

This note describes the analysis of the literature and interviews. It is structured as follows: first, an overview is given of the background of regulatory toxicity testing and the issues that have emerged in the selection of dose levels. Next, it describes the NCad's findings based on the literature and interviews, including implications of the ECHA advise on animal testing. These findings have formed the basis for the recommendations given by the NCad in this signal report.

Background

Regulatory toxicity testing of chemicals

The existing legislation on chemicals in the European Union is designed to prevent substances from causing harm to humans, animals and the environment. The basis for the European laws and regulations on chemicals is the REACH Regulation (Regulation (EC) No 1272/2008) and the CLP Regulation (Regulation (EC) No 1272/2008) (see Annex 2 for further explanation). REACH registration is mandatory for all chemicals of which at least 1 tonne per year is produced or imported. Registrants (manufacturers or importers) submit information on intrinsic hazard properties to ECHA through a mandatory 'robust study summary' of each toxicological study, including a detailed summary of methods, results and conclusions. A significant proportion of these studies involve animal studies (see Annex 3 for an overview of the standard information requirements). The common denominator in these studies is to determine whether a substance causes toxicity, what the associated effects are, and at what dose toxicity occurs. When it comes to harm to humans (in other words toxicity), the aim of this assessment is to protect both workers and consumers.

The selection of dose levels in regulatory toxicity testing

For the assessment of potential adverse effects of a substance, the REACH regulation requires that, if standard information requirements cannot be met with existing information and non-testing methods, (animal) studies must be conducted in accordance with standardised test guidelines approved by the OECD and the EU. The test guidelines describe the design and execution of the study with some flexibility, e.g. in determining substance dose levels. Only if preliminary studies, in the form of dose range-finding studies[9] or existing data, show that there is no observable toxicity after administration of at least the limit dose of 1000 mg/kg bw/day, selection of only one dose level equal to the limit dose is sufficient. In all other cases, toxicity studies are performed with a minimum of three dose levels of the chemical. An optimal dose range is used for effect characterisation (the dose-response relationship) and consists of a low dose level that shows no toxicity, a middle dose level that induces low toxicity, and a high dose level with marked toxic effects. The registrant determines how hazardous the substance is and whether a classification and labelling of the substance according to the CLP regulation is applicable. With classification and labelling, any hazard classification is made known to users so that risk management measures can be taken. The registrant also determines the maximum exposure level to which people may be exposed where no adverse effects are expected.

The ECHA advice addresses specific endpoints of toxicity, namely repeated dose toxicity (subacute and subchronic toxicity) and reproductive and developmental toxicity. Regarding the highest dose, or the top dose, the OECD test guidelines for these endpoints (see list of consulted sources) indicate that it should be '*chosen with the aim of inducing some toxicity, but not death or severe suffering*'. Thereby, some form of minimal toxicity serves as a bottom limiter for the highest dose level to avoid underdosing and consequently underestimating toxicity of a substance.
The individual OECD test guidelines give different interpretations of minimal toxicity, such as developmental and/or maternal toxicity in the form of clinical signs or a decrease in body weight (OECD Test Guideline 414) or some systemic toxicity (OECD Test Guideline 443). At the same time, the OECD test guidelines specifically mention that inducing toxicity does not equate inducing severe suffering or death.

In recent years the RIVM Bureau REACH has addressed an apparent international trend of underdosing in regulatory toxicity testing (annual reports Bureau REACH 2019-2022). Following this, ECHA came to a similar conclusion in a review of

[9] Dose range-finding studies are done in preparation for the main study, in this case the subacute, subchronic, reproductive or developmental toxicity study. Dose range-finding studies have a similar design to the main study but with a shorter duration and with fewer animals. The results of these studies aid informed selection of dose levels in the main study. Dose range-finding studies are not mandatory but are always performed in well-designed studies.

55 available summaries of reproduction toxicity studies[10] (ECHA, 2023). It was noted by ECHA that this finding is also relevant for other toxicity studies. In addition, a number of supplementary publications were prepared by Bureau REACH (Van Berlo et al., 2022; Heringa et al., 2020; Woutersen et al., 2020) that critically discuss aspects that are considered when selecting the highest dose level in practice, such as effects on body weight gain and toxicokinetic data. Informed by these findings too, ECHA issued advice in 2022 for subacute, subchronic, reproductive and developmental toxicity studies to clarify the requirements when selecting the highest dose level (ECHA 2022a, b). The ECHA advice states that 'it should be demonstrated that the aim is that it is the **highest possible dose level** without severe suffering or death, or the limit dose concept shall be used'.

Findings

To make a scientifically justified assessment of possible toxic effects of a substance, the selection of appropriate doses in animal studies is of great importance. Incorrect selection can lead to either underdosing or overdosing. The NCad's investigation shows that the problem leading to the increase in severe suffering is complex and has several facets that are directly or indirectly related conducting preliminary research. These factors are discussed below in relation to findings from the literature and interviews conducted. In doing so, the findings are divided into thematic blocks.

Underdosing and overdosing

- To make a scientifically justified assessment of appropriate dose levels, preliminary research in the form of dose range-finding studies using animal testing is of great importance. Incorrect selections can lead to either underdosing or overdosing. Both lead to invalid tests and therefore problematic animal use. In its recent advice, ECHA calls for dose range-finding studies to be reported comprehensively in the registration dossier. It appears that this is not always done. One of the reasons given for this is that it is not a standard information requirement and ECHA's registration system is not designed to incorporate this information. Supplying this information therefore requires an additional investment of time and money.
- Several reasons may underlie the underdosing. The literature and interviews indicate that preliminary work in the form of dose range-finding studies, is in fact generally conducted, even though it is not reported in detail (Knight et al., 2023). From the interviews it appears that a common practice has emerged whereby, based on the results from a dose range-finding study, the principal investigator at the laboratory tries to find a maximum dose at which clear toxic effects are observed with the lowest possible suffering for the animal, either with or without consultation with the registrant. However, selecting the right dose levels requires a tailor-made approach and the substance may behave differently than anticipated. In rare cases (for instance with some small manufacturers/importers) there is insufficient in-house expertise to conduct a proper dose-range finding study, the necessity is not recognised, or the aim is to estimate the lowest possible toxicity; this can result in underdosing.
- Overdosing, for example as a result of implementation of ECHA advice, may result in severe suffering in animals in the middle- and/or high-dose level groups. Part of the resulting toxicity may not be specifically related to the substance under investigation but may result from an overall disruption of the homeostasis of the animal (see, e.g. for example, OECD Guideline NO.35; Yu-Mei Tan et al., 2021; Lewis et al.,2024). This amplifies the animal's suffering without added value in the hazard assessment of the substance.

The ECHA advice on dose selection

- The OECD test guidelines define the highest dose as that dose at which some toxicity occurs but not death or severe suffering, if needed through the application of humane endpoints.[11] The ECHA advice further specifies 'high' as being the highest dose possible *before* severe distress or mortality occurs: ' it should be demonstrated that the highest dose was chosen with the aim of selecting the **highest possible dose level** without severe suffering or death'. The difference between the OECD and ECHA descriptions is subtle but effectively implies that minor or moderate suffering is sufficient according to the OECD test guidelines, while the suffering according to the ECHA advice must be at the borderline of moderate-to-severe.
- For ECHA, the classification of substances into hazard classes as part of the CLP

[10] Specifically: the Extended One Generation Reproductive Toxicity Study, which was implemented in 2015. This reproductive toxicity test was included as a REACH information requirement in 2015, replacing the two-generation reproductive study. The Extended One Generation Reproductive Toxicity Study has a clear added value: far fewer animals are needed, and more parameters are assessed.

[11] A humane endpoint is applied when an animal is suffering and the scientific endpoint is reached or there are ethical or welfare-related reasons to stop the experiment for that animal, leading to humane killing or treatment of the animal.

regulation is an important reason to prevent underdosing. The reason for this is twofold. First, CLP classification secures hazard communication with relevant labelling on risk management measures, which is necessary for a safe working environment and in consumer communication on chemicals. In addition, classification of a substance may trigger a generic risk management approach, which may result in restrictions on the use of a substance. For example, the use of substances classified as reprotoxic is not allowed in cosmetics or toys. It is pointed out that there is uncertainty because the nature and level of exposure of a substance cannot be known in advance. For a generic risk management approach, the aim is therefore to cover all possible exposure scenarios, ranging from long-term exposure to low amounts to unforeseen incidental high exposures. The use of long-term exposure and as-high-as-possible dose levels in toxicity testing is considered a way to overcome this uncertainty. However, experts question the relevance of very high doses that (may) lead to overdose, in relation to realistic human exposure scenarios. The possible worst-case scenario for which these exposures may be relevant, according to experts, is prolonged and oral exposure of humans to very high amounts of a substance. This is said to be a highly undesirable scenario that should not be anticipated in animal tests but should be avoided at all costs by using preventive measures and taking adequate risk management policies.

- ECHA's advice on dose selection aims to avoid unnecessary execution of invalid studies due to underdosing. It should be noted that studies where overdosing occurs can also be deemed invalid.

The ECHA advice and effects on animal welfare

- In the issues surrounding the use of maximum dose levels, the aspect of refinement in the form of the lowest possible suffering appears to be secondary to the desire to overcome said uncertainties surrounding human exposure with the highest possible doses where overdose cannot be precluded. For example, it is stated by Bureau REACH that 'While refinement, reduction and replacement of animal tests are very important objectives to strive for, proper hazard assessment is indispensable for a reliable safety assessment for worker and public health' (Heringa et al., 2020).
- The fact that animals must be exposed to the highest possible dose level in a dose-range-finding study before severe suffering occurs implies in practice that at least some of the animals will experience severe suffering. It is indicated from the field that this is indeed the case because the maximum dose level is being targeted. Moreover, because the choice of dose levels for the main study seeks the borderline with severe suffering, there is a greater risk of the selected dose turning out to be too high, resulting in severe suffering even in the main study, which lasts longer and uses more animals. This development is at odds with ECHA's ambition to reduce animal use and reduce animal suffering as much as possible (ECHA 2016)[12] .
- A specific aspect of animal welfare concerns the 'body weight' parameter. In practice and in accordance with OECD testing guidelines, the criterion '10% decrease in body weight gain' is used along with other factors to assess the animal's clinical condition and physical well-being. A recent publication (van Berlo et al., 2022) suggests that this criterion should be removed from OECD test guidelines, except in the study of carcinogenic effects where it should be replaced by the criterion '10% decrease in body weight'. In pregnant animals, which should in fact be gaining weight, a 10% decrease in body weight can be considered a significant welfare impairment (Lewis, R.W. at all. 2024; Arts et al., 2023)
- The ECHA advice on reproductive toxicity studies only considers mortality or severe suffering in the parental P0 generation as relevant aspects for dose level selection: 'the highest dose should be as high as possible without causing death or severe suffering in parental P0 generation' (ECHA 2022a). The advice explicitly mentions that these effects in the foetuses or offspring *are not* taken into account when selecting the highest dose level. This is because the dose selected should be as high as possible to identify any effects on fertility in the first generation of animals, and effects in the offspring should not be limiting. The welfare implications of this requirement can be significant. Interviewed stakeholders involved in animal research confirm that there is an increase in the number of offspring animals reaching a humane endpoint. There is also an increase of severe suffering in offspring. Cases cited as examples were scoliosis in offspring and high neonatal mortality. A striking note is that foetal forms of animals are only part of the annual statistical reporting on numbers of animals in Europe if they are born alive, although EU Directive 2010/63/EU on the protection of animals used for scientific purposes does apply to foetal forms of mammals as from the last third of their normal development (EC 2023).

[12] ECHA describes this in its Practical guide on the use of alternatives to animal testing as: The least severe test that uses the fewest animals needs to be employed and conducted in a way that causes the least pain, suffering, distress and lasting harm.'

Additional preliminary research

- To support dose range-finding studies in animals, there are innovative methods in preliminary research to make a more informed selection of dose levels and avoid unnecessary suffering. Results from these methods can sometimes even be used to replace animal testing. A widely used approach is read-across, in which effects of a substance are predicted based on available information from substances with a related structure. QSARs (Quantitative Structure-Activity Relationship) are mathematical models that establish a quantitative relationship between the structure of a substance and its toxicity. Interviewees had the impression that these innovative methods are more readily accepted by ECHA if this leads to identification of a hazardous substance, and to a much lesser extent if the conclusion is that a substance is not hazardous. Scientific publications from bureau REACH are in line with this reasoning (Heringa et al., 2020).
- Toxicokinetic studies provide insight into how substances behave in the body, e.g. concentrations of the substance or its metabolites in different bodily organs or tissues and guide dose selection in dose range-finding studies. These studies are generally done to arrive at the 'minimal toxic dose' for pharmaceuticals. Here the starting point is not *as high as possible* but *as little suffering as possible*. In toxicity testing of chemical substances, ECHA is in principle receptive to the utilisation of toxicokinetic data. The Guidance on Information Requirements and Chemical Safety Assessment, published by ECHA states: 'Information on the toxicokinetics of a substance may identify the optimal study type and design including dose settings, or even make further testing unnecessary ' (ECHA 2011). However, ECHA now finds that information on toxicokinetics is generally not generated because it is not required under the REACH regulation or is of insufficient quality. Both the ECHA advice and Bureau REACH rule out the use of toxicokinetic studies in dose selection, ECHA citing the notion that 'the available toxicokinetic data is typically insufficient to conclude on toxic dose levels and, therefore, guide on dose-level selection'. The additional argument here is that exposure scenarios should not play a role in dose selection because they are unpredictable and not based on biological parameters (Heringa et al., 2020).

With regard to laws and regulations

Among other things, the NCad's investigation resulted in identifying a number of inconsistencies between the ECHA advice and existing guidelines. There are also developments in laws and regulations that may amplify the identified issues around animal welfare impairment.

- The ECHA advice states that the highest possible dose level should be selected without causing severe suffering or death. The implication is that this dose level is on the borderline of severe suffering or death and that this borderline will easily be surpassed in practice. As such, this advice is not in line with OECD guidance document No.19, which describes clinical signs and humane endpoints and states: '*there is strong scientific evidence that pain and distress are present in animals in comparable situations as they occur in humans*' and '*studies must be designed to minimise any pain, distress or suffering experienced by the animals, consistent with the scientific objective of the study*'.
- Discussions on dose levels are now focussed on the areas of reproductive and developmental toxicity and sub-acute and sub-chronic toxicity. However, the topic of 'underdosing' has been addressed by ECHA as non-specific for these endpoints. It is therefore conceivable that this topic will also become relevant for other disciplines within toxicology.
- The research covered by the ECHA advice requires the greatest proportion of the total number of experimental animal used to comply with the REACH regulation. A recent study (Knight J. et al. 2023) estimates the number of experimental animals used to date at 2.9 and, based on test proposals submitted to date, expects at least another 1.9 million to be used in the near future. Moreover, the number of animal studies for determining reproductive and developmental toxicity is expected to increase substantially due to recent[13] and upcoming[14] amendments to the REACH regulation. This means that an increase can also be expected in the number of animals used in dose range-finding studies, and in the number of animals that involve an increase in suffering as a result of the modification made by the ECHA advice to the criteria for dose-level selection.

[13] A recent amendment to the REACH regulation (Commission Regulation (EU) 2022/477 of March 2022 amending Annexes VI to X of Regulation (EC) No 1907/2006) reduces the possibilities to waive reproductive toxicity studies and further extends the obligation to conduct these studies.

[14] In the upcoming revision of the REACH regulation, information requirements for reproductive and repeated dose toxicity testing are expected to be further extended by the inclusion of endocrine disruption as a mandatory endpoint and a possible extension of standard information requirements for substances produced or imported in a volume of 1-10 tonnes per year.

List of consulted sources and literature

Official publications

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Annex 1 Experts consulted

When drafting its recommendations of counsel, the NCad gratefully uses experts from home and abroad. Stakeholders and chain partners were also consulted. The experts consulted are not co-authors of this NCad signal report and may have opinions that differ from those presented by the NCad in this report. For purposes of this signal report the following experts and organisations were consulted:

Prof. Aldert Piersma (former RIVM, Utrecht University)
Dr Manon Beekhuijzen (Charles River)
Drs Steffi Vermeulen-Bressers (Charles River)
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Dr. Betty Hakkert (bureau REACH)

Annex 2 Legal frameworks and guidelines

REACH Regulation (EC) No 1907/2006. REACH stands for Registration, Evaluation, Authorisation and Restriction of Chemicals and aims to protect humans and the environment from unintended effects of chemicals. REACH sets standard information requirements that depend on the production or import volume. This is the minimum information required to fulfil the registration obligation and includes a broad set of studies related to the production or import volume of the substance, the route of expected exposure and to specific intrinsic hazard properties such as genotoxicity or damage to the unborn child. REACH requires registrants (manufacturers or importers) to generate and assess information on intrinsic hazard properties of substances. A large part of this information is obtained using animal studies. ECHA has a coordinating and implementing role within the REACH regulation. Research conducted to comply with the REACH regulation is an important source of information for CLP.

CLP Regulation (EC) No 1272/2008. CLP stands for Classification, Labelling and Packaging and aims to ensure a high level of health and environmental protection by clearly communicating potential hazards of chemicals through standardised labelling and packaging. Thereby, hazard classification is made known to users so that risk management measures can be taken. If the relevant information on hazard properties of a substance meets the classification criteria in CLP, the relevant hazard class (type of hazard) and hazard category (severity of hazard) are assigned.

OECD testing guidelines. New animal studies should be conducted in accordance with the principles of good laboratory practice (GLP) and according to regulatory accepted test guidelines such as the Organisation for Economic Cooperation and Development (OECD) guidelines. OECD regulations are leading in many parts of the world, including the European Union (EU).

Directive 2010/63/EU of the European Parliament and of the Council. All scientific research using experimental animals must be conducted in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes. The directive requires test procedures to be designed to prevent or minimise any pain, suffering, distress and lasting harm. If this cannot be avoided, a harm-benefit analysis should be done.

Annex 3 Standard information requirements on human toxicity

Information requirements 1 to 10 tonnes per year

Endpoints - *in vitro* research	Endpoints - vertebrates
In vitro skin irritation/corrosion	Acute toxicity: oral
In vitro eye irritation	
Skin sensitisation	
In vitro gene mutation in bacteria	

Additional information requirements 10 to 100 tonnes per year

Endpoints - *in vitro* research	Endpoints - vertebrates
	In vivo skin irritation (if you cannot classify your substance based on the *in vitro* results).
	In vivo eye irritation (if you cannot classify your substance based on the *in vitro* results).
	Testing proposal for *in vivo* genotoxicity (if any of the *in vitro* tests are positive)
	Acute toxicity: inhalation
	Short-term repeated dose toxicity (≥8 days)
	Screening for reproductive/developmental toxicity*

** test covered by ECHA advice, guidance of recommendations*

Additional information requirements 100 to 1,000 tonnes per year

Endpoints - *in vitro* research	Endpoints - vertebrates
	Subchronic toxicity (90 days) *
	Prenatal developmental toxicity in one species*
	Extended One-Generation Reproductive Toxicity (if triggered) *

** test covered by ECHA advice, guidance of recommendations*

Additional information requirements 10 to 100 tonnes per year

Endpoints - *in vitro* research	Endpoints - vertebrates
	Long-term repeated dose toxicity (≥12 months) if triggered*
	Developmental toxicity in a second species*
	Extended One-Generation Reproductive Toxicity *
	Carcinogenicity if triggered

** test covered by the ECHA advice, guidance of recommendations*

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