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27 January 2017

Re: Public Consultation in relation to the REACH REFIT evaluation

To whom it may concern:

Thank you for the opportunity to respond to the public consultation in relation to the REACH REFIT evaluation.

Enclosed are a position paper from myself on behalf of the PETA International Science Consortium Ltd. A strategy to phase out chemical tests on animals is appended.

If you require any further information, please do not hesitate to contact me.

Yours sincerely,

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Consultation response in relation to the REACH REFIT evaluation

Position paper from the PETA International Science Consortium regarding REACH

Contents

1. Executive Summary

Europe must adopt an approach to chemicals regulation based on human-relevant, 21st century toxicology methods that do not rely on animal tests. The PETA International Science Consortium Ltd. offers its expertise towards developing an appropriate strategy to phase out tests on animals but, in the meantime, this position paper makes recommendations to ensure that tests on animals for the REACH registration 2018 deadline are minimised.

A key objective of REACH is to promote alternatives to testing on animals for hazard assessment. However, tests on animals continue to occur even when alternatives are available and without prior approval of testing proposals. Furthermore, Board of Appeal cases and decisions taken by the European Ombudsman indicate that ECHA has not prioritised alternatives to testing on animals wherever possible. ECHA and the European Commission must learn from these cases and update their legislative and administrative practices to ensure that tests on animals are minimised in preparation for the REACH 2018 deadline.

ECHA must amend its administrative processes relating to adopting evaluation decisions by considering dossier updates after sending a draft decision to the registrant. In addition to comments on proposals for amendment, registrants' comments on all versions of draft decisions should be considered by ECHA and the MSC. Once a decision has been adopted ECHA should, at the registrants' request, examine updated dossiers prior to the date in the decision if the registrant has proposed adaptations to the requested data. These steps are essential to ensure compliance with REACH and to provide legal certainty to registrants, because a failure to take such information into account could lead to registrants conducting tests on animals when a valid adaptation is available.

When conducting substance evaluations, ECHA must adopt a step-wise approach before requesting tests on animals. Specifically, ECHA must demonstrate that there is a potential risk that needs to be clarified, that any requested tests use the fewest possible animals, and that the information generated has a realistic possibility of leading to improved risk management measures. ECHA must not use substance evaluations to make excessive requests for information beyond the scope of the objectives pursued.

ECHA should take a proactive approach to accepting non-animal methods and adaptations to the standard information requirements. ECHA must pay particular attention to read-across justifications and check substance and analogue dossiers if the read-across justification requires further development. Ongoing training will improve acceptance and use of non-animal methods.

The legislation should be amended to require testing proposals for Annexes VII and VIII endpoints as well as Annex IX and X endpoints and, the scope of scientifically valid information and studies proposed by third parties should be expanded to include testing strategies, read-across justifications and other approaches that avoid testing on animals.

ECHA must update its endpoint specific guidance and the European Commission must update the REACH Annexes and Test Method Regulation expeditiously to reflect advances in non-animal methods. In particular, the standard requirement for prenatal development toxicity in a second species in any annex must be removed.

Adopting these recommendations will help minimise tests on animals for the REACH 2018 deadline but ultimately, Europe must base its chemicals legislation on 21st century toxicology methods that do not rely on animal tests. The use of non-animal methods will enhance the competitiveness of the chemicals sector by lowering costs while ensuring better protection of human health and the environment.

2. Introduction

Europe should adopt an approach to chemicals regulation based on human-relevant, 21st century toxicology methods that do not rely on animal tests. To achieve this goal, a strategy for phasing out tests on animals must be implemented in Europe's chemicals legislation. The PETA International Science Consortium Ltd. offers its expertise towards developing an appropriate strategy (see appended strategy). In the meantime this position paper addresses areas of concern relating to the use of animals in testing and proposes updates to the legislation and improvements to the way that REACH is administered to ensure that tests on animals for the REACH 2018 deadline are minimised.

3. Compliance with Article 25(1), the last resort principle

A key objective of REACH is to promote alternatives to testing on animals for hazard assessment (Article 1). In particular, REACH states that "for human toxicity, information shall be generated *whenever possible* by means other than vertebrate animal tests, through the use of alternative methods . . . " (Article 13(1), emphasis added) and "[i]n order to avoid animal testing, testing on vertebrate animals for the purposes of this Regulation shall be undertaken only as *a last resort*." (Article 25(1), emphasis added). It is the responsibility of *all* those acting under REACH, including ECHA, Member States and registrants, to ensure that these provisions are adhered to.

However, the Article 117(3) reports on The Use of Alternatives to Testing on Animals for the REACH Regulation (2011[1] and 2014[2]), previous communication with ECHA, and evidence that tests on animals are conducted without approval of testing proposals,[3] indicate that more must be done before the REACH 2018 registration deadline to ensure tests on animals be conducted only as a last resort, as required by law.

Furthermore, investigations conducted by the European Ombudsman and appeals brought before the Board of Appeal (the Board) have clarified certain aspects of the REACH regulation and identified areas where its administration can be improved. The European Chemicals Agency (ECHA), the European Commission and Members States must act on the recommendations of the Board and the Ombudsman to ensure tests on animals are truly minimised.

3.1. ECHA's administrative processes must be amended to reduce animal tests

REACH is an extremely complex piece of EU legislation, and it is to be expected that ECHA would implement processes to ensure its efficient administration. While the Science Consortium agrees that such processes are necessary and should be complied with wherever possible, a degree of flexibility is necessary in some circumstances to ensure compliance with Article 25(1). **The self-imposed limitations of ECHA's administrative processes should**

[1] ECHA (2011) The Use of Alternatives to Testing on Animals for the REACH Regulation. Available at https://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2011_en.pdf (accessed 16 January 2017)

[2] ECHA (2014) The Use of Alternatives to Testing on Animals for the REACH Regulation Second report under Article 117(3) of the REACH Regulation. Available at https://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2014_en.pdf (accessed 16 January 2017)

[3] ECHA (2015) Survey results - Analysis of higher tier studies submitted without testing proposals. Available at https://echa.europa.eu/documents/10162/13628/analysis_higher_tier_without_tp_results_en.pdf (accessed 16 January 2017)

not be strictly adhered to when the likely outcome is potentially avoidable tests on animals.

Below we outline several administrative processes that have been adopted by ECHA which are not dictated by the legal text, and which have a significant impact on animal testing. Of particular concern are the arbitrary cut-off dates set by ECHA relating to the adoption of draft decisions, the restrictions placed on registrants when commenting on draft decisions and ECHA's refusal to review updated dossiers prior to the deadline for compliance in the adopted decisions.

3.1.1. Arbitrary cut-off dates must be flexible

ECHA's cut-off point for new information to be considered in the decision-making process, specifically the date when ECHA first notifies the registrant of its draft decision[4] is a measure of administrative convenience and not defined in REACH. This arbitrary cut-off date means registrants are unable to have updated dossiers reviewed from the date they receive ECHA's first draft decision, until the deadline for compliance in the adopted decision has passed. The current inflexibility of ECHA's administrative practices is a breach of Article 25 and could lead to an increased number of avoidable tests on animals being conducted and an increased number of appeals to the board (with the associated administrative burden) by appellants who are placed in a position of unacceptable legal uncertainty.

In the *CINIC* appeal case, ECHA claimed that if it is required to perform checks of information submitted after the draft decision was sent to the Member State Competent Authorities (MSCAs), this would render its work unmanageable.[5] However, ECHA acknowledged that there is no legal obstacle to restarting the decision-making process in such circumstances.[6,7] The Board agreed that ECHA's refusal to consider new information received after the cut-off point has passed, and before the deadline set in the Contested Decision, potentially risks, contrary to Article 25(1), tests on vertebrate animals solely to ensure compliance with an ECHA decision.[8]

However, the Board's interpretation of what constitutes substantial new information is interpreted narrowly and to date no other appeal has met the criteria.[9] **To minimise tests on animals the Board and ECHA must adopt a broad interpretation of what constitutes "substantial new data" and include elements such as updated read-across and (Q)SAR justifications.**

The last resort principle contained in Article 25 should take precedence over measures of simple administrative convenience. ECHA must exercise a degree of flexibility in its administrative processes by reviewing updated dossiers and giving the Member States Committee (MSC) the opportunity to also review updates prior to adopting decisions. This flexibility is essential to ensure that registrants do not conduct tests on animals when they are not required, out of concern with the possible consequences of non-compliance (e.g., fines or

[4] 'ECHA tightens its practice on dossier updates' ECHA/NA/15/02 http://echa.europa.eu/view-article/-/journal_content/title/echa-tightens-its-practice-on-dossier-updates
[5] Appeal Decision A-001-2014, *CINIC*, paragraph 89
[6] Appeal Decision A-001-2014, *CINIC*, paragraph 89
[7] When the decision in the *CINIC* case was adopted ECHA would not consider updates after the draft decision had been referred to the MSCA. Since then ECHA's administrative practices have become even more restrictive and ECHA will not take into account dossier updates after the draft decision has been referred to the registrant.
[8] Appeal Decision A-001-2014, *CINIC*, paragraphs 101
[9] See for example Appeal Decision A-017-2014, *BASF SE*, paragraph 68

penalties). **ECHA should be provided with clear guidance to amend its administrative practices and introduce flexibility.**

3.1.2. Comments on draft decisions must be considered throughout the process

As noted by the Board in *Ablemarle*,[10] Article 51(5) of REACH must be understood as giving the Appellants the right to comment on any Proposal for Amendments (PfAs) to the draft decision and not once more on the draft decision itself. Currently, once a draft decision has been circulated to the MSC, ECHA only allows registrants to comment on PfAs (if any) and not the draft decision itself. However, in *Ablemarle*, the Board acknowledged that, in certain circumstances it is possible that the addressee of a decision should be given the opportunity to be heard beyond the opportunities foreseen in Articles 51(2)-(8) and 52.[11] Further, the Board considers that although the right to comment on amendments to a draft decision made by the MSC is not foreseen in the REACH Regulation, it is possible that the addressee of a decision could, depending on the nature of those amendments, potentially be entitled to make known their views on them.[12] Nevertheless, ECHA does not appear to have changed its current practice yet. Furthermore, as the MSC meeting is the first opportunity that third parties have to comment on draft substance evaluation and compliance check decisions, the ability of stakeholders to comment only on PfAs, and not the entire draft decision, severely limits stakeholder involvement (see Section 4.1).

We believe that allowing registrants to comment on the draft decision at the same time as the PfAs would not create an unmanageable administrative burden; ECHA (and the Member States) would simply have to take the comments on the draft decision into consideration when updating the draft decision in the same way that comments on the PfAs are considered. It should never be considered too late to comment on draft decisions circulated to registrants if the result would be to avoid tests on animals. **Guidance should be provided to ECHA and registrants regarding situations when the registrant may comment on updates to the draft decisions throughout the decision making process and even after decisions have been discussed at the MSC.**

3.1.3. Updated dossiers must be considered prior to deadline for compliance in adopted decisions

Currently ECHA will not assess dossier updates before the deadline for compliance set in the adopted decision has passed. This places registrants in a position of unacceptable legal uncertainty. If the registrant does not conduct the requested test(s) and adapts the information requirement requested in the decision using, for example, a (Q)SAR, read-across or other method, it will not know until after the deadline for compliance has passed whether ECHA considers that the information provided complies with the Decision. If ECHA does not review the updated dossier prior to the date in the Decision, the registrant has no ability to respond to ECHA's decision by then carrying out the required test before ECHA informs the relevant MSCAs and invites them to take appropriate action. This policy and risk of enforcement action, with no opportunity for remedy will almost certainly lead to some registrants choosing to conduct required tests rather than submitting justifications as to why such tests are not required, even where such justifications exist. As recognised by the Board in *CINIC,* this leaves the registrant in a position of considerable legal uncertainty and may

[10] Appeal Decision A-009-2014, *Ablemarle*, paragraph 225
[11] Appeal Decision A-009-2014, *Ablemarle*, paragraph 225
[12] Appeal Decision A-009-2014, *Ablemarle*, paragraph 229

lead to a number of unnecessary vertebrate animal tests being carried out and accordingly breaches the last resort principle.[13]

The Board's decision in *Solutia*[14] highlights the problems caused by not considering dossier updates until after the deadline for compliance has passed. In *Solutia* the registrant submitted a read-across adaptation proposal, which was reviewed by ECHA. ECHA claimed that the read-across justification did not meet the relevant information requirements and immediately issued a "Statement of Non-Compliance" (a "SONC") to the relevant Member State. The Appellant argued that its read-across proposal did meet the relevant information requirements and appealed the issue of the SONC, partly on the grounds that ECHA had not adopted the correct procedure prior to issue of the SONC. Nevertheless, due to its concern over potential enforcement action, the Appellant, whilst appealing, also carried out a requested animal test "*to be on the safe side*" notwithstanding the fact that the Appellant did not think it should have been required.[15] In its decision, the Board held that ECHA had not followed the correct procedure when issuing the SONC and annulled it. As such the Appellant conducted an animal test, which may, when ECHA re-evaluates the case, no longer have been required. This highlights that the risk of enforcement action is a key driver in registrants carrying out animal tests, even where they consider that they have a valid adaptation to the required test, for example a read-across argument.

It is critical that ECHA's administrative practices be flexible enough to ensure that updated dossiers are assessed prior to the deadline for compliance in the decision at the registrant's request when the registrant adapts the data requirements requested in the decision.

3.1.4. Proposal for improved flexibility in ECHA's administrative processes

ECHA is at liberty to introduce a degree of flexibility on a case by case basis (unless the REACH regulation is directly breached) to ensure that animal testing is conducted only as a last resort.

To comply with the last resort principle, The Science Consortium submits that ECHA's administrative procedures should be amended to avoid legal uncertainty for registrants without placing undue administrative burden on ECHA. This could be achieved by ECHA reviewing justifications from registrants proposing to adapt required tests ahead of deadlines set in adopted decisions and prior to notification to MSCA's of non-compliance. Any such change in administrative procedure must also allow for sufficient time for the required test to be carried out if ECHA finds that the justification provided by the registrant does not comply with the adopted decision, for example, by extending the deadline for compliance by the time taken to complete the assessment process.

In the absence of such an amendment to its administrative procedures, ECHA must be prepared to reopen compliance check, substance evaluation and testing proposal proceedings at the request of an applicant as a result of new information (including updates to Annex XI adaptations such as read-across justifications) becoming available after the arbitrary cut-off

[13] Appeal Decision A-001-2014, *CINIC*, example paragraphs 100 and 101

[14] Appeal Decision A-019-2013, *Solutia*. *Solutia* relates to the requirements of Directive 67/548/EEC rather than the REACH Regulation. However it is of relevance because Article 135(1) REACH Regulation provides that "[t]he requests to notifiers to provide further information to the competent authority in accordance with Article 16(2) of Directive 67/548/EEC, shall be considered as decision adopted in accordance with Article 51 of [the REACH Regulation]."

[15] Appeal Decision A-019-2013, *Solutia*, paragraph 36

point or after a decision has been adopted. We submit that there is no provision within the REACH regulation that prevents ECHA from reviewing new information once it becomes aware of it. Compliance with the spirit of the REACH regulation demands such an approach.

3.2. ECHA's must comply with Article 25(1) when requiring tests

Both the Board and the European Ombudsman have clarified that ECHA has a duty to be compliant with Article 25(1). For example, the Board noted in *Honeywell* that when ECHA has identified an information gap under Section 8.6.4 of Annex X it in effect assumes the responsibility (which in most cases belongs primarily to the registrant) of ensuring that tests on vertebrate animals are used only as a last resort. Furthermore, the Board indicated that ECHA must ensure that the test using the fewest animals is employed[16] and must give sufficient consideration to the precise objectives pursued when requesting tests, i.e., what information ECHA seeks and sufficiently clear objectives so the registrant can comment in a meaningful way during the decision making process. When there is uncertainty or doubt regarding which test may fulfil the data required then the least onerous option available must be selected by ECHA[17] and, in certain circumstances it falls on ECHA to advocate an appropriate integrated and step-wise approach to testing to achieve the object pursued.[18] Furthermore, ECHA must consider the adequacy of risk management measures with regard to the protection of human health and the environment when considering whether to employ a stepwise approach, with the consequent timing implications.[19] The Board further clarified that it is unreasonable of ECHA to require a test using vertebrate animals when considerable uncertainty remains over whether the information generated will be useful.[20]

ECHA and the Member States must be sure to implement these directives when adopting compliance check, substance evaluation and testing proposal decisions. **Oversight, for example from the Board, the Commission or another independent body, should be provided to ensure ECHA's compliance with Article 25(1).**

3.3. Substance evaluation

Whilst REACH contains no explicit requirement that dossier evaluation should precede substance evaluation, the Board observes that there are a number of indications in the REACH Regulation which suggest that ECHA should carry out a compliance check prior to substance evaluation.[21] The substance evaluation procedure should not, ordinarily, be used in place of a compliance check to fill data gaps.[22] If data gaps in dossiers could be filled through substance evaluation and directed at several registrants (regardless of the registration type and tonnage) with associated cost sharing consequences, this could undermine the balance achieved in the legislation, for example between cost and information.[23] ECHA should not, therefore, extend the standard information requirements without clear and appropriate justification. [24]

Under the substance evaluation procedure greater clarity regarding the potential risks to human health and the environment are required to substantiate a request for further

[16] Appeal Decision A-005-2011, *Honeywell*, paragraph 110.
[17] Appeal Decision A-005-2011, *Honeywell*, paragraph 184.
[18] Appeal Decision A-005-2011, *Honeywell*, paragraph 193.
[19] Appeal Decision A-005-2011, *Honeywell*, paragraph 194.
[20] Appeal Decision A-005-2011, *Honeywell*, paragraph 201.
[21] Appeal Decision A-005-2014, *Azko Nobel*, paragraph 77
[22] Appeal Decision A-009-2014 *Albemarle*, paragraph 90
[23] Appeal Decision A-005-2014, *Azko Nobel*, paragraph 88
[24] Appeal Decision A-005-2014, *Azko Nobel*, paragraph 86

information.[25] ECHA must be able to demonstrate that there is a potential risk, that this risk needs to be clarified, and that the requested information has a realistic possibility of leading to improved risk management measures. If these conditions cannot be met the information requested would not meet real information needs for the protection of human health and the environment pursuant to substance evaluation.[26] ECHA and the MSCAs must ensure that these criteria are met whenever adopting substance evaluation decisions to ensure that excessive data requests are not made.

3.4. A proactive approach to accepting adaptations to standard information requirements is required

ECHA must take a proactive approach to ensuring that tests on animals are conducted only as a last resort. There are a number of actions that ECHA can take to fulfil this responsibility. For example, ECHA must share relevant information regarding the potential availability of non-animal methods with registrants. The decision in case 1606/2013/AN[27] related to a testing proposal and checks which ECHA should carry out of other dossiers in its possession for the same substance. ECHA accepted the European Ombudsman's proposed solution in this case and agreed that ECHA should adopt the practice of sharing "with the registrant any relevant information concerning the potential availability of alternative testing methods for the registered substance". This decision indicates that whilst it was not ECHA's role to put forward adaption arguments on behalf of registrants or identify, in every case, the most appropriate alternative testing method when ECHA is aware, or can easily become aware, of the existence of alternative means of generating missing information without animal testing, ECHA should share any relevant information with the registrant. Furthermore, as mentioned above, the Board's decision in *Honeywell,*[28] highlights the additional responsibilities ECHA assumes in respect of Article 25, when requiring a test to meet an information requirement it has itself identified under a compliance check.

3.4.1. Read-across

Although Annex XI provides general adaptations for waiving tests on animals and permits a weight-of-evidence approach, regulatory acceptance of adaptations not identified in Annexes VII-X remains problematic. One area of particular concern is ECHA's reluctance to accept read-across and this issue must be a key focus for the 2018 REACH deadline.

Read-across is the mechanism by which the largest number of tests on animals can be avoided for REACH and up to 75% of dossiers include a read-across or category approach for at least one endpoint.[29] Yet, ECHA has demonstrated a very restrictive approach to accepting read-across waiving justifications and has often called for registrants to improve their read-across/grouping arguments. The Board stresses that ECHA must act within the margins of its discretion and cannot make disproportionate information requests that would be detrimental to the objectives of read-across set out in the REACH regulation.[30] Further, ECHA needs to balance the objectives of the read-across provisions in REACH with the

[25] Appeal Decision A-005-2014, *Azko Nobel,* paragraph 73
[26] Appeal Decision A-005-2014, *Azko Nobel,* paragraph 73
[27] Ombudsman case 1606/2013/AN
[28] Appeal Decision A-005-2011, *Honeywell Belgium N.V.*
[29] ECHA (2014) The Use of Alternatives to Testing on Animals for the REACH Regulation Second report under Article 117(3) of the REACH Regulation. Available at https://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2014_en.pdf (accessed 16 January 2017)
[30] Appeal Decision A-006-2012, *Momentive,* paragraph 62.

"inherent uncertainty in any read-across adaptation and the need for predictive (eco)toxicology to be alert to the unusual or unexpected".[31] To date, ECHA has provided very few read-across case studies, did not publish the Read-Across Assessment Framework (RAAF) until May 2015 and only recently consulted on an update to the RAAF for environmental fate and environmental hazard properties. **Because read-across is a critical way to avoid large number of new tests on animals, ECHA's lack of appropriate guidance on the use of read-across is unacceptable and must be urgently addressed.**

Although ECHA is not expected to develop, or improve, read-across adaptations on the registrant's behalf,[32] ECHA must take a proactive approach in order to help registrants develop their read-across arguments. For example, it is not disproportionate to expect ECHA to check substance and analogue substance dossiers at the outset of its consideration of a read-across argument if it considers that further justification is required before the read-across adaptation can be accepted. Doing so would ensure that ECHA is aware of and can communicate to the registrant, additional information that may justify read-across proposals, thus enabling registrants to update their dossier prior to referral of the draft decision to the MSCAs.

Failure to adopt these approaches is a clear breach of Article 25 as well as of the spirit of the European Ombudsman decision in case 1606/2013/AN.

3.4.2. Nanomaterials

The draft Appendices on ECHA's Guidance on Information Requirements and Chemical Safety Assessment regarding recommendations for nanomaterials[33] and multiple research publications[34,35] suggest that the majority of the ECHA Guidance Documents and OECD Test Guidelines used for traditional chemicals are applicable to the hazard assessment of nanomaterials, occasionally with adaptations required or with certain limitations.[36] However, within the draft Appendices, ECHA highlights these limitations much more for non-animal methods than for tests on animals, which inevitably encourages registrants to use tests on animals as standard. This is counterproductive, as it is widely accepted within the nanoscience community that the extensive use of non-animal methods will expedite the understanding of the mechanisms of nanomaterial toxicity, which is necessary for the sustainable growth of the nanotechnology industry and the protection of human health.[37]

Several appeals against decisions taken by ECHA regarding nanomaterials have revealed that read across proposals between similar nanomaterials have been rejected.[38,39] There are also

[31] Appeal Decision A-006-2012, *Momentive,* paragraph 62.
[32] Appeal Decision A-004-2015) *Polypnt S.p.A.,* paragraph 123
[33] Available at: https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach
[34] Arts, Josje HE, *et al.* A decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping). Regulatory Toxicology and Pharmacology. 2015;71(2):S1-27;
[35] Kühnel D, Nickel C. The OECD expert meeting on ecotoxicology and environmental fate—towards the development of improved OECD guidelines for the testing of nanomaterials. Science of the Total Environment. 2014;472:347-53.
[36] Schwirn K, Tietjen L, *et al*. Why are nanomaterials different and how can they be appropriately regulated under REACH?. Environmental Sciences Europe. 2014;26(1):1-9.
[37] Savolainen, K., et al., Nanosafety in Europe 2015–2025: towards safe and sustainable nanomaterials and nanotechnology innovations. Finnish Institute of Occupational Health, Helsinki, 2013; George S, Ho SS, et al. The multi-facets of sustainable nanotechnology–Lessons from a nanosafety symposium. Nanotoxicology. 2015;9(3):404-6; Murphy CJ, Vartanian AM, et al. Biological responses to engineered nanomaterials: Needs for the next decade. ACS central science. 2015;1(3):117-23.
[38] Announcement of Appeal A-014-2015, *Grace GmbH & Co. KG and Advanced Refining Technologies GmbH*.

cases where tests on animals have been requested for each product grade, despite the registrant arguing that this testing is unnecessary.[40] Requesting tests for every nanomaterial type or "form" will result in an unprecedented number of tests on animals, which is ethically, logistically and economically unfeasible.

ECHA must strive to be more accepting of scientifically sound non-animal methods, such as read-across, so that the hazard assessment of nanomaterials can progress in a way that is pragmatic and uses tests on animals only as a last resort.

3.5. Animal tests must not be conducted without prior approval of testing proposals

REACH contains a mechanism to bar registrants from conducting tests required under Annexes IX and X without prior approval of a testing proposal by ECHA. Registrants must also show that they have considered adaptations to proposed tests. Importantly, all such testing proposals must be published by ECHA with opportunity for third parties to submit "scientifically valid information and studies that address the relevant substance and hazard endpoint, relating to the testing proposal" (REACH, Article 40 (2)).This helps ensure that the best use has been made of existing information, particularly information from existing tests on animals. Despite the importance of testing proposals, registrants have conducted tests on animals without prior approval of a testing proposal and have often failed to give appropriate justification for why they have conducted the tests.[41] **Through appropriate guidance and severe penalties to act as a deterrent, ECHA and MSCAs must ensure that registrants do not conduct tests on animals without the required testing proposal.**

3.5.1. Testing proposals should be required for all tests on animals

Currently, testing proposals are only required for endpoints included in Annexes IX and X and third parties can only submit "scientifically valid information and studies." However, **the REACH regulation should be updated to require testing proposals for *any* new tests on animals, including short term tests.** This is especially relevant given that non-animal methods are available for skin and eye irritation and skin sensitisation and that tests on animals are a Column 2 adaptation. In addition, the scope of scientifically valid information and studies proposed by third parties should be expanded to include testing strategies, read-across justifications and other approaches that avoid testing on animals.

As noted by the Board,[42] although ECHA is not legally obliged to do so, ECHA "should consider, in certain cases, making third party consultations more explanatory so that all possibly relevant data is made available to help in deciding whether to approve, modify or reject testing proposals. In certain circumstances this could entail publishing in the third party consultation the actual test proposed, as well as the hazard endpoint in question. This could also contribute to fulfilling the Agency's obligations under Article 25(1) to ensure that testing on vertebrate animals is only undertaken as a last resort."

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[39] Announcement of Appeal A-015-2015; *Evonik Degussa GmbH and Others*
[40] Appeal Decision A-010-2015, *Rhodia Operations SAS*
[41] ECHA (2015) Survey results - Analysis of higher tier studies submitted without testing proposals. Available at https://echa.europa.eu/documents/10162/13628/analysis_higher_tier_without_tp_results_en.pdf (accessed 16 January 2017)
[42] Appeal Decision A-001-2014 *CINIC*, paragraph 48

3.6. The last resort principle must be enforced

It is unacceptable that Member States have varying approaches to the enforcement of Articles 13 and 25[43] and that the Enforcement Forum has not identified the last resort principle as a priority for enforcement. REACH was adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals *and* to promote alternative methods for the hazard assessment of substances in order to minimise the number of tests on animals. It is a glaring shortcoming that the promotion of alternatives has not been prioritised. **The REACH legislation must be amended so that Article 13(1) and 25(1) are adequately and uniformly enforced across Europe, with severe penalties (for example fines or market restrictions) for registrants who fail to comply with the last resort principle and for Member States who fail to enforce the principle.**

In addition, enforcement authorities within Member States for Directive 2010/63/EU on the protection of animals used for scientific purposes are often different from those responsible for REACH. Therefore, the authorities responsible for Directive 2010/63/EU will frequently be dependent on ECHA to evaluate when a breach of Article 4 of Directive 2010/63/EU has occurred regarding testing for REACH because, in many circumstances, only ECHA can determine whether use of a non-animal approach in place of a prescribed animal test is acceptable. The European Ombudsman[44] clarified that ECHA has an obligation to verify that data submitted to it is in line with the last resort principle, as required by Article 13(1) and 41(1)(a) of the REACH Regulation and this may, in part, be achieved through direct cooperation with the relevant enforcement authorities of the Member States. **In accordance with the Ombudsman's decision, ECHA must be required to inform Member States of *all* possible instances of non-compliance, not just proven violations and Member States should investigate and sanction non-compliance with the animal protection provisions of REACH.**

3.7. Updates to Annexes, guidance and Test Method Regulation must be expeditious

ECHA and the Commission must ensure that non-animal methods are implemented expeditiously. **To date, the lag between validation of new methods and incorporation in REACH annexes, updates to ECHA's endpoint specific guidance and the Test Method Regulation have been unacceptably slow and this has likely led to numerous animals being used in avoidable testing**. For example, the OECD Test Guideline on the extended one-generation reproductive toxicity tests has the potential to halve the number of animals used per chemical in reproductive toxicity testing (from approximately 2600 to 1400). Yet although this test guideline was adopted by the OECD in 2012, it took a full three years for the REACH annexes to be updated in 2015.

ECHA must also ensure that information on its website regarding the use of alternatives can be easily accessed, for example, by including a prominent link on the homepage.

As noted by the Board in *BASF Pigments GmbH* communications by ECHA may be considered to give rise to legitimate expectations to third parties[45] and it is therefore essential that the advice be accurate and up-to-date. One particular concern regarding EHCA and the

[43] *Report on penalties applicable for infringement of the provisions of the REACH Regulation in the Member States* (2009), Milieu http://ec.europa.eu/environment/chemicals/reach/pdf/report_reach_penalties.pdf (accessed 23 January 2017)

[44] Decision of the European Ombudsman closing the inquiry into complaint 1568/2012(FOR)AN against the European Chemicals Agency (ECHA). Available at: http://www.ombudsman.europa.eu/cases/decision.faces/en/58549/html.bookmark (accessed 23 January 2017)

[45] Appeal Decision A-014-2014, *BASF Pigments GmbH*, paragraph 40

Commission's guidance relates to the interplay between the Cosmetics Regulation[46] and REACH (discussed in Section 5).

3.7.1. Testing requirements for prenatal development toxicity (PNDT) must be updated

Current guidance from ECHA calls for a PNDT test in one species under Annex IX and another under Annex X. However, other European legislation aimed at protecting human health and the environment does not require a PNDT on a second species as a standard information requirement. To meet the requirements for reproductive toxicity testing under the Biocidal Products Regulation (BPR), a PNDT in rabbits and an extended one-generation reproductive toxicity tests (EOGRTS) in rats is considered sufficient. We recommend that this approach be taken to meet the Annexes IX and X REACH data requirements. **The appended letters demonstrates that testing for PNDT in a second species does not provide any additional information and should, therefore, be removed from the standard information requirements.**

Further, the REACH Annex IX Column 2 adaptations are ambiguous and state that "[a] decision on the need to perform a study at this tonnage level *or the next* on a second species should be based on the outcome of the first test and all other relevant available data" [*emphasis added*]. This language has led to confusion regarding the requirements for PNDT studies under Annex IX and X. Differing requirements under the REACH and biocides regulations and the ambiguity of the legal text within Annexes IX and X of REACH are likely to lead to conflict and confusion over the requirement to minimise the number of animals used and the tests required at the higher tonnage levels. Whilst tests on animals are currently required by law to assess PNDT, we strongly recommend that REACH Annexes IX and X be updated to remove the cumulative requirement for a PNDT study in a second species. A PNDT study in a second species should not be a default requirement in either Annex IX or X.

3.8. Ongoing training regarding non-animal methods is essential

Ensuring that ECHA reviewers are familiar with the most up-to-date non-animal methods will improve acceptance of these methods and therefore, non-animal methods are more likely to be used by registrants. Regulatory authorities, including ECHA, MSCAs and Member State Helpdesks must participate in continued education and training regarding the use of non-animal methods. The Science Consortium offers to organise training sessions for ECHA, and other agencies, with the Institute for In Vitro Sciences, a global leader in the advancement of non-animal methods.

3.9. The Substances Database must be used to its full extent to avoid animal tests

The diverse and extensive toxicological and physicochemical information held within the REACH substances database makes it one of the most valuable resources in the world for aiding systematic analyses, computational studies, and evaluation of study protocols and, as such, minimising the use of animals in toxicity tests. To serve that purpose, it must be searchable and machine-readable, but there appear to be restrictions that limit the use of these data for minimising animal testing, which is under the purview of ECHA. Restrictions that limit the use of the REACH substances database must be lifted and must be done to ensure that the substances database is used to the full extent possible to minimise tests on animals.

[46] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32009R1223.

An article published in *ALTEX* in February details efforts led by researchers from the Centre for Alternatives to Animal Testing (CAAT) to extract publicly available information from the substances database and convert it into a more searchable and easy-to-use database.[47] The creation of this new searchable database has "the potential to save millions of animals and reduce testing costs by hundreds of millions".[48] We are fully supportive of this initiative but are deeply concerned that we have yet to see the new database in the public domain. Recent media reports suggest that this is the result of various ECHA-related legal issues encountered by the researchers in making their searchable database public.[49,50]

As data contained within the REACH registered substances database are publicly available and the REACH legislative text provides for the protection of confidential information, it is unclear what factors limit the use of publicly available information held by ECHA and the database developed by CAAT. ECHA must resolve this critical issue and promote the use of data from the substances database.

Given the clear mandate in REACH, laid out in Article 1(1), to promote the use of non-animal test methods, it is obviously critical that this database immediately be made searchable and user-friendly.

3.10. Communication with stakeholders can reduce animal tests

In general, ECHA communicates regularly with stakeholders through its weekly newsletter, stakeholder days, etc. However, it is anticipated that thousands of chemicals will be registered for the 2018 deadline, many of which will be registered by small and medium enterprises (SMEs) who have little experience with REACH. ECHA must do more, for example by setting up an SME network, to reach these registrants to ensure that they are aware of their legal requirement to test on animals only as a last resort.

4. Transparency

There is insufficient transparency regarding some aspects of the administration of REACH. We are particularly concerned about the limited opportunities for stakeholder engagement in MSC and CARACAL meetings.

4.1. Member States Committee

The Member States Committee meetings are particularly important to animal welfare stakeholders. Understanding decisions made by the MSC relating to choice of test method and the extent of animal testing required, and hearing Member States' concerns about how best to protect human health and the environment while minimising animal use, are critical to

[47]Luechtefeld T, Maertens A, Russo DP, Rovida C, Zhu H, Hartung T. Global analysis of publicly available safety data for 9,801 substances registered under REACH from 2008-2014. *ALTEX*. 2016;33(2):95-109.
[48]Gilbert N. Legal tussle delays launch of huge toxicity database. *Nature*. 11 February 2016. http://www.nature.com/news/legal-tussle-delays-launch-of-huge-toxicity-database-1.19365?WT.mc_id=TWT_NatureNews (accessed 18 November 2016)
[49]Davies, E. US database needs permission to access data, claims Echa. *Chemical Watch*. 18 February 2016. https://chemicalwatch.com/45222/us-database-needs-permission-to-access-data-claims-echa. Accessed 4 November 2016.
[50]Jacobs S. This database could help end animal testing – if only scientists had access to it. *Grist*. 16 February 2016 (accessed 4 November 2016)

our work. Article 11 of the rules of procedure[51] indicates that the MSC should operate with a high level of transparency, and the presence of the accredited stakeholder organisations (ASOs) at MSC meetings is key to achieving this.

The purpose of ASO observer participation at the MSC is to represent concerns within civil society – in our case, citizens concerned about the use of animals for toxicity testing – and observe that REACH is being implemented in a transparent fashion. As ASO observers at the MSC we have had the opportunity to participate in MSC discussions, where appropriate, as third party experts. However, our ability to provide an appropriate level of scrutiny is severely hampered by the large number of closed sessions. Ideally, for complete transparency there is a need for ASO presence at all MSC sessions, however we understand that some sessions must remain closed, in accordance with Article 6(12) of the Rules of Procedure. **Nevertheless, more sessions at the MSC should be open to ASOs including sessions where decisions regarding tests on animals are adopted.**

ASO participation in MSC meetings is also restricted by the fact that comments can only be made on parts of decisions where PfAs have been made by Member States and ASOs do not have access to the complete draft decision. As noted by the European Ombudsman in case 2186/2012/FOR regarding third party access to ECHA draft decisions, scientific advice can only be reliable if it is open to scientific review by others, including academics and specialised third parties, such as NGOs. **Therefore, the Science Consortium requests access for ASOs to draft decisions discussed at MSCs and the ability to comment on all sections of the draft decision and not just sections covered by PfAs.**

4.2. Competent Authorities for REACH and CLP

There is insufficient stakeholder engagement in the Competent Authorities for REACH and CLP (CARACAL) meetings. While some animal protection groups are CARACAL observers the PETA International Science Consortium has been denied access to these meetings on several occasions. Given the complementary technical and policy expertise that different animal protection organisations possess, additional seats at the CARACAL should be offered to animal protection groups to facilitate optimal engagement and contribution by concerned organisations.

5. Interface between REACH and the Cosmetics Regulation

The key objectives of the Cosmetics Regulation are to facilitate the free circulation within the EU of cosmetics products whilst ensuring a high level of protection of human health. These objectives are to be achieved through safety assessment of cosmetics products and ingredients by means of approved non-animal methods. Tests on animals are prohibited throughout the development of a product or ingredient, from the research and development phase through to regulatory testing, as well as during the period when the product or ingredient is available for marketing.

However, through a Joint Statement[52] and associated guidance, the European Commission and ECHA purport to provide a settled legal interpretation of the interface between REACH[53]

[51] ECHA (2013) Rules of procedure for the Member State Committee. Available at: https://echa.europa.eu/documents/10162/13578/msc_procedure_rules_en.pdf/03e16e67-d1c2-4f58-9ceb-74fd1e099e (accessed 20 January 2017)

[52] Clarity on interface between REACH and the Cosmetics Regulation (ECHA/NA/14/46**)**. Available at http://echa.europa.eu/view-article/-/journal_content/title/clarity-on-interface-between-reach-and-the-cosmetics-regulation.

and the Cosmetics Regulation[54] in the form of "soft law" guidance which narrows the circumstances in which the clear prohibition on animal testing applies in relation to cosmetics products and their ingredients. **This is contrary to the express wording of both pieces of legislation and a recent judgement by the Court of Justice of the European Union regarding the interpretation of the marketing ban laid down in Article 18(1)(b) of the Cosmetics Regulation (Case C-592/14).**

A complaint[55] submitted by Science Consortium member, PETA UK, to the European Ombudsman sets out these concerns and recommendations to resolve the issues raised.

6. Strategy to phase out tests on animals for chemical safety assessments

As a result of REACH, Europe has lost its position as a world leader in the protection of animals in laboratory experiments, with hundreds of thousands of animals being killed in unreliable tests. Europe now has the opportunity to once again be a global leader in progressive and innovative science by phasing out chemical testing on animals.

REACH continues to develop a huge volume of data but it is failing to generate *useful* data for human hazard/risk assessment because it requires tests on animals to predict human health effects rather than requiring the use of human-relevant non-animal methods. Therefore, various processes under REACH (e.g. registration, evaluation) which are expected to generate data that can be used by public authorities to adopt risk management measures are inadequate.

The ultimate goal of European chemicals legislation that protects humans and the environment should be to phase out tests on animals for chemical safety assessment in favour of more sophisticated and relevant animal-free science. The Netherlands National Committee for the protection of animals used for scientific purposes (NCad) recently published a report entitled "Transition to animal-free science – on opportunities for the phasing out of animal procedures and the stimulation of innovation without laboratory animals"[56] which concludes that this is a realistic goal by 2025:

> "In the case of *regulatory research*, the NCad sees potential for a significant reduction in the use of laboratory animals. The use of laboratory animals in regulatory safety testing of chemicals, food ingredients, pesticides and (veterinary) medicines can be phased out by 2025, whilst maintaining the existing safety level."

One strategy for phasing out tests on animals is the approach adopted by the Cosmetics Regulation (EC No. 1223/2009) which prohibits certain tests on animals after specified cut-off dates. The cosmetics ban led to a boom in investment in the development of non-animal

[53] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02006R1907-20140410.
[54] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32009R1223.
[55] Complaint to European Ombudsman from PETA UK. Ref 1130/2016/JAS. Available at http://www.ombudsman.europa.eu/en/cases/case.faces/en/48472/html.bookmark (accessed 27 January 2017)
[56] NCad (2016) *Transition to non-animal research* Available at: https://www.ncadierproevenbeleid.nl/documenten/rapport/2016/12/15/ncad-opinion-transition-to-non-animal-research (accessed 20 January 2017)

methods; a similar ban for chemicals legislation would see the development of more humane, human- and environment-relevant alternatives that would better protect human health and the environment. In its current form the REACH legislation has led to little to no innovation in the development of alternatives to testing on animals.

Testing on animals for short-term endpoints, such as skin and eye irritation and skin sensitisation, must be prohibited immediately, as non-animal methods are available. Repeated dose toxicity testing, carcinogenicity and reproductive toxicity are complicated endpoints that require focused development of alternatives and should be phased out by 2025, as indicated in the NCad report. To achieve this goal, adequate resources must be dedicated to the development and implementation of non-animal test methods.

The appended paper entitled "Developing a Strategy to Phase Out Chemical Tests On Animals" for a recommended strategy for phasing out tests on animals for regulatory purposes.

7. Conclusions

The legal provisions under REACH regarding its administration are broadly adequate; however, the way in which ECHA implements REACH seriously compromises the underlying principle that tests on animals must be conducted as a last resort. In order to address these concerns ECHA must:

- Update its administrative processes to introduce flexibility when adopting substance evaluation, compliance check and testing proposal decisions
- Ensure that, when requiring tests on animals, it considers the precise objectives pursued, ensures that where necessary an integrated or step-wise approach is implemented and that it requests tests that require the fewest animals
- Take a proactive approach to accepting non-animal methods and adaptations to standard information requirements, especially with regard to the use of read-across
- Not exceed the objectives of substance evaluation by making excessive data requests
- Ensure that use of the Substances Database is maximised to ensure that tests on animals are avoided, for example by making it machine-readable
- Participate in ongoing training in the use of non-animal methods

Member States must ensure that the last resort principle is adequately and uniformly enforced across Europe and the Enforcement Forum must ensure enforcement of this principle is prioritised.

Great transparency at the MSC and CARACAL can be achieved by having fewer closed sessions and allowing participation of more accredited stakeholders.

Many registrants will be registering chemicals for the first time for the 2018 deadline; therefore, adequate communication with them regarding the use of non-animal methods is essential.

The recommendations outlined in this position paper must be implemented with all due speed in order to reduce the vast numbers of animals who will be used prior to the REACH 2018 deadline.

8. Contact details

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Annex: Developing a strategy to phase out chemical tests on animals

As a result of REACH, Europe has lost its position as a world leader in the protection of animals in laboratory experiments, with hundreds of thousands of animals being killed in unreliable tests. Europe now has the opportunity to once again be a global leader in progressive and innovative science by phasing out chemical testing on animals. Harmonisation and international acceptance of non-animal testing methods for regulatory toxicity testing requirements must be a priority and funds should be diverted from animal studies for the development of non-animal methods.

Harmonisation and international acceptance of non-animal testing methods for regulatory toxicity testing requirements

The past quarter century has seen a revolution in the way in which chemicals are tested. This is the result of our better understanding of biological processes which has allowed for the development of testing methods that can look directly at cellular mechanisms rather than at the crude "black box" results that come from using animals. Mechanistic information about the potential toxicity of a chemical, such as the potential for receptor binding or gene or pathway activation, is more readily obtained *in vitro* than *in vivo*.

Concurrently, there has been a growing recognition among regulators and the regulated community that the animal methods have not been protecting either human health or the environment as well as the fact that "the current approach is time-consuming and costly, resulting in an overburdened system that leaves many chemicals untested, despite potential human exposure to them," (National Academies of Sciences, 2007a). In 2007, the U.S. National Academies of Sciences published a landmark report entitled "Toxicity Testing in the 21st Century: A Vision and a Strategy":

> "Toxicity testing is approaching a scientific pivot point. It is poised to take advantage of the revolutions in biology and biotechnology. Advances in toxicogenomics, bioinformatics, systems biology, epigenetics, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on *in vitro* methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin."
>
> "The proposed changes will generate better data on the potential risks humans face from environmental agents, building a stronger scientific foundation that can improve regulatory decisions to mitigate those risks, and reducing the time, money, and animals needed for testing."
>
> "The report recommends an approach that would take advantage of rapidly evolving scientific understanding of how genes, proteins, and small molecules interact to maintain

> normal cell function and how some of these interactions can be perturbed in ways that could lead to health problems. Specifically, the new testing approach would focus on toxicity pathways -- cellular pathways that, when sufficiently perturbed, are expected to lead to adverse health effects."
>
> "The committee recommends the use of high-throughput assays -- rapid, automated experiments that can test hundreds or thousands of chemicals over a wide range of concentrations -- to evaluate chemicals' effects on these toxicity pathways. On the basis of data from these and other experiments, researchers could develop models to describe responses in toxicity pathways, and other models to estimate the human exposure necessary to produce responses in these pathways." (National Academies of Sciences, 2007b).

The U.S. Environmental Protection Agency has made major advances in implementing these "Tox21" and "ToxCast" programmes.

By eliminating the use of tests on animals for regulatory purposes where full replacements exist and promoting the acceptance of methods currently in development, Europe has the opportunity to further shift the regulatory testing paradigm towards innovative non-animal techniques, and become a world leader in the application of these methods. In the appendices we have elaborated on opportunities where the use of animals for regulatory testing should be ended immediately or within the next two to five and five to ten years; these include acute systemic toxicity testing, genotoxicity testing, pyrogenicity testing, vaccine and biologics testing, endocrine disruption and carcinogenicity. Furthermore, we recognize that regulatory acceptance of non-animal techniques in one region or country is an open door to international harmonisation and the wider statutory elimination of animal testing methods. Therefore we advocate that the European Commission liaise with industry, regulatory and research agencies worldwide to establish and promote clear paths to validation and harmonisation of non-animal techniques for regulatory testing requirements.

Funds should be diverted from animal studies for the development of non-animal methods

Forward-thinking scientists are developing and implementing methods for studying and treating diseases and testing products that do not entail the use of animals and are relevant to *human* health. Researchers have developed human cell-derived skin models, "organs-on-chips," *in silico* models and other methodologies that can replicate human physiology, diseases, drug responses, and chemical exposure more accurately than experiments on animals.

Studies have repeatedly shown that these new methodologies are better at modelling human diseases than crude experiments on animals. Indeed, the NIH in its most recent 5-year strategic plan announced that it would reduce and replace animal experiments, stating:

> Petri dish and animal models often fail to provide good ways to mimic disease or predict how drugs will work in humans, resulting in much wasted time and money while patients wait for therapies. To address that challenge, NIH, DARPA [Defense Advanced Research

> Projects Agency], and FDA [Food and Drug Administration] are collaborating to develop 3D platforms engineered to support living human tissues and cells, called tissue chips or organs-on-chips. An integrated body-on-a-chip is the ultimate goal.

With greater investment in exciting and progressive non-animal methods and bold policy initiatives of the sort recently demonstrated by the Netherlands (Netherlands National Committee, 2016), we will have far more promising cures and treatments for humans and more effective and reliable methods for toxicity assessment, while also alleviating unimaginable suffering of animals.

Please find appended further identification of areas of research where opportunities lie for the development, validation and implementation of non-animal methods for regulatory research.

Cited References:

National Academies of Sciences. *Report calls for new directions, innovative approaches in testing chemicals for toxicity to humans* [Press release]. 2007a http://www8.nationalacademies.org/onpinews/newsitem.aspx?RecordID=11970.

National Academies of Sciences. *Toxicity testing in the 21st Century: A vision and a strategy*. 2007b. https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a.

National Institutes of Medicine. *NIH-wide strategic plan: fiscal years 2016-2020*. 2015. https://www.nih.gov/sites/default/files/about-nih/strategic-plan-fy2016-2020-508.pdf.

Netherlands National Committee for the protection of animals used for scientific purposes (2016) *Transition to non-animal research.* https://www.ncadierproevenbeleid.nl/documenten/rapport/2016/12/15/ncad-opinion-transition-to-non-animal-research

APPENDICES

Please find below further detail on opportunities for replacing the use of animals in regulatory testing.

SKIN IRRITATION / CORROSION

Recommendation: Immediately eliminate the use of animals for skin irritation / corrosion testing.

Non-Animal Approaches Available Now

The Organisation for Economic Cooperation and Development (OECD) has developed step-wise guidance for an integrated testing strategy using *in vitro* skin irritation methods that avoids or minimizes animal use (OECD 2014).

OECD Test No. 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis (RHE) Test Method. May be used for the hazard identification of irritant chemicals (substances and mixtures) in accordance with the UN Globally Harmonized System of Classification and Labelling (GHS) category 2, category 3 or non-classified chemicals. May be used as a stand-alone test or in a tiered testing strategy.

a) **OECD Test No. 430: *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER).** Allows the identification of non-corrosive and corrosive test chemicals in accordance with the UN GHS but does not allow the sub-categorization of corrosive substances and mixtures.

b) **OECD Test No. 431: *In Vitro* Skin Corrosion: RHE Test Method.** Allows the identification of corrosive chemical substances and mixtures and enables the identification of non-corrosive substances and mixtures when supported by a weight of evidence determination using other existing information. The test protocol may also provide an indication of the distinction between severe and less severe skin corrosives.

c) **OECD Test No. 435: *In Vitro* Membrane Barrier Test Method for Skin Corrosion.** Allows for subcategorization of corrosive chemicals into the three UN GHS sub-categories of corrosivity.

Methods are generally validated for use with cosmetics and industrial chemicals registered under the European Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) regulation. Efforts are ongoing to validate the reconstructed human epidermis (RHE) method as an acceptable alternative to the ISO 10993-required rabbit skin irritation test for assessing medical device biocompatibility (Casas, Lewerenz and Rankin). Likewise, some of the above methods are currently undergoing evaluation in a joint effort by the US Environmental Protection Agency (EPA), industry, and the US NTP Interagency Centre for the Evaluation of Alternative Toxicological Methods (NICEATM) for use with pesticide products. This consists of side-by-side comparison and analysis of existing *in vitro*/*in vivo* data generated by pesticide companies for their products. *Depending on the outcome of these efforts, additional work may be*

needed to further validate use of these methods with certain classes of chemicals that were not covered during OECD validation efforts.

Additionally, there are opportunities available to waive these tests based on criteria described in OECD Guidance document on considerations for waiving or bridging of mammalian acute toxicity tests (OECD 2016).

Cited References:

Casas, J W, et al. "In vitro human skin irritation test for evaluation of medical device extracts." *Toxicology In Vitro* 27.8 (2013): 2175-2183.

OECD 2014. "OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 203: Guidance Document on an Integrated Approach On Testing and Assessment (IATA) for Skin Corrosion and Irritation." 2014. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)19&doclanguage=en.

OECD 2016. "Guidance Document on Considerations for Waiving or Bridging of Mammalian Acute Toxicity Tests. Series on Testing & Assessment No. 237." 2016. http://www.oecd.org/env/ehs/testing/mono%202016%2032.pdf.

EYE IRRITATION / CORROSION

Recommendation: Immediately eliminate the use of animals for eye corrosion / irritation testing and validate a non-animal method that can directly predict GHS category 2 (irritant) substances for use in a regulatory setting.

Non-Animal Approaches Available Now

The general consensus is that no single *in vitro* alternative test can be used to replace the *in vivo* rabbit eye test to predict across the full range of serious eye damage / eye irritation responses for different chemical classes. However, by employing combinations of alternative test methods used in a tiered testing strategy replacement may be accomplished. A top-down approach is used when chemicals are expected, based on existing information, to have a high irritancy potential or induce serious eye damage. Conversely, a bottom-up approach may be used when chemicals are expected, based on existing information, not to cause sufficient eye irritation to require a classification. A guidance document on an integrated approach to testing and assessment (IATA) of serious eye damage and irritation is underway at the OECD (OECD 2016a).

a) **OECD Test No. 491: Short Time Exposure (STE) Test Method.** This method can identify, without further testing, either a chemical causing serious eye damage (GHS category 1) or one not requiring classification (GHS No category). The test guideline states that it cannot be used to define GHS category 2 substances (moderate/mild irritants) and further testing for definitive classification is needed. **However, this test method has been shown to be capable of classifying irritants as minimal, moderate, or severe, although results are not accepted for regulatory use** (Institue for In Vitro Sciences). *Additional work in this area may allow this method to be acceptable for classifying GHS category 2 irritants for regulatory purposes and should be pursued.*

b) **OECD Test No. 492: Reconstructed human Cornea-like Epithelium (RhCE) Test Method (EpiOcular™, MatTek, Corp.).** This method can identify those chemicals not classified for eye irritation or causing serious eye damage (GHS No category), but cannot differentiate between GHS category 1 and GHS category 2, and thus a positive finding would require additional testing.

c) **OECD Test No. 460: Fluorescein Leakage Test Method.** This method can identify, without further testing, either a chemical causing serious eye damage (GHS category 1) or one not requiring classification (GHS No category). It is recommended as an initial step within a top-down approach to identify ocular corrosives / severe irritants, specifically for limited types of chemicals (i.e., water soluble substances and mixtures).

d) **OECD Test No. 437: Bovine Corneal Opacity and Permeability (BCOP) Test Method.** This method has undergone international validation by the US Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the

European Union Reference Library for alternatives to animal testing (EURL ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM). The method is capable of accurately predicting chemicals (both substances and mixtures) that induce serious eye damage (GHS category 1) as well as those not requiring classification for eye irritation or serious eye damage (GHS No category) without further testing. OECD does not recommend its use to classify category 2 substances. However, the EPA has worked with industry and a private *in vitro* contract laboratory to develop a system whereby the BCOP can be used to identify moderate / mild irritants in AMCPs as defined by EPA's system of classification, which is similar but not completely analogous to the GHS. *Further work in this area may lead to acceptable use of this method to classify category 2 irritants.*

e) **OECD Test No. 438: Isolated Chicken Eye Test Method.** This method has undergone international validation by ICCVAM, EURL ECVAM and JaCVAM. The method is capable of accurately predicting chemicals (both substances and mixtures) that induce serious eye damage (GHS category 1) as well as those not requiring classification for eye irritation or serious eye damage (GHS No category) without further testing. It is recommended as the first step within a top-down or a bottom-up testing strategy approach. It cannot be used to classify category 2 substances.

Methods are generally validated for use with cosmetics and industrial (REACH) chemicals, and there may be limitations with some methods with certain types of chemicals (e.g., surfactants, solids, etc.). None of the current OECD-approved assays is recommended to be used to directly determine category 2 eye irritants in a regulatory setting (i.e., most have been validated to determine GHS category 1 (severe eye damage) or No category). ***There is a vital need for validation of a non-animal method that can directly predict category 2 (irritant) substances for use in a regulatory setting.***

The EPA currently accepts the use of *in vitro* methods for determination of eye irritation / corrosion when classifying pesticidal anti-bacterial cleaning products (AMCPs) and has published a guidance document that describes the testing framework industry can use for this endpoint (USEPA). Also, the EPA in collaboration with NICEATM and industry members is currently engaged in evaluating these methods for use with conventional pesticides through a side-by-side comparison of *in vitro*/*in vivo* data for representative pesticide chemical classes. A report on this effort is expected within the next six months.

Additionally, there are opportunities available to waive these tests based on criteria described in OECD Guidance document on considerations for waiving or bridging of mammalian acute toxicity tests (OECD 2016b).

Cited References:

OECD 2016a. "Work plan for the Test Guidelines Programme." 2016. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)6&doclanguage=en.

OECD 2016b. "Guidance Document on Considerations for Waiving or Bridging of Mammalian Acute Toxicity Tests. Series on Testing & Assessment No. 237." 2016. http://www.oecd.org/env/ehs/testing/mono%202016%2032.pdf.

SKIN SENSITISATION

Recommendation: Immediately eliminate the use of animals for skin sensitisation testing.

Non-Animal Approaches Available Now

Testing on animals for skin sensitisation can be fully replaced with three in vitro / in chemico assays that each address a different key event in the adverse outcome pathway for this endpoint (OECD 2012). The methods distinguish between sensitizers and non-sensitizers and are recommended to be used in an integrated approach to testing and assessment.

a) **OECD Test No. 442C: *In Chemico* Skin Sensitisation Direct Peptide Reactivity Assay (DPRA).** The DPRA addresses the molecular initiating event of the skin sensitisation AOP.

b) **OECD Test No. 442D: *In Vitro* Skin Sensitisation ARE-Nrf2 Luciferase (KeratinoSens) Test Method.** This method addresses the second key event of the skin sensitization AOP.

c) **OECD Test No. 442E: *In Vitro* Skin Sensitisation Human Cell Line Activation Test (h-CLAT).** This method addresses the third key event of the skin sensitization AOP. While none of the methods is endorsed for potency determination, the h-CLAT shows promise in this regard. *Further efforts are required to explore this potential.*

In general, the methods can be used to test industrial (REACH) chemicals and cosmetics although they may not be suitable for testing some substance types. There is an ongoing effort to validate non-animal skin sensitisation methods to replace the ISO 10993-required guinea pig skin sensitization test for assessing medical device biocompatibility (Coleman, McNamara and Grailer; McKim, Keller and Gorski). In the US, an integrated testing strategy that draws on *in silico*, *in chemico*, and *in vitro* approaches has been developed by ICCVAM, in collaboration with Procter and Gamble, to determine skin sensitisation potential (ICCVAM). The EPA is currently collecting and analysing paired *in vitro*/*in vivo* skin sensitisation data to assist in moving towards non-animal approaches to evaluating pesticides (USEPA). With a successful outcome, this work could lead to regulatory acceptance by the EPA within the next two years.

Additionally, there are opportunities available to waive these tests based on criteria described in OECD Guidance document on considerations for waiving or bridging of mammalian acute toxicity tests (OECD 2016).

Cited References:

Coleman, K P, et al. "Evaluation of an in vitro human dermal sensitization test for use with medical device extracts." *Applied In Vitro Toxicology* 1.2 (2015): 118-130.

ICCVAM. "Integrated Testing Strategies to Identify Potential Skin Sensitizers." 2016. http://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/immunotoxicity/nonanimal/index.html.

McKim, J M Jr, D J 3rd Keller and J R Gorski. "An in vitro method for detecting chemical sensitization using human reconstructed skin models and its applicability to cosmetic, pharmaceutical, and medical device safety testing." *Cutaneous and Ocular Toxicology* 31.4 (2012): 292-305.

OECD 2012. "The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. Series on Testing and Assessment No. 168." 2012. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)10/part1&doclanguage=en.

OECD 2016. "Guidance Document on Considerations for Waiving or Bridging of Mammalian Acute Toxicity Tests. Series on Testing & Assessment No. 237." 2016. http://www.oecd.org/env/ehs/testing/mono%202016%2032.pdf.

USEPA. "Strategic Vision for Adopting 21st Century Science Methodologies." 2016. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science.

ACUTE SYSTEMIC TOXICITY

Acute systemic toxicity testing includes acute oral, dermal, and inhalation toxicity testing. Several international efforts have focused on non-animal replacements for acute systemic toxicity testing including the Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) program (Ekwall, Barile and Castano) and the EU Framework Programme (FP) 6 ACuteTox project (http://www.acutetox.eu/). In addition, a 2015 series of webinars and a workshop hosted by the PETA International Science Consortium Ltd., the Physicians Committee for Responsible Medicine, and NICEATM presented a strategy for replacing acute systemic toxicity testing (Hamm, Sullivan and Clippinger; PISC 2016a). A 2016 series of webinars and a workshop hosted by the PETA International Science Consortium Ltd. and NICEATM focused specifically on alternative approaches for acute inhalation toxicity testing (workshop report draft in progress, PISC 2016a). As a result of these and other efforts, there are alternative approaches that can be currently used to reduce/ replace or waive these tests, and other approaches undergoing further development.

Non-Animal Approaches Available Now

GENERAL WAIVING OF ACUTE TOXICITY TESTS

Waivers for acute toxicity testing in animals may be issued by regulatory authorities if certain criteria can be met (this includes the three topical skin and eye endpoints discussed above as well as acute systemic toxicity testing). The EPA issued guidance for waiving or bridging acute toxicity tests for pesticides or pesticide products (USEPA 2016a) and the OECD recently issued guidance for waiving or bridging acute toxicity testing (OECD 2016). The guidance includes use of existing data for read-across and the consideration of the physicochemical properties of the test substance.

Another approach that can be currently used to avoid certain acute toxicity testing is the use of the GHS Additivity Equation for classifying formulations and mixtures for acute systemic toxicity tests (United Nations). This is a method that essentially “adds” the toxicities of known ingredients in pesticide mixtures without the need for animal testing of those mixtures. It has been demonstrated to accurately predict acute systemic toxicity for agrochemical formulations (Corvaro et al.) The GHS Additivity Equation is currently usable as stand-alone replacement method in some geographies, such as for the purposes of the European Classification, Labelling and Packaging regulation. The EPA is currently involved in a pilot study to evaluate use of this method in its pesticide registration program (USEPA 2016b;).

Retrospective analyses of data that have been traditionally required by regulatory agencies are needed to determine how the data generated are actually being used. This analysis will provide

evidence of redundant or valueless animal tests that can be waived or completely eliminated without endangering public health and safety. Information on mechanisms of acute systemic toxicity will improve the development of assays for key events, predictive models, and integrated approaches to testing and assessment. The OECD's AOP framework can facilitate the systematic reporting, curating, and integrating of this information (OECD 2013).

ACUTE DERMAL TOXICITY

Recommendation: Acute dermal toxicity testing should be avoided.

Non-Animal Approaches Available Now

Acute dermal toxicity testing is often a regulatory requirement in addition to acute oral and acute inhalation toxicity testing and it focuses on determination of lethal doses through the dermal route. There has been general consensus that testing through the dermal route is essentially redundant if there is available data on oral toxicity. EPA and NICEATM analysed the relative contribution of data from acute oral and dermal toxicity tests to pesticide hazard classification and labelling. Data were collected from about 600 paired acute lethality dermal and oral toxicity studies in rats used to assess pesticide formulations. Finding that the dermal study for formulations provided little to no added value in regulatory decision making, EPA has issued guidance allowing registrants to submit waiver requests for this study (USEPA 2016c).

Further assessments of existing data are needed for other classes of chemicals to determine if acute dermal toxicity testing can be subject to waiver or be eliminated altogether.

Furthermore, there were recommendations to waive dermal studies for substances that are non-classified by the oral route as well as testing dermal absorption prior to conducting acute dermal toxicity studies. As a result, in Europe REACH Annex VIII has already been amended so that substances that are non-classified by the oral route do not require dermal data (The European Commission).

ACUTE INHALATION TOXICITY

Recommendation: Acute inhalation toxicity testing should be avoided through waivers or use of the GHS Additivity Equation; further work is needed to develop non-animal testing approaches.

Non-Animal Approaches Available Now

As described above, the EPA and OECD have guidance documents for waiving or bridging acute toxicity testing, including acute inhalation toxicity (USEPA 2016a) (OECD 2016). For example, in the case of acute inhalation toxicity, if the substance demonstrates low volatility, is not aerosolized or otherwise made inhalable as a gas or vapour under conditions of use, storage, handling, or transport then the test can be waived.

Non-Animal Approaches Likely to be Available Within 2-5 years

Numerous promising research efforts are underway in both the US and Europe to develop non-animal methods for acute inhalation toxicity. A recent series of webinars and a workshop hosted by the PETA International Science Consortium and NICEATM presented several approaches that could eventually replace animal testing for this endpoint, including use of human "lung-on-a-chip" and "Metabo-Lung" models as well as QSARs and read-across predictors (PISC 2016b). The drafting of a workshop report and establishment of specific working groups to carry out workshop recommendations are underway. Developing an integrated approach to replacing animals in inhalation toxicity testing will likely be needed and include the use of *in silico, in chemico,* and *in vitro* methods. Continued development of AOPs and increased understanding of toxicity mechanisms will be important in defining human-relevant testing batteries. *For use by regulatory authorities, more research, proof of concept, evaluation with different classes of chemicals, and validation is needed.*

ACUTE ORAL TOXICITY

Recommendation: Acute oral toxicity testing should be avoided through waivers or use of the GHS Additivity Equation; however, further work is needed to develop non-animal testing approaches.

Non-Animal Approaches Available Now

EURL ECVAM's strategy to replace, reduce, and refine the use of animals in the assessment of acute mammalian systemic toxicity focuses on the *in vitro* 3T3 neutral red uptake cytotoxicity assay, which can be used in a weight-of-evidence approach to support the identification of non-classified substances (acute oral LD_{50}>2000mg/kg b.w.) (EURL ECVAM). *In vitro* tests such as 3T3 NRU and normal human keratinocyte (NHK) assays that measure basal cytotoxicity can also be useful in determining starting doses in animal tests, but cannot be used to fully assess systemic toxicity as a stand-alone test.

Non-Animal Approaches Likely to be Available Within 2-5 years

EURL ECVAM is currently working to improve confidence in the 3T3 NRU through the use of quantitative structure-activity relationships (QSARs), and by accounting for target organ information and the lack of metabolism in 3T3 cells (Hamm, Sullivan and Clippinger; Prieto).

QSAR models have been developed for predicting rodent oral toxicity (The National Academies of Sciences, Engineering and Medicine). Information on repeated dose toxicity, if available, can also be used to predict acute effects. EURL ECVAM has proposed an approach to identify non-classified substances using information from 28-day repeated dose toxicity studies, thereby avoiding acute systemic toxicity testing (Bulgheroni, Kinser-Overskainen and Hoffmann; Graepel, Asturiol and Prieto).

Promising approaches are under development that involve use of both *in silico* and *in vitro* methods. One approach that uses mitochondrial membrane potential inhibition as measured in the ToxCast™ suite of assays has been investigated for 1,800 compounds as a predictor of mammalian and fish acute toxicity (USEPA 2016d) (Bhhatarai, Wilson and Bartels). When compared to curated data from regulatory and literature studies, the method did well for predicting fish toxicity and rat intravenous toxicity, but did not perform well for rat oral toxicity. After inclusion of a model that first accounted for bioavailability after passage through the gut, the ToxCast results had much better correlation to rat oral data.

Further work, particularly at the international level, is needed to develop a reliable non-animal approach to predicting acute oral toxicity.

Cited References:

Bhhatarai, B, et al. "Acute toxicity prediction in multiple species by leveraging mechanistic ToxCast mitochondrial." *Toxicological Sciences* 147.2 (2015): 386-396.

Bulgheroni, A, et al. "Estimation of acute oral toxicity using the No Observed Adverse Effect Level (NOAEL) from the 28 day repeated dose toxicity studies in rats." *Regulatory Toxicology and Pharmacology* 53 (2009): 16-19.

Corvaro, et al. *Regulatory Toxicology and Pharmacology*. 2016. 82:99-110 .

EURL ECVAM. "EURL ECVAM strategy to replace, reduce and refine the use of animals in the assessment of acute mammalian systemic toxicity." 2013.

Ekwall, B, et al. "MEIC Evaluation of Acute Systemic Toxicity: Part VI. The prediction of human toxicity by rodent LD50 values and results from 61 in vitro methods." *ATLA* 26.Suppl 2 (26): S617-658.

Graepel, R, et al. "Exploring waiving opportunities for mammalian acute systemic toxicity tests." *ATLA* 44.3 (2016).

Hamm, J, et al. "Alternative approaches for identifying acute systemic toxicity: moving from research to regulatory testing." *Toxicology In Vitro* (2016): In press.

OECD 2016. "Guidance Document on Considerations for Waiving or Bridging of Mammalian Acute Toxicity Tests. Series on Testing & Assessment No. 237." 2016. <http://www.oecd.org/env/ehs/testing/mono%202016%2032.pdf>.

OECD 2013. "Guidance Document on Developing and Assessing Adverse Outcome Pathways. Series on Testing and Assessment No. 184." 2013. September 2016. <http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)6&doclanguage=en>. 55

PISC 2016a. *Acute Systemic Toxicity*. 2016. http://www.piscltd.org.uk/acute-systemic-toxicity/.

PISC 2016b. *Webinar series on Alternative Approaches for Acute Inhalation Toxicity Testing to Address Global Regulatory and Non-regulatory Data Requirements*. 2016. http://www.piscltd.org.uk/acute-inhalation-toxicity/.

Prieto, P. "The value of selected in vitro and in silico methods to predict acute oral toxicity in a regulatory context: Results from the European Project ACuteTox." *Toxicology in vitro* (2013): 357-376.

The European Commission. "Commission Regulation (EU) 2016/863 of 31 May 2016 amending Annexes VII and VIII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards s." 2016. September 2016. http://eur-lex.europa.eu/eli/reg/2016/863/oj.

The National Academies of Sciences, Engineering and Medicine. *Application of Modern Toxicology Approaches for Predicting Acute Toxicity for Chemical Defense*. Washington DC: The National Academies Press, 2015.

United Nations. "Part 3 Health Hazards." 2009. https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev03/English/03e_part3.pdf.

USEPA 2016a. "Bridging or waiving data requirements." 2016. https://www.epa.gov/pesticide-registration/bridging-or-waiving-data-requirements.

USEPA 2016b. "Strategic Vision for Adopting 21st Century Science Methodologies – GHS Dose Additive Mixtures Equation Pilot." https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science.

USEPA 2016c. "Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations & Supporting Retrospective Analysis - November 9, 2016." https://www.epa.gov/sites/production/files/2016-11/documents/acute-dermal-toxicity-pesticide-formulations_0.pdf.

USEPA 2016d. "Toxicity Forecasting: Advancing the Next Generation of Chemical. https://www.epa.gov/chemical-research/toxicity-forecasting.

GENOTOXICITY

Recommendation: In light of existing non-animal methods and weight-of-evidence approaches, the use of animals in genotoxicity testing can be dramatically reduced.

Non-Animal Approaches Available Now

Currently, the assessment of genotoxicity typically follows a step-wise approach, beginning with a core battery of *in vitro* tests. The major endpoints that must be evaluated are gene mutation, structural chromosome aberrations and numerical chromosome aberrations. In its Strategy to Avoid and Reduce Animal Use in Genotoxicity Testing, EURL ECVAM recommends the Ames test to identify gene mutations combined with the *in vitro* micronucleus test to identify both structural and numerical chromosome aberrations (EURL ECVAM). If a substance produces negative results in both tests, it can be categorized as having no genotoxic potential and no further testing is indicated. If a substance produces positive results in either test, certain regulatory applications currently specify *in vivo* tests as the next step. This is because while these *in vitro* tests are highly sensitive, producing false negative results at a low rate, they are less specific, producing false positive results at a higher rate. The number of false positive results can be reduced by using p53-competent human cells, evaluating cytotoxicity based on cell proliferation, and testing at reduced maximum concentrations (Corvi and Madia). These considerations have been incorporated into recent revisions of OECD Test Guidelines.

To better assess the genotoxic potential of substances that produce positive results in the core battery, additional *in vitro* tests can be used in place of *in vivo* tests. In its Notes of Guidance for Testing Cosmetic Ingredients and Their Safety Evaluation, the SCCS recommends using a micronucleus test on 3D-reconstructed human skin or a comet assay in either mammalian cells or on 3D-reconstructed human skin (SCCS). However, negative results produced in these alternative tests do not necessarily rule out genotoxic potential. In such cases, expert judgement as well as mechanistic investigations may be helpful to evaluate the weight–of-evidence. For example, *in vitro* toxicogenomics-based tests can provide information on the mode of action of potential genotoxicants by identifying global gene expression changes.

Non-Animal Approaches Likely to be Available within 2-5 years

Validation studies of the micronucleus test and comet assay on 3D-reconstructed human skin are currently being conducted by Cosmetics Europe providing further opportunities for phasing-out the use of animals for genotoxicity testing (Pfuhler).

Cited References:

Corvi, R and F Madia. "In vitro genotoxicity testing - Can the performance be enhanced? Food and Chemical." *Food and Chemical Toxicology* (2016): In Press.

EURL ECVAM. "EURL ECVAM Strategy to Avoid and Reduce Animal use in Genotoxicity Testing." 2013. http://publications.jrc.ec.europa.eu/repository/bitstream/JRC86616/jrc_report_en_34844_online.pdf.

Pfuhler, P et al. The Cosmetics Europe strategy for animal-free genotoxicity testing: Project status up-date. *Toxicology In Vitro* 28 (2014): 18-23.

SCCS. "SCCS's Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation, 9th revision, 25 April 2016, SCCS/1564/15." n.d. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf.

CARCINOGENICITY

Recommendation: In light of existing non-animal methods and weight-of-evidence approaches, the use of animals in carcinogenicity testing can be dramatically reduced.

Non-Animal Approaches Likely to be Available within 2-5 years

a) Mouse carcinogenicity study:

It is essential that data from animal tests undergo systematic review for their value in protecting human health and the environment. In an assessment of 202 pesticide evaluations from the European Union review program, Billington et al. (2010) demonstrated the mouse carcinogenicity study contributed little or nothing to either derivation of an acceptable daily intake (ADI) for assessment of chronic risk to humans, or hazard classification for labelling purposes. In terms of pesticide approvals, the authors showed the mouse study did not influence a single outcome. ***Additional reviews of this kind may show that the mouse carcinogenicity study provides little value in assessment of other classes of chemicals as well. Further investigation into this area could yield results that would argue for elimination of this study entirely.***

b) Cell transformation assays:

In vitro cell transformation assays (CTAs) recapitulate a multistage process that closely models *in vivo* carcinogenesis and have the potential to detect both genotoxic and non-genotoxic carcinogens. In its recommendation on the CTA based on the Bhas 42 cell line, EURL ECVAM notes that information on the transforming potential of substances generated by CTAs may be sufficient for decision-making (EURL ECVAM). The Bhas 42 cell line was developed from BALB/c 3T3 cells through transfection with a Harvey ras sarcoma viral mutated oncogene homolog (v-Ha-ras). Since Ha-ras is involved in multistage carcinogenesis, Bhas 42 cells are more predisposed to transformation than the original cells BALB/c 3T3. In a validation study, the Bhas 42 CTA was tested with 98 substances including carcinogens and non-carcinogens. For predicting carcinogenicity, its performance was equivalent or superior to conventional genotoxicity assays (Hayashi, Kojima and Corvi). As the protocols were transferable and reproducible between laboratories, they are recommended for routine use. In addition, because the Bhas 42 CTA is based on a cell line rather than primary cells, no animals are required.

In its Guidance Document on the Bhas 42 CTA, the OECD recommends that the assay be used as part of a testing strategy rather than as a stand-alone assay. When combined with other information such as genotoxicity data, structure-activity analysis and toxicokinetic information, CTAs in general, and the Bhas 42 CTA specifically, can contribute to the assessment of carcinogenic potential and may provide an alternative to the use of *in vivo* testing (OECD 2015). The structural alerts (SAs) rulebase has recently been expanded with a large number of new SAs for non-genotoxic carcinogenicity and has been implemented into the OECD (Q)SAR Toolbox

version (Benigni, Bossa and Tcheremenskaia). The identification of DNA-reactive chemicals with the Ames test or genotoxic SAs can be combined with the identification of non-genotoxic carcinogens with non-genotoxic SAs leaving CTAs to model most of what is left unexplained.

There is an effort underway at the OECD level to generate an integrated approach to testing and assessment for non-genotoxic carcinogens (OECD 2016).

Cited References:

Benigni, R, C Bossa and O Tcheremenskaia. "In vitro cell transformation assays for an integrated, alternative assessment of carcinogenicity: a data-based analysis." *Mutagenesis* 28.1 (2013): 107-116.

EURL ECVAM. "EURL ECVAM recommendation on the cell transformation assay based on the Bhas 42 cell line. JRC Reference Report." 2013. http://dx.doi.org/10.2788/42908.

Hayashi, M, et al. "Bhas 42 cell transformation assay validation study report submitted to JaCVAM." 2012.

OECD. “Guidance document on the in vitro Bhas 42 cell transformation assay." 2016. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2016)1&docLanguage

OECD 2016. "Work plan for the Test Guidelines Programme." 2016. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)6&doclanguage=en.

ENDOCRINE DISRUPTION

Recommendation: In light of existing non-animal methods and weight-of-evidence approaches, the use of animals in endocrine testing can be substantially reduced.

Non-Animal Approaches Available Now

ToxCast for Oestrogen and Androgen Pathways:

EPA's Toxicity Forecaster (ToxCast) uses more than 700 high-throughput screening assays, which cover a range of high-level cell responses and approximately 300 signalling pathways, and computational toxicology approaches to rank and prioritize chemicals. Data have already been generated on thousands of chemicals of interest to the EPA.

A subset of the ToxCast assays are devoted to evaluating possible effects on various hormone pathways including the oestrogen, androgen, and thyroid systems. These are being used successfully in the EPA's Endocrine Disruption Screening Program (EDSP) to rank and prioritize chemicals. After a comparative study of ToxCast oestrogen pathway assay results to uterotrophic assay results (Browne, Judson and Casey), EPA has announced that it will accept ToxCast data as an alternative to at least one animal test, the uterotrophic assay that screens for effects on the oestrogen pathway (USEPA).

EPA recently published results of work using ToxCast and a computational model to screen chemicals for effects on the androgen pathway (Kleinstreuer et al. 2016) and is working on use of this method as an alternative for the rat Hershberger assay that is currently used to screen for androgen effects. Work is expected to be completed within the coming year.

Non-Animal Approaches Likely to be Available Within 2-5 years

ToxCast and other approaches for the Thyroid Pathway:

The thyroid pathway has more complexity than either the oestrogen or androgen pathways. Though ToxCast is showing promising results, more research is required in this area and use of this system to replace tests in animals is still several years away. There are complementary efforts at the international level. An OECD scoping document for *in vitro* approaches to the

thyroid signalling pathway was published in 2014 (OECD). The OECD Molecular Screening Group's *in vitro* Thyroid Subgroup is working to bring relevant *in vitro* thyroid assays to the attention of OECD member countries and provide recommendations for their development / use.

More research and development is needed in this area to obtain non-animal approaches to screening for thyroid disruption potential in humans and wildlife populations.

Cited References:

Browne, P, et al. "Screening chemicals for estrogen receptor bioactivity using a computational model." *Environmental Science and Technology* 49 (2015): 8804-8814. 61

Kleinstreuer, N et al. "Development and Validation of a Computational Model for Androgen Receptor Activity" Chem. Res. Toxicol., Just Accepted Manuscript • DOI: 10.1021/acs.chemrestox.6b00347 • Publication Date (Web): 18 Nov 2016.

OECD. "New scoping document on in vitro and ex vivo assays for the identification of modulators of thyroid hormone signaling, Series on Testing and Assessment No. 207." 2014. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)23&doclanguage=en.

USEPA. *Use of high throughput assays and computational tools; Endocrine Disruptor Screening Program.* 2015. https://www.epa.gov/endocrine-disruption/use-high-throughput-assays-and-computational-tools-endocrine-disruptor.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY

Recommendation: Immediately fund and support the development of innovative non-animal methods for assessing developmental and reproductive toxicity.

Reproductive and developmental toxicity testing is not just one of the most animal intensive areas of regulatory toxicology, where just one test can consume more than a thousand animals, but it is also time- and cost-intensive. None of the *in vivo* methods used for testing reproductive and developmental toxicity have been validated for their relevance to humans (Rovida, Longo and Rabbit). There are considerable limitations surrounding the *in vivo* methods, with a predictivity of only around 60 per cent and large interspecies variations (Hartung; Bouvier d'Yvoire, Bremer and Casati).

There are many promising ongoing efforts within the area of development and reproductive toxicity, and as such, it is important that the European Commission maintains contact with the relevant institutes and organisations involved. In particular, EU-ToxRisk is an integrated European 'Flagship' program-driving mechanism-based toxicity testing and risk assessment for the 21st Century (EU-ToxRisk).

Additionally, the EPA is moving forward with 21st century toxicology methods to screen and prioritize chemicals for endocrine-disrupting potential (see above section) (EDSP). Data from this effort should be used along with other existing data to evaluate aspects of reproductive toxicity in a weight of evidence approach (Martin, Knusden and Reif).

Non-Animal Approaches Likely to be Available within 5-10 years

EURL ECVAM has investigated the validation of *in vitro* reproductive toxicity test methods and is leading the development of an AOP for an aspect of reproductive toxicity, i.e. PPARγ activation leading to impaired fertility (Rolaki, Nepelska and Bremer; AOP Wiki). The EU FP6 project, ReProTect, has also investigated possible strategies to cover the entire mammalian reproductive cycle, resulting in a series of published works (ReProTect). Furthermore, the ChemScreen FP7 project has been designed to generate a rapid screening system that is relatively simple and cost-effective (van der Burg, Bay Wedebye and Dietrich). The US EPA's National Center for Computational Toxicology is also exploring *the potential for chemicals to disrupt prenatal development through the use of its v*irtual embryo model, v-Embryo™ which integrate *in vitro* and *in silico* modelling approaches (USEPA). While the field is gradually moving toward integrated testing and assessment strategies in order to cover the majority of possible mechanisms driving a broader range of potential adverse outcomes, the European Commission could accelerate work in this area by promoting and funding projects aimed at developing additional AOPs of developmental and reproductive toxicity, developing rapid validation testing procedures and by promoting regulatory acceptance of non-animal testing strategies.

Cited References:

AOP Wiki. *Aromatase (Cyp19a1) reduction leading to impaired fertility in adult female*. 2016. https://aopkb.org/aopwiki/index.php/Aop:7.

Bouvier d'Yvoire, J P, et al. "ECVAM and new technologies for toxicity testingECVAM and new technologies for toxicity testing. In: New Technologies for Toxicity Testing, eds Balls M, et al." *Adavnces in Experimental Medicine and Biology* 745 (2012): 154-180.

EDSP. *Comprehensive Management Plan*. 2014. http://www.epa.gov/endo/pubs/EDSP_Comprehesive_Management%20Plan_%20021414_f.pdf.

EU-ToxRisk. 2016. http://www.eu-toxrisk.eu/.

Hartung, T. "Toxicology for the twenty-first century." *Nature* 460 (2009): 208-212.

Martin, M T, et al. "Predictive model of rat reproductive toxicity from ToxCast high throughput screening." *Biology of Reproduction* 85 (2011): 327-339.

ReProTect. *Development of a Novel Approach in Hazard and Risk Assessment or Reproductive Toxicity by a Combination and Application of In Vitro, Tissue and Sensor Technologies 2009*. 2009. https://ec.europa.eu/research/endocrine/pdf/reprotect_final_report_summary.pdf.

Rolaki, A, et al. "Reproductive toxicity – effects on fertility and developmental toxicity. In: JRC Science and Policy Reports: Alternative Methods for Regulatory Toxicology: A State-of-the-Art Review, eds Worth, A., et al." 2014. https://echa.europa.eu/documents/10162/13634/echa_jrc_sla_report_en.pdf.

Rovida, C, F Longo and R R Rabbit. "How are reproductive toxicity and developmental toxicity addressed in REACH dossiers?" *ALTEX* 28 (2011): 273-294.

USEPA. *Virtual Tissue Models: Predicting How Chemicals Impact Development*. 2016. https://www.epa.gov/chemical-research/virtual-tissue-models-predicting-how-chemicals-impact-development.

van der Burg, B, et al. "The ChemScreen project to design a pragmatic alternative approach to predict reproductive toxicity of chemicals." *Reproductive Toxicology* 55 (2015): 114-123.

EXPOSURE-BASED ASSESSMENT

Recommendation: Immediately promote the use of exposure-based waiving as an opportunity to dramatically reduce the use of animals.

Non-Animal Approaches Likely to be Available within 5-10 years

This approach to reducing animal testing focuses on shifting from hazard-based approaches to exposure-driven approaches for regulatory decision making. It promotes "fit-for-concern" assessments rather than "box-checking" regulatory testing approaches to human safety assessment. It explores safety based on real concern rather than characterizing hazard(s) that will not be relevant for human safety assessment. There is also movement in the pesticide industry to explore ways to further the approach of exposure-based assessment efforts.

Further work and collaboration by all involved stakeholders will be necessary to determine if exposure-based waiving can be accepted and approved by regulatory authorities and the public.