Non-Animal Testing: It's Within REACH

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Introduction

REACH contains measures designed to establish and enforce the principle that animals must be used only as a last resort. Adapting standard information requirements using alternative methods can provide an assessment of a toxicity endpoint that is more predictive of human health effects than animal testing, reduces the number of animals used and fosters efficient use of resources. Here we outline strategies to minimise testing on animals for REACH under Annexes VII and VIII in preparation for the 2018 deadline.

Before testing on animals, the general procedures under Annex XI of REACH for adapting the standard information requirements should be considered. These approaches include the use of physicochemical data, QSARs, read-across, consideration of all existing data and the development of a weight of evidence approach. Furthermore, testing can be waived if it is technically not possible to perform or, in some cases, based on the exposure scenario for the chemical. ECHA's "Practical Guide 10: How to Avoid Unnecessary Testing on Animals"[1] should be consulted.

Skin Irritation and Corrosion

- OECD-accepted *in vitro* methods can be used in an integrated approach to testing and assessment (IATA) to predict skin irritation and corrosion for most chemical classes and, importantly, to predict non-irritancy.
- *In vivo* testing for skin irritation/corrosion is no longer a standard requirement (the updated Annexes are due to be published at the end of May or the beginning of June 2016).[2]

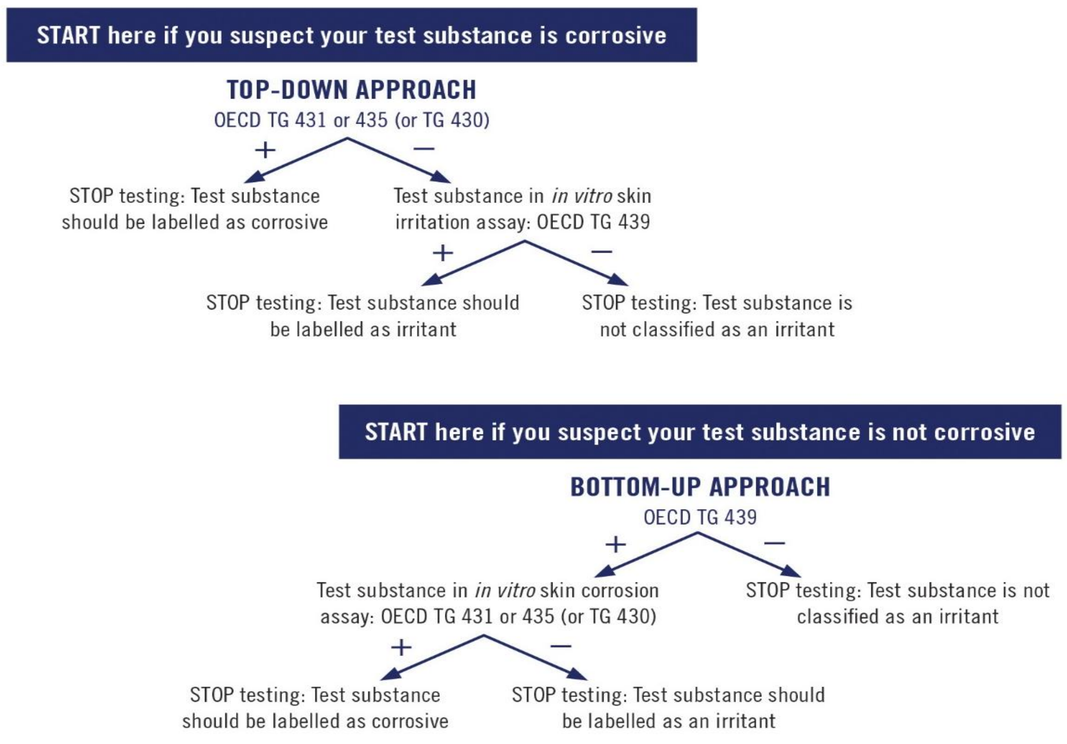

Figure 1. *In vitro* skin corrosion/irritation testing strategy[3]

Repeated Dose and Reproductive Toxicity

- If screening is required by regulators but repeated dose data are available, conduct the OECD TG 421 reproduction/developmental toxicity screening test.
- If both repeated dose and reproductive toxicity studies are required by regulators, conduct the combined study (OECD TG 422).

Severe Eye Damage and Eye Irritation

- *In vitro* methods used in a bottom-up and top-down approach can replace *in vivo* testing.
- *In vivo* testing for severe eye damage and irritation is no longer a standard requirement. (the updated Annexes are due to be published at the end of May or the beginning of June 2016).[2]
- See EURL ECVAM's "Alternative Methods for Regulatory Toxicology – a State-of-the-Art Review".[4]

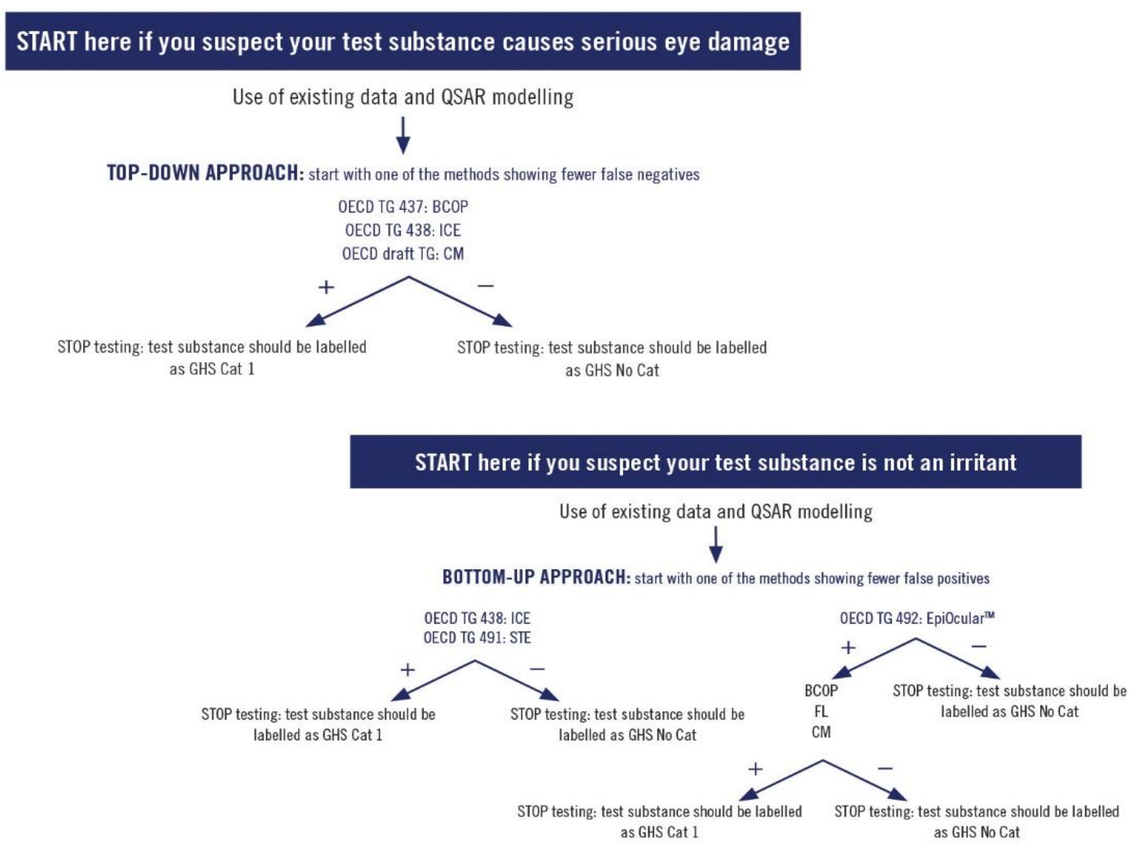

Figure 2. Top-down and bottom-up testing strategies for serious eye damage/eye irritation.

Skin Sensitisation

- OECD-adopted *in vitro* and *in chemico* methods predict key steps in the OECD adverse outcome pathway[5] (AOP) and should be used in an IATA to predict skin sensitisation.
- Updated REACH Annexes including the *in vitro* and *in chemico* methods will be published in September 2016.[6]
- The human cell line activation test was adopted by the OECD in April 2016 and will be published later in the year.

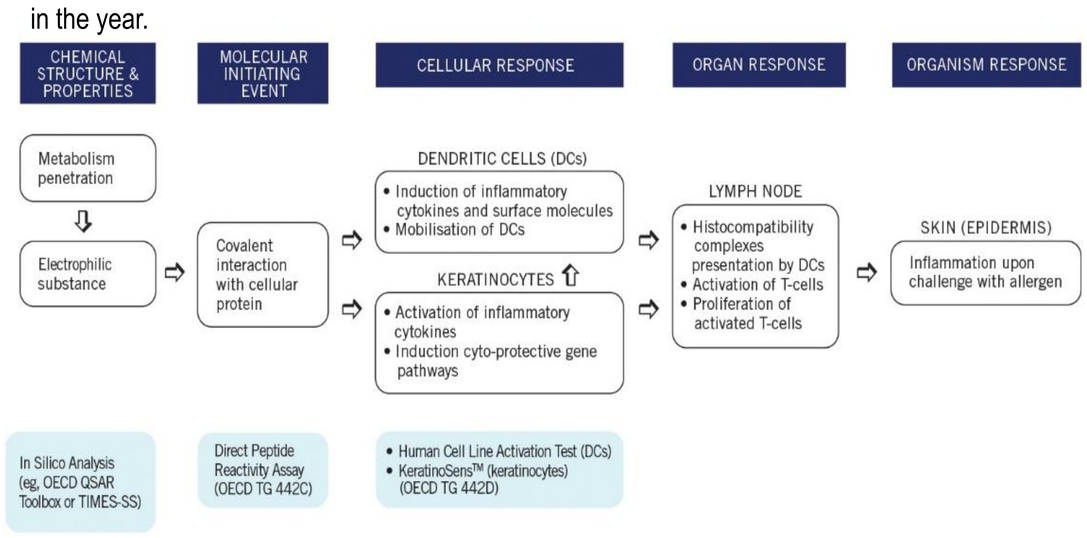

Figure 3. AOP for skin sensitisation initiated by covalent binding to proteins and non-animal methods to predict key events.

Mutagenicity

- Approved *in vitro* methods include OECD TG 471, 473, 476, 487 and 490.
- If *in vitro* testing triggers *in vivo* mutagenicity testing, submit a testing proposal to ECHA.
- If *in vivo* testing is required by regulators, tests should be combined.

Acute Toxicity

All: ensure that all modern toxicology approaches[7] and strategies to waive testing are considered.[8,9]

Oral:

Use the 3T3 neutral red uptake (NRU) cytotoxicity test in a weight of evidence approach to predict nontoxic chemicals and support read-across arguments.[10] If *in vivo* acute oral toxicity testing is required by regulators, use the 3T3 NRU assay to set starting doses (OECD GD 129). See OECD GD 24 for a comparison of OECD acute toxicity tests, and consult OECD GD 19 on humane endpoints.

Dermal:

Substances that do not meet the criteria for classification as acutely toxic or specific target organ toxicity, single exposure (STOT SE), by the oral route do not require testing for acute dermal toxicity as a second route of administration (the updated REACH Annex is due to be published at the end of May or the beginning of June 2016).[2] If dermal toxicity testing is required, consider whether testing can be waived based on low dermal penetration[11] (OECD TG 428 and OECD GD 28).

Inhalation:

Consider waiving based on substance vapour pressure and whether aerosols, particles or droplets are an inhalable size. If inhalation toxicity testing is required, use the acute toxic class method (OECD TG 436) instead of OECD TG 403.

Short-Term Aquatic Toxicity

- Acute toxicity testing using adult fish is not always required for REACH; the fish embryo toxicity test (FET; OECD TG 236) can be used if the chemical is within the applicability domain.
- If acute fish toxicity according to TG 203 is required, use the threshold approach.[12]

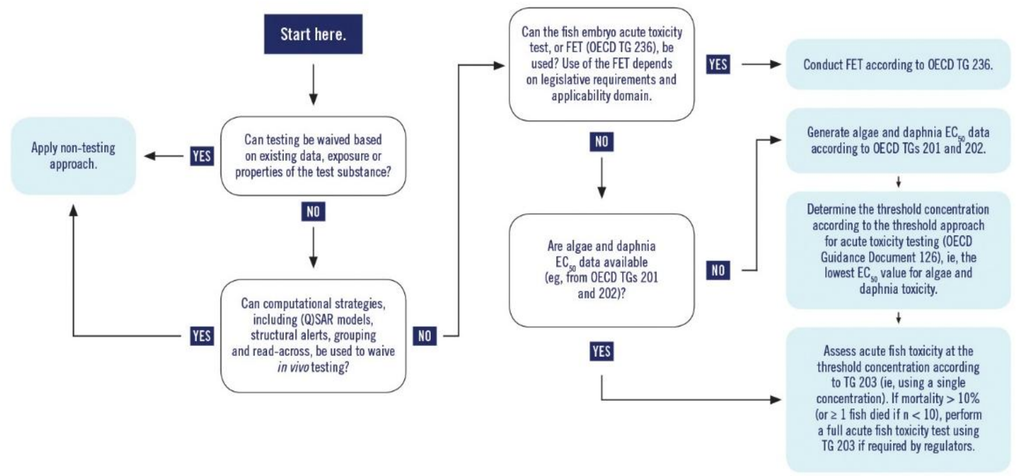

Figure 4. Strategy to minimise the number of fish used to assess short-term aquatic toxicity

Key Messages

- IATA provide opportunities for minimising the use of animals and foster the efficient use of resources.
- IATA are available or in development for many REACH Annex VII and VIII endpoints.
- Registrants are legally obligated to consider whether animal testing can be omitted using general adaptations under Annex XI of REACH.
- If adaptation is not possible and a new animal test is explicitly required by regulators, use the least severe test with the fewest animals possible.

References and abbreviations are available at PISCLtd.org.uk.

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