HOW TO MINIMISE ANIMAL TESTING FOR REACH ANNEX VII AND VIII ENDPOINTS

Skin irritation/ corrosion	May 2016 updates to Annex VIII state that *in vivo* testing for skin irritation/corrosion is required only when *in vitro* studies are not applicable or the results of the *in vitro* studies are not adequate for classification and labeling. *In vivo* testing is a Column 2 adaptation and not a standard information requirement.[1] If testing is required, registrants should use OECD-accepted *in vitro* methods using an integrated approach to testing and assessment (IATA)[2] to predict skin irritation, corrosion and non-irritancy. OECD test guidelines (TGs) include: • OECD TG 430: *In vitro* transcutaneous electrical resistance test method for skin corrosion[3] • OECD TG 431: *In vitro* reconstructed human epidermis test method for skin corrosion[4] • OECD TG 435: *In vitro* membrane barrier test method for skin corrosion[5] • OECD TG 439: *In vitro* reconstructed human epidermis test method for skin irritation[6]
Serious eye damage/ eye irritation	May 2016 updates to Annex VIII state that *in vivo* testing for serious eye damage or eye irritation is required only if the *in vitro* studies are not applicable, the results obtained from the studies are not applicable, or the results obtained from the *in vitro* studies are not adequate for classification or labeling. *In vivo* testing is a Column 2 adaptation and not a standard information requirement.[7] If testing is required, registrants should apply a top-down or bottom-up approach[8] using OECD-accepted *in vitro* methods in an integrated testing strategy to eliminate animal testing for serious eye damage and non-irritancy. OECD TGs include: • OECD TG 460: Fluorescein leakage test method for identifying ocular corrosives and severe irritants[9] • OECD TG 437: Bovine corneal opacity and permeability test method[10] • OECD TG 438: Isolated chicken eye test method[11] • OECD TG 491: Short time exposure *in vitro* test method for identifying i) chemicals inducing serious eye damage and ii) chemicals not requiring classification for eye irritation or serious eye damage[12] • OECD TG 492: Reconstructed human cornea-like epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage[13] • OECD draft TG: The cytosensor microphysiometer test method: an *in vitro* method for identifying ocular corrosive and severe irritant chemicals as well as chemicals not classified as ocular irritants[14,15]
Acute systemic toxicity	Studies do not generally need to be conducted if the substance is classified as corrosive to the skin. Registrants should use the 3T3 neutral red uptake (NRU) cytotoxicity[16] test to predict non-toxic chemicals. Consult ECHA's Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance[17] for information on using a weight-of-evidence approach, which includes the use of existing data, exposure information, read-across, *in silico* methods, and/or *in vitro* methods, to fulfil data requirements for acute systemic toxicity. If 28-day oral toxicity test data exist or registrants are required to conduct this testing, registrants should use the results in a weight-of-evidence approach for waiving acute toxicity when the substance is anticipated to be non-toxic (as per Annex XI, section 1.2).[18] Substances that do not meet the criteria for classification as acutely toxic or toxic to a specific target organ with single exposure (STOT ET) by the oral route no longer require testing for acute dermal toxicity as a second route of administration.[19] Testing can also be waived if the substance is not permeable to the skin.[20] Registrants should use the OECD QSAR toolbox[21] to fill any data gaps and use results from the 3T3 NRU to support read-across. If an *in vivo* test is perceived to be required, 3T3 NRU test results should be used to set starting doses[22] using the most relevant route.

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Mutagenicity	If *in vitro* testing under Annex VIII triggers *in vivo* mutagenicity testing, registrants must submit a testing proposal for prior approval by ECHA. *In vitro* OECD TGs include: • OECD TG 471: Bacterial reverse mutation (Ames) test[23] • OECD TG 476: *In vitro* cell gene mutation test in mammalian cells[24] • OECD TG 473: *In vitro* chromosomal aberration test in mammalian cells[25] • OECD TG 487: *In vitro* mammalian cell micronucleus test[26] • OECD TG 490: *In vitro* mammalian cell gene mutation assays using the thymidine kinase gene[27] Registrants should use the OECD QSAR toolbox.[28]
Skin sensitisation	Annex VII was amended in September 2016 to include *in vitro* methods for skin sensitisation.[29] Registrants should use the OECD adverse outcome pathway for skin sensitisation,[30] which describes the key events in an adverse response from the molecular initiating event to adverse health effects in humans. OECD TGs include: • OECD TG 442C: *In chemico* skin sensitisation: direct peptide reactivity assay[31] • OECD TG 442D: *In vitro* skin sensitisation: ARE-Nrf2 luciferase test method[32] • OECD TG442E: *In vitro* skin sensitisation: human cell line activation test (h-CLAT)[33] • OECD draft TG: *In Vitro* Skin Sensitisation: IL-8 Luc assay[34] • OECD draft TG: *In Vitro* Skin Sensitisation: U937 Skin Sensitisation Test (U-SENS™)[35] Registrants should use the OECD QSAR toolbox[36] to fill data gaps.
Short-term aquatic toxicity	Testing does not need to be conducted if the substance is highly insoluble in water or unlikely to cross biological barriers, or if a long-term fish toxicity study is already available. If testing is required: • The fish embryo toxicity test (OECD TG 236)[37] should be used whenever possible (in a weight-of-evidence approach, if necessary) as an alternative to the fish acute toxicity test (OECD TG 203).[38] • If OECD TG 203 is required, the threshold approach[39] for acute fish toxicity testing should be used. Registrants should use the OECD QSAR toolbox[40] to fill data gaps.

For all endpoints	• Consult the ECHA Read-Across Assessment Framework,[41] which includes the scientific assessment of environmental fate and hazards of chemicals to avoid testing on animals. • Check the OECD website[42] for the most up-to-date versions of all documents. • Consider other QSAR tools on the EURL ECVAM website.[43]
Contract research organisations offering *in vitro* services	• Cyprotex, Cheshire, UK (www.cyprotex.com) • Institute for *In vitro* Sciences, Gaithersburg, MD, US (www.iivs.org) • VitroScreen, Milan, Italy (www.vitroscreen.com) • XCellR8, Cheshire, UK (www.x-cellr8.com) • MatTek Corporation, Ashland, MA, US and MatTek *In vitro* Life Science Laboratories, Bratislava, Slovak Republic (www.mattek.com)

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