GUIDANCE FOR USM IACUC COMMITTEE MEMBERS AND RESEARCHERS FOR INTERNATIONAL REGULATORY ACCEPTANCE OF ALTERNATIVES METHODOLOGY AS A REPLACEMENT TEST TO ANIMAL METHOD FOR SAFETY (TOXICITY) ASSESSMENT

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The table below includes a list of the methods for chemical safety testing that are accepted by U.S. and international regulatory authorities as replacement alternatives (*in vitro* method) to required animal tests.

TOXICITY AREA	METHOD	REGULATORY ACCEPTANCE/ENDORSEMENT & APPLICABLE REGULATIONS
Acute Dermal Systemic Toxicity	*In vitro* dermal absorption methods	U.S.: Accepted *via* OECD Test Guideline 428 (2004) EU: Accepted *via* OECD Test Guideline 428 (2004)
Acute Oral Systemic Toxicity	*In vitro* assays for paralytic shellfish toxin detection	U.S.: Receptor binding assay listed as approved method in National Shellfish Sanitation Program Guide for Control of Molluscan Shellfish (2013)
Biologics Testing	Cell-based potency assay for stability and potency of botulinum neurotoxin type A products	U.S.: Allergan, Inc., method accepted by FDA in 2011
	ELISA test for batch potency testing of erysipelas vaccines (antibody quantification)	U.S.: USDA SAM 613 (2008) EU: Published in European Pharmacopeia
	ELISA test for batch potency testing of Leptospira interrogans serovar Canicola (antigen quantification)	U.S.: USDA SAM 625 (2008, updated 2017)
	ELISA test for batch potency testing of Leptospira interrogans serovar Icterohaemorrhagiae (antigen quantification)	U.S.: USDA SAM 627 (2008, updated 2017)

	ELISA test for batch potency testing of Leptospira interrogans serovar Pomona (antigen quantification)	U.S.: USDA Supplemental Assay Method (SAM) 624 (2008, updated 2017)
	ELISA test for batch potency testing of Leptospira kirschneri serovar Grippotyphosa (antigen quantification)	U.S.: USDA SAM 626 (2009, updated 2017)
	USDA guidelines for validation of *in vitro* potency assays	U.S.: Veterinary Services Memorandum 800.112 (updated 2015): clarifies information found in 9 CFR 102.3, 9 CFR 113.8, and VS Memo 800.50.
Dermal Corrosivity and Irritation	Corrositex® *in vitro* membrane barrier skin corrosivity test	U.S.: Accepted by U.S. agencies in 1999; 49 CFR 173.137 (2011) EU: Accepted *via* OECD Test Guideline 436 (2006, updated 2015)
	EpiDerm™ *in vitro* human skin model skin corrosivity test	U.S.: Accepted *via* OECD Test Guideline 431 (2004, updated 2013, 2014, 2015, 2016); 49 CFR 173.137 (2011) EU: Accepted *via* OECD Test Guideline 431 (2004, updated 2013, 2014, 2015, 2016)
	EpiDerm™ *in vitro* human skin model skin irritation test	U.S.: Accepted *via* OECD Test Guideline 439 (2010, updated 2013, 2015) EU: Accepted *via* OECD Test Guideline 439 (2010, updated 2013, 2015)
	EpiSkin™ *in vitro* human skin model skin corrosivity test	U.S.: Accepted *via* OECD Test Guideline 431 (2004, updated 2013, 2014, 2015, 2016); 49 CFR 173.137 (2011) EU: Accepted *via* OECD Test Guideline 431 (2004, updated 2013, 2014, 2015, 2016)
	EpiSkin™ *in vitro* human skin model skin irritation test	U.S.: Accepted *via* OECD Test Guideline 439 (2010, updated 2013, 2015) EU: Accepted *via* OECD Test Guideline 439 (2010, updated 2013, 2015)
	Guidance document on an integrated approach for testing and assessment for skin corrosion and irritation	U.S.: Accepted *via* OECD Guidance Document 203 (2014)

		EU: Accepted *via* OECD Guidance Document 203 (2014)
	Rat TER *in vitro* skin corrosivity test	U.S.: Accepted *via* OECD Test Guideline 430 (2004, updated 2013, 2015) EU: Accepted *via* OECD Test Guideline 430 (2004, updated 2013, 2015)
	Reconstructed human epidermis *in vitro* test method for skin corrosivity testing	U.S.: Accepted *via* OECD Test Guideline 431 (2004, updated 2013, 2014, 2015, 2016) EU: Accepted *via* OECD Test Guideline 431 (2004, updated 2013, 2014, 2015, 2016)
	SkinEthic™ *in vitro* human skin model skin corrosivity test	U.S.: Accepted *via* OECD Test Guideline 431 (2004, updated 2013, 2014, 2015, 2016) EU: Accepted *via* OECD Test Guideline 431 (2004, updated 2013, 2014, 2015, 2016)
	SkinEthic™ *in vitro* human skin model skin irritation test	U.S.: Accepted *via* OECD Test Guideline 439 (2010, updated 2013, 2015) EU: Accepted *via* OECD Test Guideline 439 (2010, updated 2013, 2015)
Dermal Phototoxicity	3T3 NRU phototoxicity test for skin photo-irritation	U.S.: Accepted *via* OECD Test Guideline 432 (2004) EU: Accepted *via* OECD Test Guideline 432 (2004)
	3T3 NRU phototoxicity test: application to UV filter chemicals	U.S.: Accepted *via* OECD Test Guideline 432 (2004) EU: Accepted *via* OECD Test Guideline 432 (2004)
	FDA guidance on photosafety evaluation of pharmaceuticals	U.S.: Accepted *via* International Conference on Harmonisation Guideline S10 (2015) EU: Accepted *via* International Conference on Harmonisation Guideline S10 (2015)
	In vitro BG1Luc ER TA agonist assay to identify substances that induce human ER activity	U.S.: Accepted by agencies in 2012 EU: Accepted *via* OECD Test Guideline 457 (2011)

Endocrine Disruptors	*In vitro* BG1Luc ER TA antagonist assay to identify substances that inhibit human ER activity	U.S.: Accepted by agencies in 2012 EU: Accepted *via* OECD Test Guideline 457 (2011)
	In vitro H295R steroidogenesis assay	U.S.: Accepted *via* OECD Test Guideline 456 (2011) EU: Accepted *via* OECD Test Guideline 456 (2011)
	Stably transfected transactivation in vitro assay to detect estrogen receptor agonists and antagonists	U.S.: Accepted *via* OECD Test Guideline 455 (2009, updated 2012, 2015, 2016); EPA OPPTS 890.1300 (2009) EU: Accepted *via* OECD Test Guideline 455 (2009, updated 2012, 2015, 2016)
Genetic Toxicity	*In vitro* mammalian cell micronucleus test	U.S.: Accepted via OECD Test Guideline 487 (2010, updated 2014, 2016) EU: Accepted *via* OECD Test Guideline 487 (2010, updated 2014, 2016)
Immunotoxicity: Allergic Contact Dermatitis	In chemico skin sensitization test (direct peptide reactivity assay)	U.S.: Accepted *via* OECD Test Guideline 442C (2015) EU: Accepted *via* OECD Test Guideline 442C (2015)
	In vitro skin sensitization test (ARE-Nrf2 luciferase test)	U.S.: Accepted *via* OECD Test Guideline 442D (2015) EU: Accepted *via* OECD Test Guideline 442D (2015)
	In vitro skin sensitization test (human cell line activation test)	U.S.: Accepted *via* OECD Test Guideline 442E (2016, updated 2017) EU: Accepted *via* OECD Test Guideline 442E (2016, updated 2017)
Multiple Toxicities	Process for evaluating and implementing alternative approaches to traditional *in vivo* acute toxicity studies for FIFRA regulatory use	U.S.: 40 CFR 158
	Bovine corneal opacity and permeability *in vitro* test method to identify severe eye irritants/corrosives or chemicals not requiring eye hazard classification	U.S.: Accepted by agencies in 2008; 2013 update accepted *via* OECD Test Guideline 437 (2009, updated 2013, 2017) EU: Accepted *via* OECD Test Guideline 437 (2009, updated 2013, 2017)

Ocular Corrosivity and Irritation	Cytosensor microphysiometer *in vitro* test method for eye safety testing	Accepted by U.S. agencies in 2011 (OECD test guideline under consideration)
	In vitro fluorescein leakage test method for identifying ocular corrosives and severe irritants	U.S.: Accepted *via* OECD Test Guideline 460 (2012, updated 2017) EU: Accepted *via* OECD Test Guideline 460 (2012, updated 2017)
	Isolated chicken eye *in vitro* test method to identify severe eye irritants/corrosives or chemicals not requiring eye hazard classification	U.S.: Accepted by agencies in 2008; 2013 update accepted *via* OECD Test Guideline 438 (2009, updated 2013, 2017) EU: Accepted *via* OECD Test Guideline 438 (2009, updated 2013, 2017)
	Reconstructed human cornea-like epithelium test for identification of substances not requiring ocular hazard labeling	U.S.: Accepted *via* OECD Test Guideline 492 (2015, updated 2017) EU: Accepted *via* OECD Test Guideline 492 (2015, updated 2017)
	Short time exposure test for identification of ocular corrosives and substances not requiring ocular hazard labeling	U.S.: Accepted *via* OECD Test Guideline 491 (2015, updated 2017) EU: Accepted *via* OECD Test Guideline 491 (2015, updated 2017)
Pyrogen Testing	Human peripheral blood mononuclear cell/interleukin-6 *in vitro* pyrogen test	U.S.: Accepted by FDA in 2009; use addressed in June 2012 FDA guidance on pyrogen testing EU: Published in European Pharmacopeia
	Human whole blood/interleukin-1β *in vitro* pyrogen test	U.S.: Accepted by FDA in 2009; use addressed in June 2012 FDA guidance on pyrogen testing EU: Published in European Pharmacopeia
	Human whole blood/interleukin-1β *in vitro* pyrogen test: application of cryopreserved human whole blood	U.S.: Accepted by FDA in 2009; use addressed in June 2012 FDA guidance on pyrogen testing EU: Published in European Pharmacopeia
	Human whole blood/interleukin-6 *in vitro* pyrogen test	U.S.: Accepted by FDA in 2009; use addressed in June 2012 FDA guidance on pyrogen testing

		EU: Published in European Pharmacopeia
	In vitro monocyte activation type pyrogen test	U.S.: FDA guidance (2012) provides for use in place of USP methods
	Monocytoid cell line Mono Mac 6/interleukin-6 *in vitro* pyrogen test	U.S.: Accepted by FDA in 2009; use addressed in June 2012 FDA guidance on pyrogen testing EU: Published in European Pharmacopeia

Regulatory requirements on animal/non-animal testing for different sectors

PHASE NO.	STUDIES	DESCRIPTION	SECTOR
1	Animal studies	Definitive safety assessment requires the result of animal studies; non animal studies used for screening and explanation.	Agrochemical/Biocides
2	Animal studies with non-animal based study waiving	Definitive safety assessments made using animal studies, but some studies will not be required if non-animal data indicate they are not needed.	Pharmaceutical General chemicals
3	Non-animal studies with animal studies with exceptional circumstances	Definitive safety assessments will be made with non-animal studies, but some animal studies will be necessary to address uncertainty	Agrochemical/Biocides
4	Non-animal studies	Definitive safety assessment requires the result of animal studies; non animal studies used for screening and explanation.	Personal care in EU

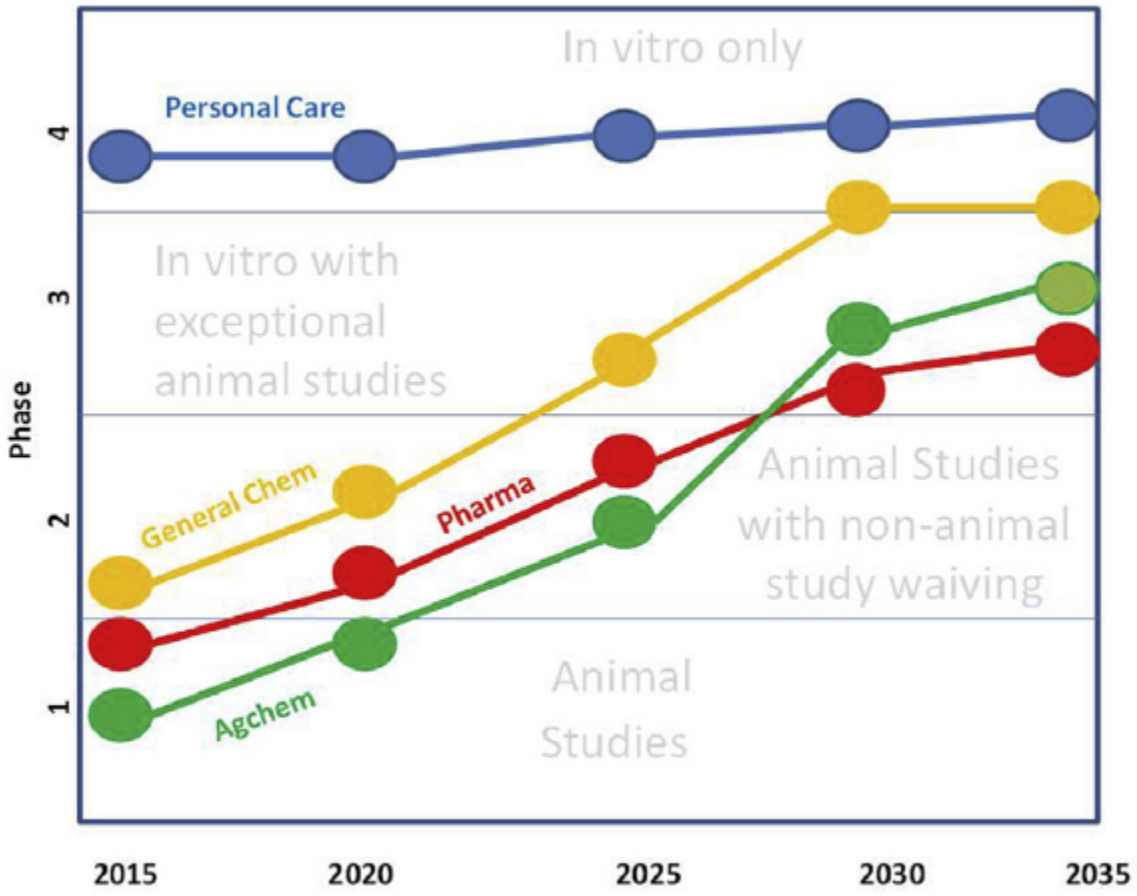

Fig. 1. Projection of rate of progress through phases towards non-animal based safety assessment for the different industry sectors.

Reference:

1. https://ntp.niehs.nih.gov/pubhealth/evalatm/accept-methods/index.html
2. F. Sewell et al (2017). Steps towards the international regulatory acceptance of non-animal methodology in safety assessment