Cross-jurisdictional Considerations for Avoiding Animal Testing When Evaluating Chemical Toxicity

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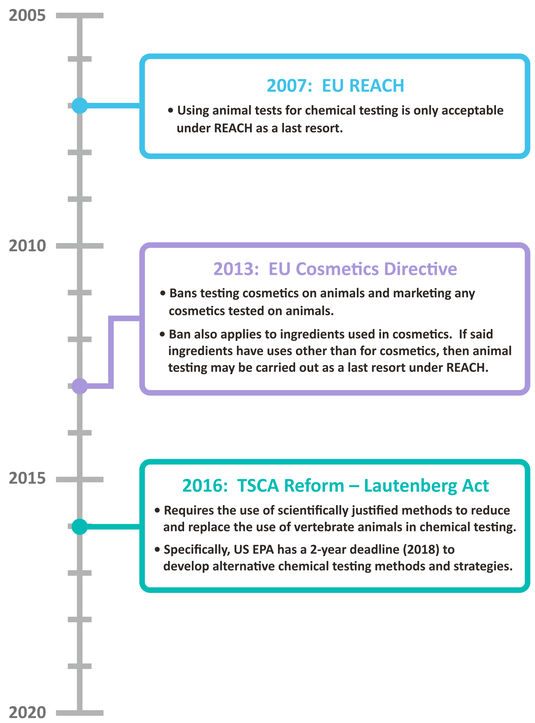
OBJECTIVE

Provide an overview of how to avoid animal testing when assessing a chemical's toxicity, to save cost and time during the chemical registration process.

BACKGROUND

In recent years, the push to use non-animal testing to assess chemical toxicity has become a focus of regulations such as the 2016 Toxic Substances Control Act (TSCA). This poster will outline the broad regulatory landscape of chemical testing requirements and acceptance of animal testing alternatives when registering new industrial chemicals with regulatory agencies around the world. Formulating a registration plan is the most important tool for executing a successful cross-jurisdictional testing strategy. When no human or animal data are readily available for a chemical, a combination of read-across, *in vitro* testing, quantitative structure-activity relationship (QSAR) models, weight-of-evidence analyses, and data waiving can be used to evaluate its potential toxicity. Understanding the methods and tools available as well as areas of collaboration (*e.g.*, data sharing) is vital to fulfilling registration data requirements while avoiding animal tests.

Figure 1 Milestones in Alternatives to Animal Testing



Notes: EU = European Union; REACH = Registration, Evaluation, Authorisation, and Restriction of Chemicals; US EPA = United States Environmental Protection Agency.

EVALUATING ALTERNATIVES TO ANIMAL TESTING

Figure 2 Testing Options

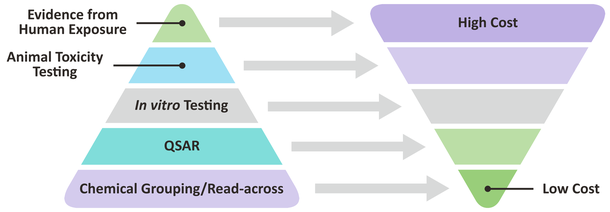


Table 1 Acceptance of Animal Testing Alternatives in New Industrial Chemical Registration

Accept	US (Lautenberg Act)	EU	Canada	China	Japan	Australia
In Vitro Data?	Yes	Yes	Yes	Yes	No[a]	Yes
QSAR?	Yes	Yes	Yes	No[b]	No[c]	Yes
Read-across?	Yes	Yes	Yes	No[b]	No[c]	Yes
Data Waiving?	Yes	Yes	Yes	Yes	No[c]	Yes

Notes: (a) Except for mutagenicity (1 of ~14 required tests). (b) Only as reference, unless testing is scientifically not feasible. (c) Except for bioaccumulation (1 of ~14 required tests).

Table 2 Cost of Animal Tests and Non-animal (*In Vitro*) Equivalents

Tests	Animal	Cost	*In Vitro*	Cost
Carcinogenicity	2-year non-genotoxic (rat)	$700,000	Syrian hamster embryo cell transformation test	$700,000
Developmental Toxicity	Embryotoxicity (rat)	$50,000	Rat limb bud test	$50,000
Skin Irritation	Draize skin test (rabbit)	$1,800	EpiDerm™ human skin model; CORROSITEX® membrane barrier	$1,800
Eye Irritation	Draize eye test (rabbit)	$1,800	Bovine corneal opacity and permeability (BCOP) test	$1,800
Mutagenicity	Bone marrow chromosome aberration (rodents)	$30,000	Micronucleus test	$30,000
Subchronic Toxicity (oral)	Repeated-dose 28-day oral toxicity (mouse)	$110,000	No *in vitro* equivalent available	
Subchronic Toxicity (inhalation)	Repeated-dose 28-day inhalation toxicity (rat)	$200,000		
Acute Toxicity (oral)	Acute toxicity (rat)	$16,000		
Acute Toxicity (dermal)	Acute toxicity (rabbit)	$4,000		
Acute Toxicity (inhalation)	Acute toxicity (rat)	$23,000		
Skin Sensitization	Guinea pig maximization test (GPMT)	$6,000		
Acute Aquatic Toxicity – Crustacean	Acute daphnia immobilization	$10,000	No *in vitro* equivalent available, but QSAR models exist	
Acute Aquatic Toxicity – Fish	Acute fish toxicity	$12,000		
Acute Aquatic Toxicity – Algae	Algae growth inhibition	$10,000		

Sources: Humane Society International (2017) and quotes provided to Gradient directly from several laboratory companies.

Table 3 Required Testing for an Industrial Chemical Manufactured or Imported at a Volume of ≥100,000 kg/year (or 100 metric tons/year)

Test	US	EU	Canada	China	Japan	Australia
Carcinogenicity	No[a]	No	No	No	Yes*	No
Reproductive Toxicity	No[a]	Yes*[,a]	No	Yes	Yes*	No
Subchronic Toxicity (28-day)	No[a]	Yes[a]	Yes[a]	Yes	Yes (must be oral)	Yes[a]
Subchronic Toxicity (90-day)	No[a]	No	No	Yes	Yes*	No
Acute Toxicity (oral, dermal, or inhalation)	No[a]	Yes[a]	Yes[a]	Yes	No	Yes[a]
Skin Irritation	No[a]	Yes[a]	Yes[a]	Yes	No	Yes[a]
Eye Irritation	No[a]	Yes[a]	No	Yes	No	Yes[a]
Skin Sensitization	No[a]	Yes[a]	Yes[a]	Yes	No	Yes[a]
Mutagenicity *In Vitro/In Vivo*	No[a]	Yes[a]	Yes[a]	Yes	Yes	Yes[a]
Biodegradation	No[a]	Yes[a]	Yes[a]	Yes[b]	Yes	Yes[a]
Bioaccumulation	No[a]	Yes*[,a]	No	Yes	Yes[a]	Yes[a]
Acute Aquatic Toxicity – Fish	No[a]	Yes[a]	Yes[a]	Yes[b]	Yes	Yes[a]
Acute Aquatic Toxicity – Crustacean	No[a]	Yes[a]	Yes[a]	Yes[b]	Yes	Yes[a]
Acute Aquatic Toxicity – Algae	No[a]	Yes[a]	Yes[a]	Yes[b]	Yes	Yes[a]
Others	No	Chronic Fish;*[,a] Chronic Crustacean*[,a]	No	Toxicokinetic; Earthworm;[a] Chronic Fish; Chronic Crustacean	Developmental;* Toxicokinetic;* Avian Reproduction*	No

Notes: Source: Ruden and Hansson (2010). = Can be waived. (a) May be informed by read-across. (b) Required to be performed by a Chinese laboratory.*

DISCUSSION

Considerations for Formulating a Non-animal Testing Plan

- What are the differences between the cost and time required for an animal test and an equivalent non-animal test?
- If a minimum set of toxicity tests is required for chemical registration, are non-animal testing methods available and allowed to be used?
- If no data are available for the substance of interest, are data-rich read-across surrogates available? Can selection of read-across surrogates and documentation be done in-house?
- Are Organisation for Economic and Co-operation Development (OECD)-validated non-animal (*in vitro*) methods available?
- Are QSAR models available and are they appropriate to use for testing the chemical of interest? For example, QSAR models are not appropriate to use for predicting the toxicity of polymers, reaction products, and Substances of Unknown or Variable Composition, Complex Reaction Products, and Biological Materials (UVCBs).
- Can a toxicity test be waived due to infeasibility, demonstrated toxicity, *etc.*? What documentation is needed to certify the claim per the registration agency?
  - Examples:
    - **Reproductive and developmental** tests can be waived if a substance is already a genotoxic carcinogen or a known germ cell mutagen.
    - **Aquatic toxicity** tests can be waived if the substance is highly insoluble.
    - **90-day subchronic repeated-dose toxicity** test can be waived if a 28-day study already showed that the chemical is toxic (and can be classified as Category 1 or 2 under the Globally Harmonized System of Classification and Labelling of Chemicals [GHS]), or if the substance undergoes immediate disintegration in water and there are sufficient data on all of the degradation products.

Figure 3 Formulate a Plan