A state-of-the-art review on the alternatives to animal testing for the safety assessment of cosmetics

Supplementary material

Table S1. Glossary of abbreviations.

3Rs	Replacement, Reduction, and Refinement
ADME	Absorption, Distribution, Metabolism, and Excretion
AMNH	American Museum of Natural History
AOP	Adverse Outcome Pathway
BCOP	Bovine Cornea Opacity Permeability
CEA	Cost-Effectiveness Analysis
CFDA	Chinese State Food and Drug Administration
CTA	Cell Transformation Assay
CTFA	Cosmetic, Toiletry and Fragrance Association
DAs	Defined Approaches
DPRA	Direct Peptide Reactivity Assay
EC	European Commission
EC JRC	European Commission's Joint Research Centre
ECHA	European Chemicals Agency
EFTA	European Free Trade Association
EPA	U.S. Environmental Protection Agency
EU	European Union
EURION	European Cluster to Improve Identification of Endocrine Disruptors
EURL ECVAM	European Centre for the Validation of Alternative Methods
FDA	Food and Drug Administration
FDP	Fixed-Dose Procedure Test
FRAME	Fund for the Replacement of Animals in Medical Experiments
GMP	Good Manufacturing Practices
GTxC	Genotoxic Carcinogens
h-CLAT	Human Cell Line Activation Test
HTS	High-Throughput Screening
IATA	Integrated Approach for Testing and Assessment
ICATM	International Cooperation on Alternative Test Methods
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Isolated Chicken Eye
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ITS	Integrated Testing Strategy
JaCVAM	Japanese Centre for the Validation of Alternative Methods

LD50	Median Lethal Dose
LOAEL	Lowest Observed Adverse Effect Level
MNvit	in vitro Micronucleus Test
MoA	Mode-of-Action
MoS	Margin of Safety
NAM	New Approach Methodology
NGOs	Non-Governmental Organizations
NGRA	Next Generation Risk Assessment
NGTxC	Non-Genotoxic Carcinogens
NOAEL	No-Observed-Adverse-Effect Level
NRU	3T3 Neutral Red Uptake test
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OI	Ocular Irritation
PBTK	Physiologically Based Toxicokinetics
PoD	Point of Departure
(Q)SAR	(Quantitative) Structure-Activity Relationship
RCB	Rodent Carcinogenicity Bioassay
REACH	Registration, Evaluation, Authorisation and Registration of Chemicals
RhCE	Reconstructed Human Cornea-like Epithelium
RHE	Reconstructed Human Epidermis
RhT	Reconstructed Human Tissue
ROS	Reactive Oxygen Species
RSMN	Reconstructed Human Skin Micronucleus
SAR	Structure-Based Approaches
SCCS	Scientific Committee on Consumer Safety
SEURAT	Safety Evaluation Ultimately Replacing Animal Testing
SHE	Syrian Hamster Embryo
STE	Short Time Exposure
STSC	Safety and Technical Standards for Cosmetics
Tox21	Toxicology in the 21st Century Program
TSAR	Tracking System for Alternative Methods Towards Regulatory Acceptance
UDP	Up-and-Down Procedure
UV-Vis	Ultraviolet Radiation/Visible Light
WoE	Weight of Evidence

Table S2. Examples of QSAR computer programmes for cosmetic-relevant endpoints (not a complete list of available programmes, nor a list of regulatory verified QSAR models), modified from [86].

Toxicological Endpoint	Software Tool	Models/Modules
Acute toxicity	Danish QSAR Database (DTU)	Models for acute toxicity in rodents from ACD/Labs
	T.E.S.T. (US EPA)	Oral rat LD50
	ACD/Percepta (ACD/Labs)	Acute Toxicity Module
	ADMET Predictor (Simulations Plus)	Toxicity module
	CASE Ultra (MultiCASE)	AcuteTox model bundle
	Discovery Studio (Accelrys)	Rat oral LD50 and rat inhalation toxicity LC50
Skin irritation or skin corrosion	Danish QSAR Database (DTU)	Skin irritation model
	OECD QSAR Toolbox	Skin irritation/corrosion Inclusion (and Exclusion) rules by BfR
	ToxTree (JRC)	Skin irritation / skin corrosion
	ACD/Percepta (ACD/Labs)	Irritation Module
	CASE Ultra (MultiCASE)	SkinEye Toxicity model bundle
	Derek (Lhasa)	Irritation (of the skin) alerts
	Discovery Studio (Accelrys)	Skin irritancy
Eye irritation	OECD QSAR Toolbox	Eye irritation/corrosion Inclusion (and Exclusion) rules by BfR
	ToxTree (JRC)	Eye irritation and corrosion
	ACD/Percepta (ACD/Labs)	Irritation Module
	CASE Ultra (MultiCASE)	SkinEye Toxicity model bundle
	Derek (Lhasa)	Irritation (of the eye) alerts
	Discovery Studio (Accelrys)	Ocular irritancy
Skin sensitisation	Danish QSAR Database (DTU)	Allergic Contact Dermatitis model
	OECD QSAR Toolbox	Protein binding alerts for skin sensitisation by OASIS
	ToxTree (JRC)	Skin sensitisation reactivity domains
	VEGA (IRFMN)	CAESAR model
	ACD/Percepta (ACD/Labs)	Irritation Module
	CASE Ultra (MultiCASE)	SkinEye Toxicity model bundle
	Derek (Lhasa)	Skin sensitisation
	Discovery Studio (Accelrys)	Skin sensitization
	TIMES (LMC)	Skin sensitization with autoxidation
Repeated dose toxicity	ADMET Predictor (Simulations Plus)	Toxicity module
	CASE Ultra (MultiCASE)	Several model bundles associated with repeated dose toxicity
	Derek (Lhasa)	Several endpoints associated with repeated dose toxicity
	Discovery Studio (Accelrys)	Rat Chronic (Oral) LOAEL
	Leadscope	Several models associated with repeated dose toxicity
In vitro gene mutation in bacteria (Ames test)	Danish QSAR Database (DTU)	Models for Ames test
	OECD QSAR Toolbox	Several profilers (alerts) associated with this endpoint
	T.E.S.T. (US EPA)	Mutagenicity
	ToxTree (JRC)	*In vitro* mutagenicity (Ames test) alerts by ISS
	VEGA (IRFMN)	CAESAR, SarPy/IRFMN, ISS and KNN/Read-Across models

	ACD/Percepta (ACD/Labs)	Genotoxicity Module
	CASE Ultra (MultiCASE)	Bacterial mutagenicity model bundle
	Derek and Sarah (Lhasa)	Mutagenicity *in vitro*
	Discovery Studio (Accelrys)	Ames Mutagenicity
	Leadscope	Genetox Expert Alerts Suite and Non-human Genetic Toxicity Suite
	TIMES (LMC)	Ames mutagenicity
Mutagenicity (other endpoints than in vitro gene mutation in bacteria)	Danish QSAR Database (DTU)	Models for genotoxicity endpoints
	OECD QSAR Toolbox	Several profilers (alerts) associated with mutagenicity
	ToxTree (JRC)	Several decision trees associated with mutagenicity
	CASE Ultra (MultiCASE)	EcoTox model bundle
	Derek (Lhasa)	Chromosome damage *in vitro*
	Leadscope	Non-human Genetic Toxicity Suite
	TIMES (LMC)	Several models associated with mutagenicity
Reproductive toxicity	Danish QSAR Database (DTU)	Models for Endocrine endpoints and model for Teratogenic Potential in Humans
	VEGA (IRFMN)	CAESAR and PG models
	ADMET Predictor (Simulations Plus)	Toxicity module
	CASE Ultra (MultiCASE)	Several model bundles associated with reproductive and developmental toxicity
	Derek (Lhasa)	Several endpoints associated with reproductive toxicity
	Discovery Studio (Accelrys)	Developmental Toxicity Potential
	Leadscope	Several models associated with reproductive and developmental toxicity
	TIMES (LMC)	Androgen, AHR and Estrogen (receptor) binding affinity models

Table S3. Acute toxicity testing waiver criteria [93].

Toxicological Endpoint	Waiver Criteria
Acute Oral Toxicity	- Human exposure is limited/not significant or technically not feasible (e.g., the test substance is a gas/vapour at ambient temperature, too large to be ingested, etc.). - The test chemical is corrosive to the skin (GHS Category 1), based on validated/accepted *in vivo*/*in vitro*/other data, or if the substance has a pH ≤ 2 or ≥ 11.5 (alongside high buffering capacity). - The oral LD50 of the substance is predicted to be >2000mg/kg bw based on results of a validated/accepted NAM or test battery.
Acute Dermal Toxicity	- The test chemical is found to be corrosive or severely irritating to the skin (GHS Category 1), based on validated/accepted *in vivo*/*in vitro*/other data, or if the substance has a pH ≤ 2 or ≥ 11.5 (alongside high buffering capacity). - Dermal exposure is unlikely due to product design. - The test chemical showed no adverse effects in an acute oral toxicity test up to 2000 mg/kg bw, as it is rare for a dermal test to generate a more severe classification. Under the same premise, a waiver may be considered if the oral LD_{50} is < 300 mg/kg bw. - The oral LD50 is between 300-2000 mg/kg bw, and dermal penetration data of the test chemical indicates low dermal absorption (<10%) when compared to oral absorption. The dermal-equivalent value of the oral LD_{50} would be 3000 mg/kg bw (300 mg/kg bw [oral value] ÷ 0.1 [10% dermal absorption]).
Acute Inhalation Toxicity	- The test chemical is of low volatility, is not aerosolized, heated, evaporated, or otherwise made inhalable as a gas/vapor (under predicted use/storage/handling/transport conditions). - The test chemical is too large to be inhaled or does not readily crumble into inhalable particles. - An aerosol will be considered non-inhalable if >99% of particles by mass are >100 μm of diameter during human exposure. - Due to significant toxicokinetic reasons as to why inhalation of a chemical may lead to acute toxicity, a waiver may be considered for test chemicals that are classified as GHS Category 1 or 2 for acute or dermal toxicity. As there is no difference in labelling for these categories in terms of inhalation hazard, further animal testing to refine the classification is not needed.

Table S4. Skin irritation/corrosion testing waiver criteria [93].

Toxicological Endpoint	Waiver Criteria
Skin Irritation/Corrosion	- Results of in vitro test methods that are validated/met regulatory acceptance are sufficient to draw an appropriate conclusion. - Test chemical is considered corrosive to skin through evaluation of existing *in vivo*, *in vitro*, or other data, or has a pH ≤ 2 or ≥ 11.5 (alongside high buffering capacity). - Test chemical is spontaneously flammable in air or water at ambient temperature. - Test chemical has been classified as an acute dermal hazard. - Test chemical cannot feasibly be made into an accessible format for a skin corrosion/irritation test. - For end-use products that contain strong dyes or pigments (which can interfere with data interpretation), an HPLC/UPLC-spectrophotometry procedure may be used instead of a standard absorbance (OD) measurement, to address colour interference. The colourant may also be removed from the product if there is evidence that the colourant is not an irritant.

Table S5. Serious eye damage/irritation testing waiver criteria [93].

Toxicological Endpoint	Waiver Criteria
Serious eye damage/irritation	- Results of in vitro test methods that are validated/met regulatory acceptance are sufficient to draw an appropriate conclusion. - Test chemical is considered corrosive or irritant to skin through evaluation of existing *in vivo*, *in vitro,* or other data, or has a pH ≤ 2 or ≥ 11.5 (alongside high buffering capacity). - Test chemical is spontaneously flammable in air or water at ambient temperature. - Test chemical has been classified as an acute dermal hazard. - Test chemical cannot feasibly be made into an accessible format for a serious eye damage/irritation test. - Test chemical is composed of granules or pellets that are too large/non-friable (cannot be lodged in the eye), and the material retains its original form throughout application.