Skin Irritation and Corrosion Webinar

11 November 2014, 4pm GMT

PETA INTERNATIONAL SCIENCE CONSORTIUM, LTD.

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Upcoming Webinars

Webinar 3: Serious Eye Damage and Eye Irritation

December 4, 2014

11am ET, 4pm GMT

Kim Norman, Institute for In Vitro Sciences

João Barroso, EURL ECVAM

Webinar 4: January 2015: Skin sensitization

Webinar 5: February 2015: Mammalian acute toxicity (3T3 neutral red assay)

Webinar 6: March 2015: Ecotoxicity (fish embryo test)

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Skin Irritation/Corrosion Webinar

IIVS
Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

Gertrude-Emilia Costin, Ph.D., M.B.A.
Institute for In Vitro Sciences, Inc. (IIVS)
Gaithersburg, MD, USA
http://www.iivs.org
ecostin@iivs.org
01-301-947-6524

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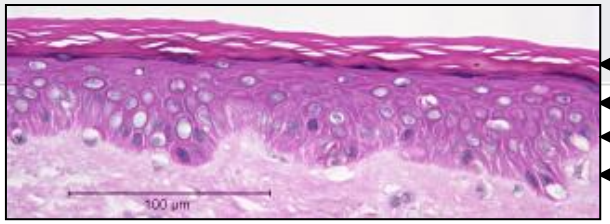
Overview

1. The impact of test substances on human skin
2. The animal test system used for skin corrosion and irritation assessment
3. Evaluation of skin corrosion and irritation potential using *in vitro* assays
4. Conclusions – *in vitro* assays validated for regulatory purposes (skin corrosion and irritation endpoints)

1. The impact of test substances on human skin

Skin corrosive/irritant

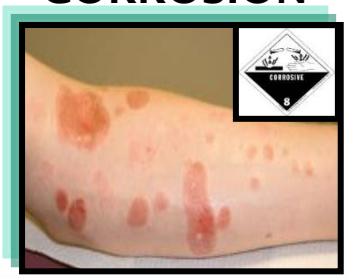
- Vast physical barrier against mechanical, chemical and microbial factors
- Immune network
- Unique defense system against UV irradiation



Stratum corneum
Stratum granulosum
Stratum spinosum
Basal layer of dividing keratinocytes

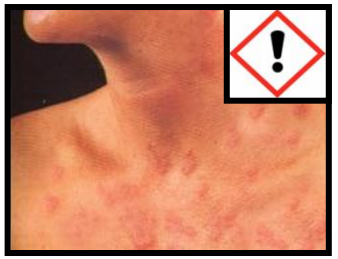
Native human skin

CORROSION



- Irreversible damage of the skin following exposure to a test substance
- Visible necrosis through the epidermis and into dermis - macroscopically typified by ulcers, bleeding, etc.

IRRITATION



- Reversible damage of the skin following exposure to a test substance
- Characterized macroscopically by erythema (redness) and oedema
- Damage to keratinocytes and dermal cells leads to inflammation

- **Registration and labelling of chemicals**
- **Transport of chemicals**
- **Occupational safety**
- **Safety of cosmetics, toiletries and household products**

2. The animal test system used for skin corrosion and irritation assessment

Acute dermal corrosion and irritation animal test (Draize)

Brief overview and current regulatory status

- **Test system**: albino rabbits
- **Assay endpoint**: erythema and eschar formation
- oedema formation
- **Assay control**: untreated skin areas of the test animal

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- **Applicability**: evaluation of the corrosion and irritation potential of test substances
- **Limitations**:
  The rabbit and human skin have different physiological properties and responses to test substances which may be more toxic to rabbits than to humans and vice versa.

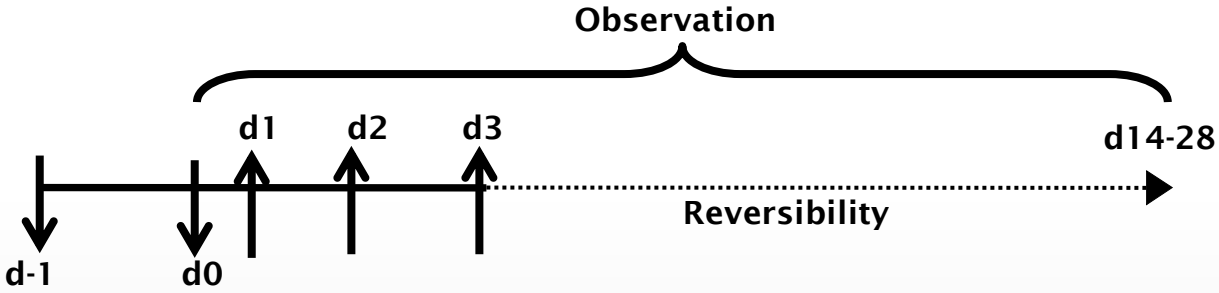
  The Draize rabbit test has been criticized for over-prediction of human skin irritation.

  A debated ethical issue of the *in vivo* test concerns the animals' suffering and discomfort.

  .....
- **Regulatory status**: OECD Test Guideline 404 (TG 404) (updated 24 April 2002)

Draize: Typical protocol

- **Animals:**1-3 rabbits (sequential testing)
- **Test substance:** solid or liquid applied on 6 cm² skin surface
- **Exposure**: 3 minutes, 1 hr or 4 hrs (skin corrosion)
  times 4 hrs (skin irritation)



Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4

Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges or area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure	4

*Draize J. H., Woodard G., Clavery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes, J. Pharmacol. Exp. Ther., **82**, 377-390 (1944).*

3. Evaluation of skin corrosion and irritation potential using *in vitro* assays

❖ *General considerations*

Assessment of skin corrosion and irritation

- The impact of test substances on skin is evaluated progressively from corrosion and irritation potential to human compatibility
- Shortest tolerated exposures are typical for corrosive or severe irritants
- Various assays are available depending on the purpose of the testing

↓

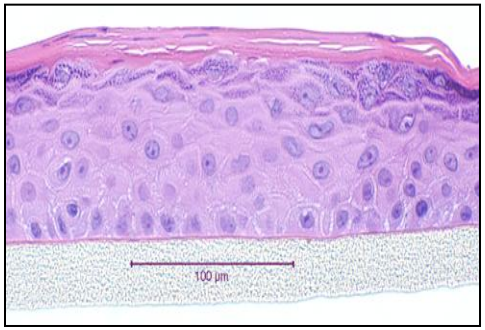
In vitro assays validated for regulatory purposes	OECD TG
SKIN CORROSION	
• Transcutaneous Electrical Resistance Test (TER) • Reconstructed Human Epidermis (RHE) Test Method • Membrane Barrier Test Method (Corrositex®)	430 431 435
SKIN IRRITATION	
• RHE Test Method (SIT)	439

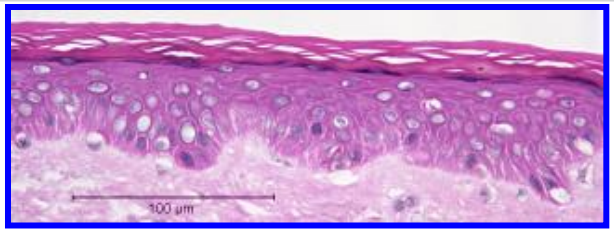
↓

Hazard identification and labelling of chemicals and finished products
Transportation of dangerous goods like industrial chemicals (neat or diluted) and their mixtures

In vitro three dimensional (3-D) reconstructed human epidermis (RHE) models validated for regulatory purposes

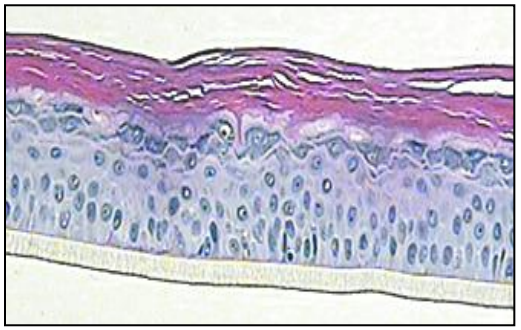
EpiDerm™ (EPI-200)



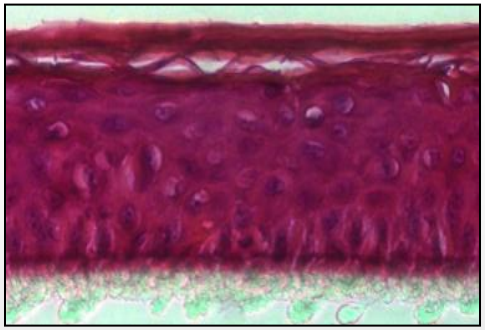


Native human skin

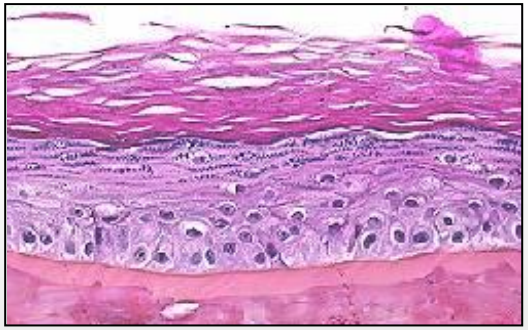
SkinEthic™ RHE



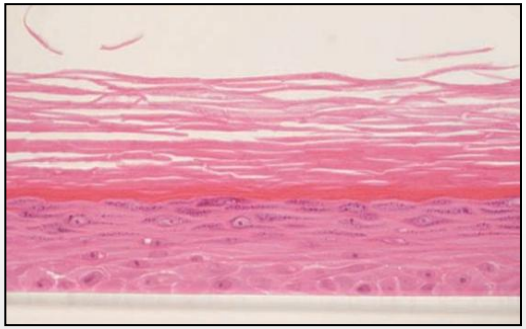
epiCS®



EpiSkin™ (SM)



LabCyte EPI-MODEL



1. **General model criteria**
   - Human keratinocytes are used for construction of the models
   - Multiple layers of viable epithelial cells

2. **Functional model criteria**
   - Tissues must be viable (QC from manufacturer, internal controls)
   - *Stratum corneum* must form sufficient barrier
   - RHE models should exhibit long term reproducibility

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3. Evaluation of skin corrosion and irritation potential using *in vitro* assays

❖ ***Assay specific considerations***

***In vitro* skin corrosion**

Transcutaneous Electrical Resistance Test (OECD 430)

Brief overview and current regulatory status

- **Test system**: rat skin discs (dorso-lateral, 20 mm each, tested in triplicate from the same animal) - from humanely killed rats aged 28-30 days; Wistar-derived or comparable strain
- **Assay endpoint**: electrical impedance of the skin expressed as a transcutaneous electrical resistance (TER) value in kilo Ohms (kΩ) – measure of barrier function
- **Assay controls**: negative (sterile, deionized water); positive (10 M hydrochloric acid)

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- **Applicability**: identification of non-corrosive and corrosive test substances and mixtures in accordance with the UN GHS (Globally Harmonized System)
- **Limitations**: does not allow the sub-categorization of corrosive substances and mixtures in accordance with the UN GHS

……

- **Regulatory status**: OECD Test Guideline 430 (TG 430) (updated 26 July 2013)

TER: Typical protocol

Tissue Preparation

Skin discs are prepared as outlined in the OECD TG 430.

Tissue Mounting

Each skin disc is placed with the epidermal surface in contact with the PTFE tube.

Tissue Treatment

Test substance is applied to the epidermal surface for 24 hrs.

Treatment Termination

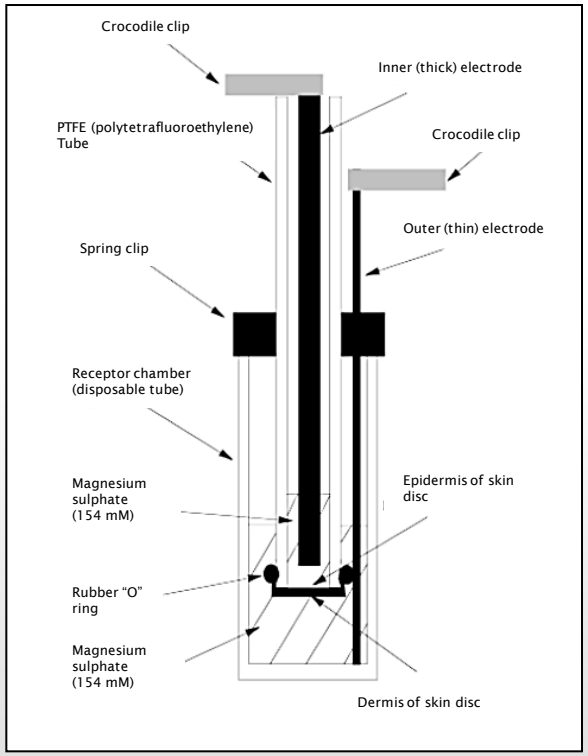
The test substance is removed with a jet of tap water.

TER Measurement

The skin impedance is measured as TER.

Dye Binding Method

For test substances that can remove the skin lipids (detergents, emulsifiers, etc.) and make the skin barrier more permeable to ions: if TER values are around 5 kΩ, the dye sulphorhodamine B is used. If the stratum corneum is compromised, the dye will rapidly penetrate and the amount of the dye bound to the skin is assessed by reading the Optical Density (OD_{565nm}).



Prediction Model

Resistance measured	Prediction to be considered (UN GHS Category)
i) If the mean TER value obtained for the test substance is ≤ 5 kΩ and the skin discs are obviously damaged (*e.g.*, perforated), OR ii) The mean TER value obtained for the test chemical is ≤ 5 kΩ, AND • The skin discs show no obvious damage (*e.g.*, perforation), BUT • The mean discs dye content is ≥ the mean disc dye content of the 10 M HCl positive control obtained concurrently)	Corrosive
i) If the mean TER value obtained for the test substance is > 5 kΩ, OR ii) The mean TER value obtained for the test substance is ≤ 5 kΩ, AND • The skin discs show no obvious damage (*e.g.*, perforation), AND • Thea mean disc dye content is < the mean disc dye content of the 10 M HCl positive control obtained concurrently	Non-corrosive

RHE Test Method (OECD TG 431)

Brief overview and current regulatory status

- **Test system**: 3-D RHE models [EpiDerm™ (EPI-200), EpiSkin™ (SM), SkinEthic™ RHE, epiCS®]
- **Assay endpoint**: tissue viability (%) – assessed by reduction of the vital dye MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) by viable cells
- **Assay controls**: negative (sterile, deionized water of NaCl solution 9 g/L); positive (8 N KOH or glacial acetic acid)

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- **Applicability**: the results can be used for regulatory purposes for distinguishing corrosive from non-corrosive test substances
- **Limitations**: currently only the EpiSkin™ model could be used to support sub-categorization of corrosive test substances.

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- **Regulatory status**: OECD Test Guideline 431 (TG 431) (updated 26 September 2014)

RHE: Typical protocol

Tissue Receipt

The tissues are incubated at standard culture conditions (at least 1 hr).

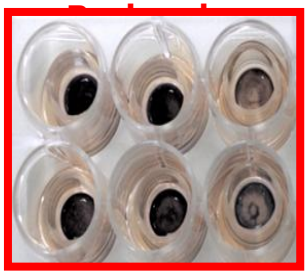
Tissue Treatment

Duplicate tissues are transferred into fresh media and treated topically with control and test substances for 3 min / 1 hr (4 hrs).

Tissue Rinsing

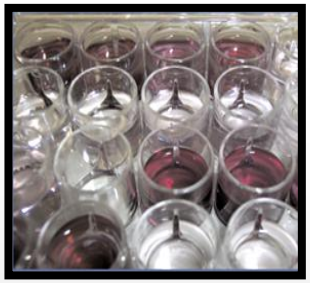
After exposure, tissues are rinsed to remove the control and test substances.

MTT



The tissues are placed into wells containing unreduced MTT and incubated at standard culture conditions (3 hrs).

Isopropanol Extraction



The tissues are placed in isopropanol (2 hrs) to extract the reduced MTT. Extracted MTT is transferred to a 96-well plate.

Spectrophotometric Quantification

Optical density values (OD_{570}) are determined using a plate reader and used to calculate viability values (presented relative to negative control tissue values).

RHE: Prediction Models

EpiSkin™ (SM)

Viability measured after exposure time points (3, 60 and 240 minutes)	Prediction to be considered (UN GHS Category)
< 35% after 3-minutes exposure	Corrosive: • Optional Sub-category 1A
≥ 35% after 3-minutes exposure AND < 35% after 60-minutes exposure OR ≥ 35% after 60-minutes exposure AND < 35% after 240-minutes exposure	Corrosive: • A combination of optional Sub-categories 1B and 1C
≥ 35% after 240-minutes exposure	Non-corrosive

EpiDerm™ (EPI-200)
SkinEthic™ RHE
epiCS®

Viability measured after exposure time points (3 and 60 minutes)	Prediction to be considered (UN GHS Category)
< 50% after 3-minutes exposure	Corrosive: • Optional Sub-category 1A
≥ 50% after 3-minutes exposure AND < 15% after 60-minutes exposure	Corrosive: • A combination of optional Sub-categories 1B and 1C
≥ 50% after 3-minutes exposure AND ≥ 15% after 60-minutes exposure	Non-corrosive

Membrane Barrier Test Method (Corrositex®) (OECD 435)

Brief overview and current regulatory status

- **Test system**: artificial membrane designed to respond to corrosive substances in a manner similar to animal skin *in situ*
- **Assay endpoint**: the time (in minutes) required for a test substance to penetrate through the Corrositex Biobarrier Membrane and produce a color change in the Chemical Detection System (CDS)
- **Assay controls**: negative (10% citric acid, 5% propionic acid); positive (sodium hydroxide)

- **Applicability**: assigns UN Packing Group to corrosives or verifies if a test substance is non-corrosive
- **Limitations**: materials with a pH of ≥ 4.5 and ≤ 8.5 generally fail to qualify for testing based on the CDS used in the kit provided by In Vitro International

- **Regulatory status**: OECD Test Guideline 430 (TG 430) (updated 19 July 2006)

Corrositex®: Typical protocol

Qualification of the Test Substance

The test substance is added the CDS containing vial to determine if it qualifies for the assay based on a color change detected when the pH of the CDS drops below 4.5 or rises above 8.5.

Categorization

The test substance is added to two tubes to determine the appropriate timetable for Packing Group Assignment as indicated by the manufacturer. A Category 1 test substance will be evaluated for up to 4 hrs; a Category 2 test substance will be evaluated for up to 1 hr.

Biobarrier Preparation

Biobarrier Placement

The biobarrier matrix powder is solubilized and added to a membrane disc containing a porous cell membrane. The biobarrier membrane is placed onto a vial of CDS.

Break Through Observations

The test substance is added onto four biobarrier membranes; the CDS vial is continuously monitored for the first 10 min. If no color change occurs, the process is repeated three times until the remaining biobarrier membranes are treated with the test substance. The vials are observed until a color change (*i.e.*, break through) occurs. The break through times are recorded.

Prediction Model

Category I

Mean Time to Produce a Change in Chemical Detection System	Packing Group
≤ 3 Minutes	I
> 3 Minutes - 1 Hour	II
> 1 - 4 Hours	III
> 4 Hours	Not Applicable

Category II

Mean Time to Produce a Change in Chemical Detection System	Packing Group
≤ 3 Minutes	I
> 3 Minutes - 30 minutes	II
> 30 - 60 minutes	III
> 60 minutes	Not Applicable

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3. Evaluation of skin corrosion and irritation potential using *in vitro* assays

❖ ***Assay specific considerations***

***In vitro* skin irritation**

RHE Test Method (SIT) (OECD TG 439)

Brief overview and current regulatory status

- **Test system**: 3-D RHE models [EpiDerm™ (EPI-200), EpiSkin™ (SM), SkinEthic™ RHE, LabCyte EPI-MODEL24]
- **Assay endpoint**: tissue viability (%) - MTT
- **Assay controls**: negative (sterile, deionized water or Calcium and Magnesium Free DPBS); positive (5% SDS)

- **Applicability**: the results can be used for regulatory purposes to determine the skin irritancy of test substances either as a stand-alone replacement for *in vivo* skin irritation testing or as partial replacement test within a tiered testing strategy
- **Limitation**: does not allow the classification of test substances to the optional UN GHS Category 3 (mild irritants)

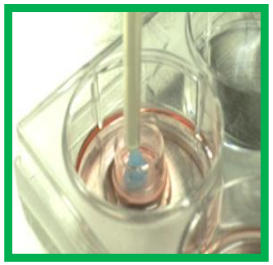
- **Regulatory status**: OECD Test Guideline 439 (TG 439) (updated 26 July 2013)

RHE: Typical protocol

Tissue Receipt

The tissues are incubated first for 1 hr and then over night (with media change) at standard culture conditions).

Tissue Treatment



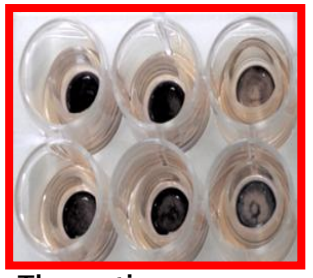
Triplicate tissues are treated topically with the control and test substances).

Tissue Rinsing

After exposure, tissues are rinsed and then placed in the incubator at standard culture conditions for two sequential post-treatment incubations (24 hrs and 18 hrs, respectively, with media change).

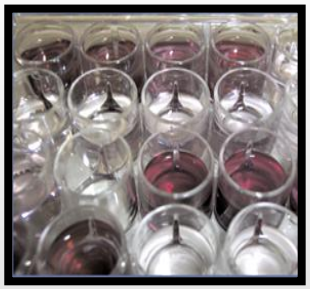
Post-treatment Incubation

MTT Reduction



The tissues are placed into wells containing unreduced MTT solution and incubated at standard culture conditions (3 hrs).

Isopropanol Extraction



The tissues are placed in isopropanol (2 hrs) to extract the reduced MTT. Extracted MTT is mixed and transferred to a 96-well plate.

Spectrophotometric Quantification

Optical density values (OD_{570}) are determined using a plate reader and used to calculate viability values (presented relative to negative control tissue values).

Prediction Model

In vitro result	*In vivo* prediction	Prediction to be considered (UN GHS CATEGORY)
Mean tissue viability ≤ 50%	Irritant (I)	Category 2
Mean tissue viability > 50%	Non-irritant (NI)	No Category

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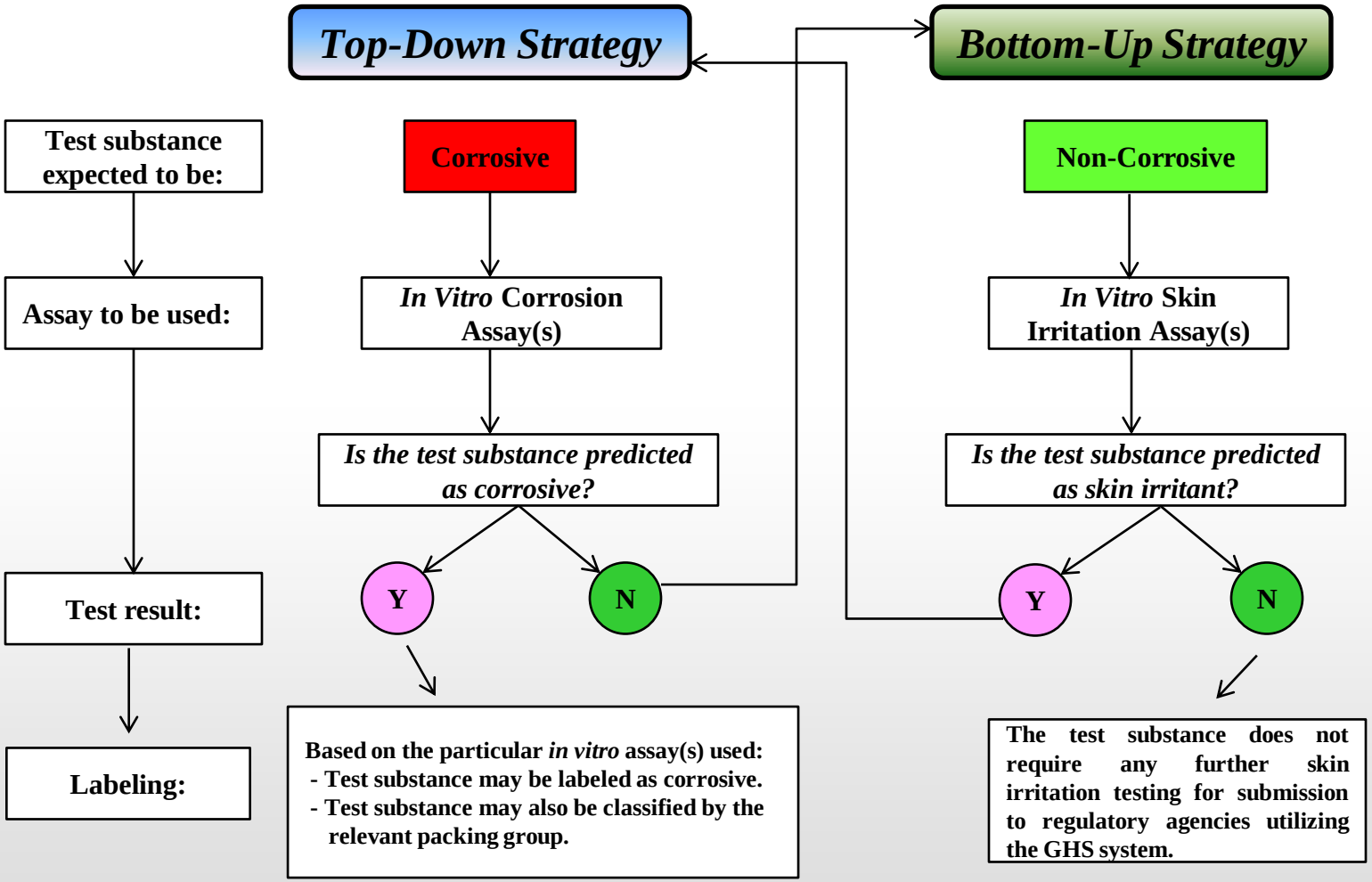
4. Conclusions – *in vitro* assays validated for regulatory purposes (skin corrosion and irritation endpoints)

Assays designed for regulatory purposes

- Often rely on a single exposure time/dose which provides a predictive response
- Limited in their predictive scope (not useful for evaluating toxic effects outside of specific predictive range)
- Are frequently ingredients-oriented
- In the interest of both sound science and animal welfare, *in vivo* testing should not be undertaken until all available data relevant to the potential dermal corrosion and irritation of the test substance have been evaluated in a weight-of-the-evidence analysis.
- Tiered testing strategy can include:
  - search for existing studies in humans and/or laboratory animals
  - evidence of corrosion and irritation of structurally related substances or mixtures
  - data demonstrating strong acidity or alkalinity of the substance
  - results from validated and accepted *in vitro* or *ex vivo* tests

Tiered testing strategies for the assessment of skin corrosion and irritation potential



SKIN IRRITATION AND CORROSION

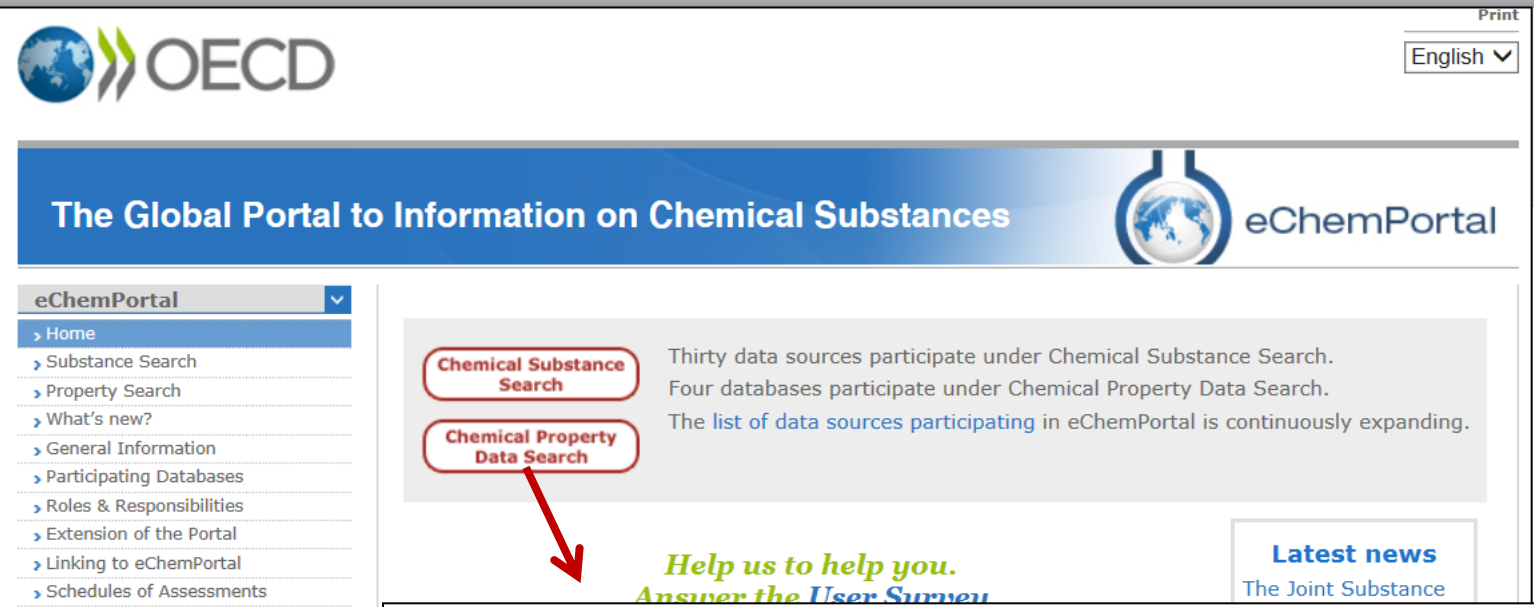
Costanza Rovida

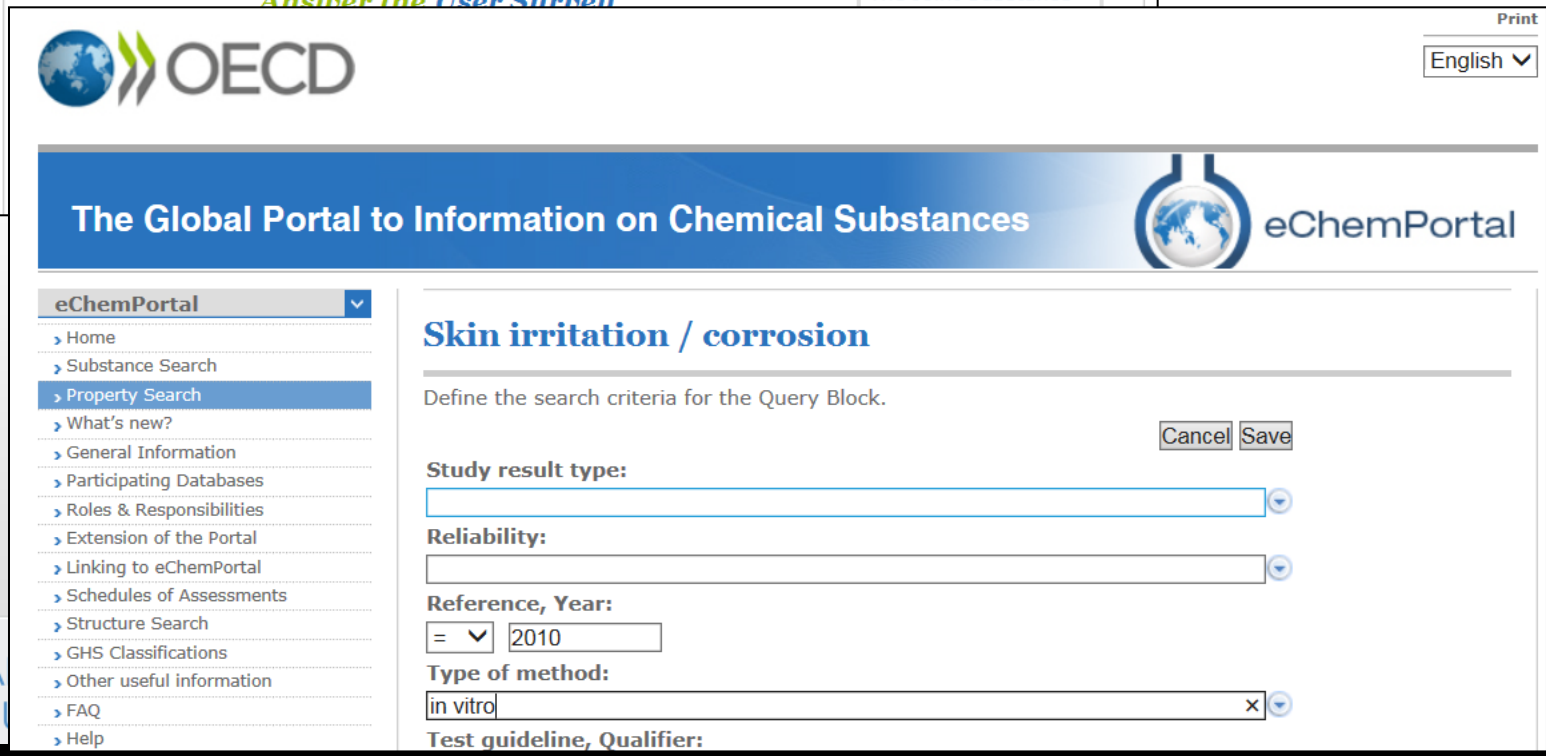
CAAT Europe

REACH mastery

costanza.rovida@chimici.it

www.echemportal.org





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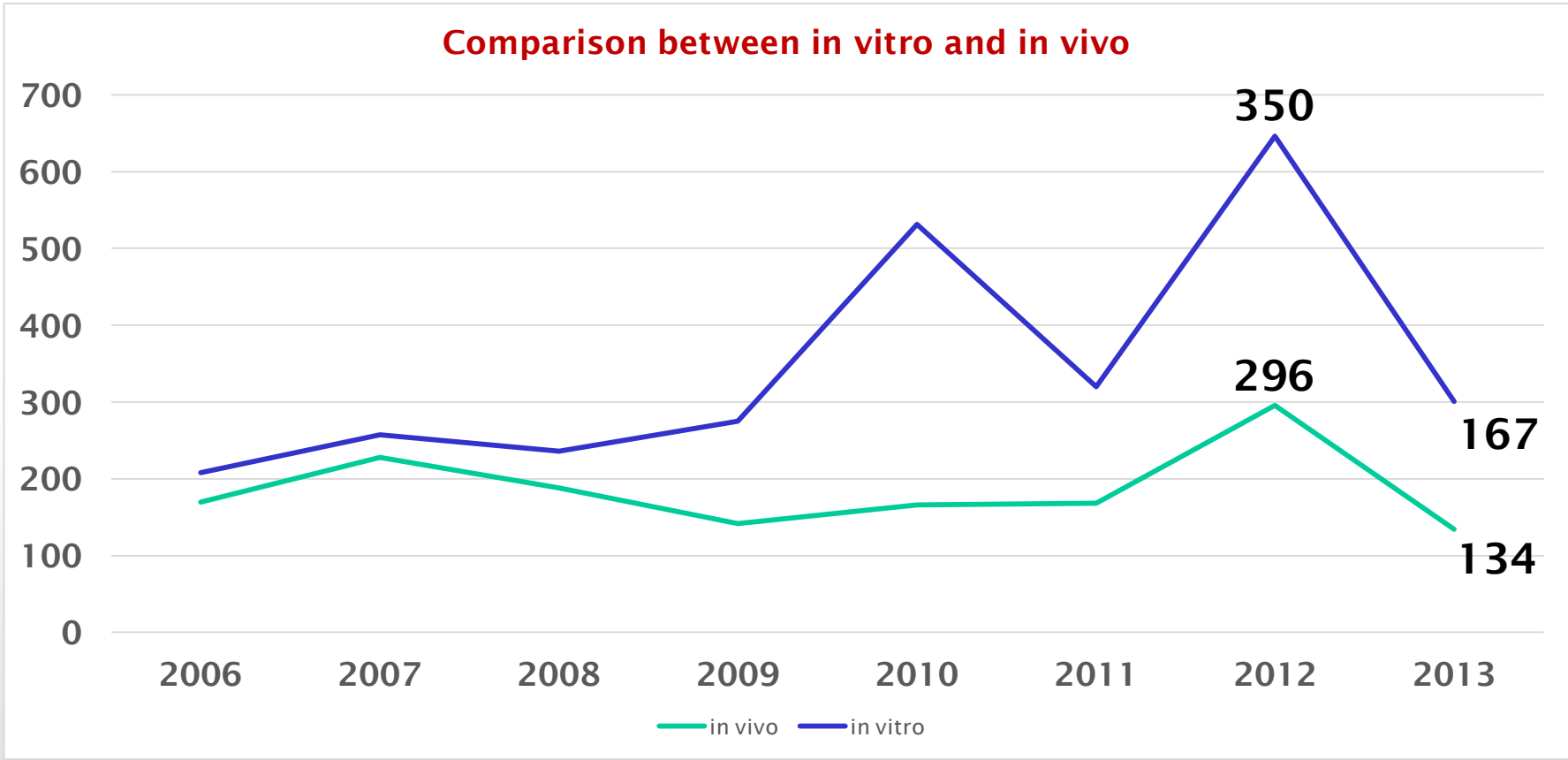
Number of tests per year



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EUROPEAN COMMISSION

REPORT FROM THE COMMISSION TO THE COUNCIL AND THE EUROPEAN PARLIAMENT

Seventh Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union

Table 7.1 Number of animals used in toxicological and other safety evaluations

Type of tests versus species

Data of 2011*

7.1 Species		7.2 Acute and sub-acute toxicity testing methods (including limit test)			7.3 Skin irritation	7.4 Skin sensitisation	7.5 Eye irritation	7.6 Sub-chronic and chronic toxicity	7.7 Carcino-genicity	7.8 Develop-mental toxicity	7.9 Muta-genicity	7.10 Repro-ductive toxicity	7.11 Toxicity to aquatic vertebrates not included in other columns	7.12 Other	7.13 Total
		7.2.1. LD50, LC50	7.2.2. Other lethal methods	7.2.3. Non lethal clinical signs methods											
1.a.	Mice (Mus musculus)	220544	51356	43637	64	16846	30	16436	5271	1188	9931	742	0	98796	464841
1.b.	Rats (Rattus norvegicus)	8376	10870	65185	1490	64	0	42274	6445	20189	11278	61209	0	45200	272508
1.c.	Guinea-Pigs (Cavia porcellus)	773	1847	1546	88	15214	0	1630	110	0	0	254	0	5270	26732
1.d.	Hamsters (Mesocricetus)	0	0	210	11	0	0	489	0	0	50	0	0	857	1617
1.e.	Other Rodents (other Rodentia)	182	4	0	0	0	0	0	0	0	0	0	0	274	460
1.f.	Rabbits (Oryctolagus cuniculus)	15	143	2947	3151	44	2080	634	0	2560	0	2978	0	8515	23067
1.g.	Cats (Felis catus)	0	0	34	0	0	0	12	0	0	0	0	0	285	331
1.h.	Dogs (Canis familiaris)	0	123	2469	0	0	0	2785	0	0	0	95	0	1903	7375
1.i.	Ferrets (Mustela putorius furo)	0	0	0	0	0	0	0	0	0	0	0	0	52	52
1.j.	Other Carnivores (other Carnivore)	0	0	0	0	0	0	0	0	0	0	0	0	11	11
1.k.	Horses, donkeys and cross-breds (Equidae)	0	0	33	0	0	0	0	0	60	0	0	0	148	241
1.l.	Pigs (Sus)	0	39	807	45	0	0	729	0	22	0	86	0	1682	3410
1.m.	Goats (Capra)	0	0	0	0	0	0	0	0	0	0	0	0	8	8
1.n.	Sheep (Ovis)	0	0	0	0	0	0	30	0	299	0	0	0	438	767
1.o.	Cattle (Bos)	0	0	45	0	0	0	24	0	230	0	0	0	488	787
1.p.	Prosimians (Prosimia)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.q.	New World Monkeys (Ceboidea)	0	0	24	0	0	0	0	0	0	0	0	0	20	44
1.r.	Old World Monkeys (Cercopithecoidea)	0	0	877	0	0	0	1306	0	266	0	15	0	927	3391
1.s.	Apes (Hominoidea)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.t.	Other Mammals (other Mammalia)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.u.	Quail (Coturnix coturnix)	329	370	0	0	0	0	45	0	0	0	0	0	2350	3094
1.v.	Other birds (other Aves)	423	182	4584	0	0	0	0	50	0	0	556	0	8492	14287
1.w.	Reptiles (Reptilia)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.x.	Amphibians (Amphibia)	0	0	1660	0	0	0	0	0	500	0	0	516	19	2695
1.y.	Fish (Pisces)	34137	11641	11898	0	0	0	13730	0	16468	29	6381	38890	45909	179083
1.z.	TOTAL	264779	76575	136956	4849	32168	2110	80124	11876	41782	21288	72316	39406	221644	1004873

(*) France reporting for 2010

REACH Regulation, EC 1907/2006
(Registration, Evaluation, Authorisation and restriction of Chemicals)

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GLOBAL RISK & REGULATION NEWS

Negative results from *in vitro* tests are not accepted by regulators and must be always confirmed *in vivo*

ANNEX XI

GENERAL RULES FOR ADAPTATION OF THE STANDARD TESTING REGIME SET OUT IN ANNEXES VII TO X

1.4. In vitro methods

…….

If the results obtained from the use of such in vitro methods do not indicate a certain dangerous property, the relevant test shall nevertheless be carried out at the appropriate tonnage level to confirm the negative result, unless testing is not required in accordance with Annexes VII to X or the other rules in this Annex.

Such confirmation may be waived, if the following conditions are met:

(1) results are derived from an in vitro method whose scientific validity has been established by a validation study, according to internationally agreed validation principles;

(2) results are adequate for the purpose of classification and labelling and/or risk assessment; and

(3) adequate and reliable documentation of the applied method is provided.

ECHA
European Chemicals Agency

Evaluation under REACH

Progress Report 2010

Page 32

Skin irritation-corrosion

Annexes VIII-X requires an *in vivo* test to assess Skin irritation/corrosion. However, there are currently several *in vitro* methods available that can be used in a weight-of-evidence approach, to fully replace animal testing.

It is generally agreed that the EU B.46 (OECD 439) *in vitro* methods for Skin irritation represent a full replacement of the respective *in vivo* method (OECD 404) in a tiered testing strategy and in conjunction with *in vitro* skin corrosivity tests, if necessary. It should be noted that B.46 method does not address corrosivity; therefore, in case of positive result in a B46 test, a test addressing skin corrosion has to be performed.

It is recommended that the following testing strategy is followed when performing in vitro tests to assess skin-irritation and corrosion (see also *Guidance on information requirements and chemical safety assessment Chapter R.7a: Endpoint specific guidance)*

- Skin corrosion shall be tested first; in case of positive results, no further testing is necessary; the substance shall be classified accordingly.
- If the results of the skin corrosion test is negative, then a skin irritation study according to EU method B.46 shall be performed; if the result is positive, no further testing is necessary but classification of the substance.
- A negative result in the B.46 test does not need to be confirmed by additional testing.

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Other official documents

ECHA
EUROPEAN CHEMICALS AGENCY

Skin irritation/corrosion

TITLE OF THE TEST GUIDELINES (YEAR OF APPROVAL)

Irritation

Reconstructed human epidermis tests, **EU B.46, OECD 439** (in EU 2009 and in OECD 2010, revised in 2013 by OECD)

Corrosion

Transcutaneous electrical resistance test (TER), **EU B.40, OECD 430**, (2000, revised in 2013 by OECD)

Human skin model test (includes more than one protocol), **EU B.40 bis, OECD 431**, (2000, revised in 2013 by OECD)

In vitro membrane barrier test method, **OECD 435** (2006)

Note: the latest version of the test guideline should always be used independent of whether it is published by EU or OECD.

http://echa.europa.eu/documents/10162/21650280/oecd_test_guidelines_skin_irritation_en.pdf

European Commission

JRC SCIENCE AND POLICY REPORTS

Alternative methods for regulatory toxicology – a state-of-the-art review

Report EUR 26797 EN
http://publications.jrc.ec.europa.eu/repository/handle/111111111/32662

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GLOBAL RISK & REGULATION NEWS

OECD 404: Acute Dermal Irritation/Corrosion *in vivo*
Description of the evaluation and testing strategy

Step 1: Evaluation of existing human and animal data

Step 2: Analysis of structure activity relationships (SAR)

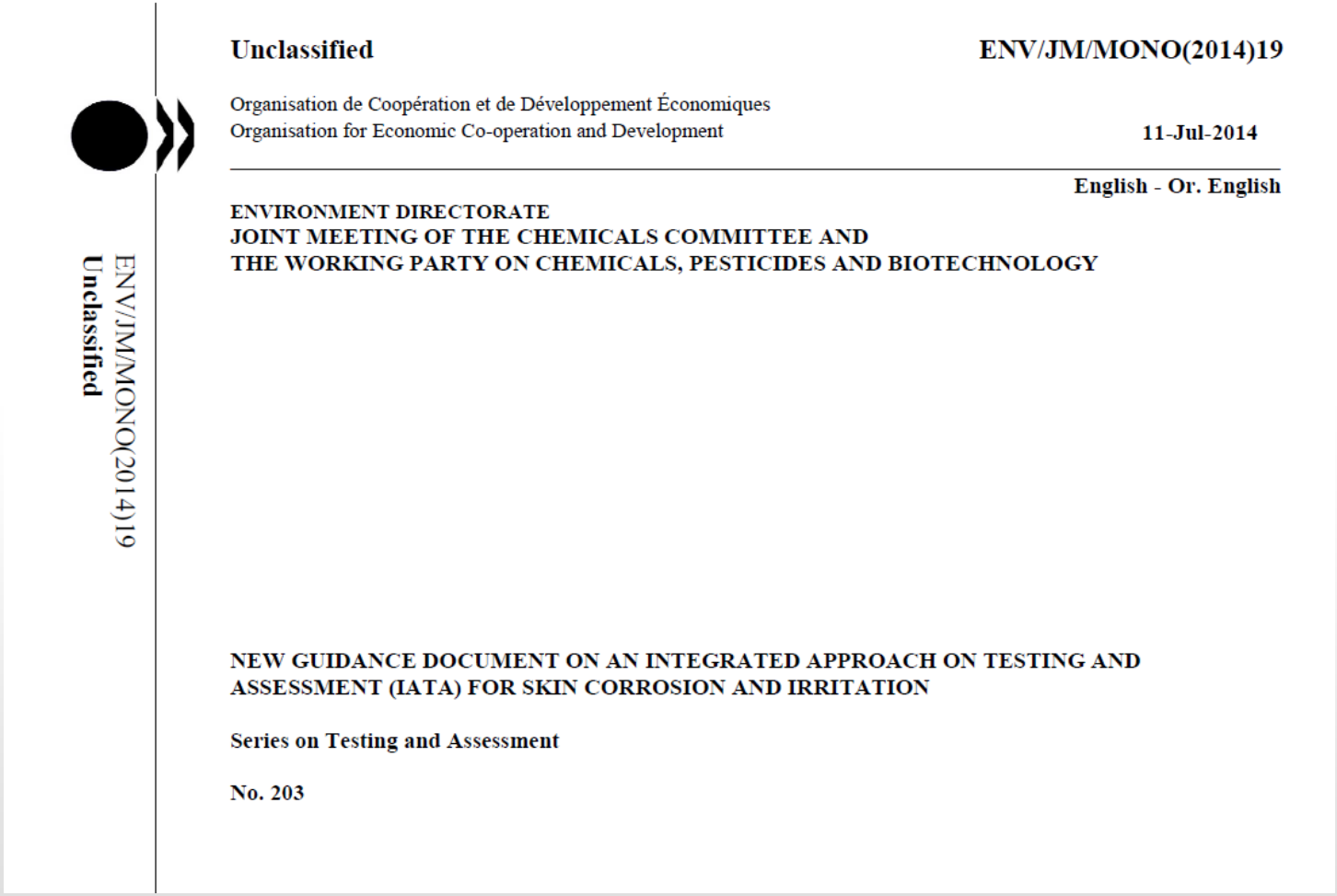
Step 3: Physicochemical properties and chemical reactivity

Step 4: Dermal toxicity

Step 5 and 6: Results from *in vitro* or *ex vivo* tests

Step 7 and 8: In vivo test in rabbits

Integrated approach to testing and assessment



ENV/JM/MONO(2014)19
Unclassified

Unclassified **ENV/JM/MONO(2014)19**

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

11-Jul-2014

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

NEW GUIDANCE DOCUMENT ON AN INTEGRATED APPROACH ON TESTING AND ASSESSMENT (IATA) FOR SKIN CORROSION AND IRRITATION

Series on Testing and Assessment

No. 203

PETA INTERNATIONAL SCIENCE CONSORTIUM, LTD.

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1. Evaluation of existing human and animal data

Human data:

a. Occupational exposure

b. (consumer exposure)

Old animal data:

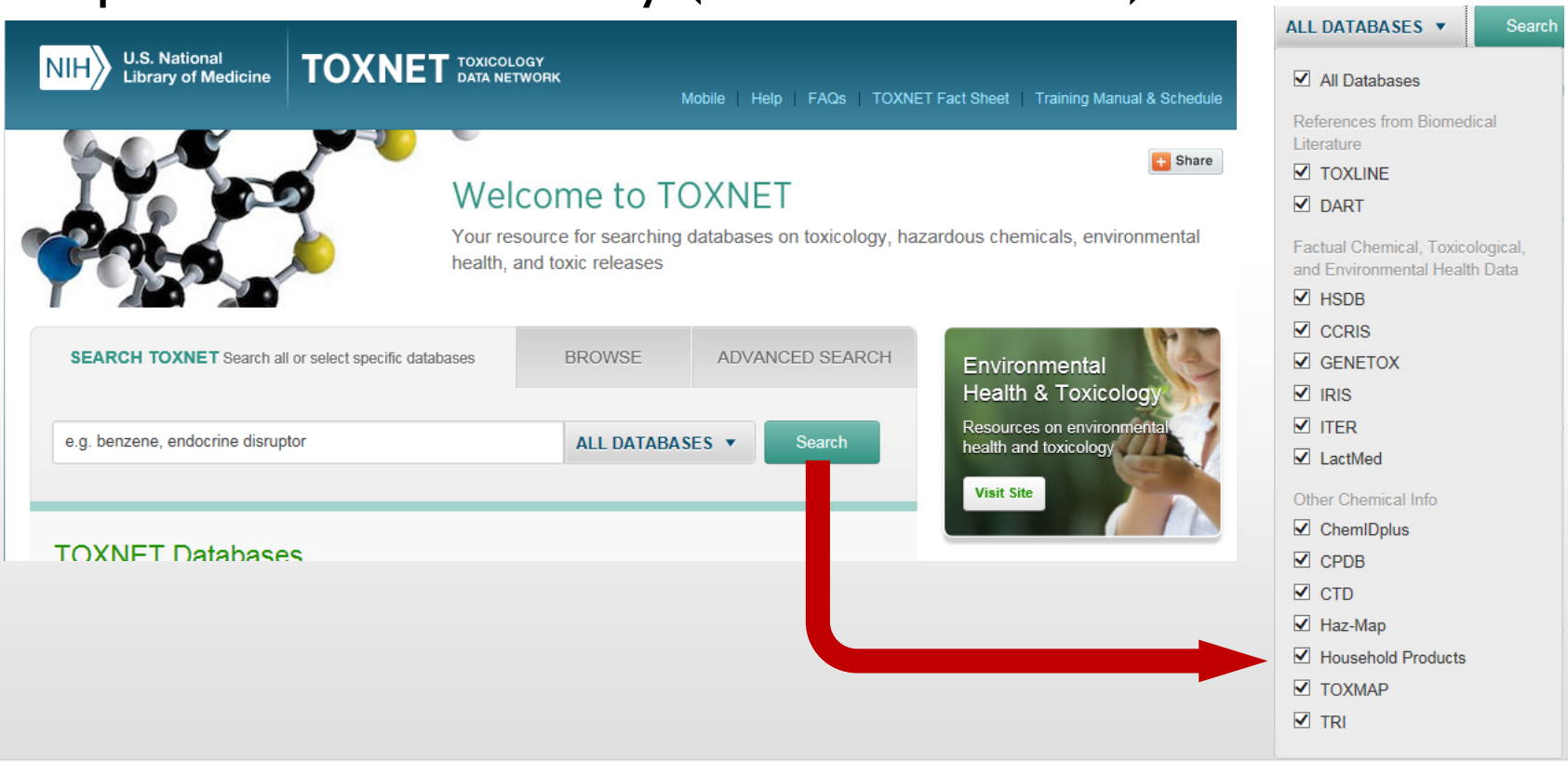
a. Impurities

b. GLP

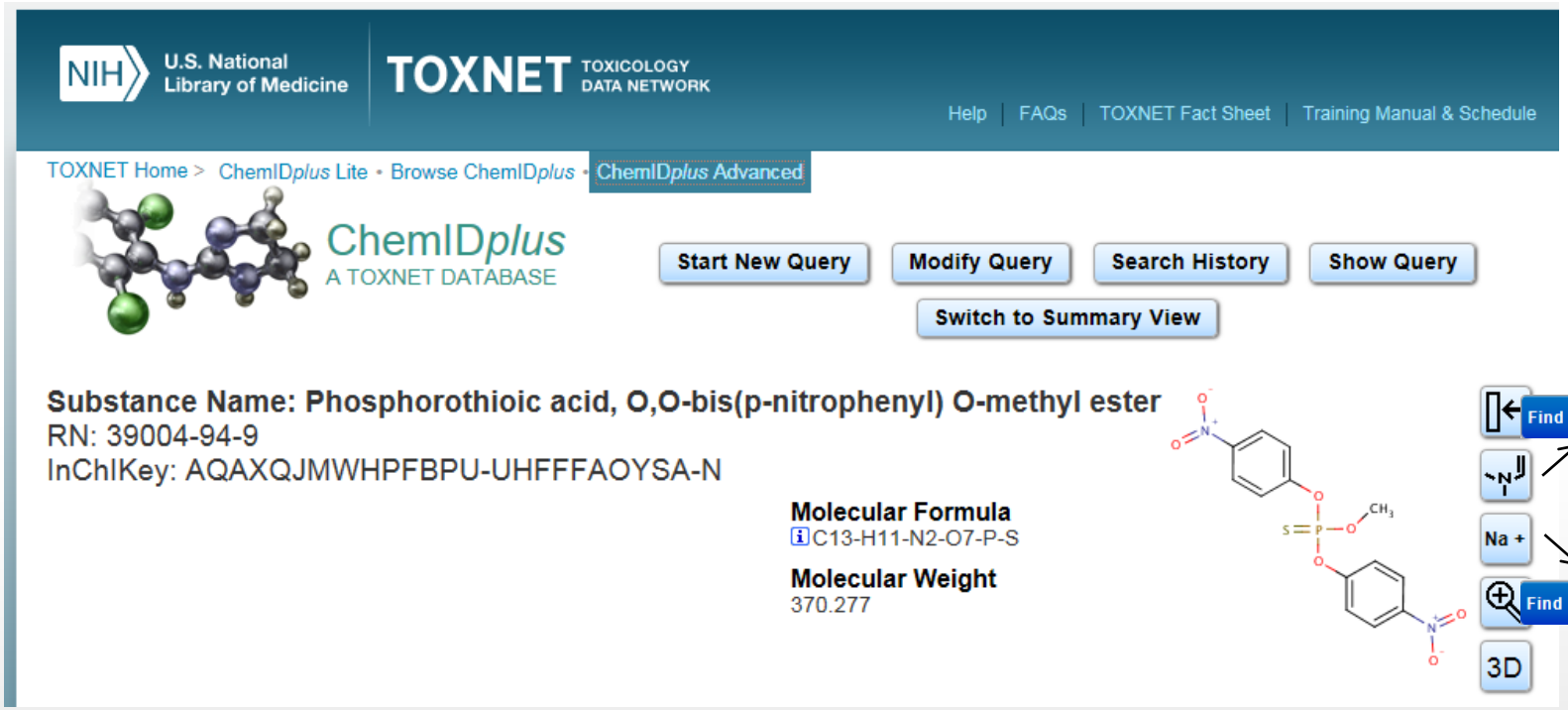
Gathering all existing information

Step 3: Physicochemical properties and chemical reactivity

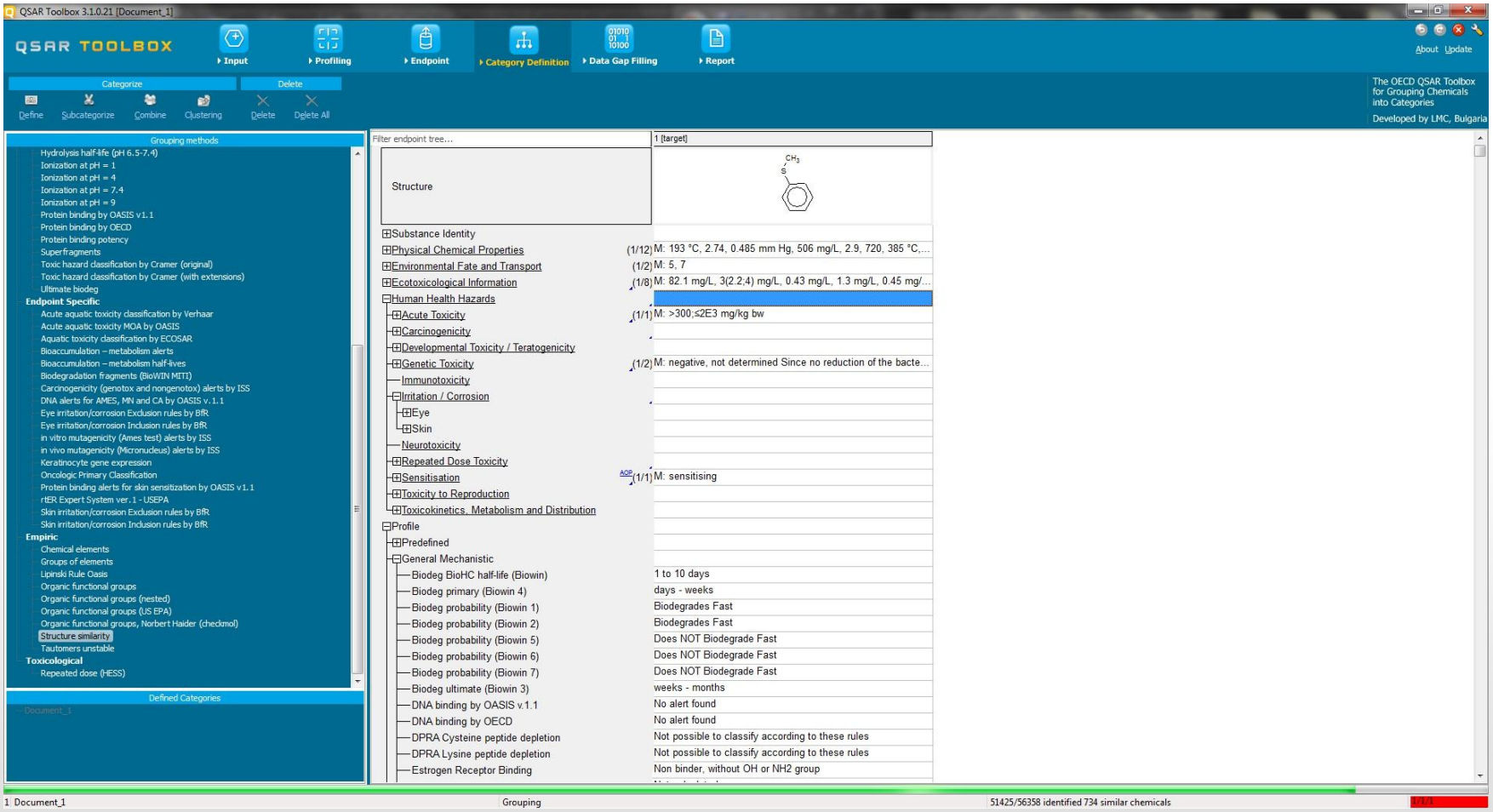
Step 4: Dermal toxicity (and other data)



Gathering all existing information



2: Analysis of structure activity relationships (SAR)



What do we need?

Skin irritation
Not irritant
Category 2 – H315 Causes skin irritation
Category 1 – H314 Causes severe skin burns and eye damage

What do we need?

Skin irritation	Eye irritation
Not irritant	Not irritant
Not irritant	Category 2 - H319 Causes serious eye irritation
Category 2 – H315 Causes skin irritation	Category 2 - H319 Causes serious eye irritation
Category 2 – H315 Causes skin irritation	Category 1- H318 Causes serious eye damage
Category 1 – H314 Causes severe skin burns and eye damage	
Category 1A / 1B / 1C (Packing group I, II, III)	

Skin Irritation - costs

Description	Guideline	Price (€)
In vivo skin irritation/corrosion on rabbits	OECD 404 (Method B.4)	1,800
In vitro Skin Corrosion - Transcutaneous Electrical Resistance Test Method (TER)	OECD 430 (Method B.40)	1,900
In vitro Skin Corrosion – Human skin model test	OECD 431 (Method B.40 Bis)	2,900
In vitro Skin Irritation - Reconstructed Human Epidermis Test Method	OECD 439 (Method B.46)	2,100

***Prices come from some CROs in Italy and it should be noted that prices vary widely depending on multiple factors, including the exact service provided and geographical location. The prices listed are for a full GLP study as requested by regulators for a REACH dossier. It is likely that the price for *in vitro* testing may be significantly less for companies that have brought the *in vitro* methods in-house.**

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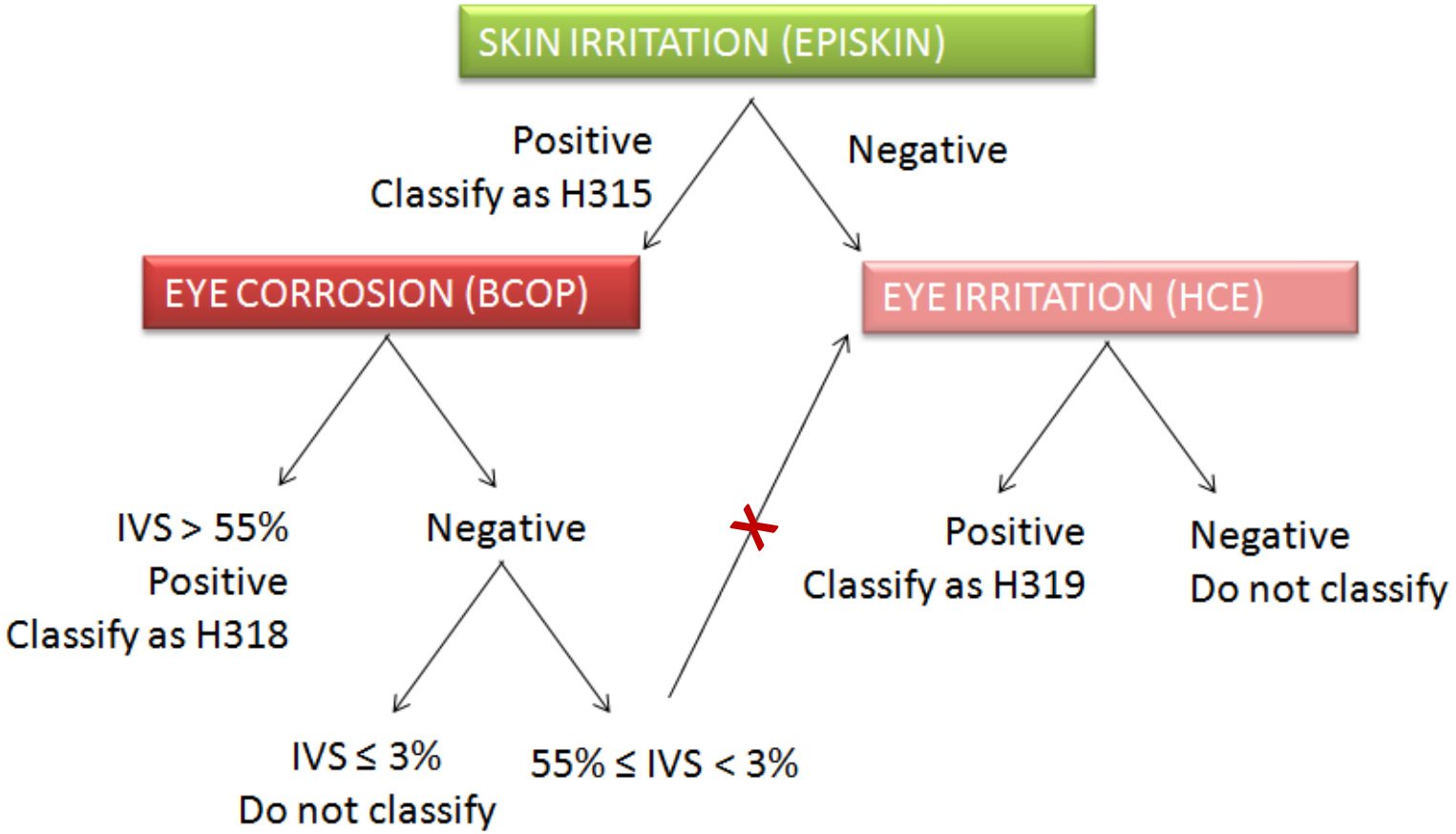
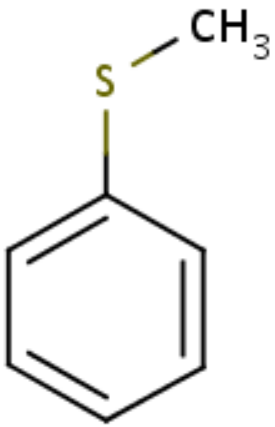
Example of strategy



Example of substance: Thioanisole



methyl phenyl sulphide
EC 202-878-2
CAS 100-68-5

TOXNET Home > Multi-database Search Results

TOXNET SEARCH RESULTS | BROWSE TOXNET | ADVANCED SEARCH

100-68-5 ALL DATABASES Search

Search Term singular/plural Records with all of the words Include Synonyms and CAS Numbers in Search

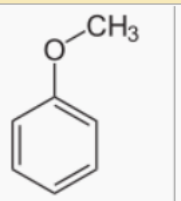
TOXNET databases use unique formats. Only one record from each of the selected resources appears below. Click on "More Results" to see all records retrieved for your search.

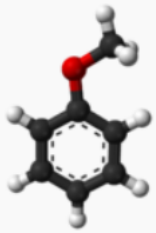
Search Details | History | My List

TOP RESULTS	DATABASE	ADD TO MY LIST
1. Co-treatment of single, binary and ternary mixture gas of ethanethiol, dimethyl disulfide and thioanisole in a biotrickling filter seeded with Lysinibacillus sphaericus RG-1. Wan S; Li G; An T; Guo B J Hazard Mater. 2011, Feb 28; 186(2-3):1050-7. [Journal of hazardous materials] [PubMed] PubMed Citation	TOXLINE More Results (59)	Select Record
2. TECNAZENE 117-18-0	HSDB More Results (6)	Select Record
3. Thioanisole 100-68-5	ChemIDplus More Results (2)	Select Record
4. Methyl phenyl sulfide 100-68-5	HAZMAP More Results (1)	Select Record

Thioanisole and similar substances: Anisole

Anisole





IUPAC name [hide]

Methoxybenzene

Other names [hide]

Phenoxymethane
Phenyl methyl ether

Identifiers

CAS number	100-66-3 ✓

anisole

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- Classification and Labelling
- Manufacture, Use & Exposure
- PBT assessment
- Physical and chemical properties
- Environmental fate and pathways
- Ecotoxicological Information
- Toxicological information
  - Toxicological information.001
  - Toxicokinetics, metabolism and distribution
  - Acute Toxicity
  - Irritation / corrosion
    - Skin irritation / corrosion
      - Exp Key Skin irritation / corrosion.001
    - Eye irritation
      - Exp Key Eye irritation.001
  - Sensitisation
  - Repeated dose toxicity
  - Genetic toxicity
  - Toxicity to reproduction
- Guidance on safe use
- Reference substances

Exp Key Skin irritation / corrosion.001

Administrative Data | Data source | Materials and methods | Results and discussions | Applicant's summary and conclusion

Any other information on results incl. tables

Table 73.1/1: Irritant/corrosive response data for each animal at each observation time up to removal of each animal from the test

Score at time point / Reversibility	Erythema	Edema
	Max. score: 4	Max. score: 2
60 min	2/1/2	0/0/2
24 h	2/1/2	2/2/2
48 h	2/2/3	2/2/2
72 h	1/2/2	0/0/0
Average 24h, 48h, 72h	1.7/1.7/2.3	1.3/1.3/1.3
Reversibility*)	c	c
Average time (unit) for reversion	7 days	72 hours

*) Reversibility: c. = completely reversible; n.c. = not completely reversible; n. = not reversible

Applicant's summary and conclusion

Interpretation of results

slightly irritating

Criteria used for interpretation of results

EU

EUH066 (repeated exposure may cause skin dryness or cracking) Observed in an in vivo skin irritation study.

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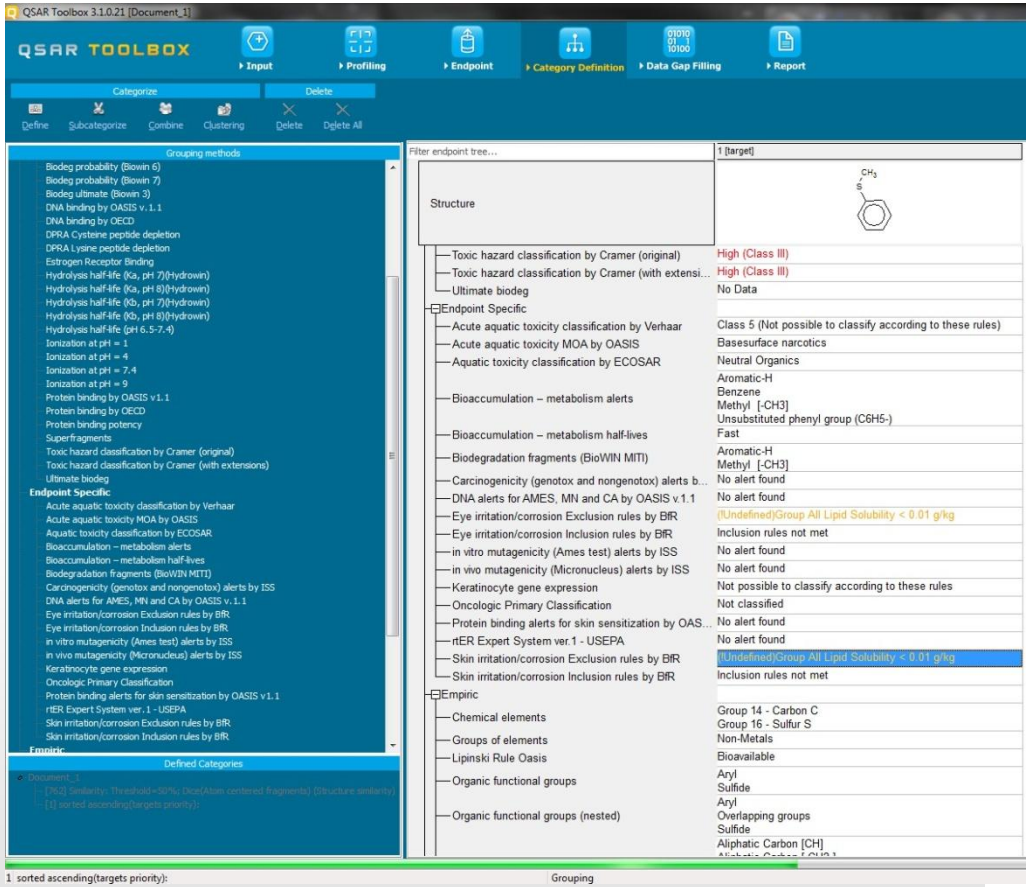
Thioanisole and QSAR Toolbox



Evaluation of (Q)SARs for the Prediction of Skin Irritation/Corrosion Potential

Physico-chemical exclusion rules

Sponsor: European Commission
Directorate General
Joint Research Centre
Institute for Health and Consumer Protection
European Chemicals Bureau

Authors: Emiel Rorije
Etje Hulzebos

National Institute of Public Health and Environment (RIVM)
Bilthoven
NL

September 2005

https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/information-sources/qsar-document-area/Evaluation_of_Skin_Irritation_QSARs.pdf

Thioanisole: mild irritant?

Details retrieved from the ECHA database

Guideline B46: In Vitro Skin Irritation:
Reconstructed Human Epidermis Model Test
GLP Study
Test substance: 10 µL undiluted
Duration of treatment:
15 minutes followed by washing and incubation for 42 hours at 37°C
Test animal: human
Species and strain, number of animals: not relevant
control animals: Other, negative control tissues treated with PBS;
positive control tissues treated with 5% SDS
Results: The relative mean tissue viability obtained after 15 minutes treatment compared to the negative controls was 11% (< 50%)

H315: Causes skin irritation

Thioanisole: eye irritant?

Details retrieved from the ECHA database

OECD Guideline 437:
Bovine Corneal Opacity and Permeability (BCOP)
GLP Study
Test substance: 750 µL undiluted
Duration of treatment: 10 minutes
Test animal: Bovine
Species and strain, number of animals: not relevant
Results: IVS range from 2.9 and 4.8, average 3.9

BCOP result very close to non classification. This should trigger further investigation
However, this substance is already classified as skin irritant

H318: Causes eye irritation

Thioanisole: corrosive?

Details retrieved from the ECHA database

Guideline B40: In Vitro Skin corrosion:
    In vitro Skin Corrosion: Human Skin Model Test
GLP Study
Test substance: 50 µL undiluted
Duration of treatment: 3 minutes and 1 hour
Test animal: human
Species and strain: not relevant
number of animals: 4 tissues
Results:
    3 minutes: viability 50%
    1 hour: viability 59%

Not Corrosive, Classification H315 confirmed

Conclusions

- Start from the regulatory framework
- There is no unique instructions and even simple tiered strategy should be tailored to the specific substance and the specific use
- Assessment of the substance should be performed globally, not endpoint by endpoint
- Interpretation of all available results, avoid just adding the conclusions from each single study report
- Look at cost and simplicity
- (Too much based on expert judgment)

Contact Information

Gertrude-Emilia Costin, Ph.D., M.B.A.
Institute for In Vitro Sciences, Inc. (IIVS)
Gaithersburg, MD, USA
http://www.iivs.org
ecostin@iivs.org
01-301-947-6524

Costanza Rovida, Ph.D.
CAAT Europe
REACH mastery
costanza.rovida@chimici.it