NICEATM

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods

The Cytosensor Microphysiometer (CM) Test Method – Validation Status and Appropriate Use

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NICEATM-ICCVAM Evaluation of CM

- Reviewed available data and information regarding the usefulness and limitations for assessing the ocular hazard potential of chemicals and products
- Determined validation status
  - Accuracy: sensitivity and specificity
  - Reproducibility for identifying ocular corrosives/severe irritants vs. all other hazard categories
  - Scope of substances tested
  - Availability of a standardized test method protocol
- Independent international scientific peer review panel

Overview of CM

- L929 mouse fibroblast cells are treated with the test substance
  - Seven concentrations (predetermined in the dose range-finding assay)
  - Diluted in low-buffered treatment medium
  - At least two independent runs
- L929 cells are treated with the positive control in each run
  - 10% (w/v) sodium lauryl sulfate (SLS)
- Time of exposure: 13 minutes 30 seconds
- Endpoint measured: Rate of pH change

Validation Database

- 53 water-soluble surfactants (32 surfactant-containing formulations and 21 surfactant substances tested across seven different laboratories)
  - Most of the 32 formulations, which are limited to cosmetic and personal care products, contain one or more surfactants at a final concentration of greater than five percent
  - No pesticide formulations included
- 29 water-soluble nonsurfactants (27 nonsurfactant chemicals and 2 nonsurfactant formulations tested in seven laboratories)
  - For example, acids, alcohols, alkalis, and ketones
- Reproducibility data from two validation studies
  - Balls et al. (1995): 4 laboratories
  - Brantom et al. (1997): 2 laboratories

Decision Criteria Proposed to Classify CM Data

MRD_{50} (mg/mL)[1]	EPA	GHS
>80	Category IV	NA
>2; ≤80	No prediction can be made	NA
>10	NA	No Category
>2; ≤10	NA	No prediction can be made
≤2	Category I	Category 1

[1]MRD_{50}: Metabolic rate decrement of 50%. The concentration of test substance (weight/volume) required to cause 50% inhibition of the basal acidification (metabolic) rate.

CM Test Method Accuracy: Ocular Corrosives and Severe Irritants[1]: Surfactant-Containing Substances

	No.	Accuracy		Sensitivity		Specificity		False Positive Rate[2]		False Negative Rate[3]	
		%	No.	%	No.	%	No.	%	No.	%	No.
EPA	52	85	44/52	78	18/23	90	26/29	10	3/29	22	5/23
GHS	53	94	50/53	91	21/23	97	29/30	3	1/30	9	2/23

[1]EPA = Cat I vs. Cat II/III/IV, GHS = Cat 1 vs. Cat2A/2B/NC
[2]The three false positives when using the EPA classification system are classified as Category II (n=2) or III (n=1) based on *in vivo* data. The one false positive when using the GHS classification system is Not Classified based on *in vivo* data.
[3]The false negative substances were classified as mild or moderate irritants *in vitro* based on the EPA and GHS classification systems (i.e., EPA Category II/III; GHS Category 2A /2B).

CM Test Method Accuracy: Ocular Corrosives and Severe Irritants[1]: Nonsurfactant Substances

	No.	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate[2]	
		%	No.	%	No.	%	No.	%	No.	%	No.
EPA	25	92	23/25	71	5/7	100	18/18	0	0/18	29	2/7
GHS	29	83	24/29	55	6/11	100	18/18	0	0/18	45	5/11

[1]EPA = Cat I vs. Cat II/III/IV, GHS = Cat 1 vs. Cat2A/2B/NC

[2] Two substances were false negatives when using the EPA classification system and were classified *in vitro* as either Category II/III (n = 1) or IV (n = 1). Five substances were false negatives using the GHS classification system and were classified *in vitro* as either Category 2A/2B (n = 4) or Not Classified (n = 1).

CM Test Method Accuracy: Substances Not Labeled as Irritants[1]: Surfactant-Containing Substances

	No.	Accuracy		Sensitivity		Specificity		False Positive Rate[2]		False Negative Rate[3]	
		%	No.	%	No.	%	No.	%	No.	%	No.
EPA	52	92	48/52	98	45/46	50	3/6	50	3/6	2	1/46
GHS	53	68	36/53	100	28/28	32	8/25	68	17/25	0	0/28

[1]EPA = Cat IV vs. Cat I/II/III; GHS = NC vs. Cat 1/2A/2B

[2]Three substances were false positive when using the EPA classification system and were classified *in vitro* as Category II/III. Seventeen substances were false positive when using the GHS classification system and were classified *in vitro* as Category 2A/2B (n=16) or Category 1 (n=1).

[3] The one false negative was EPA Category III based on *in vivo* data. For this substance, six test animals were included in the *in vivo* test. One test animal had no observable effects, three test animals had conjunctival redness (score = 1), and two test animals had corneal opacity (score = 1) that cleared after one day.

CM Test Method Accuracy: Substances Not Labeled as Irritants[1]: Nonsurfactant Substances

	No.	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate[2]	
		%	No.	%	No.	%	No.	%	No.	%	No.
EPA	29	66	19/29	67	16/24	60	3/5	40	2/5	33	8/24
GHS	25	64	16/25	62	13/21	75	3/4	25	1/4	38	8/21

[1]EPA = Cat IV vs. Cat I/II/III; GHS = NC vs. Cat 1/2A/2B

[2]Eight substances were false negative when using the EPA and GHS classification systems. In the EPA system, they were classified *in vivo* as Category 1 (n = 1) and Category II (n = 3) and Category III (n = 4). In the GHS system, they were classified *in vivo* as Category 1 (n = 1) and Category 2A (n = 7).

CM Interlaboratory Reproducibility

Material Type	Number of Laboratories	Agreement Among Laboratories	Percentage (# correct/total)	Maximum Mean CV	Study
Surfactants	4	100%	55% (6/11)	37%	EC/HO – Balls et al. (1995)
		75%	27% (3/11)		
		50%	18% (2/11)		
Nonsurfactants		100%	48% (11/23)	51%	
		75%	22% (5/23)		
		67%	4% (1/23)		
		50%	13% (3/23)		
Surfactants	2	100%	90% (9/10)	23%	COLIPA – Brantom et al. (1997)
		0%	10% (1/10)		
Surfactant-based formulations and mixtures		100%	100% (7/7)	16%	
Nonsurfactants		100%	78% (7/9)	51%	
		0%	22% (2/9)		

ICCVAM Recommendations for CM[1]: Usefulness and Limitations – Ocular Corrosives and Severe Irritants

Usefulness

- Can be used for identification of ocular corrosives and severe irritants (EPA Category I, GHS Category 1) in appropriate circumstances and with certain limitations

Limitations

- Limited to water-soluble substances (i.e., water-soluble surfactants, surfactant-containing formulations, and nonsurfactants)

[1] ICCVAM. 2010. Test Method Evaluation Report. NIH publication No. 10-7553A. Available: http://iccvam.niehs.nih.gov/methods/ocutox/MildMod-TMER.htm

ICCVAM Recommendations for CM[1]: Usefulness and Limitations – Substances Not Labeled as Irritants

Usefulness

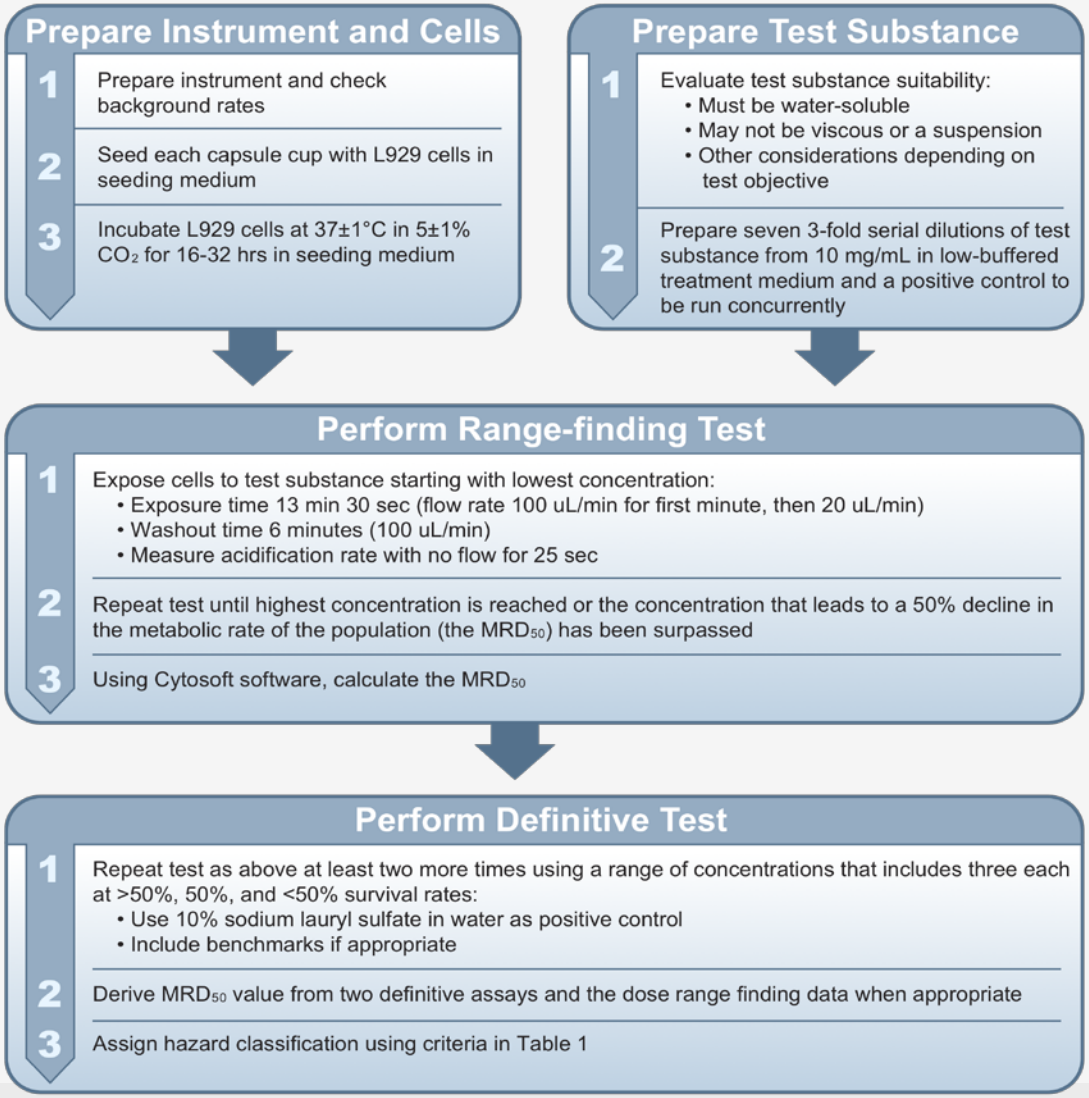
- Can be used for identification of substances not labeled as irritants (EPA Category IV) in appropriate circumstances and with certain limitations

Limitations

- Restricted to water-soluble surfactant chemicals and certain types of surfactant-containing formulations (e.g., cosmetics and personal care product formulations, but not pesticide formulations), but **not** nonsurfactants

[1] ICCVAM. 2010. Test Method Evaluation Report. NIH publication No. 10-7553A. Available: http://iccvam.niehs.nih.gov/methods/ocutox/MildMod-TMER.htm

ICCVAM-Recommended CM Protocol[1]



[1] Available at http://iccvam.niehs.nih.gov/docs/protocols/IVOcular-CM.pdf

ICCVAM Recommendations: Future Studies

- Additional studies to expand the applicability domain of CM for ocular corrosives and severe irritants and for substances not labeled as irritants
  - Use ICCVAM-recommended reference substances[1] or a reference set from this list
- Optimization studies to increase performance of CM for identifying all categories of ocular irritancy hazard classification
- ICCVAM encourages users to provide all data from future studies to further evaluate the usefulness and limitations of CM

[1] Available at http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_tmer.htm

Draft OECD Test Guideline Currently Under Consideration

- Draft OECD Guideline for the Testing of Chemicals

  The Cytosensor Microphysiometer Test Method: An *In Vitro Method for Identifying Chemicals Not Classified as Irritant, as well as Ocular Corrosive and Severe Irritant Chemicals*

  - Based on international validation study by ECVAM, in collaboration with ICCVAM and JaCVAM

[1]Available at http://www.oecd.org/document/55/0,3343,en_2649_34377_2349687_1_1_1_1,00.html

2010 ICCVAM Evaluation of CM

- In 2010, ICCVAM also evaluated CM for identifying nonsevere irritants
- ICCVAM concluded that CM is **not** recommended to identify moderate and mild ocular irritants as defined by the EPA and GHS classification systems

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