Usefulness and Limitations of the Cytosensor® Microphysiometer (CM) Test Method for Ocular Safety Testing

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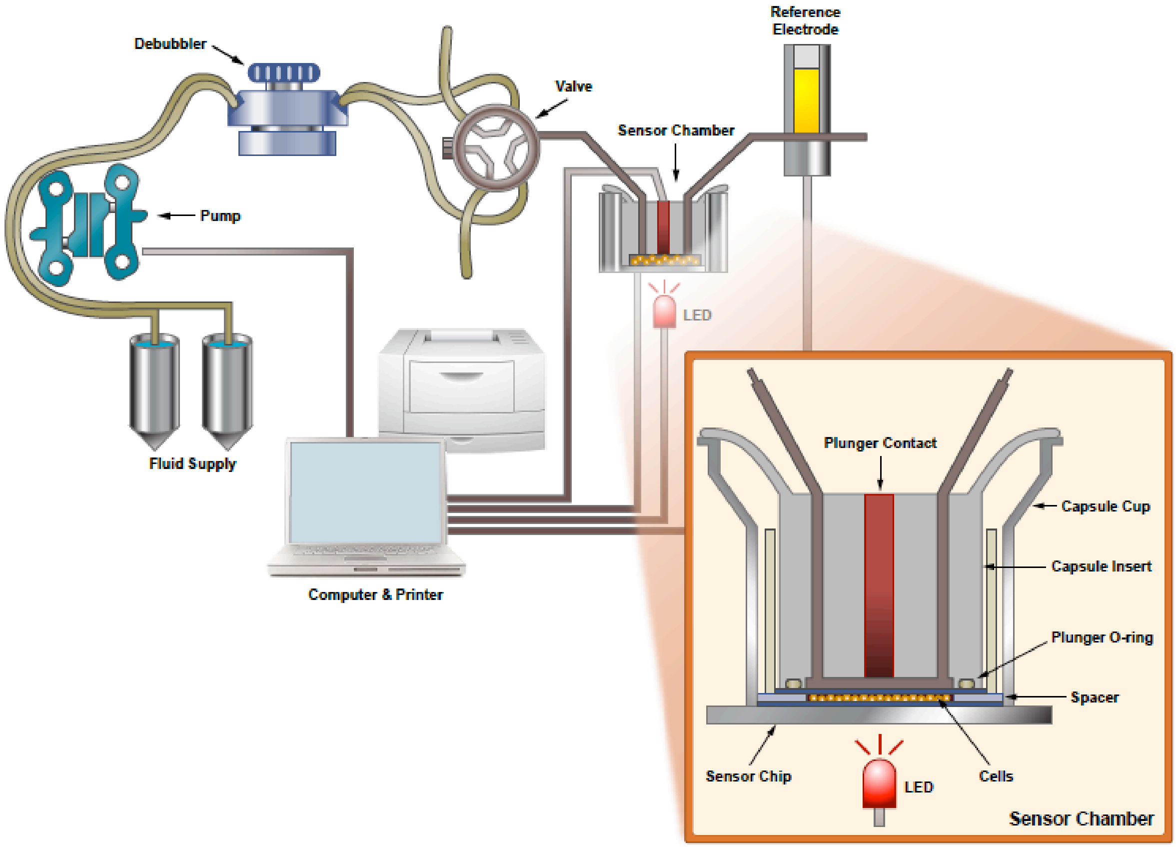
Introduction

- The Cytosensor microphysiometer (CM) test method models damage to corneal and conjunctival epithelial cells.
- Use of CM is restricted to water-soluble substances.
- CM estimates changes in cellular metabolism (i.e., glucose utilization rate) of mouse L929 fibroblasts by monitoring the rate of excretion of acid byproducts as measured by the resulting decrease in pH of the surrounding medium in an enclosed chamber (**Figures 1 and 2**):
  - Rate of pH change per unit time approximates the metabolic rate of the cell population.
- The test substance concentration that results in a 50% reduction in acidification rate (i.e., MRD_{50} [metabolic rate decrement of 50%]) is the endpoint used as a correlate to potential eye irritation (**Figure 3**).

Figure 1. Diagram of the Operating Components of CM[1]



[1] This illustration was modified from a figure from the CM manual. Original illustration was courtesy of Dr. Rodger Curren (Institute for In Vitro Sciences, Inc.).

Figure 2. ICCVAM-Recommended Protocol for CM[1]

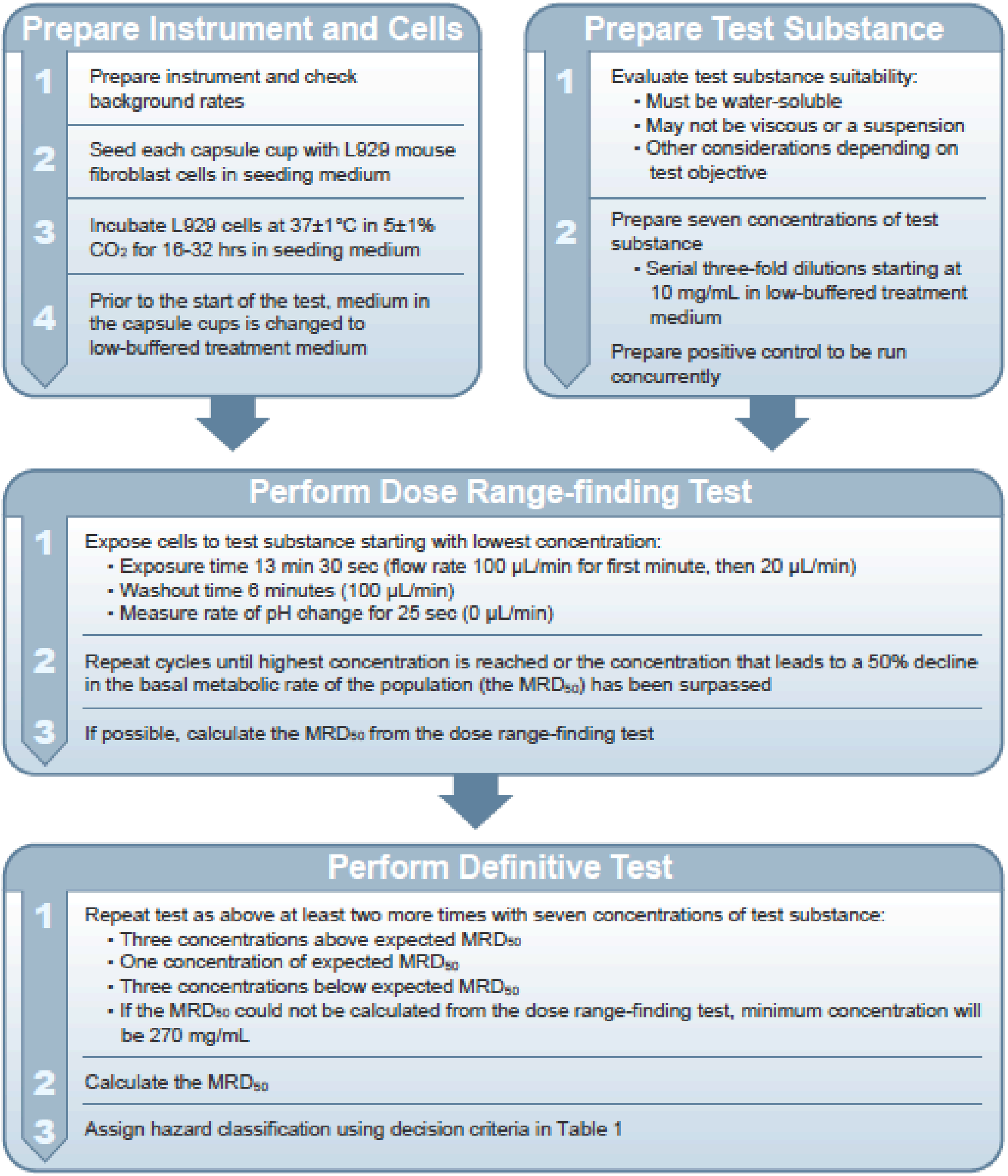
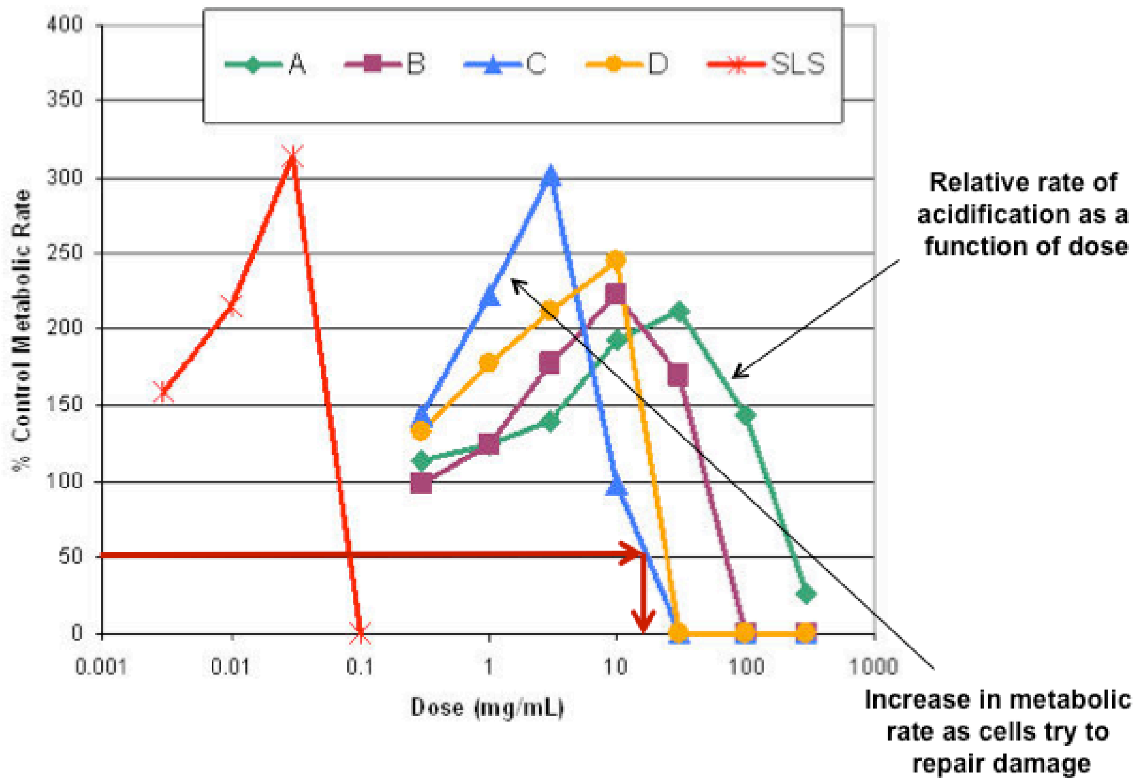


Table 1. Decision Criteria for the EPA and GHS Classification Systems Used for CM Evaluation

MRD_{50} (mg/mL)	EPA (EPA 2007)	GHS (UN 2009)
>80	Category IV (No hazard label required)	NA
>2; ≤80	No prediction can be made	NA
>10	NA	Not Classified
>2; ≤10	NA	No prediction can be made
≤2	Category I (Severe/corrosive)	Category 1 (Severe/corrosive)

Abbreviations: MRD_{50} = metabolic rate decrement of 50%; NA = not applicable for this particular classification and labeling system

Figure 3. Example of CM Data and MRD_{50} Calculation[1,2]



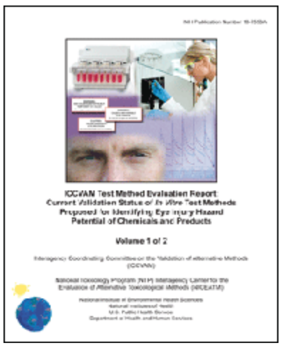
Abbreviations: MRD_{50} = metabolic rate decrement of 50%; SLS = 10% (w/v) sodium lauryl sulfate (positive control).

[1] Figure courtesy of Dr. Rodger Curren (Institute for In Vitro Sciences, Inc.).

[2] Letters A, B, C, and D represent different test substances.

ICCVAM Evaluation of CM

- ICCVAM evaluated the usefulness and limitations of CM for identifying ocular corrosives/severe irritants and substances not labeled as irritants.
- The ICCVAM evaluation process of CM included scientific peer review by an international independent panel, review by the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), and multiple public commenting opportunities.
- ICCVAM recommendations were published in September 2010 (ICCVAM 2010).



- ICCVAM recommendations were accepted in March 2011 by some U.S. Federal agencies.

Validation Database

- Accuracy assessments were conducted for each of two distinct databases.

  1. 53 surfactant substances (tested in seven different laboratories) included:
     - 21 surfactant chemicals
     - 32 surfactant-containing formulations
  2. 29 nonsurfactant substances (tested in seven different laboratories) included:
     - 27 nonsurfactant chemicals, which included a range of chemical classes (e.g., acids, alcohols, alkalis, and ketones)
     - 2 nonsurfactant formulations

Test Method Accuracy

Distinguishing Substances Not Labeled as Irritants From All Other Hazard Categories

- For surfactant-containing substances (**Table 2**), accuracy was 68% (36/53) for the GHS and 92% (48/52) for the EPA classification system. False negative rates were 0% (0/28) for the GHS classification system and 2% (1/46) for the EPA classification system.
  - The one false negative substance for the EPA classification system was Category III based on a 6-animal 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.
- For nonsurfactant substances (**Table 3**), accuracy was 64% (16/25) for the GHS classification system and 66% (19/29) for the EPA classification system. False negative rates were 33% (8/24) for the EPA classification system and 38% (8/21) for the GHS classification system.

Distinguishing Ocular Corrosives and Severe Irritants From All Other Hazard Categories

- For surfactant-containing substances (**Table 4**), accuracy was 85% (44/52) for the EPA classification system and 94% (50/53) for the GHS classification system. False positive rates were 3% (1/30) for the GHS classification system and 10% (3/29) for the EPA classification system.
- For nonsurfactant substances (**Table 5**), accuracy was 83% (24/29) for the GHS classification system and 92% (23/25) for the EPA classification system. False positive rates were 0% (0/18) for both the GHS and EPA classification systems.

Table 2. Accuracy of CM for Distinguishing Substances Not Labeled as Irritants From All Other Irritant Classes for Surfactant-Containing Substances

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

Table 3. Accuracy of CM for Distinguishing Substances Not Labeled as Irritants[1] From All Other Irritant Classes for Nonsurfactant Substances

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

Abbreviations: CM = Cytosensor microphysiometer; N = number of substances included in this analysis; No. = data used to calculate the percentage

[1] EPA classification system (EPA 2007): Category IV vs. Category I/II/III

[2] GHS classification system (UN 2009): Not Classified vs. Category 1/2A/2B

Table 4. Accuracy of CM for Distinguishing Corrosives/Severe Irritants[1] From All Other Irritant Classes for Surfactant-Containing Substances

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

Table 5. Accuracy of CM Distinguishing Corrosives/Severe Irritants[1] From All Other Irritant Classes for Nonsurfactant Substances

Classification System	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
EPA[1]	25	92	23/25	71	5/7	100	18/18	0	0/18	29	2/7
GHS[2]	29	83	24/29	55	6/11	100	18/18	0	0/18	45	5/11

Abbreviations: CM = Cytosensor microphysiometer; N = number of substances included in this analysis; No. = data used to calculate the percentage

[1] EPA classification system (EPA 2007): Category I vs. Category II/III/IV

[2] GHS classification system (UN 2009): Category 1 vs. Category 2A/2B/NC

ICCVAM Recommendations: Usefulness and Limitations

Evaluation as a Screening Test to Identify Substances Not Labeled as Irritants

- Water-soluble surfactant chemicals and certain types of surfactant-containing formulations:
  - Accuracy and reliability of CM are sufficient to support its use as a screening test to identify these types of substances (e.g., cosmetics and personal care product formulations, but not pesticide formulations) as substances not labeled as irritants and distinguish them from all other hazard categories when results are to be used specifically for hazard classification and labeling purposes.
- Water-soluble nonsurfactant substances and formulations:
  - CM is **not** recommended for these types of substances due to the high false negative rate.

Evaluation as a Screening Test to Identify Ocular Corrosives and Severe Irritants

- Water-soluble surfactants, surfactant-containing formulations, and nonsurfactants:
  - CM can be used as a screening test to identify these types of substances as ocular corrosives and severe irritants in a tiered-testing strategy, as part of a weight-of-evidence approach.
    - A substance that tests negative with CM would need to be tested in the rabbit eye test to confirm whether the substance is or is not a corrosive/severe eye irritant, and if it is not, to distinguish between moderate and mild ocular irritants.

ICCVAM Recommendations: Future Studies

- Conduct studies to expand the applicability domain of CM for the identification of ocular corrosives and severe irritants and substances not labeled as irritants.
- For these studies, select from the list of ICCVAM-recommended reference substances for validation of *in vitro* ocular safety test methods for the evaluation of ocular corrosives and severe irritants (ICCVAM 2006).
- Similarly, a set of reference substances could also be selected from this list for the evaluation of substances not labeled as irritants.
- Identify and test substances in the moderate and mild ocular irritant categories to further evaluate the performance of CM for the identification of all ocular hazard categories.
- Encourage users to provide ICCVAM with all data generated from future studies to assist with further characterization of the usefulness and limitations of CM for the evaluation of all ocular hazard categories.

Conclusions

- ICCVAM recommended CM as an *in vitro* alternative to the rabbit eye test for:
  - Identifying substances within a limited applicability domain as ocular corrosives/severe irritants
  - Identifying substances within an even more restricted applicability domain as substances not labeled as irritants
- While not a complete replacement for the rabbit eye test, CM can be used as a screening test in a tiered-testing strategy, as part of a weight-of-evidence approach.
- CM is the first *in vitro* test method available in the U.S. for identifying a subset of substances that do not require ocular hazard labeling.
- An OECD Expert Group is currently developing a draft test guideline for CM.

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