Cytosensor Microphysiomer

Best Practices for Ocular Safety Testing
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Overview of Cytosensor™ Microphysiometer

- Machine developed in late 1980s by Molecular Devices, Sunnyvale, CA
- Intended purpose was to conduct real time measurements of cellular metabolism rate
- Receptor-activated metabolism changes of most interest for drug discovery
- However, additional uses were proposed to be measures of cell toxicity not receptor related, e.g. eye irritation
- Procter & Gamble (Bruner, *et al*.) took the lead on ocular irritation (collaborated with IIVS)

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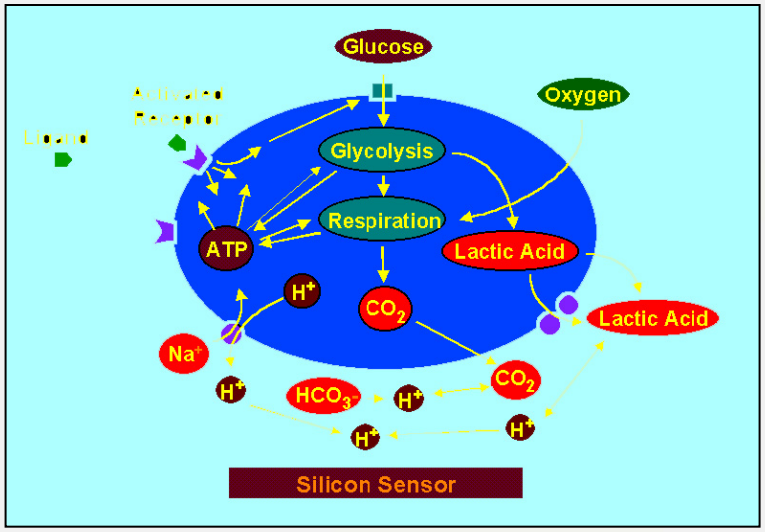
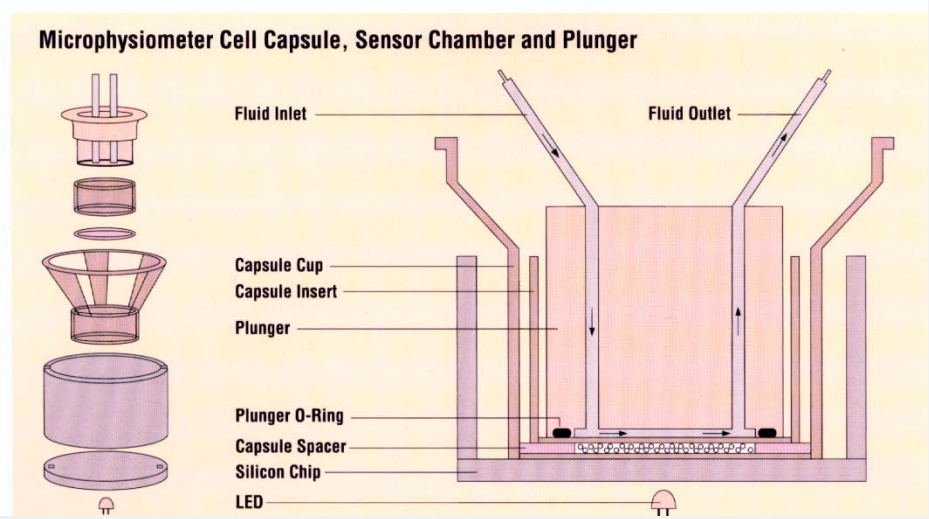
Overview of Cytosensor™ Microphysiometer

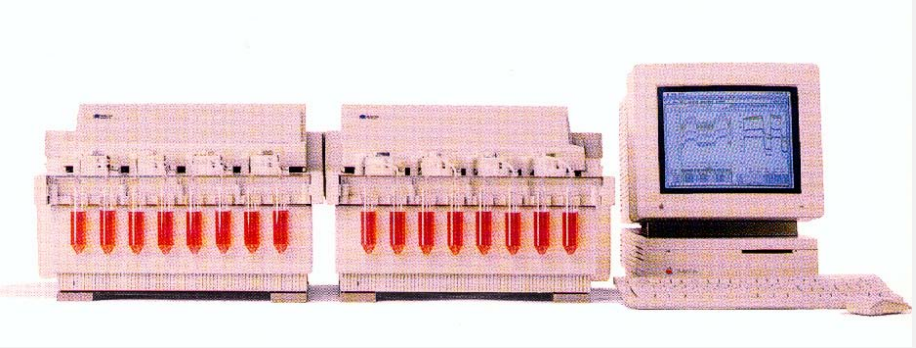
- Original instrument – Silicon Microphysiometer – replaced by similar Cytosensor Microphysiometer
- Included in many evaluations from 1992 on
- ECVAM retrospective review (using extensive BRD) 2007-2009
- ECVAM SAC report in early 2009 on validated uses
- Popular review (full of drama & suspense) published Spring 2010

Cytosensor™ Microphysiometer





Cell viability measured by the continued production and secretion of hydrogen ions from glucose metabolism



SCIENCE

EDUCATION

OUTREACH

Cytosensor™ Microphysiometer

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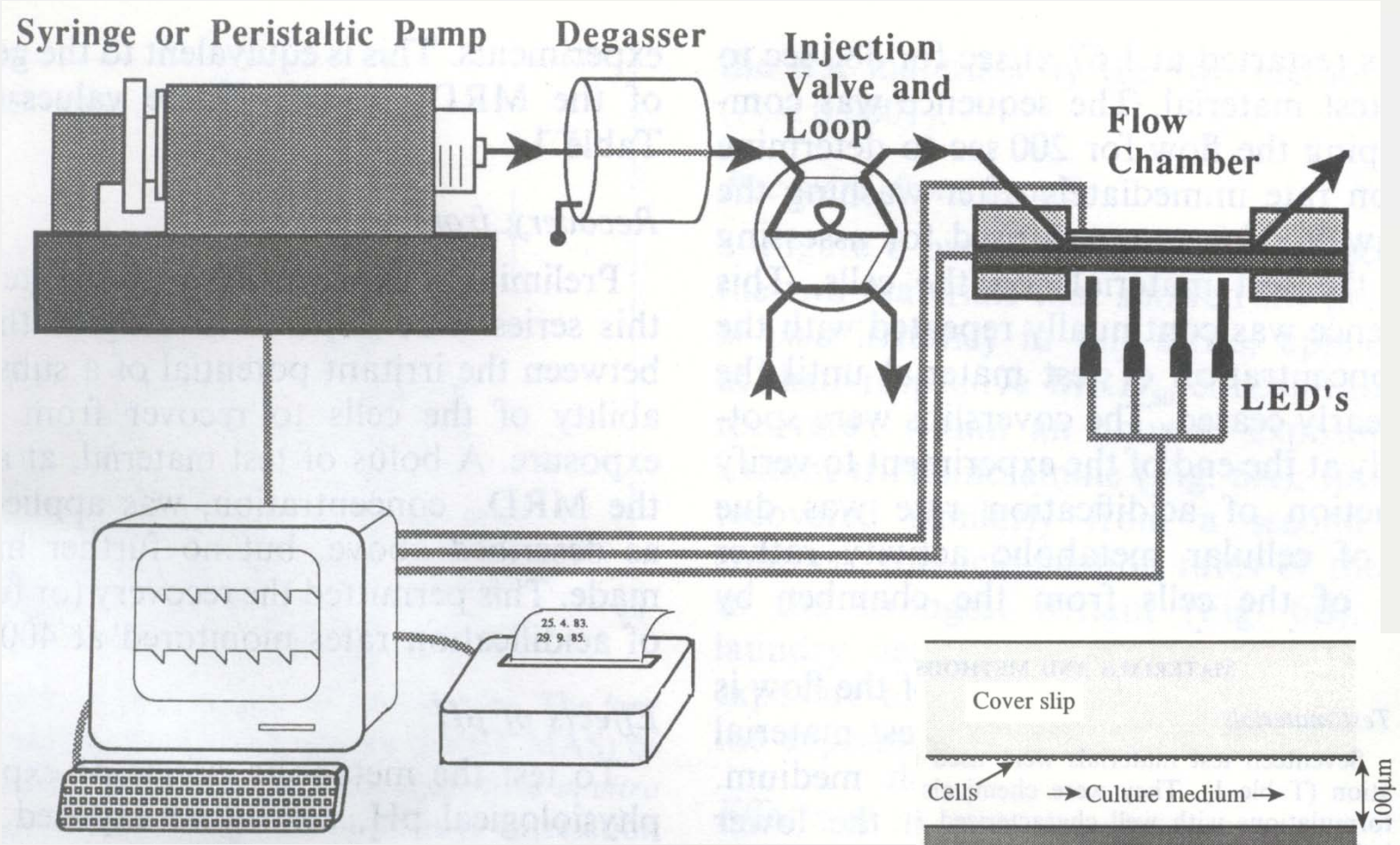
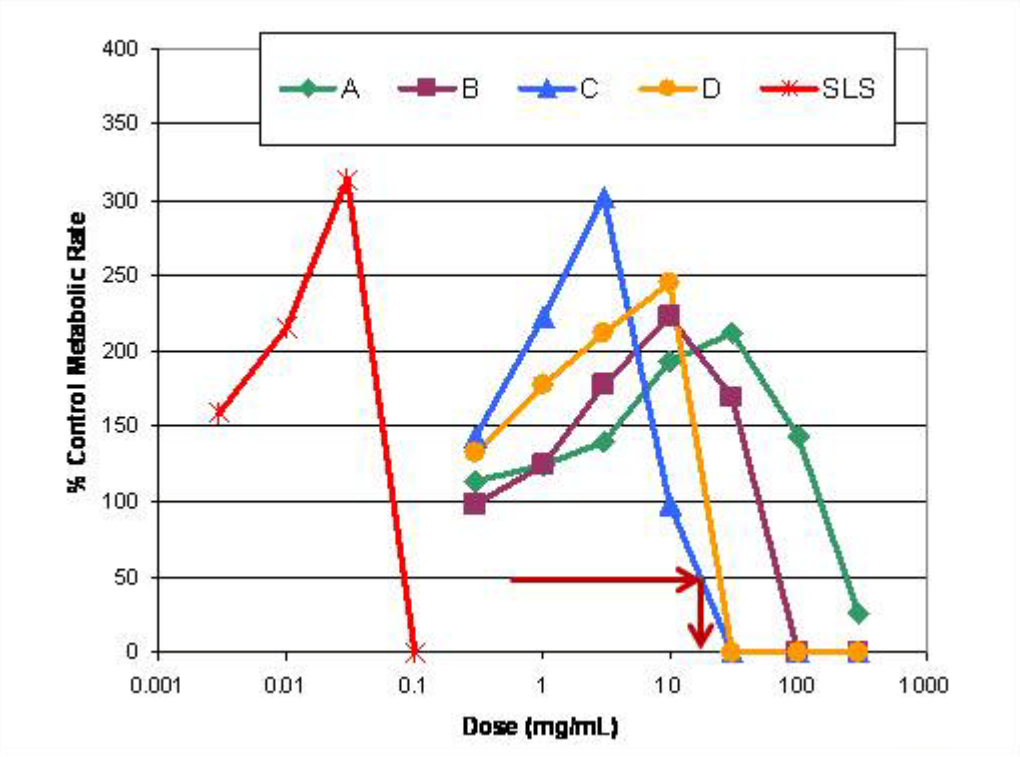
Instrument Operation



Data Presentation

- Assay endpoint: Dose calculated to reduce the metabolic rate to 50% of the initial rate (MRD50)
- Positive Control: SLS
  - MRD_{50} = 80±11 μg/ml
    CV=14%
    n=640 trials



Main Studies Utilized To Understand Cytosensor Performance

Major Validation Studies

- EC/HO
- CTFA Phase III
- COLIPA

Multi-lab studies

- Bagley et al. (2 labs for SM)

Single lab sponsorship

- L'Oreal data in Catroux, et al.
- P&G data for Bruner et al. and unpublished data
- IIVS historic positive control data, and technician training data.

Study Design

Applicability Domain

- For technical reasons (small bore tubing), only fully water-soluble test materials can be used with the Cytosensor!
- Majority of work has been conducted with water-soluble surfactants or surfactant based products
- High and Low pH materials not excluded because exposure medium is very weakly buffered

Study Design (2)

Test Method

- Mouse L929 cells seeded into chambers
- Chambers placed in Cytosensor, program started with defined start/stop points for medium flow
- Dose range finding conducted with wide concentrations of test article
- Doses on either side of an LC50 chosen for definitive studies
- Test materials applied using increasing doses
- Final LC50 calculated
- Data interpreted relative to benchmark or to standard hazard classifications

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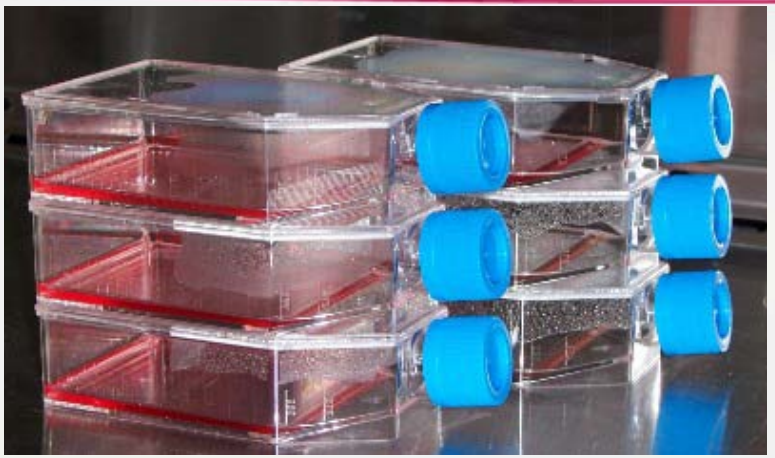
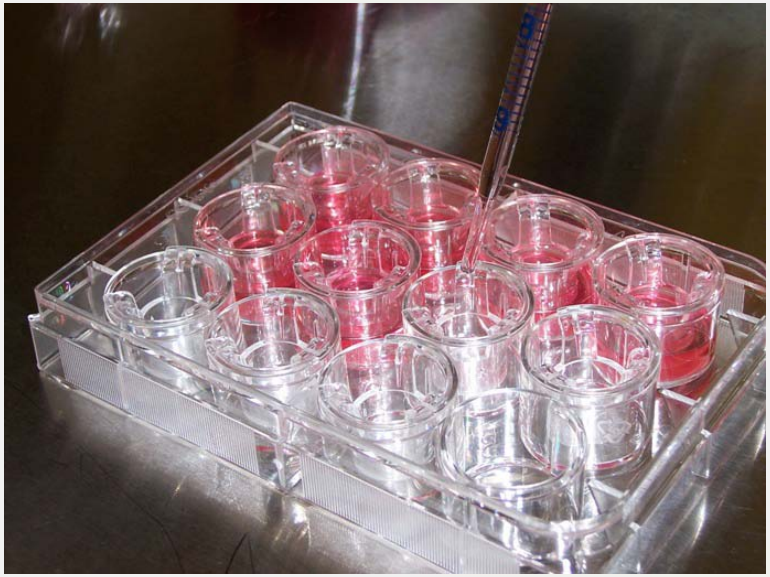
Cell Growth

- L929 cells grown to confluency





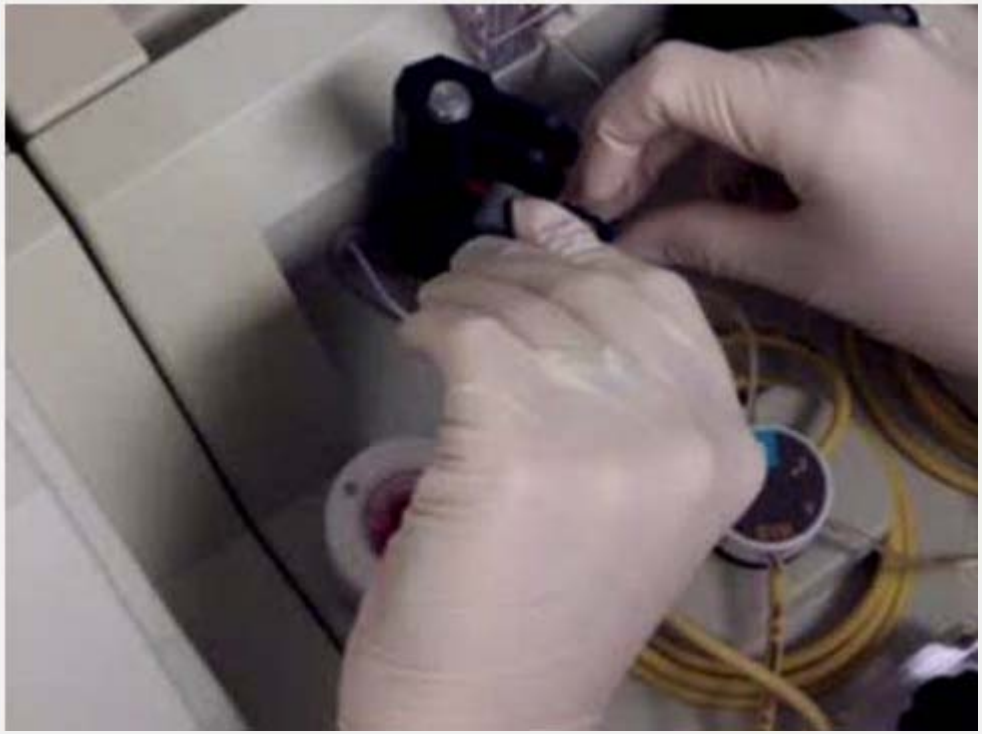
- Cells seeded onto 0.3µm pore transwells at 600,000 cells per well
- Incubated 18-36 hours in transwell

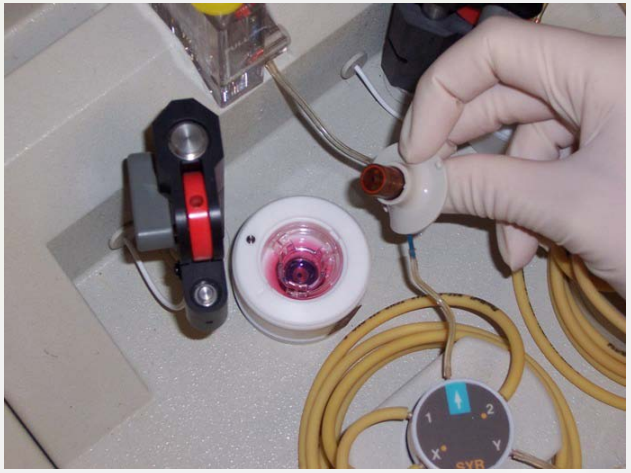
Preparation of Chambers

- Refresh cell media
- Place spacer in transwell over cell
- Place transwell in sensor chamber

Final Chamber Assembly

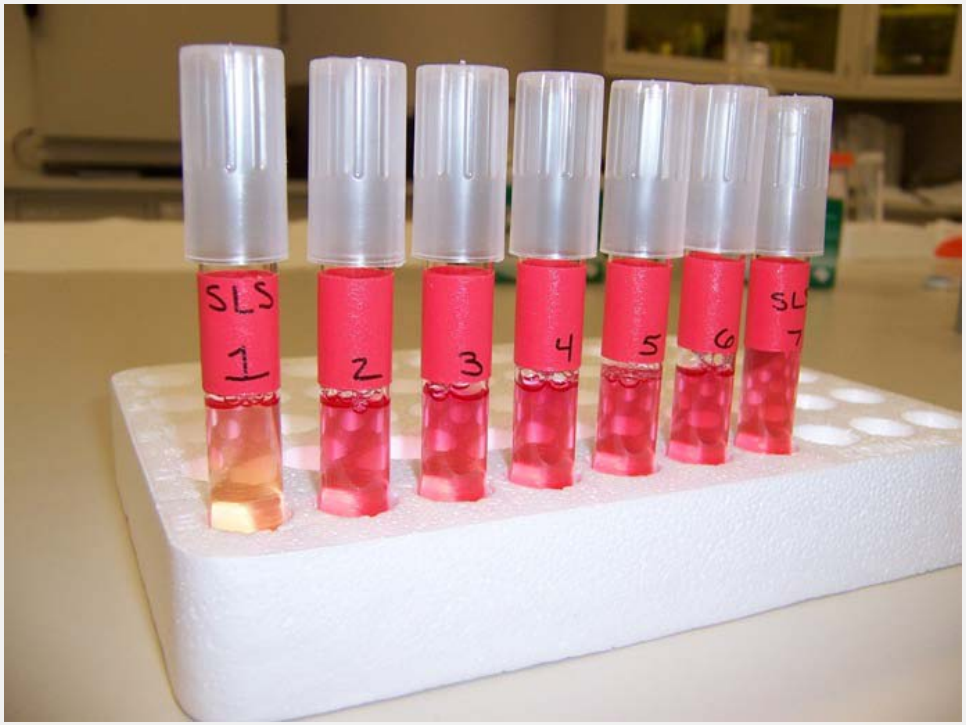
- Replace dummy chamber with sensor chamber





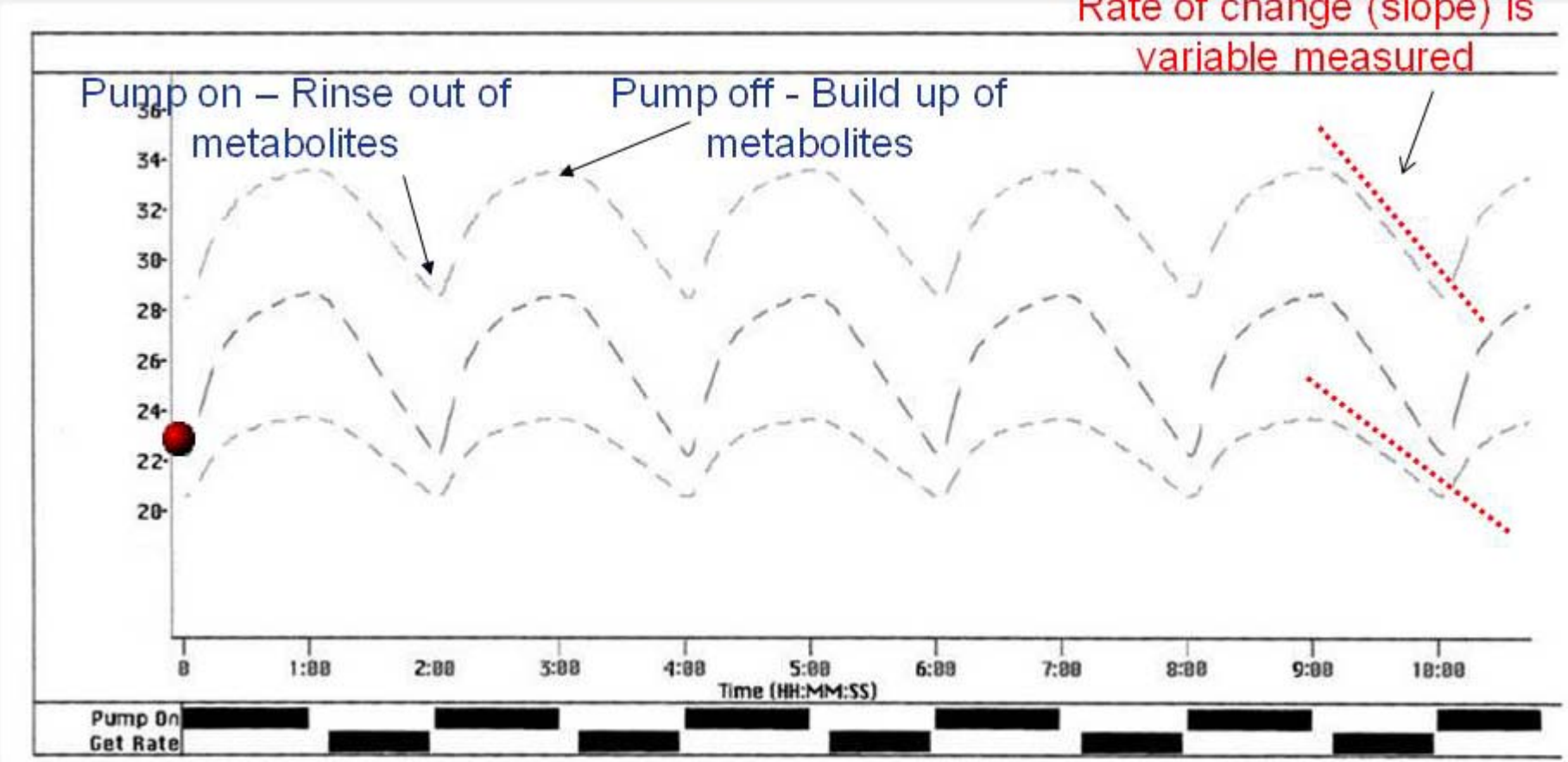
Test Article Preparation

- Serial dilutions of test articles
  - Personal care products
    - surfactants
  - Household cleaning products
  - Cosmetics
  - Pharmaceuticals



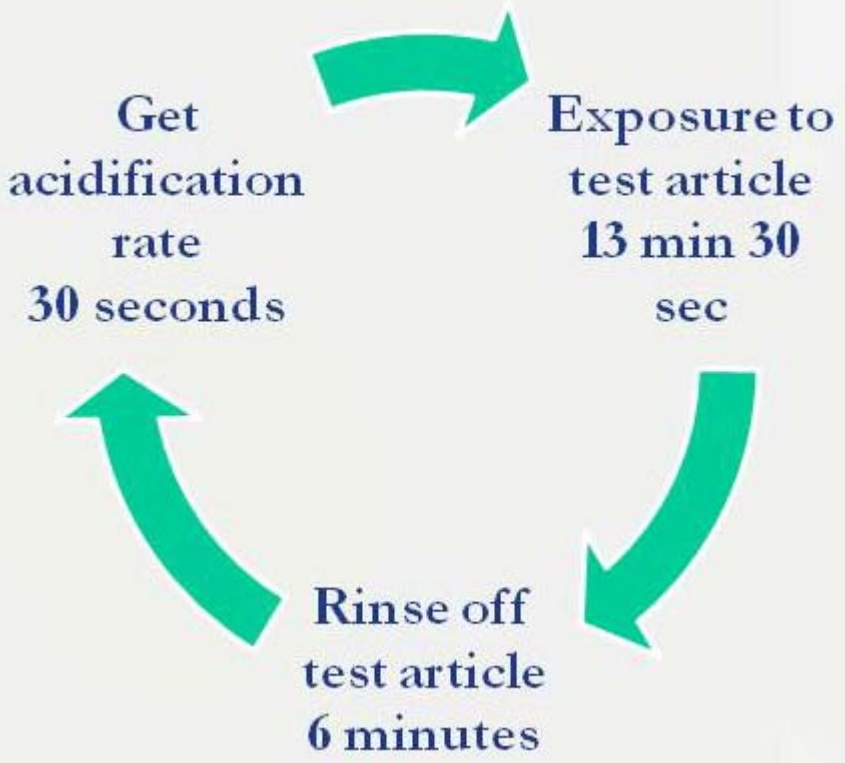
Calibration of Microphysiometer

- Allow cells to stabilize for 1 hour before testing

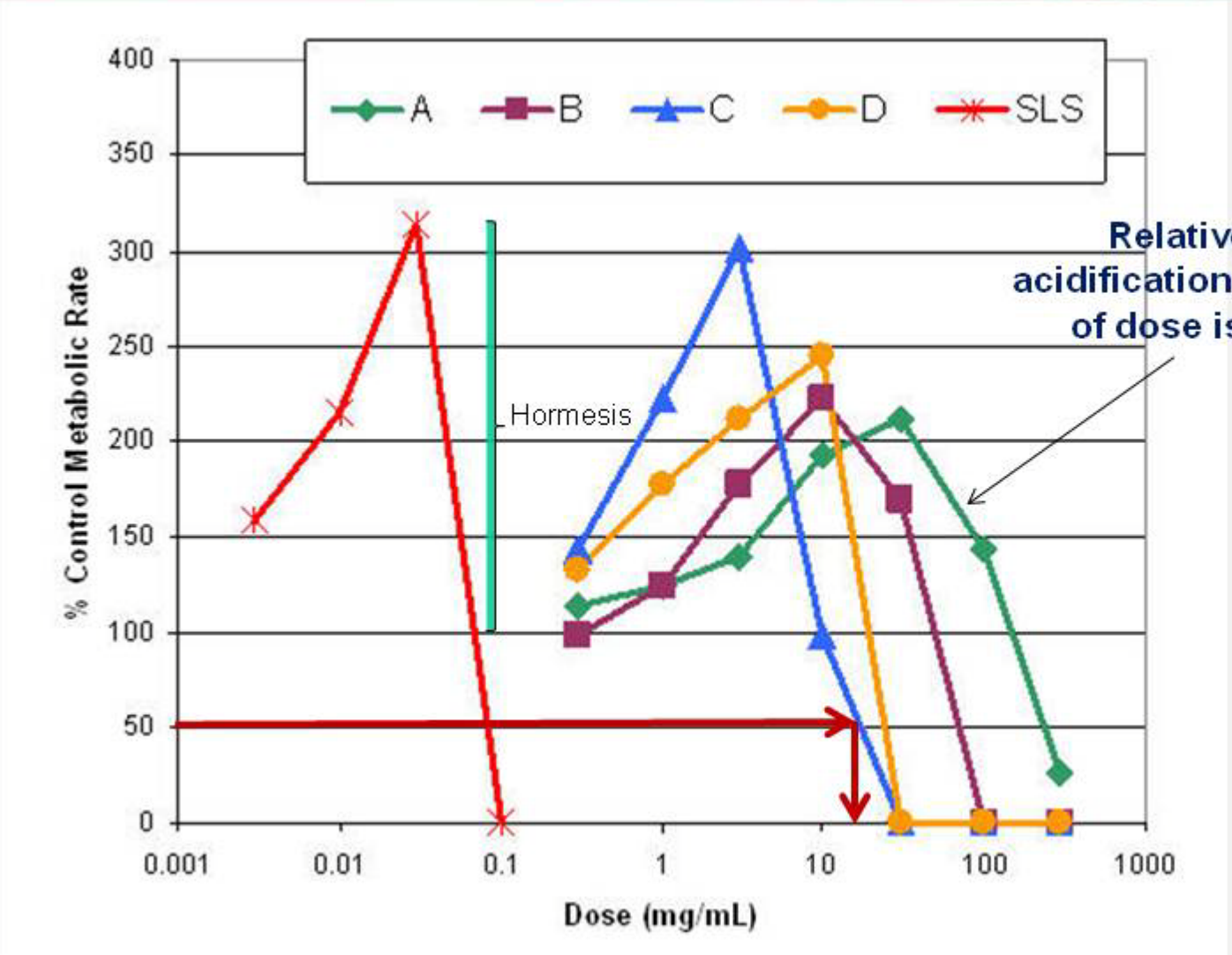


Exposure Parameters

- Total dosing time is 20 minutes
- Begin with lowest dose and work towards highest dose until cells have an acidification rate near zero

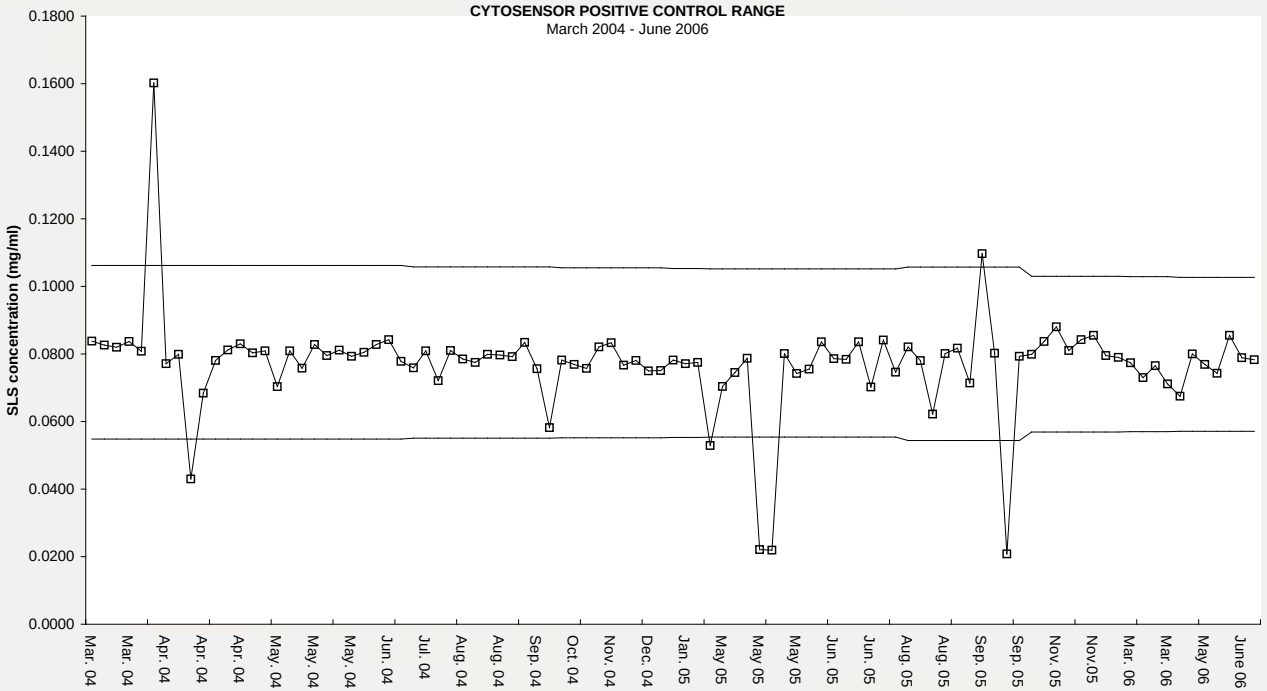


Data Presentation



Within-Laboratory Reproducibility

Substance	Dates	No. of Assays	Mean MRD_{50} (mg/mL)	SD	CV
SLS	April, 14 1994 – June 30, 2006	629	0.0799	0.011	14.3%
SLS	March 2, 2004 - June 30, 2006	285	0.0792	0.022	28.0%



Summary Of Within-Lab Reproducibility

Study	# of materials	# of Replicates	CV	Com-ments
Bruner et al.	17	3-11	0.6%-16.9%	SM, 320s
L'Oreal	19	3	5.7%-64.6%	SM, 410s
EC/HO	60	≥3	Data not yet obtained	SM
CTFA	25	3(30); 4(5)	0.0%-61.4%	CM
COLIPA	55	≥3	1.3%-55.9%; 0.0%-59.4%	CM
Common EC/HO & COLIPA	20	≥3	Data not yet obtained	CM
SM/CM comparison	11	≥3	1.7%-41.5% (SM); 3.7%-47.7% (CM)	
IIVS Positive control	1	629	14.3%	CM

Between-Laboratory Reproducibility

Studies	chemicals tested	products tested	Instrument	Coded?	Results reported (Range)	Replicates			CV's	Comments	Testability	
						labs	expts	reps				
Several companies [5] (Bagley, et al. 1992)	12	20	SM (2 Protocols)	Yes	Log MRD_{50} - 0.398 to 4.467 (log µg/ml), interlab correlation and Draize correlation	2	32	3	81% , 20%			
EC/HO[1] (Balls, Botham et al. 1995)	31 – 38 (depending on lab) of 60 were compatible	0	SM	Yes	MRD_{50} - 2.41 to 5.56 (log µg/ml)	4	28	3	Ave. = 6.7%(4); Hi 23.5%		73.7% Agreement	
COLIPA[1] (Brantom, P.G., et al., 1997)	17-20 (depending on lab) of 23 were compatible	9 of 32 were compatible	CM	Yes	MRD_{50} - 2.48 to 5.5 (log µg/ml) , interlab correlation and Draize correlation	2	29	3	Ave. = 3.1%; Hi 18.9%		94.5% agree	
14 common EC/HO & COLIPA	14		CM&SM	Yes		5(6)	14	3	Not Completed	Also correlation analysis	NA	

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Data Interpretation (ESAC Statement)

- The Cytosensor Microphysiometer test method can be used for two of the three EU and GHS classification categories used for the endpoint of ocular irritation:
- A. The **Cytosensor Microphysiometer test method (INVITTOX Protocol 102 modified)** is considered to have been scientifically validated and to be ready for consideration for regulatory use as an initial step within a **Top-Down Approach** to identify ocular corrosives
- and severe irritants (EU R41, GHS Category 1, and EPA Category I) from all other classes for the chemical applicability domain of water-soluble chemicals (substances and mixtures).
- B. Furthermore, the **Cytosensor Microphysiometer test method (INVITTOX Protocol 102 modified)** is considered to have been scientifically validated and to be ready for consideration for regulatory use as an initial step within a **Bottom-Up Approach** to identify non-irritants (EU:NC; GHS: NC; EPA: cat IV) from all other classes only for water-soluble surfactants and water-soluble surfactant-containing mixtures.

Data Interpretation

- Identifying Severe Irritants (water-soluble substances and mixtures):
  - If MRD50 ≤ 2 mg/ml, then Category I for EPA (and GHS,EU CLP)
- Identifying Non-Irritants (water-soluble surfactants and surfactant-containing mixtures):
  - If MRD50 >80 mg/ml, then Category IV for EPA
  - If MRD50 > 10 mg/ml, then No Category for GHS and EU CLP
- Use own company benchmarks for other, non-regulatory, interpretations

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