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# *In Vitro* Lecture and Luncheon for Students

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# *In Vitro* Lecture and Luncheon for Students



Leveraging *In Vitro* Methods to  
Advance Microbial Product  
Safety

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## Disclaimer

The opinions expressed in this presentation are those of the presenter and do not necessarily reflect those of The Clorox Company or its affiliates.



1. What are antimicrobial products?
2. What alternatives are there to traditional methods?
3. Assessing new methods for regulatory use.
4. Case study: Playing the regulator—is the method acceptable for use?



# What Are Antimicrobial Products?

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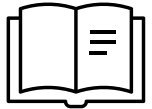
It's All In The Claim: Antimicrobial products “kill” bacteria, viruses, and other microbial pathogens.

**Kills 99.9% of Bacteria\* and Viruses†**





- Authority: US Environmental Protection Agency (EPA)



- Law: Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)



- Product: Disinfecting and sanitizing

**\*\*Require pre-market review and registration\*\***  
Ingredients and finished product



# Data Supporting Antimicrobial Product Registrations Historically Generated Using Animals

Endpoint	Animals Used* per product tested
Acute Oral Toxicity	5 Rats
Acute Dermal Toxicity	5 Rats
Acute Inhalation Toxicity	5 Rats
Eye Irritation	3 Rabbits
Skin Irritation	3 Rabbits
Skin Sensitization	
• Local Lymph Node Assay (LLNA)	15 Mice
• Guinea-Pig Maximization Test (GPMT)	15 Guinea Pigs
• Buehler test	30 Guinea Pigs



\*Minimum

\*\*Six Acute Toxicity Studies = "6-Pack"\*\*

- Historically animal testing has been thought of as the gold standard... that thought is now changing:

	<b>Animal</b>	<b><i>In vitro</i></b>
<b>Human-Relevant</b>	Animal Species Differences	Humanized models
<b>Reliable</b>	High Variability	Controlled/ Reduced Variability
<b>Subjective</b>	Yes: Endpoints requiring expert judgement	No: Analytical endpoints

- New approach methods (NAMs) have the opportunity to be designed to better protect the consumer and environment: THE NEW GOLD STANDARD!

# Alternative Methods Accepted By US EPA Supporting Antimicrobial Product Registrations

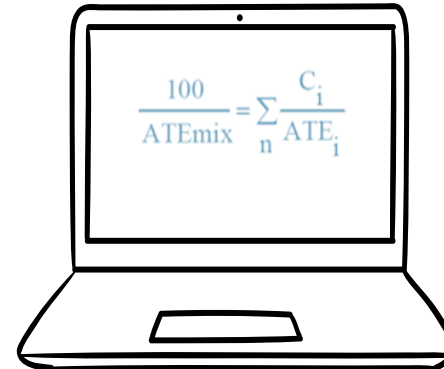
## Data Waiver



## Bridging



## Computational Modelling



## Alternative Testing



*EPA (2012) Guidance for Waiving or Bridging*

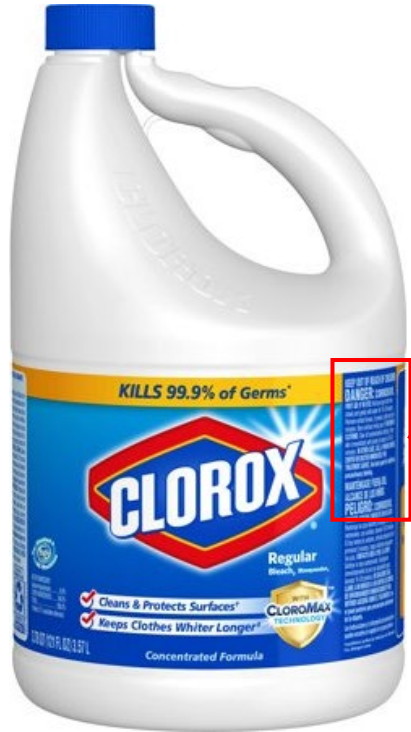
*EPA (2016) Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations and Supporting Retrospective Analysis*

*United Nations (2023) Globally Harmonized System of Classification and Labelling of Chemicals (GHS): Tenth Edition*

*Hamm et al (2021) Performance of the GHS Mixtures Equation for Predicting Acute Oral Toxicity*

*US EPA (2015) Use Of An Alternate Testing Framework For Classification Of Eye Irritation Potential Of EPA Pesticide Products*

# “6-Pack” Purpose: Safety Label Language



**KEEP OUT OF REACH OF CHILDREN**  
**DANGER: CORROSIVE.**  
**FIRST AID: IF IN EYES:** Hold eye open and rinse slowly and gently with water for 15–20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. **IF ON SKIN OR CLOTHING:** Take off contaminated clothing. Rinse skin immediately with plenty of water for 15–20 minutes. **IN EITHER CASE, CALL A POISON CONTROL CENTER OR DOCTOR IMMEDIATELY FOR TREATMENT ADVICE. See back panel for additional precautionary labeling.**



Toxicity Testing Results



Hazard Categories



Safety Labeling  
 Precautionary  
 First Aid

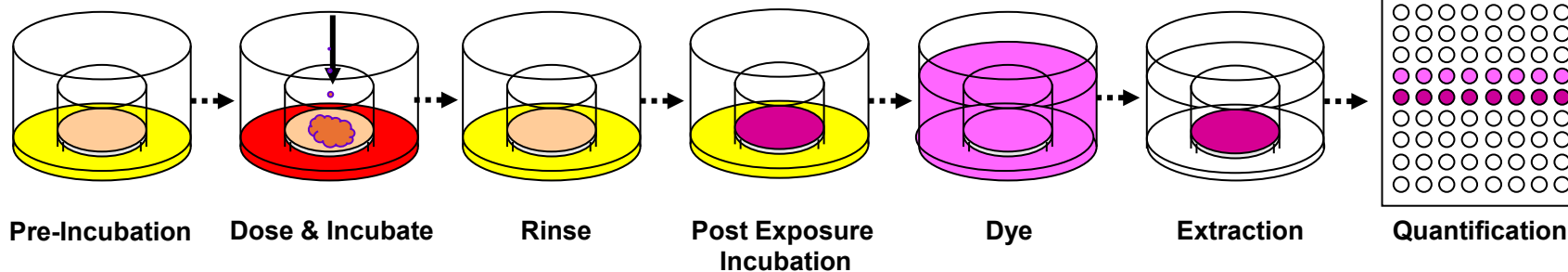
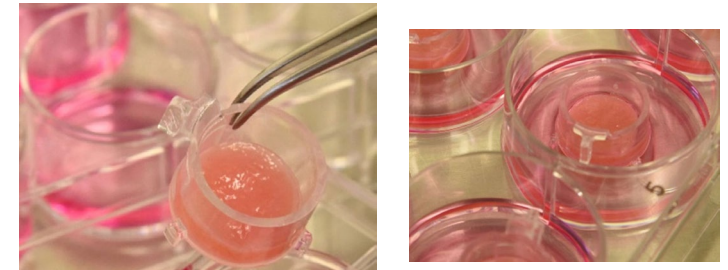
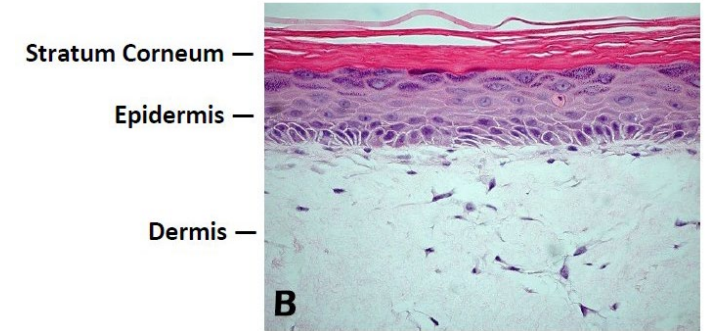
**PRECAUTIONARY STATEMENTS: HAZARDS TO HUMANS AND DOMESTIC ANIMALS. CAUTION:** Causes moderate eye irritation. Avoid contact with eyes, skin or clothing. Wash thoroughly with soap and water after handling. Avoid contact with foods. **FIRST AID: If in eyes:** Hold eye open and rinse slowly and gently with water for 15–20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. **If on skin or clothing:** Take off contaminated clothing. Rinse skin immediately with plenty of water for 15–20 minutes. Call a poison control center or doctor for treatment advice. Have the product container or label with you when calling a poison control center or doctor, or going for treatment.

# A Novel Approach To Fulfill Skin Irritation Data Requirements For Antimicrobial Products With No/Low Irritation Potential



# A New *In Vitro* Antimicrobial Skin Irritation Test

- Human 3D-reconstructed full thickness skin model for use with antimicrobial products
- Developed by testing 23 antimicrobial formulations
- Protocol determines non-irritating (EPA Category IV)

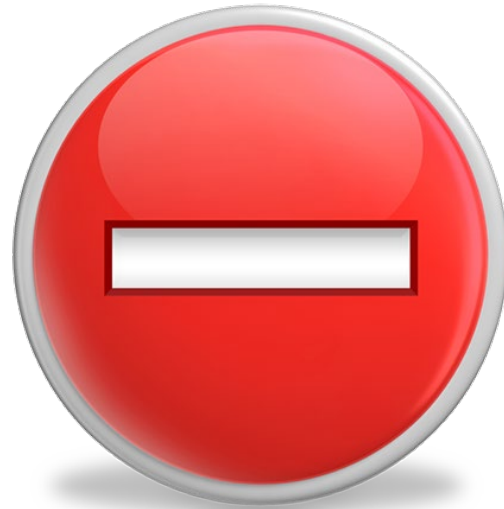


# Test Performance Metrics: How Good Is My Test?



## **Sensitivity**

*Identify Positives*



## **Specificity**

*Identify Negatives*



## **Accuracy**

*Identify BOTH Positives & Negatives*

- Animal (*in vivo*) test
  - Reproducibility (predict itself): 40-60%
- *In Vitro* test



Sensitivity	Specificity	Accuracy	n
78%	83%	79%	23

Photo supplied by  
<https://www.phenion.com>

“n” is the number of substances analyzed



# Assessing New Methods for Regulatory Use: A Case Study

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# Case Study Background

- You are a regulator being asked to approve a new alternative method for skin sensitization.
- You are provided with the following data and asked your opinion if this should be approved.

Method	Sensitivity	Specificity	Accuracy	n
A	91%	64%	82%	111
B	81%	83%	82%	103
C	74%	73%	74%	103

- *Sensitivity measures the ability of a method to identify a true positive*
- *Specificity measures the ability of a method to identify a true negative*
- *Accuracy measures the ability of a method to identify both a true positive and negative (combined)*
- *“n” is the number of substances analyzed*

**NOTE:** Everyone will assess method A and your table will assess EITHER B or C (assigned)

1. What is your group's perspective on the assigned methods (A and B, or A and C): would you have approved them initially?
2. What was the group reaction to the animal test (LLNA) being Method A?
3. How (if at all) did the data on the LLNA help with the "acceptance" of Methods B and C?
4. How (if at all) did the data comparison to human help with the "acceptance" of Methods B and C?
5. What was the group reaction to the "reveal" that ALL the methods are approved for global use?
6. Any additional group perspectives on additional information would you like to know (as the regulator) to increase confidence in accepting these methods (or others)?

- Validation has been a HOT topic in recent years.
- Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Report on Validation (2024) 6 Key Concepts for Validation:
  1. Context of use
  2. Biological Relevance
  3. Technical Characterization
  4. Data Integrity
  5. Information Transparency
  6. Independent Review
- It is important that we have a transparent approach to accept new methods for use... AND that we are not unnecessarily holding ourselves to a higher standard than necessary!