



THE SCIENCE OF BEER

# THRESHOLD TESTING & THE ASBC FLAVOR SPIKING CALCULATOR

ASBC/MBAA 2014 Annual Meeting  
Chicago, IL

**Ian McLaughlin**

Craft Brew Alliance  
Technical Service Data Analyst  
QC/Sensory



# THRESHOLD TESTING

- What are thresholds?
- How do you test them?
- What's the catch?
- ASBC Flavor Spiking Calculator



# THRESHOLD TESTING

- Types of thresholds:
  - Absolute (detection) threshold
    - “Something is there...”
  - Recognition threshold
    - “I know what’s there!”
  - Difference threshold (JND - just noticeable difference)
    - “Now there’s more/less of it!”
  - Terminal threshold
    - “There’s so much, I can’t tell anymore!”



# THRESHOLD TESTING

- How to test for thresholds
  - Ascending Method of Limits
    - 6 sequential difference (triangle) tests
    - Each set doubles the intensity of the previous
    - Set expected result near the third test level



# THRESHOLD TESTING

- How to test for thresholds, cont'd
  - Ascending Method of Limits
    - Present 'odd' samples in each position equally across all sets
    - Always evaluate from low-to-high (left-to-right)
    - Guessing is required when answer is unclear
    - Keep retasting to a minimum to avoid fatigue



# THRESHOLD TESTING



# THRESHOLD TESTING

- Threshold value

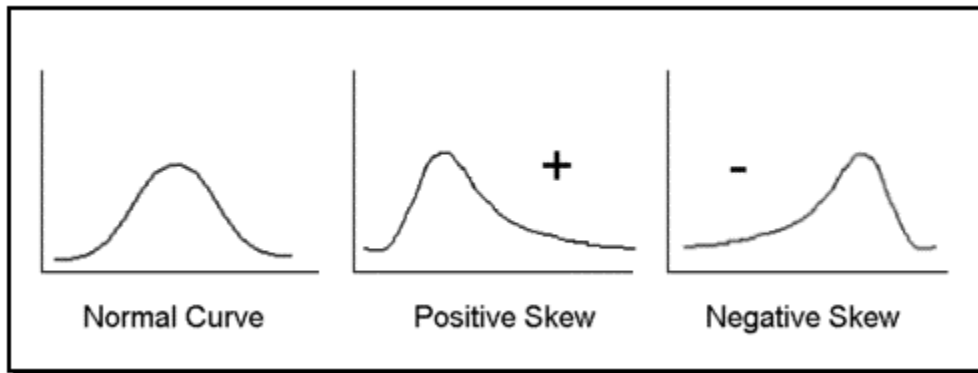
- Population: the concentration at which 50% of the population can detect the compound
- Individual: the geometric mean of the last\* incorrect answer's concentration and the first\* correct answer's concentration

TESTS	1	2	3	4	5	6
Correct?	√	√	√	X	√	√



# THRESHOLD TESTING

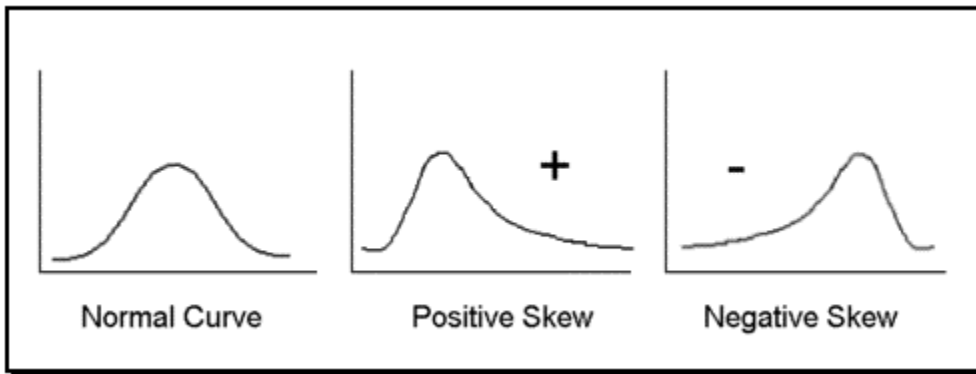
- What is the geometric mean?
  - Measure of “central tendency”, resists skewing
  - Arithmetic mean =  $(a_1 + a_2 + a_3 \dots + a_n) / n$
  - Geometric mean =  $(a_1 * a_2 * a_3 \dots * a_n)^{(1/n)}$
  - 30, 35, 45, 50
    - Arithmetic mean: 40
    - Geometric mean: 39.2





# THRESHOLD TESTING

- What is the geometric mean?
  - Measure of “central tendency”, resists skewing
  - Arithmetic mean =  $(a_1 + a_2 + a_3 \dots + a_n) / n$
  - Geometric mean =  $(a_1 * a_2 * a_3 \dots * a_n)^{(1/n)}$
  - 30, 35, 45, 2000
    - Arithmetic mean: 527.5
    - Geometric mean: 98.6



# THRESHOLD TESTING

- Difficulties:
  - With the idea
    - Ill-defined, in theory
    - Results do not reproduce well
      - +/- 20% reproducibility for given panel, +/- 50% between large panels
    - Published values can vary enormously
      - Little consistency in testing matrix
    - Accuracy depends on analyte measurement
      - Otherwise, results vary along with your precision at lab bench



# THRESHOLD TESTING

- Difficulties, cont'd:
  - With the test
    - 6 triangle tests, 3+ replications = lots of tasting
      - Potential for fatigue
      - Panelist burnout



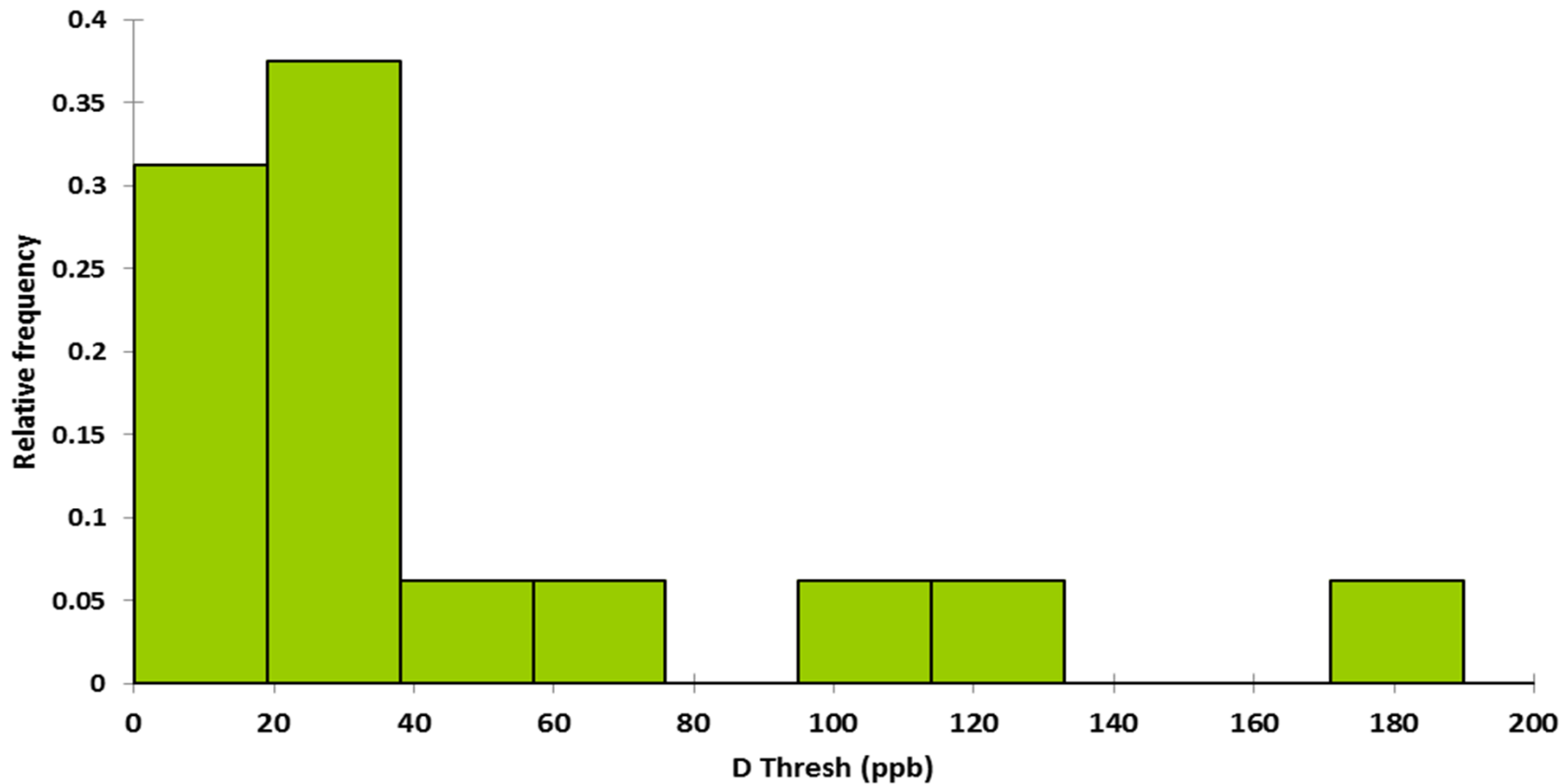
# THRESHOLD TESTING

- Difficulties, cont'd:
  - With the people
    - Anosmia
      - When a person's threshold exceeds 2 st.dev above the pop. mean
    - Lots of variation in population
      - Different distributions for each compound
    - Day-to-day influences on your sensitivity
      - Illness, injury, allergies, medication, etc
      - Ergo, tasting reps, throwing out outliers



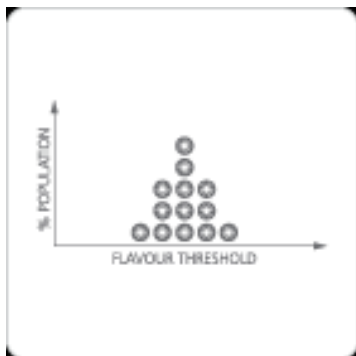
# THRESHOLD TESTING

Histogram - Distribution of Diacetyl Thresholds (ppb)  
16 CBA panelists

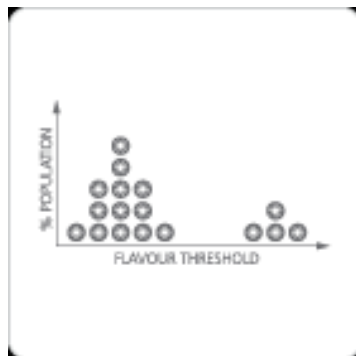


# THRESHOLD TESTING

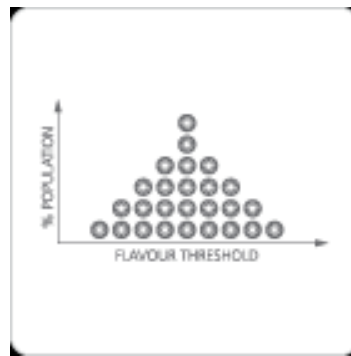
## Threshold Distribution Patterns of various beer flavors



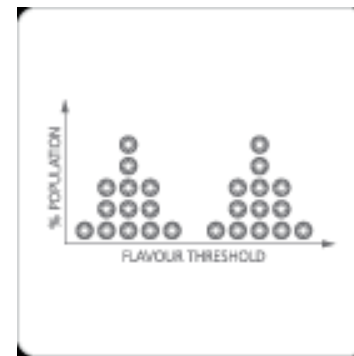
Ethyl acetate



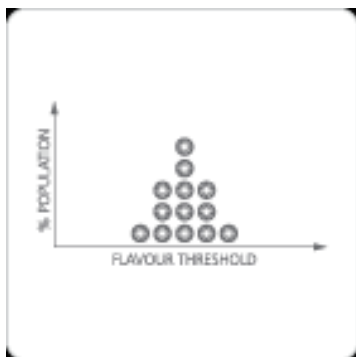
Isovaleric



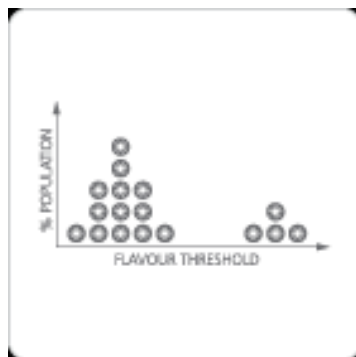
DMS



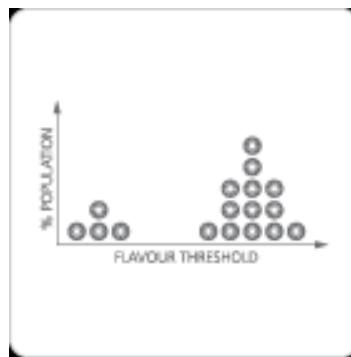
Indole



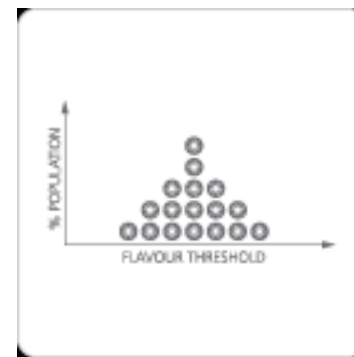
Isoamyl acetate



Isobutyraldehyde



Geraniol



Butyric acid



\* Histograms courtesy of Aroxa.com

# THRESHOLD TESTING

- When to deploy threshold testing?
  - Setting production specifications
  - Selecting and screening panelists
  - “Backup analytical instrument”
    - CBA tests many panelists for diacetyl threshold
    - Used to clear D-rest samples if gas chromatograph for VDK is disabled



# THRESHOLD TESTING

- How to create the test samples
  - Consult the ASBC Flavor Standards webinar for details
  - Use “Flavor Capsules” from Aroxa/FlavorActiv
    - Expensive, due to amount needed
    - Uses lots of beer at sub-3x levels
  - Use chemical supply company products
    - ie, Sigma Aldrich’s food grade product collection
    - [ASBC Flavor Calculator](#)





# THRESHOLD TESTING



## Methods of Analysis

### Flavor Standard Spiking Calculator

STEP

1	CHOOSE COMPOUND:	Acetaldehyde
2	CHOOSE INTENSITY FACTOR:	1 x
3	ENTER FINAL VOLUME IN MILLILITERS:	1000 mL

4	Add	257.4 $\mu$ L
	of	5.0000% aqueous stock solution
	into	1000 mL of a low-flavor base beer
	using the	99% pure compound

#### Stock Solution Preparation:

To make a 5.0000% stock solution of Acetaldehyde add:

*5 mL of acetaldehyde into ~50 mL of chilled reagent water in a 100 mL volumetric flask. Bring to dilution line with more water.*

*Acetaldehyde* smells or tastes like *green apple, solvent, paint, pumpkin* and it usually comes from *yeast*.

#### SPECIAL NOTES:

*Highly volatile and dissolves plastic at high concentrations. Use chilled glass pipettes.*

*6 month shelf life.*

For internal consumption of flavor standards, use only food-grade materials.

When possible, use a fume hood, gloves, and dispose of waste materials in air-tight containers.

***Some flavor compounds can be hazardous at high concentrations: consult and post all relevant MSDS materials.***

*The flavor standard additions calculated here should be considered as starting points for your own training regimen.*

*Every person is unique in their responses to stimuli, and we vary the most in our responses to smell and taste.*


*The list of flavors in this calculator is not intended to be exhaustive, but rather focused on the flavors available in food-grade form.*

*Please consult the ASBC Methods of Analysis for information about basic lab practices and equipment.*

<http://www.asbcnet.org/MOA/LabBasics.aspx>



# THRESHOLD TESTING


Methods of Analysis

Flavor Standard Spiking Calculator

STEP		
1	CHOOSE COMPOUND:	Acetaldehyde
2	CHOOSE INTENSITY FACTOR:	1
3	ENTER FINAL VOLUME IN MILLILITERS:	1000
4	Add <b>257.4</b> $\mu$ L of 5.0000% aqueous stock solution into 1000 mL of a low-flavor base beer using the 99% pure compound	<div style="border: 1px solid black; padding: 2px; font-size: 0.8em;">           Ethyl acetate            Ethyl butyrate            Ethyl decanoate  <b>Ethyl hexanoate</b>            Ethyl octanoate            Eugenol            Ferrous sulfate (7H2O)            Geraniol         </div>

Stock Solution Preparation:

To make a 5.0000% stock solution of Acetaldehyde add:

*5 mL of acetaldehyde into ~50 mL of chilled reagent water in a 100 mL volumetric flask. Bring to dilution line with more water.*

*Acetaldehyde smells or tastes like green apple, solvent, paint, pumpkin and it usually comes from yeast.*

SPECIAL NOTES:

*Highly volatile and dissolves plastic at high concentrations. Use chilled glass pipettes.*

*6 month shelf life.*

For internal consumption of flavor standards, use only food-grade materials.

When possible, use a fume hood, gloves, and dispose of waste materials in air-tight containers.

***Some flavor compounds can be hazardous at high concentrations: consult and post all relevant MSDS materials.***

*The flavor standard additions calculated here should be considered as starting points for your own training regimen.*

*Every person is unique in their responses to stimuli, and we vary the most in our responses to smell and taste.*

*The list of flavors in this calculator is not intended to be exhaustive, but rather focused on the flavors available in food-grade form.*

*Please consult the ASBC Methods of Analysis for information about basic lab practices and equipment.*

<http://www.asbcnet.org/MOA/LabBasics.aspx>



# THRESHOLD TESTING



## Methods of Analysis

### Flavor Standard Spiking Calculator

STEP

1	CHOOSE COMPOUND:	Ethyl hexanoate
2	CHOOSE INTENSITY FACTOR:	1
3	ENTER FINAL VOLUME IN MILLILITERS:	4
4	Add <b>293.6</b> $\mu\text{L}$ of 0.1000% ethyl hexanoate into 1000 mL of a low-flavor base beer using the 98% pure compound	

*Ethyl hexanoate* smells or tastes like *red apple, black licorice* and it usually comes from *yeast*.

**SPECIAL NOTES:**

*Long shelf life.*

For internal consumption of flavor standards, use only food-grade materials.

When possible, use a fume hood, gloves, and dispose of waste materials in air-tight containers.

**Some flavor compounds can be hazardous at high concentrations: consult and post all relevant MSDS materials.**

*The flavor standard additions calculated here should be considered as starting points for your own training regimen.*

*Every person is unique in their responses to stimuli, and we vary the most in our responses to smell and taste.*

*The list of flavors in this calculator is not intended to be exhaustive, but rather focused on the flavors available in food-grade form.*

*Please consult the ASBC Methods of Analysis for information about basic lab practices and equipment.*

<http://www.asbcnet.org/MOA/LabBasics.aspx>



# THRESHOLD TESTING



## Methods of Analysis

Flavor Standard Spiking Calculator

STEP

1	CHOOSE COMPOUND:	<b>Ethyl hexanoate</b>
2	CHOOSE INTENSITY FACTOR:	4 x
3	ENTER FINAL VOLUME IN MILLILITERS:	355 mL

4	Add	<b>416.9</b> $\mu$ L
	of	0.1000% ethanol stock solution
	into	355 mL of a low-flavor base beer
	using the	98% pure compound

### Stock Solution Preparation:

To make a 0.1000% stock solution of Ethyl hexanoate add:

*100  $\mu$ L of ethyl hexanoate into ~50 mL of 95% ethanol in a 100 mL volumetric flask. Bring to dilution line more ethanol.*

*Ethyl hexanoate* smells or tastes like  
*red apple, black licorice*  
and it usually comes from *yeast*.

### SPECIAL NOTES:

*Long shelf life.*

For internal consumption of flavor standards, use only food-grade materials.

When possible, use a fume hood, gloves, and dispose of waste materials in air-tight containers.

***Some flavor compounds can be hazardous at high concentrations: consult and post all relevant MSDS materials.***

*The flavor standard additions calculated here should be considered as starting points for your own training regimen.*

*Every person is unique in their responses to stimuli, and we vary the most in our responses to smell and taste.*

*The list of flavors in this calculator is not intended to be exhaustive, but rather focused on the flavors available in food-grade form.*

*Please consult the ASBC Methods of Analysis for information about basic lab practices and equipment.*

<http://www.asbcnet.org/MOA/LabBasics.aspx>



# THRESHOLD TESTING

Thank you!

Questions?

