Chasing Zero – How Changes in Methodology Contribute to the Food Safety Conundrum

JIFSAN APRIL 18, 2013

Greenbelt, MD

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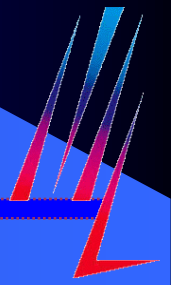
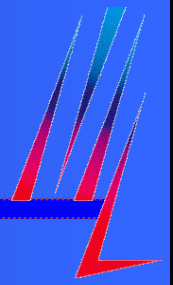
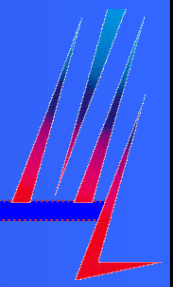


Practical Analytical Aspects of Analyses of Low Level Components in Food



From Alcohol to Sudan Dyes
or
From Absolute to Zero

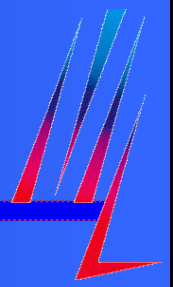


My Presentation

- **A bit of philosophy**
- **A bit of history**
  - **Where greater sensitivity led to lower risk**
  - **How scientists create some of the confusion**
  - **Examples for consideration**
- **Thoughts regarding the analytical chemist's role in the future.**

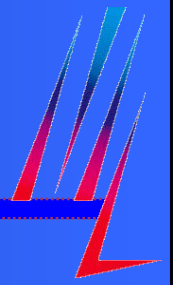
Public Policy from Food Safety Research

- **Support ethical research**
- **Promote significant reproducible research findings to public (share the benefits of research)**
- **Prevent abusive use of information**
- **Establish regulations to achieve above**
- **Regulations-Science Based Decisions**



Regulation for Food Safety Improvement

- **Improved diets/Reduced risks**
- **Enjoy benefits of health/safety research**
  - **Healthier, more comfortable living**
  - **Longer life span**
  - **Secure food supply**
- **Minimizes unfair competition**
- **Opportunities for enhanced products**
- **Requires relevant analyses**

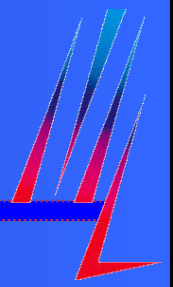


Regulation for Food Safety Improvement

- **Safety**
- **Security**
- **Comfort**

Nature is Often Not so Nice

- **Seeds in Foods**
  - **Broken Teeth**
  - **Appendicitis**
- **Food Spoilage**
  - **Unpleasant flavors and odors**
  - **Reduced food supply**
  - **Economic impacts**



Nature is Often Unkind

- **Salmonella**
- **Influenza**
- **Norwalk viruses**
- **Bee/Wasp/Hornet stings**
- **Raspberry thorns**

Nature is Often Downright NASTY

- **Botulinum Toxin**
- **Ricin**
- **Black Widow Spiders**
- **Snake Venum**
- **Molds/Mycotoxins**
- **Toxic Organisms**
  - **Illness**
  - **Death**

Human Intervention (Processing) to Reduce Nature's Negative Impact Mechanized Farming

- **Reduced Human Contact with Hazards**
- **Snakes**
- **Insects**
- **Thorns**
- **Molds/Mycotoxins (breathing/skin absorption)**

Human Intervention (Processing) to Reduce Nature's Negative Impact Chemical Interventions

- **Insects**
- **Molds/Mycotoxins**
- **Noxious weeds**

Human Intervention (Processing) to Reduce Nature's Negative Impact Chemical Interventions

- Sanitary water for drinking/processing
- Microbial control
  - Sanitation
  - Preservation-Water Activity-Sterilants-pH

Human Intervention (Processing) to Reduce Nature's Negative Impact Thermal Interventions

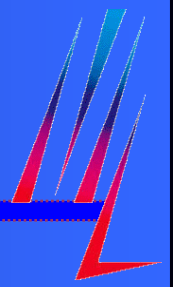
- Sterilization
- Pasteurization
- Chemical Stabilization

Human Intervention (Processing) to Reduce Nature's Negative Impact

- **HUMANS ARE CONSTANTLY TRYING TO:**
- **INCREASE FOOD AVAILABILITY**
- **INCREASE FOOD SAFETY**
- **INCREASE HUMAN COMFORT**

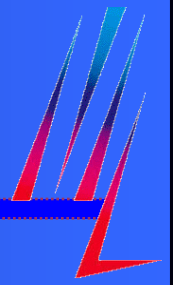
NITROSAMINES

- **Late 1970's, early 1980's**
- **Dimethylnitrosamine in Malt Beverages**
- **Direct Gas Fired Drying of Barley Malts (Destined for Brewing Malt Beverages)**
- **Addition of sulfur during kilning**



NITROSAMINES

- **Rubber Baby Bottle Nipples**
- **Nitrites for Preservation of Bacon**
- **AOAC 986.01- GC with Thermal Energy Analyzer Detection**
- **Current Analytical Method(s)-GC/MS**
- **10 ppb Compliance guidelines**



TRIHALOMETHANES

- **Chlorination of water**
  - **Past Standard-Minimum 1 PPM Cl_2 end of the line**
  - **Reduced cholera, typhoid and other diseases**
- **Humus plus chlorine ====➔ Trihalomethanes**
- **$CHCl_3$, $CHBr_3$, $CHCl_2Br$, $CHClBr_2$**
- **Analysis by GC/ECD detection**

TRIHALOMETHANES

- **Decaying vegetation (humic acid) and chlorine**
- **1991 Peru stops chlorination (based on USEPA conclusion showing increase in cancer)**
- **Cholera sets in**
- **800,000 to 1,000,000 sick-6,000 to 11,000 deaths**

TRIHALOMETHANES

- **Now Minimum 0.2 PPM Cl_2 end of the line**
  - **Adequate?**
  - **Odor/Flavor**
  - **Microbial?**

HETEROCYCLIC AROMATIC AMINES

- High Protein Foods
- Grilled Foods (Cajun Style)
- Analysis by HPLC-MS
- Balance between adequate cooking and HAAs

FURAN(s)

- Heat processing drives formation
- Found in canned, jarred, and roasted foods
- Reaction of ascorbic acid or polyunsaturated fats

FURAN(s)

- Analysis by headspace GC/MS and monitor m/z 39 and 68.
- Internal std *d*-4 furan at m/z 72.
- Modulated by cooking in open vessels, oxygen exclusion, amino acids, and sugars.

MONOCHLOROPRANDIOL (MCPD)

- Acid hydrolysis-Vegetable proteins
- Hydrochloric acid plus glycerides
- Analysis by GC/ECD on carbowax with chlorotetradecane IS.
- Enzymatic hydrolysis, adequate neutralization

BENZENE

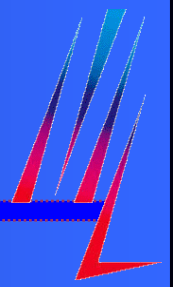
- Soft drinks question/issue
- Aqueous reaction of Benzoate and Ascorbate
- Benzoate effective preservative.
- Ascorbate (vitamin C) essential for health.
- Analysis by GC/MS m/z 78, 77, and 51.

AGEs

- AGE's (Advanced Glycation End Products)
- Heat formed compounds
- React with hemoglobin
- Analysis of blood-modified hemoglobin species

ACRYLAMIDE

- Thermal processing
- Reaction of glucose and asparagine/high temp
  - Glucose source
  - Asparagine source
  - Trapping matrix
- Analysis by LC/MS/MS



ACRYLAMIDE

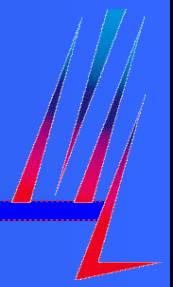
- Reduced time/temp of cook
  - Safety concern
  - Flavor loss
- Removal of glucose
- Reduction in Asparagine
  - Plant Breeding
  - Asparaginase

ABSOLUTE to “ZERO”??

- Thoughts on the Future

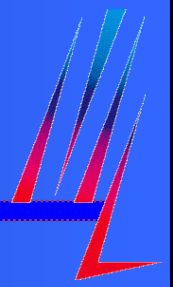
Absolute

- **Rare Occasions**
- **Chemical Reagents**
- **Alcohol/Sucrose/Salt/Lactose**

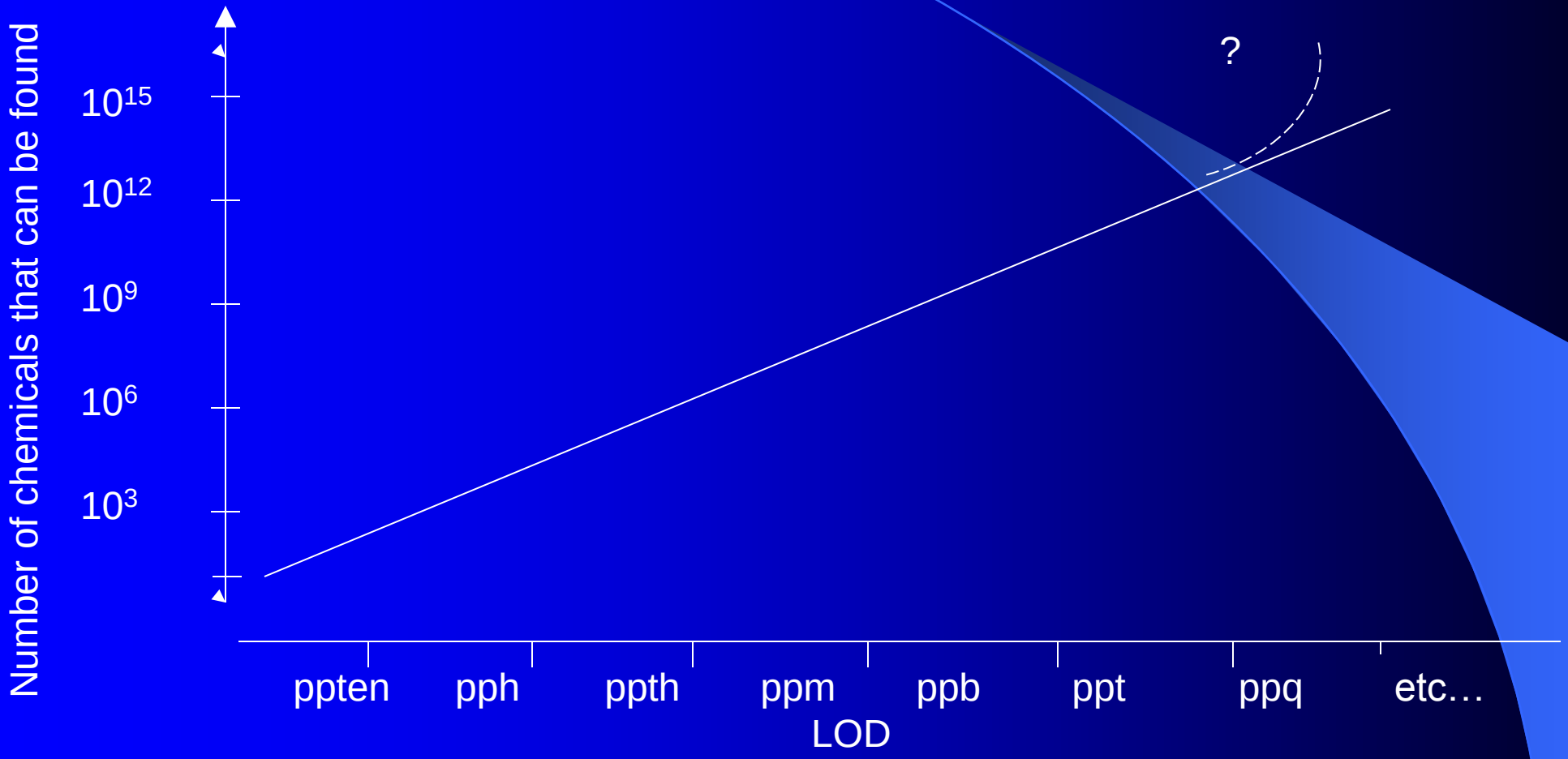


Absolute

- **Absolute Purity usually determined by lack of contaminants**
- **Pure Food and Drug Law**
- **How low to go?**
- **Approaching “Zero”**

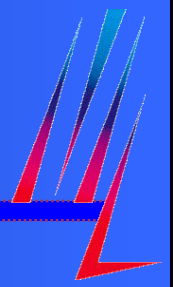


Zero is a small number with a BIG Impact



How small?

Unit	1ppm	1ppb	1ppt
Length	1 in/16 mi	1 in/16,000 mi	1 in/16 million mi (a 6” leap toward the sun)
Time	1 min/ 2 yrs	1 sec/ 32 yrs	1 sec/ 320 centuries
Money	1ct/$10,000	1ct/$10 million	1ct/$10 billion



How small?

Unit	1ppm	1ppb	1ppt
Area	1 ft^2 /23 acres	1ft^2 /36 mi^2	1in^2 / 250 mi^2
Volume	1 drop v'mouth in 80 L gin	1 drop/500 barrels gin	1 drop/pool of gin covering football field 43 feet deep or 1 drop in 520 tanker cars of 30,000 gal capacity.

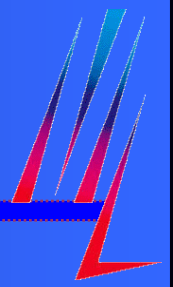
A Reverse Look
Water in Alcohol Example

- Want Pure Everclear?
- 99.9% EtOH, 0.1% water contains 0.001 g/g H_2O
- Avagadro:

  6.023 x 10^23 Molecules/18 g H_2O
- Therefore:

  1 gram of 99.9% pure EtOH still contains

  3.35 x 10^19 molecules of H_2O

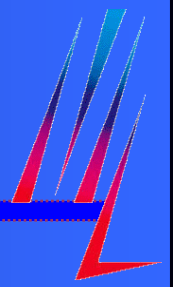


A Reverse Look
Water in Alcohol Example

- Want Pure Everclear?
- 1 ppt of water contains 0.000000001g/g H_2O
- Therefore

  1 gram of 99.999999% pure EtOH still contains

  3.35 x 10^14 molecules of water/gram.



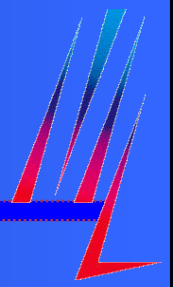
A Reverse Look
Water in Alcohol Example

- Typical analysis uses a 1 uL (0.000789 g) injection.
- Therefore:

  Inject 1 uL of ETOH

  99.999999% pure

  Injecting **2.64 x 10^11** molecules of water.
- Still have **264 Billion** water molecules to pursue in a 1 uL sample.



Requirements of Low Level Assay

- **Higher Selectivity**
- **Higher Sensitivity**
- **How much is enough?**

Zero is a small number

- 1950s & 1960s – parts per thousand, ppm
- 1970s & 1980s – ppm, ppb
- 1990's & 2000s – ppt, ppq

Analytical technology has advanced faster than our ability to interpret findings

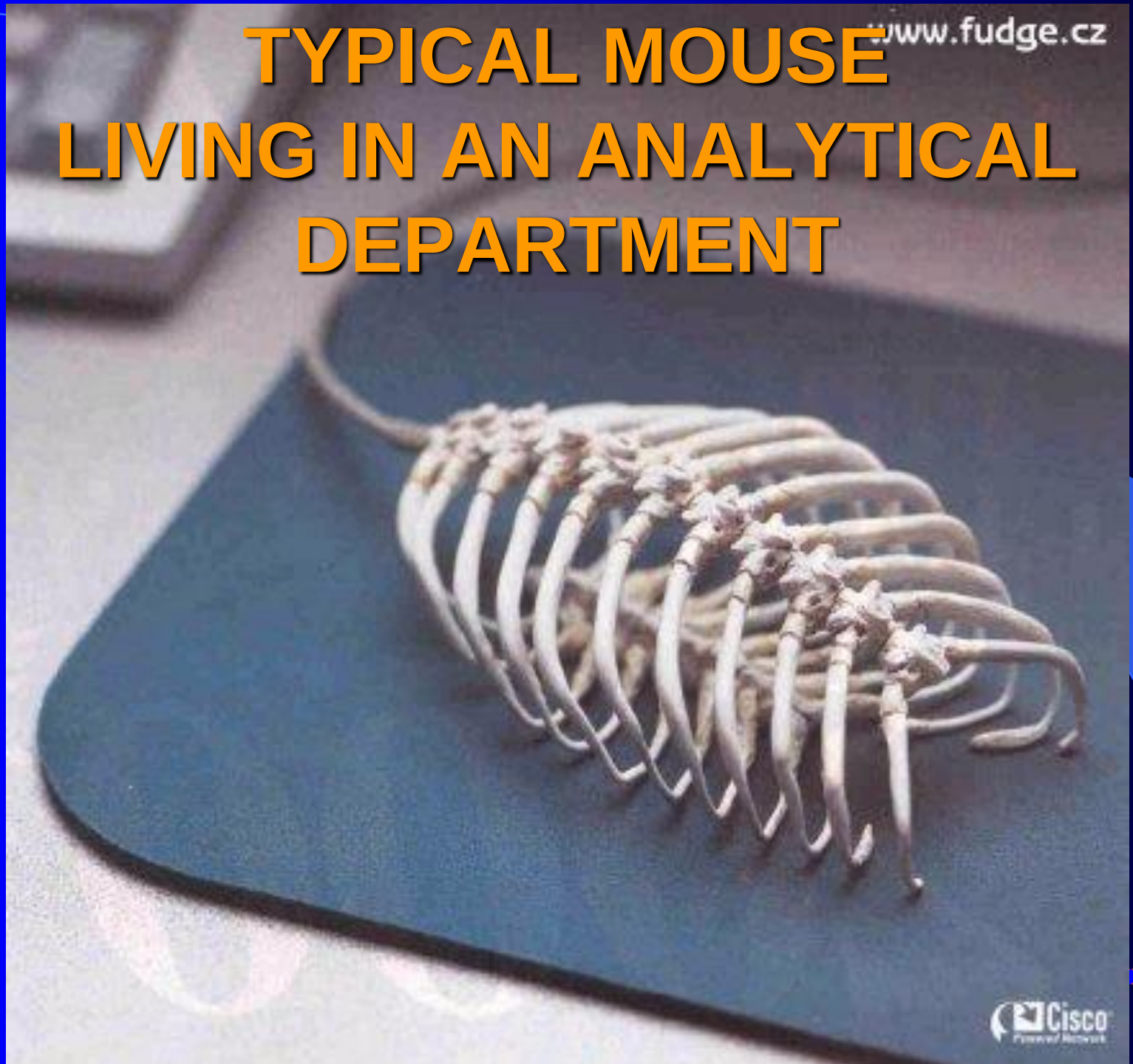
COSTS of TESTING

Concentration	Approach	Cost/Skill
1-100 %	Titration, Gravimetric, Standard HPLC, Flame Photometry, AA, FTIR UV-VIS	Low
.01 to 1%	GC, HPLC, AA, ICP, UV-VIS	Modest

COSTS of TESTING

Concentration	Approach	Cost/Skill
1-100 ppm	GC, HPLC, AA, ICP	Modest-High
1 ppb- 1ppm	GC/MS, HPLC/MS/MS, ICP/MS	High
<1 ppb	GC/TOFMS, HPLC/MS/MS ICP/MS	Very High

TYPICAL MOUSE LIVING IN AN ANALYTICAL DEPARTMENT



Medallion

How we chase zero

- A contaminant is found in food and deemed to be unacceptable.
- Zero = the current limit of detection (LOD)
- A new instrument or method drops the LOD

How we chase zero

- **The contaminant is found again & new ones may appear on the scene for the first time.**
- **Society feels obliged to chase the receding "zero", often confused about the meaning of reported results.**

- **Q. Can science draw a line?**

KNOWLEDGE

- It is best to know the facts so informed decisions can be made.
- On the other hand, we often panic over the "known" because we can measure it while ignoring the "unknown".

PPB

EXPRESSING RESULTS

- Can be confusing to the average scientist!!

ug/kg

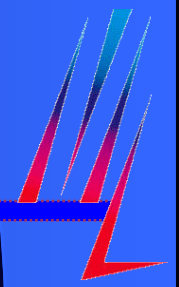
0.00000000X g/g

ppb

ng/g

X x 10^{-9}

To Say Nothing About the Consumer



EXPRESSING RESULTS

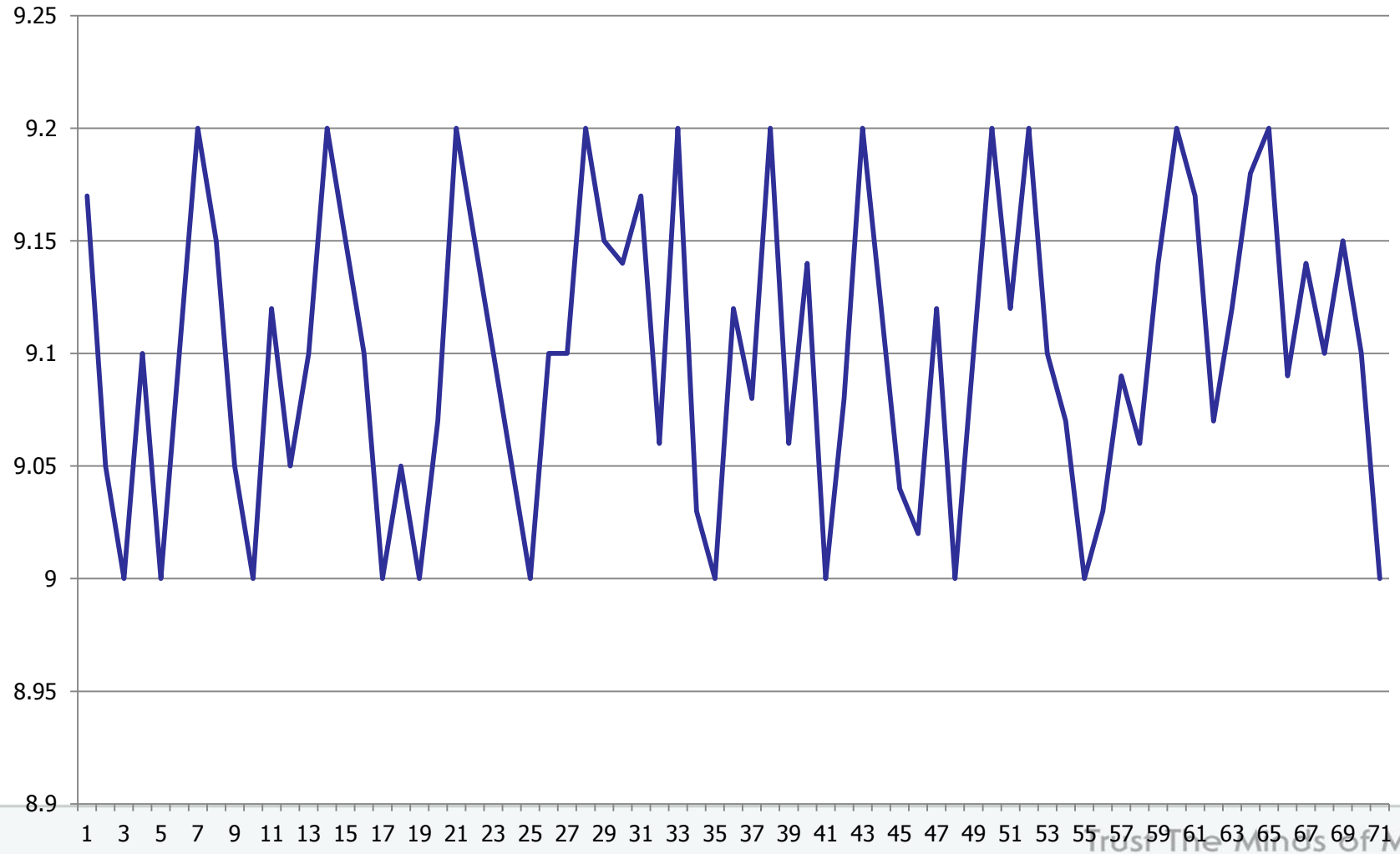
- What is published: 308 parts per billion (ppb).
- What the value represents: 0.000000308 g/g
- What the scientist pictures: 0.000000308 or 308 ug/kg
- What the consumer sees: **308**

Would Consumer Anxiety Decrease if we published **0.000000308 g/g**?

MEDALLION LABS

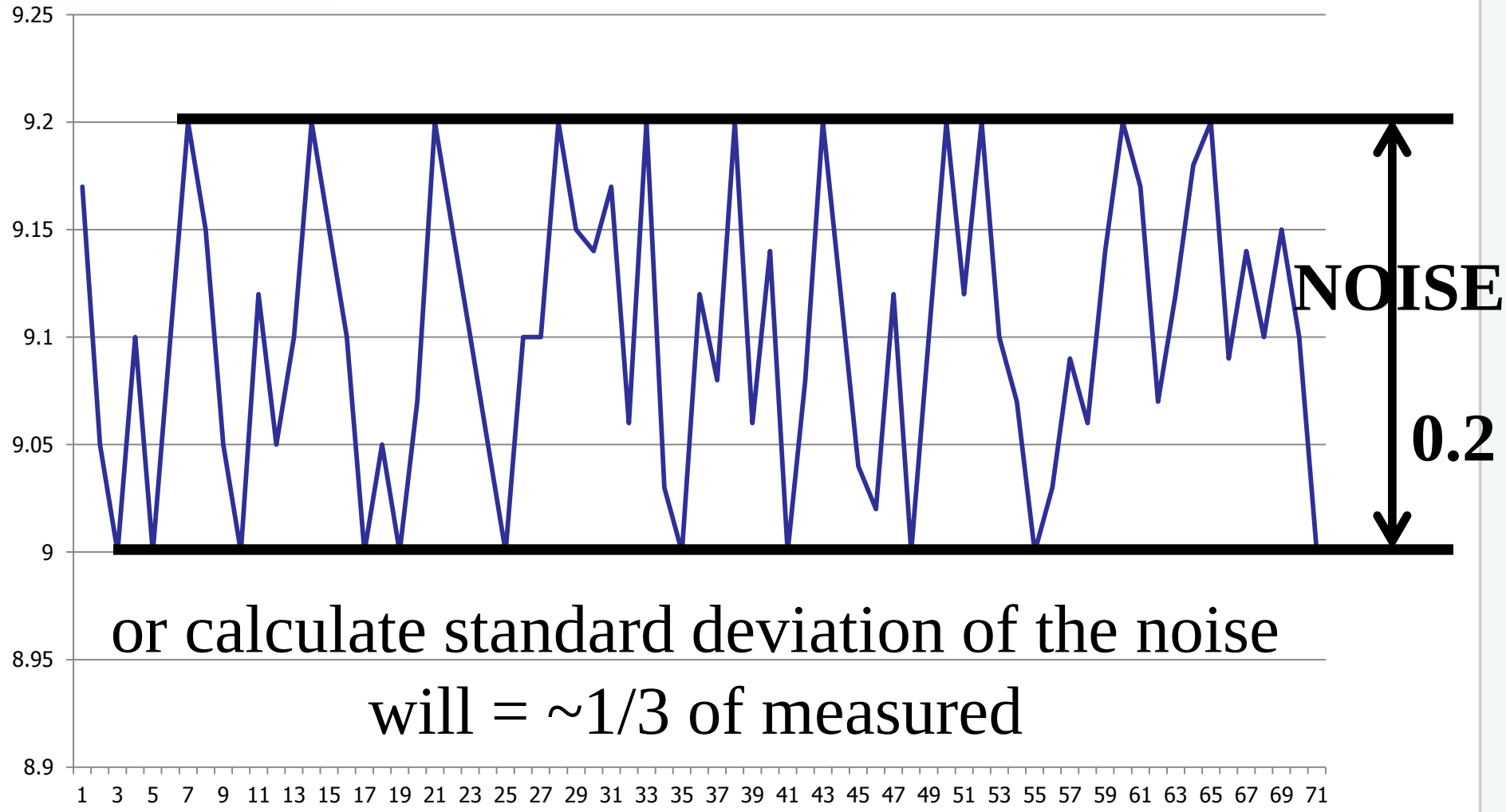
Scientifically Induced Confusion

Limit of Detection and Limit of Quantitation



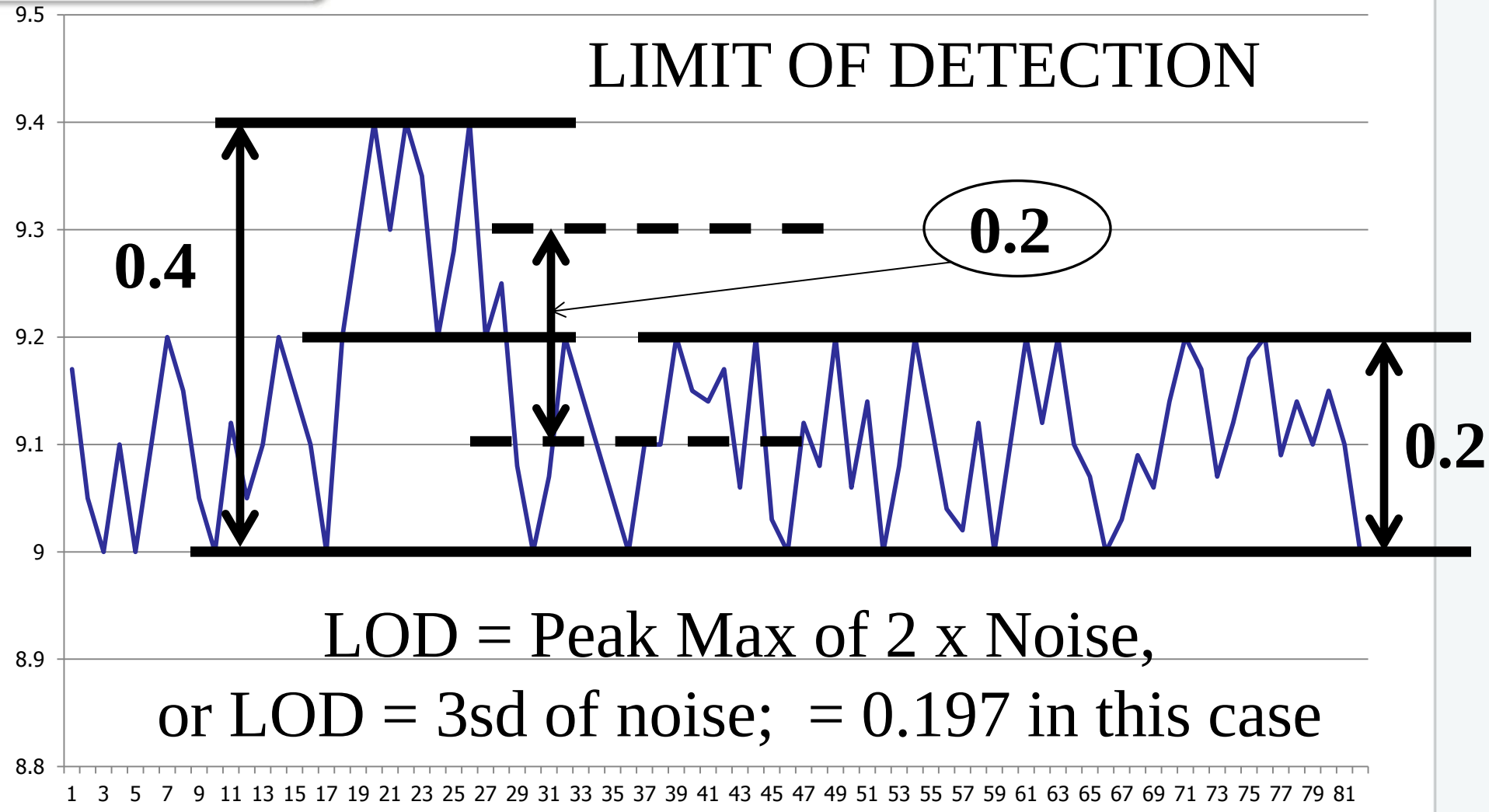
Scientifically Induced Confusion

Limit of Detection and Limit of Quantitation

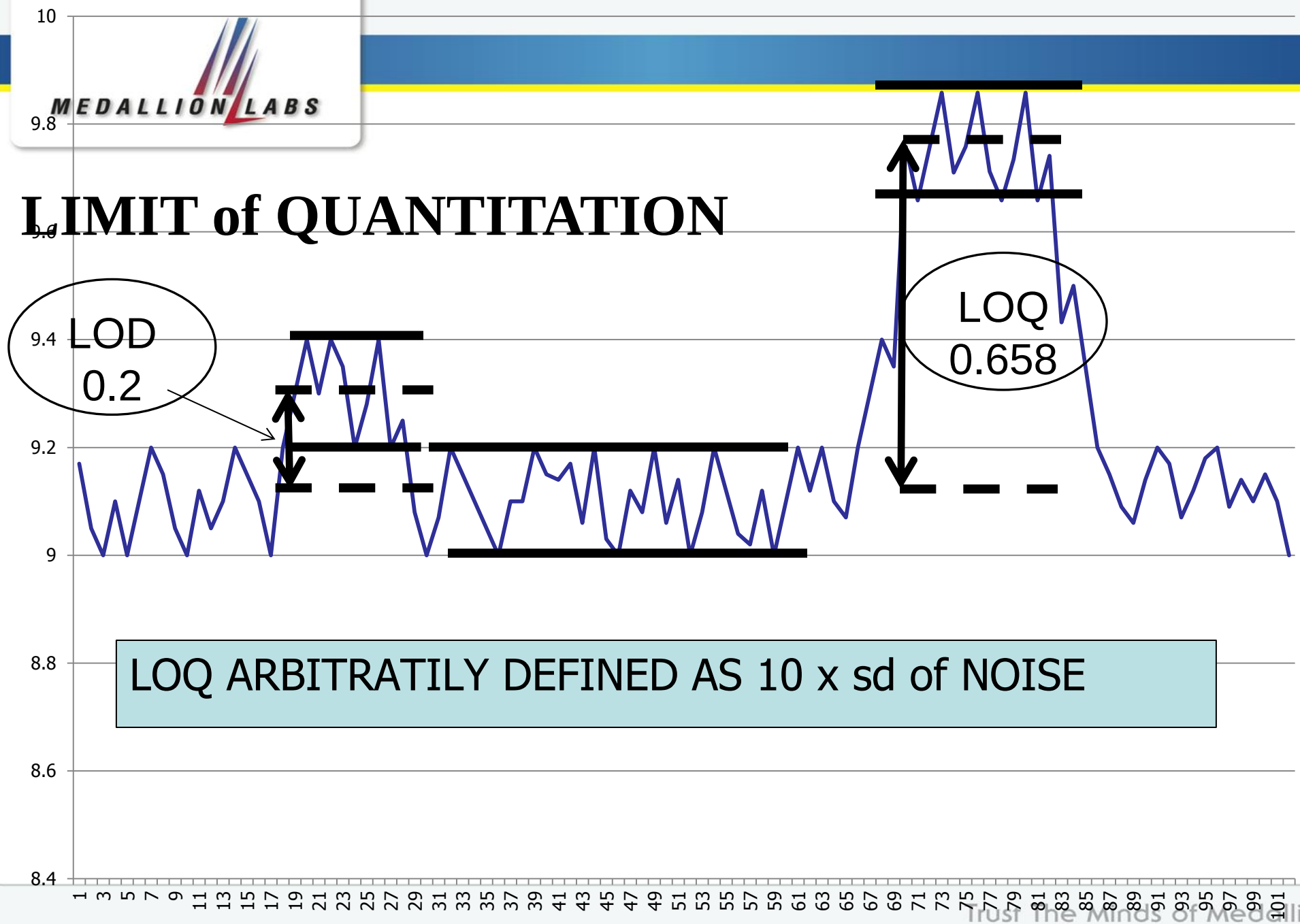


or calculate standard deviation of the noise

will = ~1/3 of measured


LIMIT OF DETECTION
0.4
0.2
0.2
LOD = Peak Max of 2 x Noise,
or LOD = 3sd of noise; = 0.197 in this case
9.5
9.4
9.3
9.2
9.1
9
8.9
8.8
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 65 67 69 71 73 75 77 79 81

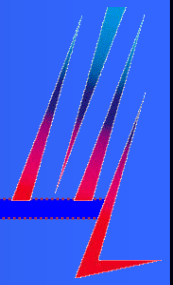
LIMIT of QUANTITATION



LOQ ARBITRATILY DEFINED AS 10 x sd of NOISE

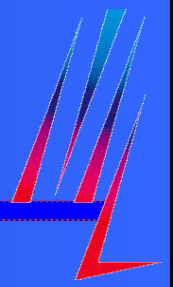
LOQ-CONFUSING CONCEPT

- LOD-Sound Science
  - 2 x Noise Easy to understand-Detection is obvious
  - 2 x Noise Easy to measure
  - For non instrumental methods
    - Plot data to get 2 x noise
    - Use 3 x sd of noise.
- ~99% confidence in result @ LOD
- >99% @ higher levels.
- Everything above the LOD is quantitatable



LOQ-CONFUSING CONCEPT

- LOQ-Arbitrary cut off.
- Everything above LOD is measurable
  - We may not like variability but numbers are real.
  - May need replicates for greater confidence
- What to do with data between LOD and LOQ
  - Real data
  - Very valuable for risk assessment
- **Let's do away with LOQ**



ADDITIONAL SOURCE OF CONFUSION

- **USE OF THE STATISTICAL TERM**

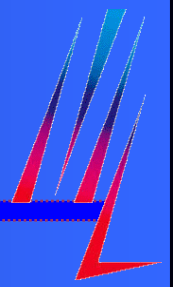
“ERROR”

- **WHEN WE REALLY MEAN**

“CONFIDENCE”

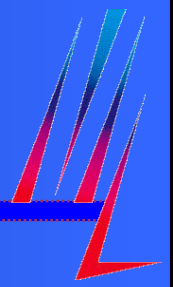
ANXIETY?

- **Closer to zero**
- **More compounds**
- **More unknowns-Less risky (low levels)**
- **Anxiety with the unknown**



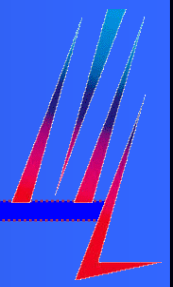
Problems Presented by Chasing Zeroes

- **Point is, do we have the resources to pursue all of these as major issues?**
- **What level should we really operate at?**
- **How shall we handle new discoveries?**



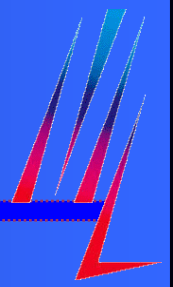
Cases of chasing zero

- **Some are an intentional part of processing/manufacture**
- **Some are naturally-occurring**
- **Some were intentional, but now are unavoidable at levels close to zero**
- **Some are carcinogens or metals**
- **Some have high exposure, some low toxicity**
- **Regulatory mandates differ, but what does the science say?**



Some cases of approaching zero Chloramphenicol

- Antibiotic used by China in bee colonies 5 or so years ago – deemed to be carcinogenic.
- Traces found in honey around the world
- “Can’t set an acceptable level” acc. to regulators
- Each lot tested down to LOD of 0.5 ppb



Chloramphenicol

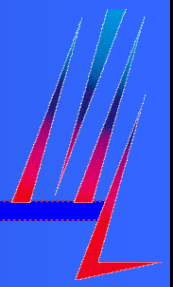
- CharmII Kit Test
- Dissolve Sample in water, add tablet
- Incubate
- Centrifuge
- Resuspend
- Read Results
- LOD of 0.43 ppb

Some cases of approaching zero Chloramphenicol

- Canada improved LOD down to 0.05 ppb (50 ppt)

Chloramphenicol in Honey

- 50:50 with Water/Extract to EtOAC (2X)
- Centrifuge/Evaporate
- Extract with Hexane/Centrifuge
- Clean up on Conditioned SPE cartridges
- Evaporate
- Take up in 0.1% formic acid



Chloramphenicol in Honey

- **LC/MS/MS**
  - **Column C18**
  - **Gradient Elution**
  - **m/z 321, 257,194,176, and 152**
  - **Quantitate off 152**

- **LOD = 0.05 ppb (50 ppt)**

Some cases of approaching zero Chloramphenicol

- **Chasing zero causes upset to business, regulation, and erodes consumer confidence**
- **Extent of Testing-Kits vs LC/MS/MS?**

Which is More Responsible?

- Test kits with Broad Application-Higher LOD
- High Sensitivity-Low LOD-Limited Usage

Kits versus Instrument

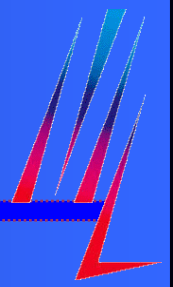
- Chloramphenicol
- Mold toxins – AFB1, FMB, OTA, DON,
- Allergens?

Some cases of approaching zero Perchlorate

- Thyroid effects, cancer possible?
- Possibly from military sites, entering ground water
- Found at ppb in lettuce irrigated with water from the Colorado river.

Some cases of approaching zero Perchlorate

- **Add $^{18}O_4$ labeled perchlorate-Internal Standard**
- **Extract sample with acetic (or nitric) acid solution**
- **Clean up on graphitized SPE cartridge as necessary**



Some cases of approaching zero Perchlorate

- LC/MS/MS
  - Mobile phase, NH_4OAC, Acetonitrile, Water
  - Electrospray ionization
  - mz 99→83*, 101→85 for native perchlorate
  - mz 107→89*, 109→91 for internal standard

    *Used for quantitation
- LOD approx 1 ppb

Some cases of approaching zero Perchlorate

- Later found in milk, etc. – even FDA's lab water
- Possibly formed naturally from salt, sunlight, & alkali pH
- NAS told EPA their RA was off (by 70x)

Some cases of approaching zero Sudan Red

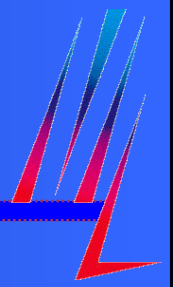
- Family of Dyes-Deemed Carcinogenic
- Disallowed for Food Use
- Purposeful Addition-Adulteration for Appearance

Some cases of approaching zero Sudan Dyes

- Sudan I
- Sudan II
- Sudan III
- Sudan IV
- Sudan-Orange G
- Sudan-Red B
- 4-(Dimethylamino)azo benzene
- Para Red

Some cases of approaching zero Sudan Dyes

- Soxhlet Extraction
- Size exclusion clean up
- LC with UV-Vis detection
- LC/MS/MS, electrospray mode
- LOD = 10 ppb for Sudan dyes and DMAAB, 100 ppb for parared

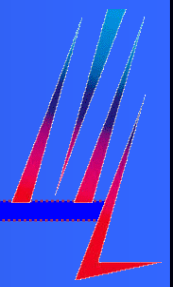


Some cases of approaching zero Sudan Dyes

- Clearly a case of stopping purposeful adulteration
- Perpetuated for economic gain

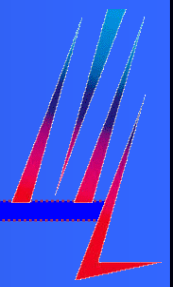
Additional cases of approaching zero

- Packaging residues – BPA, ITX, butadiene
  Processing residues – chloropropanols from acid-hydrolyzed vegetable proteins
- Heavy metals in foods – Hg in fish; Cd in veggies; Pb in chocolate, water
- Mold toxins – AFB1, FMB1, OTA, DON, in cereal grains, nuts, oil seeds
- Allergens?



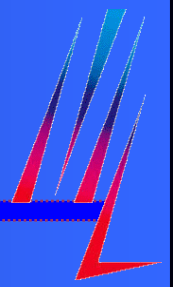
Cases of chasing zero

- **Some are an intentional part of processing/manufacture**
- **Some are naturally-occurring**
- **Some were intentional, but now are unavoidable at levels close to zero**
- **Some are carcinogens or metals**
- **Some have high exposure, some low toxicity**
- **Regulatory mandates differ, but what does the science say?**



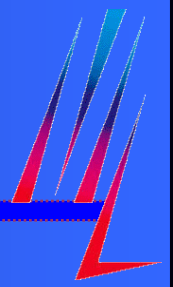
ANXIETY?

- **Closer to zero**
- **More compounds**
- **More unknowns-Less risky (low levels)**
- **Anxiety with the unknown**



Problems Presented by Chasing Zeroes

- **Point is, do we have the resources to pursue all of these as major issues?**
- **What level should we really operate at?**
- **How shall we handle new discoveries?**



Closing Thoughts

- Problems

  ==========➔ Emotional

- Solutions

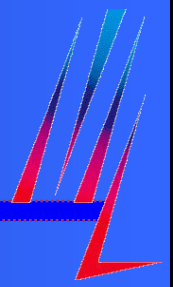
  ==========➔ Technical

- Decisions

  ==========➔ Political

NEEDED

- Solid Science to set Realistic Limits
- Methods to Conform to Limits
- Spend Resources on other Issues

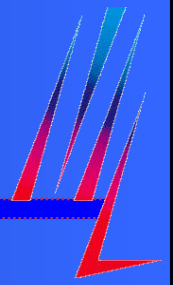


Problems Presented by Chasing Zeroes

- Consumer confidence in food is eroded
- Scarce resources do not always go to the most critical risks
- Disruption of business, international trade
- No end in sight…zero rushes ahead of us as sensitivities make quantal gains
- Global sourcing, advances in methods, sensitivity around food defense…will make issue more acute in future

Problems Presented by Chasing Zeroes

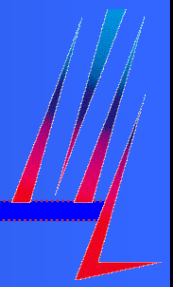
- Point is, do we have the resources to pursue all of these as major issues?
- Can society afford to continue to operate using the toxicology model of the mid-20th century? (Foreign chemicals are rare in pure food; when found, we chase to zero.)
- Can't toxicology guide us to agree on some toxicologically insignificant exposure level?



Last Thoughts

- I want to end by emphasizing that we need policies and activities that result in new discoveries.

- I also want to emphasize that these new discoveries must be handled in a sensible fashion.



Opportunities?