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 $\longrightarrow$ Trihalomethanes
- CHCl$_3$, CHBr$_3$, CHCl$_2$Br, CHClBr$_2$
- Analysis by GC/ECD detection
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 Cl₂ 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.
MONOCHLOROPRANANDIOL (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

Number of chemicals that can be found

LOD

ppten pph ppth ppm ppb ppt ppq etc…
# How small?

<table>
<thead>
<tr>
<th>Unit</th>
<th>1 ppm</th>
<th>1 ppb</th>
<th>1 ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>1 in/16 mi</td>
<td>1 in/16,000 mi</td>
<td>1 in/16 million mi</td>
</tr>
<tr>
<td></td>
<td>(a 6” leap toward the sun)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>1 min/ 2 yrs</td>
<td>1 sec/ 32 yrs</td>
<td>1 sec/ 320 centuries</td>
</tr>
<tr>
<td>Money</td>
<td>1 ct/$10,000</td>
<td>1 ct/$10 million</td>
<td>1 ct/$10 billion</td>
</tr>
</tbody>
</table>
# How small?

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<tbody>
<tr>
<td>Area</td>
<td>1 ft² / 23 acres</td>
<td>1 ft² / 36 mi²</td>
<td>1 in² / 250 mi²</td>
</tr>
<tr>
<td>Volume</td>
<td>1 drop v’mouth in 80 L gin</td>
<td>1 drop/500 barrels gin</td>
<td>1 drop/pool of gin covering football field 43 feet deep or 1 drop in 520 tanker cars of 30,000 gal capacity.</td>
</tr>
</tbody>
</table>
A Reverse Look
Water in Alcohol Example

- Want Pure Everclear?
- 99.9% EtOH, 0.1% water contains 0.001 g/g H₂O
- Avagadro:
  6.023 × 10²³ Molecules/18 g H₂O
- Therefore:
  1 gram of 99.9% pure EtOH still contains
  3.35 × 10¹⁹ molecules of H₂O
Want Pure Everclear?

1 ppt of water contains 0.000000001g/g H$_2$O

Therefore

1 gram of 99.999999% pure EtOH still contains $3.35 \times 10^{14}$ 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

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Approach</th>
<th>Cost/Skill</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-100 %</td>
<td>Titration, Gravimetric, Standard HPLC, Flame Photometry, AA, FTIR UV-VIS</td>
<td>Low</td>
</tr>
<tr>
<td>.01 to 1%</td>
<td>GC, HPLC, AA, ICP, UV-VIS</td>
<td>Modest</td>
</tr>
</tbody>
</table>
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<tr>
<td>1-100 ppm</td>
<td>GC, HPLC, AA, ICP</td>
<td>Modest-High</td>
</tr>
<tr>
<td>1 ppb- 1 ppm</td>
<td>GC/MS, HPLC/MS/MS, ICP/MS</td>
<td>High</td>
</tr>
<tr>
<td>&lt;1 ppb</td>
<td>GC/TOFMS, HPLC/MS/MS, ICP/MS</td>
<td>Very High</td>
</tr>
</tbody>
</table>
TYPICAL MOUSE LIVING IN AN ANALYTICAL DEPARTMENT
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”.
EXPRESSING RESULTS

- Can be confusing to the average scientist!!

$0.00000000X \text{ g/g}$

$X \times 10^{-9}$

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?
Scientifically Induced Confusion
Limit of Detection and Limit of Quantitation
or calculate standard deviation of the noise
will = \sim \frac{1}{3} \text{ of measured}
LOD = Peak Max of 2 x Noise, or LOD = 3sd of noise; = 0.197 in this case
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 $^{18}\text{O}_4$ 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$_4$OAC, Acetonitrile, Water
  - Electrospray ionization
  - mz 99 $\rightarrow$ 83*, 101 $\rightarrow$ 85 for native perchlorate
  - mz 107 $\rightarrow$ 89*, 109 $\rightarrow$ 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?
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- Point is, do we have the resources to pursue all of these as major issues?
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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?