Significance of Low Analytical Limits of Component Detection

John Szpylka[1]
Mérieux NutriSciences, Crete, IL, U.S.A.

When you hear the term, "food safety," an immediate thought concerns reducing the risk of microbiological contamination; a logical thought since microbial contamination can cause acute illness and has the potential to quickly cause fatalities. Another critical component of food safety concerns chemical contamination, as exemplified by allergens, which are now the number one cause of food recalls (4). Although this is another example of an immediate-impact contaminant, many chemical contaminants can also impact health over a longer period of exposure, and they must, therefore, also be assessed and monitored.

The U.S. Food and Drug Administration (FDA) recently published the Food Safety Modernization Act (FSMA) guidance document, "Draft Guidance for Industry: Hazard Analysis and Risk-Based Preventative Controls for Human Food" (8), which lists potential chemical hazards to consider when designing a risk-based safety program. The list contains both immediate and long-term chemical risks:

- Undeclared allergens
- Drug residues
- Heavy metals
- Industrial chemicals
- Mycotoxins/natural toxins
- Pesticides
- Unapproved colors and additives
- Radiological elements

As analytical instruments become more sensitive, the corresponding test methods are able to detect lower and lower levels of these contaminants. In fact, there seems to be a race to see who can create the most sensitive instrument or measure "the analyte du jour" at the lowest possible level. To properly use this additional information we must factor in scientifically sound reasoning on the impact of contaminants on short- and long-term health.

Measuring Lower Levels of Targeted Compounds

When we measure an analyte at a low concentration, how much are we actually seeing? To put this in analogous terms, standard analytical units of measurement are expressed in more common units in Table I.

Analytical instrumentation has evolved over time and can now measure compounds at the lower levels (ppb and ppt) shown in the farthest right columns of Table I. Early measurements were originally performed using gravimetric analyses, titrations, and some basic chromatographic techniques. Measurement levels were steadily decreased by using more stable instruments to quantify compounds using more precise weighing and more sensitive absorption, emission, fluorescence, and mass measuring instruments. Hybrid techniques such as ICP-MS (inductively coupled plasma–mass spectrometry), HPLC-MS (high-performance liquid chromatography–mass spectrometry), HPLC-MS/MS (high-performance liquid chromatography–tandem mass spectrometry), GC-MS (gas chromatography–mass spectrometry), GC-MS/MS (gas chromatography–tandem mass spectrometry), and 2-D chromatography have further lowered the limits of detection. There has also been much work conducted to significantly concentrate analytes before introduction into the instruments listed above. These technologies are summarized in Table II. As can be seen, the capability to measure extremely low levels of contaminants has steadily improved.

Measuring Levels of Contaminants

When a chemical found in a food is designated as a contaminant, the allowable level is ideally at least one or two orders of magnitude lower than the amount found to be harmful in the short-term (contaminants causing acute toxicity) or long-term (contaminants that accumulate over time). Many times these safety levels are unknown, especially for a newly identified contaminant. In this case, the default allowable level is typically set at "zero," or in general terms, "not detectable by the most sensitive method." This approach mirrors the FDA "*Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed*" (6), which lists allowable levels for specific contaminants as, "action levels and tolerances [that] represent limits at or above which FDA will take legal action to remove products from the market. Where no established action level or tolerance exists, FDA may take legal action against the product at the minimal detectable level of the contaminant."

Bearing in mind the ongoing lowering of limits of detection, let's consider the following scenario. A chemical is recognized as a contaminant on January 1, and on that day, the contaminant can be detected down to a level of 100 ppm. Because no established tolerance level exists, 100 ppm would be the actionable level. Manufacturers in the food supply chain use this analytical method as their tool to minimize the risk of the contaminant being present in their products. On June 1, a group develops and releases a fully validated method that can accurately measure the contaminant down to 1 ppm. According to the wording in the FDA guidance document (6), the actionable level has now dropped by two orders of magnitude, which from a safety standpoint is appropriate and defensible because no proven health-affecting level has been established. Because the potential effect on monitoring of the food supply chain can be dramatic, this typically forces decisions to be made concerning the definition of a safe or allowable level. The good news is this series of events does accelerate the pace for proper, "safe" levels to be scrutinized and defined.

[1] E-mail: john.szpylka@mxns.com

https://doi.org/10.1094/CFW-63-1-0026

Table I. Measurement units expressed in common terms

	Unit				
	% (parts per hundred)	ppth (parts per thousand)	ppm (parts per million)	ppb (parts per billion)	ppt (parts per trillion)
Distance	1 yd in 1 football field	1 yd in 10 football fields	1 yd between Orlando, FL, and Gulfport, MS	1 yd in the diameter of the orbit of Earth's moon	1 yd between the sun and Jupiter
Money	1¢ in $1	1¢ in $1,000	1¢ in $10,000	1¢ in $10 million	1¢ in $10 billion
Volume	1 drop of vermouth in 99 drops of gin	1 drop of vermouth in 2 oz of gin	1 drop of vermouth in 64 qt of gin	1 drop of vermouth in 500 bbl of gin	1 drop of vermouth in a football field 43 ft deep in gin
Mass	1 pinch of salt on 16 potato chips	1 pinch of salt in 8 single-serve bags of potato chips	1 pinch of salt in 553 lb of potato chips	1 pinch of salt in 276 tons of potato chips	1 pinch of salt in potato chips equal in weight to 5 QE2 ocean liners

Table II. Effects of the evolution of technology on detection limits

Technologies[a]	Concentrations	Costs	Comments
Wet chemistry and original LC	1–100%	Low	Instruments can be portable
GC, HPLC, AA, and ICP	0.01–1%	Medium	Instruments became more complex
GC, AA, and ICP	1–100 ppm	Medium/high	Instrument improvements
UHPLC-MS/MS, GC-MS, and ICP-MS	ppb to ppm	High	More sensitive detectors
UHPLC-MS/MS, GC-MS, and ICP-MS	ppt	Higher	Instrument improvements
Preanalysis concentration of analytes	<ppt	Higher+	Add-ons to already sensitive instruments
What's next?	Even lower?	???	???

[a] LC: liquid chromatography; GC: gas chromatography; HPLC: high-performance liquid chromatography; AA: atomic absorption spectroscopy; ICP: inductively coupled plasma spectroscopy; UHPLC-MS/MS: ultra high-performance liquid chromatography tandem mass spectrometry; GC-MS: gas chromatography–mass spectrometry; ICP-MS: inductively coupled plasma–mass spectrometry.

Currently, this process is occurring in the field of allergen testing. In 2004, the Food Allergen Labeling and Consumer Protection Act (FALCPA) was enacted, and eight foods and food groups were identified as major food allergens: milk, eggs, fish (e.g., bass, flounder, cod), crustacean shellfish (e.g., crab, lobster, shrimp), tree nuts (e.g., almonds, walnuts, pecans), peanuts, wheat, and soybean (7). FALCPA requires a food to be labeled if it includes an ingredient containing protein from one or more of the foods listed above (7). Because no allowable limits were included in the act, the existing ELISA methods used to monitor foods and ingredients for allergens set the action limit based on their detection limits, which range from 10 to 20 ppm. Recently, LC-MS/MS (liquid chromatography–tandem mass spectrometry) analysis for allergens has been shown to provide greater sensitivity in allergen detection (3). The improvement in testing sensitivity is facilitating discussions on reevaluation of acceptable levels for foods to be declared allergen-free.

Using Data from Improved Detection Limit Technology

It is important that the information obtained using instruments with increased sensitivity be placed in the proper context. The additional data we collect must be carefully interpreted, as was demonstrated to me while I was waiting for a flight at an airport. A gentleman seated next to me was upset over an article reporting lead being found in a food he eats—the level was 1 ppt. The main point he focused on was lead being detected in the food, not the fact that the measured 1 ppt level is 1,000 times lower than the action level. This is a clear example of a quote from science fiction writer Douglas Adams, "Nothing travels faster than the speed of light, with the possible exception of bad news, which obeys its own special laws" (1).

As summarized in English in the *Beverage Daily* (2) in 2002, *Oeko-Test*, an ecology-friendly publication, reported acrylamide was present in several brands of roasted coffee. The article described the health issues as, "German researchers said they found traces of the potentially cancer-causing chemical acrylamide, although not in as high concentrations as in fatty foods such as potato crisps, french fries, or bread" (2). What was not noted was that the measured levels in the coffee were below legal action levels. This is a clear example of the need to place the information gained from the ability to detect lower concentrations of contaminants in the proper and scientifically sound context.

More recently, in 2009 *CNN News* reported "90 percent of U.S. bills carry traces of cocaine" (5). The average amount of 28 μg/bill was measureable due to instrument improvements. What was great to see in this article were the following sentences that placed this information in the proper context: "Research presented this weekend reinforced previous findings that 90 percent of paper money circulating in U.S. cities contains traces of cocaine. Scientists say the amount of cocaine found on bills is not enough to cause health risks." And later, "When the ATM machine gets contaminated, it transfers the cocaine to the other bank notes. These bills have fewer remnants of cocaine. Some of the dollars in his experiment had 0.006 micrograms, which is several thousands of times smaller than a single grain of sand."

Lower Detection Limits Also Mean Obtaining Useful Information

Let's now look at lower limits of detection from a different angle. As the ability to measure lower levels of compounds evolves and increases, we are able to gain a deeper understanding of the structure and identity of a material because the amount of information we gain about the material rises at a dramatic rate. Using a simplified example for illustration, let's test for the components in a sample of brown sugar.

Let's assume the lowest level we can measure of any component in the brown sugar is 1%. Sample testing would only be

Table III. Analysis of brown sugar sample with increasingly lower limits of detection

Limit of Detection	Measureable Components in Brown Sugar Sample
1.0%	Sucrose, water(?)
0.1%	Sucrose, water, protein, something unexpected(?)
1 ppm	Sucrose, water, protein, Ca, something unexpected, something else(?)
1 ppb	Sucrose, water, protein, Ca, Fe, K, Na, something unexpected, something else, or worse(?)
1 ppt	Sucrose, water, protein, Ca, Fe, K, Na, who knows what else(?)

able to measure sucrose and perhaps water, with any additional compounds being "not detected" because of the 1% detection limit. As the technology improves and enables lower levels of components to be detected and measured, additional information concerning the brown sugar sample is obtained (Table III).

As lower limits of detection are attained, more compounds will be detected in materials, and this additional knowledge can be used to create a detailed "fingerprint" of each material. This approach is generally described as "nontargeted testing" and is being explored by a number of organizations, instrument suppliers, software developers, and academia. The development of a well-defined fingerprint or "identity standard" allows comparisons with newly obtained or purchased materials (such as food ingredients) to be made. If the data profile for the material being examined is within normal variation when compared with an identity standard, the risk of contamination (or adulteration) is reduced. If the test material differs significantly from the fingerprint, a deeper examination can be performed. Detecting lower levels of components generates an increased amount of information, and new approaches to using this information can further improve the safety and integrity of the food supply chain.

Summary

Analytical instrumentation and corresponding methods of analysis are constantly improving, allowing lower concentrations of contaminants to be detected in foods. With some compounds now being detected at the parts per trillion level, the impact of finding lower levels of contaminants must be properly interpreted so that sound regulations and the correct food safety protocols can be constructed. This will involve effective communication between scientists, regulators, instrument suppliers, food producers, consumers, and media participants.

John Szpylka, scientific affairs director, chemistry N.A., for Mérieux NutriSciences, manages nutritional analytical method development for Mérieux NutriSciences and is a technical leader for chemistry testing in North America. John is a representative to key scientific organizations and regulatory agencies, including active involvement with AOAC International, AACC International, AOCS, AAFCO, ASTA, and GMA. John is a Fellow of AOAC International and past chair of the AOAC Official Methods Board. He currently serves on numerous AOAC stakeholder panels and expert review panels concerning analyses of nutrients and contaminants. Before joining Mérieux NutriSciences, John was a principal scientist with General Mills and Medallion Laboratories. John received his doctorate in analytical chemistry from The Ohio State University after receiving a B.S. degree in chemistry from Rensselaer Polytechnic Institute. John is an AACCI member and can be reached at john.szpylka@mxns.com.

References

1. Adams, D. *A Hitchhiker's Guide to the Galaxy*. Ballantine Books, New York, 1979.
2. BeverageDaily.com. Acrylamide found in coffee. Published online at www.beveragedaily.com/Article/2002/08/28/Acrylamide-found-in-coffee. William Reed Business Media Ltd., Crawley, England, 2008.
3. Christofakis, M., and Xila, A. LC–MS/MS techniques for food allergen testing. Page 49 in: *Food Allergen Testing: Molecular, Immunochemical and Chromatographic Techniques*. G. Siragakis and D. Kizis, eds. John Wiley & Sons, Ltd., Hoboken, NJ, 2014.
4. Maberry, T. A look back at 2016 food recalls. Published online at www.foodsafetymagazine.com/enewsletter/a-look-back-at-2016-food-recalls. FSM: eDigest, FoodSafetyMagazine.com, 2017.
5. Park, M. 90 percent of U.S. bills carry traces of cocaine. Published online at www.cnn.com/2009/HEALTH/08/14/cocaine.traces.money. CNN U.S. Edition. Cable News Network, Atlanta, 2009.
6. U.S. Food and Drug Administration. *Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed*. Published online at www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/chemicalcontaminantsmetalsnaturaltoxinspesticides/ucm077969.htm. FDA, Silver Spring, MD, 2000.
7. U.S. Food and Drug Administration. Food Allergen Labeling and Consumer Protection Act of 2004 questions and answers. Published online at www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Allergens/ucm106890.htm. FDA, Silver Spring, MD, 2006.
8. U.S. Food and Drug Administration. Draft guidance for industry: Hazard analysis and risk-based preventive controls for human food. Published online at www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm517412.htm. FDA, Silver Spring, MD, 2017.