ILSI North America

Food Safety Briefs

July 2015

E. coli

Effect of Sampling Plans on the Risk of Escherichia coli O157 Illness

A. Kiermeier, J. Sumner, I. Jenson

Journal of Food Protection, Vol. 78, No. 7; pp. 1370–1374, 2015

DOI: 10.4315/0362-028X.JFP-14-558

Link to full text: Click here

Significance: Increasing the sample size or sample amount of beef from the current amount would have a very small public health effect.

Australia exports about 150,000 to 200,000 tons of manufacturing beef to the United States annually. Each lot is tested for Escherichia coli O157 using the N-60 sampling protocol, where 60 small pieces of surface meat from each lot of production are tested. A risk assessment of E. coli O157 illness from the consumption of hamburgers made from Australian manufacturing meat formed the basis to evaluate the effect of sample size and amount on the number of illnesses predicted. The sampling plans evaluated included no sampling (resulting in an estimated 55.2 illnesses per annum), the current N-60 plan (50.2 illnesses), N-90 (49.6 illnesses), N-120 (48.4 illnesses), and a more stringent N-60 sampling plan taking five 25-g samples from each of 12 cartons (47.4 illnesses per annum). While sampling may detect some highly contaminated lots, it does not guarantee that all such lots are removed from commerce.

Foodborne Pathogens

Inactivation of Escherichia Coli O157:H7 and Salmonella Enterica on Blueberries in Water Using Ultraviolet Light

C. Liu, Y. Huang, H. Chen

Journal of Food Science, Vol. 80, No. 7; pp. M1532–M1537, 2015

DOI: 10.1111/1750-3841.12910

Link to full text: Click here

Significance: Ultraviolet light treatment could be used as an alternative to chlorine washing for blueberries and potentially for other fresh produce.

In this study, ultraviolet light (UV) processing in agitated water was developed to inactivate Escherichia coli O157:H7 and Salmonella on blueberries. Blueberries were dip- or spot-inoculated with E. coli or Salmonella. Blueberries inoculated with E. coli were treated for 2 to 10 min with UV directly (dry UV) or immersed in agitated water during UV treatment (wet UV). E. coli was most easily killed on spot-inoculated blueberries with a 5.2-log reduction after 10-min wet UV treatment. Dip-inoculated blueberries were the most difficult to be decontaminated with only 1.6-log reduction after 10-min wet UV treatment. Wet UV treatment generally showed higher efficacies than dry UV treatment,

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achieving an average of 1.4 log more reduction for spot-inoculated blueberries. For dip-inoculated blueberries, chlorine washing and UV treatments were less effective, achieving <2 log reductions of E. coli. Thus, the efficacy of combinations of wet UV with sodium dodecyl sulfate (SDS), levulinic acid, or chlorine was evaluated. Inoculated blueberries were UV-treated while being immersed in agitated water containing 100 ppm SDS, 0.5% levulinic acid or 10 ppm chlorine. The 3 chemicals did not significantly enhance the wet UV treatment.

Efficacy of Sanitizer Treatments on Survival and Growth Parameters of Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes on Fresh-Cut Pieces of Cantaloupe During Storage

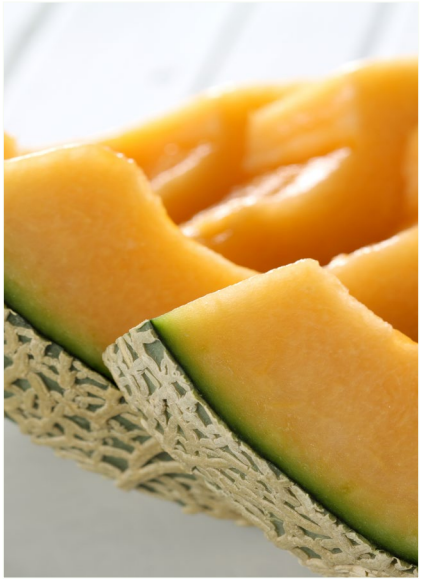
D.O. Ukuku, L. Huang, C. Sommers

Journal of Food Protection, Vol. 78, No. 7; pp. 1288–1295, 2015

DOI: 10.4315/0362-028X.JFP-14-233

Link to full text: Click here

Significance: A nisin-based sanitizer prevented transfer of human bacteria from melon rind surfaces to fresh-cut pieces, and the populations in fresh-cut pieces were below detection even by enrichment.



This study investigated survival and growth parameters of Escherichia coli O157:H7, Salmonella, Listeria monocytogenes, and aerobic mesophilic bacteria transferred from cantaloupe rind surfaces to fresh-cut pieces during fresh-cut preparation. All human bacterial pathogens inoculated on cantaloupe rind surfaces averaged ~4.8 log CFU/cm2, and the populations transferred to fresh-cut pieces before washing treatments ranged from 3 to 3.5 log CFU/g for all pathogens. A nisin-based sanitizer developed in our laboratory and chlorinated water at 1,000 mg/liter were evaluated for effectiveness in minimizing transfer of bacterial populations from cantaloupe rind surface to fresh-cut pieces. Inoculated and uninoculated cantaloupes were washed for 5 min before fresh-cut preparation and storage of fresh-cut pieces at 5 and 10°C for 15 days and at 22°C for 24 h. In fresh-cut pieces from cantaloupe washed with chlorinated water, only Salmonella was found (0.9 log CFU/g), whereas E. coli O157:H7 and L. monocytogenes were positive only by enrichment. Storage temperature affected survival and the growth rate for each type of bacteria on fresh-cut cantaloupe. Specific growth rates of E. coli O157:H7, Salmonella, and L. monocytogenes in fresh-cut pieces were similar, whereas the aerobic mesophilic bacteria grew 60 to 80 % faster and had shorter lag phases.

Listeria

The Elimination of Listeria Monocytogenes Attached to Stainless Steel or Aluminum Using Multiple Hurdles

A.W. Mertz, C.A. O'Bryan, P.G. Crandall, S.C. Ricke, R. Morawicki

DOI: 10.1111/1750-3841.12926

Journal of Food Science, Vol. 80, No. 7; pp. M1557–M1562, 2015

Link to full text: Click here

Significance: This study provides a better understanding and potential methods for the sanitization of industrial deli meat slicers, which can reduce the risk of contamination and outbreaks of L. monocytogenes and other food-borne pathogens for consumers.

Ready-to-eat luncheon meats sliced in retail delis have been found to pose the greatest risk of foodborne illness from Listeria monocytogenes among all

ready-to-eat foods. Standard cleaning and sanitizing practices of slicers used by deli employees may not eliminate Listeria in the equipment. The effects of moist heat, which is known to be more effective against L. monocytogenes than dry heat at the same temperature and time, combined with quaternary ammonium compounds (5 or 10 ppm), chlorine (10 or 25 ppm) or peracetic acid (10 or 25 ppm) on inactivating L. monocytogenes attached to stainless steel or aluminum coupons cut from commercial deli meat slicer components were investigated. All sanitizers when used alone resulted in a 2- to 3-log reduction of L. monocytogenes on stainless steel or aluminum surfaces, while moist heat alone resulted in a 3- to 4-log reduction. When combined with heat the quaternary ammonium was used at 5 ppm, peracetic acid at 10 ppm and chlorine at 10 ppm. When the 2 lethal treatments were combined there was a 5- to7-log reduction as compared to initial inoculation.

Selection and Characterization of Phage-Resistant Mutant Strains of Listeria monocytogenes Reveal Host Genes Linked to Phage Adsorption

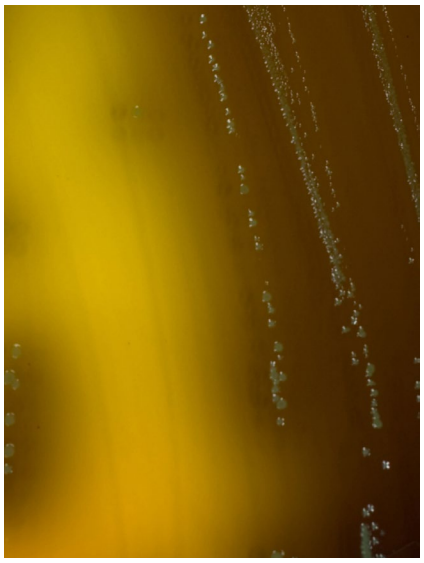
T. Denes, H.C. den Bakker, J.I. Tokman, C. Guldimann, M. Wiedmann

Applied and Environmental Microbiology, Vol. 81, No. 13; pp. 4295–4305, 2015

DOI: 10.1128/AEM.00087-15

Link to full text: Click here

Significance: Mutant strains resistant to only LP-125 lack terminal N-acetylglucosamine in their wall teichoic acid (WTA), whereas mutant strains resistant to both phages have disruptive mutations in their rhamnose biosynthesis operon but still possess N-acetylglucosamine in their WTA.



Listeria-infecting phages are readily isolated from Listeria-containing environments, yet little is known about the selective forces they exert on their host. Here, we identified that two virulent phages, LP-048 and LP-125, adsorb to the surface of Listeria monocytogenes strain 10403S through different mechanisms. We isolated and sequenced 69 spontaneous mutant strains of 10403S that were resistant to either one or both phages. Mutations from 56 phage-resistant mutant strains with only a single mutation mapped to 10 genes representing five loci on the 10403S chromosome. An additional 12 mutant strains showed two mutations, and one mutant strain showed three mutations. Two of the loci, containing seven of the genes, accumulated the majority (n = 64) of the mutations. A representative mutant strain for each of the 10 genes was shown to resist phage infection through mechanisms of adsorption inhibition. Complementation of mutant strains with the associated wild-type allele was able to rescue phage susceptibility for 6 out of the 10 representative mutant strains. Wheat germ agglutinin, which specifically binds to N-acetylglucosamine, bound to 10403S and mutant strains resistant to LP-048 but did not bind to mutant strains resistant to only LP-125.

VirR-Mediated Resistance of Listeria monocytogenes against Food Antimicrobials and Cross-Protection Induced by Exposure to Organic Acid Salts

J. Kang, M. Wiedmann, K.J. Boor, T.M. Bergholz

Applied and Environmental Microbiology, Vol. 81, No. 13; pp. 4553–4562, 2015

DOI: 10.1128/AEM.00648-15

Link to full text: Click here

Significance: VirRS-mediated regulation of dltABCD is the major resistance mechanism used by Listeria monocytogenes against cell envelope-damaging food antimicrobials.

This study screened in-frame deletion mutants of two-component system response regulators associated with the cell envelope stress response for increased sensitivity to commercially available antimicrobial compounds (nisin, lauric arginate, ε-polylysine, and chitosan). A virR deletion mutant showed increased sensitivity to all antimicrobials and significantly greater loss of membrane integrity when exposed to nisin, lauric arginate, or ε-polylysine ($P < 0.05$). The VirR-regulated operon, dltABCD, was shown to be the key contributor to resistance against these antimicrobial compounds, whereas another VirR-regulated gene, mprF, displayed an antimicrobial-specific contribution to resistance. An experiment with a β-glucuronidase (GUS) reporter fusion with the dlt promoter indicated that nisin does not specifically induce VirR-dependent upregulation of dltABCD. Lastly, prior exposure of L. monocytogenes parent strain H7858 and the ΔvirR mutant to 2% potassium lactate enhanced subsequent resistance against nisin and ε-polylysine ($P < 0.05$).

Salmonella

Inoculation Preparation Affects Survival of Salmonella enterica on Whole Black Peppercorns and Cumin Seeds Stored at Low Water Activity

L.S. Bowman, K.M. Waterman, R.C. Williams, M.A. Ponder

Journal of Food Protection, Vol. 78, No. 7; pp. 1259–1265, 2015

DOI: 10.4315/0362-028X.JFP-14-483

Link to full text: Click here

Significance: The most stable inoculum strategies were dry transfer, 24-h incubation of Salmonella and spices in tryptic soy broth, and inoculation of Salmonella cells grown on tryptic soy agar subsequent to drying.

The effects of inoculation preparation on the recoverability of Salmonella enterica from dried whole peppercorns and cumin seeds were examined. Whole black peppercorns and cumin seeds were inoculated with S. enterica using one dry transfer method and various wet inoculation methods: immersion of spice seeds in tryptic soy broth (TSB) plus Salmonella for 24 h, application of cells grown in TSB, and/or application of cells scraped from tryptic soy agar (TSA). Salmonella cells were enumerated by serial dilution and plated onto xylose lysine Tergitol (XLT4) agar and TSA. Recovery of Salmonella was high after 28 days of storage but was dependent on inoculation method, with 4.05 to 6.22 and 3.75 to 8.38 log CFU/g recovered from peppercorns and cumin seeds, respectively, on XLT4 agar. The changes in surviving Salmonella (log CFU per gram) from initial inoculation levels after 28 days were significantly smaller for the biofilm inclusion method (+0.142pepper, +0.186cumin) than for the other inoculation methods (–0.425pepper, –2.029cumin for cells grown on TSA; –0.641pepper, –0.718cumin for dry transfer; –1.998pepper for cells grown in TSB). The inoculation method influenced the recoverability of Salmonella from whole peppercorns and cumin seeds after drying.

Preharvest Salmonella Detection for Evaluation of Fresh Ground Poultry Product Contamination

N.P. Evans, R.D. Evans, J. Regalado, J.F. Sullivan, V. Dutta, F. Elvinger, et al.

Journal of Food Protection, Vol. 78, No. 7; pp. 1266–1271, 2015

DOI: 10.4315/0362-028X.JFP-14-509

Link to full text: Click here

Significance: Preharvest screening provides a meaningful evaluation of product contamination.

The goal of this study was to develop a preharvest diagnostic protocol for the evaluation of ground product contamination. The turkey processing plant where this research was conducted had previously established Salmonella screening (BAX system) of ground product, thus providing an opportunity for preharvest sample comparison. Drag swabs were collected from live-haul trailers entering the processing plant over a 12-month period. The swabs were added to modified buffered peptone water and incubated at 40°C. After incubation for 6 h or overnight, samples were tested for the presence of Salmonella with the DNAble assay and related to ground turkey samples from corresponding lots. The linear relationship for the percentage of Salmonella-positive live-haul trailers was significant for both the 6-h (slope = 1.02, R 2 = 0.96, and $P < 0.0001$) and overnight (slope = 0.35, R 2 = 0.93, and $P = 0.0015$) incubations, with the percentage of Salmonella-positive ground turkey samples. Additionally, the 6-h incubation protocol is rapid enough to allow for product mitigation and could potentially aid in the reduction of future salmonellosis outbreaks.

Efficacy of Lytic Bacteriophage Preparation in Reducing Salmonella In Vitro, on Turkey Breast Cutlets, and on Ground Turkey

C.S. Sharma, J. Dhakal, R. Nannapaneni

Journal of Food Protection, Vol. 78, No. 7; pp. 1357–1362, 2015

DOI: 10.4315/0362-028X.JFP-14-585

Link to full text: Click here

Significance: The bacteriophage preparation was effective in reducing Salmonella on turkey breast cutlets as a surface treatment but did not cause any reduction of Salmonella Heidelberg in ground turkey.

The efficacy of the recently approved Salmonella lytic bacteriophage preparation (SalmoFresh) in reducing Salmonella enterica serotype Heidelberg on turkey breast cutlets and ground turkey was evaluated. In a broth model assay, the phage preparation completely inhibited the growth of four S. enterica serotypes (Salmonella Enteritidis, Salmonella Heidelberg, Salmonella Kentucky, and Salmonella Typhimurium) at 37°C at a multiplicity of infection of 10,000 PFU/CFU. At 4°C in 0.1% peptone water (PW), phage treatment at a multiplicity of infection of 10,000 resulted in ca. 4.0-log CFU/ml reductions of Salmonella Enteritidis, Salmonella Heidelberg, and Salmonella Typhimurium. When raw turkey breast cutlets inoculated with Salmonella Heidelberg (~103 CFU/g) were treated with phage preparation (107 PFU/g) and stored at 4°C, the phage treatment caused reductions of 0.8, 0.6, and 1.3 log CFU/g ($P \leq 0.05$) of Salmonella Heidelberg on day 0, 1, and 7, respectively, compared with the counts in the control. However, no significant reduction of Salmonella Heidelberg ($P > 0.05$) was observed in ground turkey when turkey meat pieces inoculated with Salmonella Heidelberg were surface treated with phage preparation (107 PFU/g) before grinding.

Combining Lactic Acid Spray with Near-Infrared Radiation Heating to Inactivate Salmonella enterica Serovar Enteritidis on Almond and Pine Nut Kernels

J-W. Ha, D-H. Kang

Applied and Environmental Microbiology, Vol. 81, No. 13; pp. 4517–4524, 2015

DOI: 10.1128/AEM.00943-15

Link to full text: Click here

Significance: Near-infrared radiation with lactic acid treatment may be a potential intervention for controlling food-borne pathogens on nut kernel products.

The aim of this study was to investigate the efficacy of near-infrared radiation (NIR) heating combined with lactic acid (LA) sprays for inactivating Salmonella enterica serovar Enteritidis on almond and pine nut kernels and to elucidate the mechanisms of the lethal effect of the NIR-LA combined treatment. Separately prepared S. Enteritidis phage type (PT) 30 and non-PT 30 S. Enteritidis cocktails were inoculated onto almond and pine nut kernels, respectively, followed by treatments with NIR or 2% LA spray alone, NIR with distilled water spray (NIR-DW), and NIR with 2% LA spray (NIR-LA). Although surface temperatures of nuts treated with NIR were higher than those subjected to NIR-DW or NIR-LA treatment, more S. Enteritidis survived after NIR treatment alone. The effectiveness of NIR-DW and NIR-LA was similar, but significantly more sublethally injured cells were recovered from NIR-DW-treated samples. We confirmed that the enhanced bactericidal effect of the NIR-LA combination may not be attributable to cell membrane damage per se. NIR heat treatment might allow S. Enteritidis cells to become permeable to applied LA solution.

Food Allergy

Peanut Oral Immunotherapy Transiently Expands Circulating Ara H 2–Specific B Cells With a Homologous Repertoire in Unrelated Subjects

S.U. Patil, A.O. Ogunniyi, A. Calatroni, V.R. Tadigotla, B. Ruiter, A. Ma, et al.

Journal of Allergy and Clinical Immunology, Vol. 136, No. 1; pp. 125–134.e12, 2015

DOI: 10.1016/j.jaci.2015.03.026

Link to full text: Click here

Significance: The early peanut oral immunotherapy-induced Ara h 2–specific B-cell receptor repertoire is oligoclonal and somatically hypermutated and shares similar clonal groups among unrelated subjects consistent with convergent selection.

The researchers hypothesized that peanut oral immunotherapy (PNOIT) induces a clonal, allergen-specific B-cell response that could serve as a surrogate for clinical outcomes. A fluorescent Ara h 2 multimer for affinity selection of Ara h 2–specific B cells and subsequent single-cell immunoglobulin amplification was used. The diversity of related clones was evaluated by means of next-generation sequencing of immunoglobulin heavy chains from circulating memory B cells with 2x250 paired-end sequencing on the Illumina MiSeq platform. Expression of class-switched antibodies from Ara h 2–positive cells confirms enrichment for Ara h 2 specificity. PNOIT induces an early and transient expansion of circulating Ara h 2–specific memory B cells that peaks at week 7. Ara h 2–specific sequences from memory cells have rates of nonsilent mutations consistent with affinity maturation. The repertoire of Ara h 2–specific antibodies is oligoclonal. Next-generation sequencing–based repertoire analysis of circulating memory B cells reveals evidence for convergent selection of related sequences in 3 unrelated subjects, suggesting the presence of similar Ara h 2–specific B-cell clones.

Heavy Metals

Market Survey and Risk Assessment for Trace Metals in Edible Fungi and the Substrate Role in Accumulation of Heavy Metals

Q. Huang, Y. Jia, Y. Wan, H. Li, R. Jiang

Journal of Food Science, Vol. 80, No. 7; pp. H1612–H1618, 2015

DOI: 10.1111/1750-3841.12923

Link to full text: Click here

Significance: Concentrations of Cd, As, Hg, Pb, Fe, and Zn were relatively high in Lentinus edodes, whereas Tremella fuciformis and Pholiota nameko had relatively low levels of trace metals.

Levels of cadmium (Cd), arsenic (As), mercury (Hg), lead (Pb), iron (Fe), and zinc (Zn) were investigated in 285 samples of 9 species of edible fungi (Lentinus edodes, Auricularia auricula, Pleurotus ostreatus, Tremella fuciformis, Flammulina velutipes, Agrocybe chaxinggu, Armillaria mellea, Agaricus bisporus, and Pholiota nameko), which were collected from markets in Beijing, China. In addition, edible fungi and culture substrates were collected from 7 cultivation bases to examine the role of the substrate in trace metal accumulation. Data showed that all the edible fungi contained trace metals and there were significant positive correlations between Cd, Pb, and As concentrations in mushrooms and their substrates. The concentrations of Cd, As, Hg, Pb, Fe, and Zn in the tested fungi ranged from 0.005 to 13.8 mg/kg, nd to 1.62 mg/kg, nd to 0.506 mg/kg, 0.011 to 22.1 mg/kg, 46.3 to 2514 mg/kg, and 14.6 to 289 mg/kg, respectively.

Mycotoxins

Infestation and Quantification of Ochratoxigenic Fungi in Barley and Wheat Naturally Contaminated with Ochratoxin A

J. Kuruc, J. Hegstad, H.J. Lee, K. Simons, D. Ryu, C. Wolf-Hall

Journal of Food Protection, Vol. 78, No. 7; pp. 1350–1356, 2015

DOI: 10.4315/0362-028X.JFP-14-578

Link to full text: Click here

Significance: Neither infestation rate nor nonribosomal peptide synthase concentration is a reliable predictor of OTA level in a sample.

This study assessed the efficacy of two ochratoxin A (OTA)-related indices for OTA level prediction. Infestation rates were determined by direct plating for freshly harvested and stored barley, durum, and hard red spring wheat samples (n = 139) with known OTA levels. Presumptive ochratoxigenic isolates were tested for their ability to produce OTA. The nonribosomal peptide synthase (otanpsPN) involved in OTA biosynthesis was used to quantify ochratoxigenic fungi in barley and wheat. Viable Penicillium verrucosum was present in 45% of the samples. In total, 62.7% (n = 110) of the P. verrucosum isolates tested produced OTA on dichloran yeast extract sucrose 18% glycerol agar. Both OTA level and infestation rate (r = 0.30), as well as OTA level and otanpsPN concentration (r = 0.56), were weakly correlated.

Mycotoxins in Plant-Based Dietary Supplements: Hidden Health Risk for Consumers

Z. Veprikova, M. Zachariasova, Z. Dzuman, A. Zachariasova, M. Fenclova, P. Slavikova, et al.

Journal of Agricultural and Food Chemistry, Vol. 63, No. 29; pp. 6633–6643, 2015

DOI: 10.1021/acs.jafc.5b02105

Link to full text: Click here

Significance: The highest mycotoxin concentrations were found in milk thistle-based supplements for the treatment of liver diseases.

The aim of this study was to assess the extent of mycotoxin contamination of dietary supplements based on analyses of a wide set of herbal-based dietary supplements intended for various purposes: (i) treatment of liver diseases (milk thistle); (ii) reduction of menopause effects (red clover, flax seed, and soy); and (iii) preparations for general health support (green barley, nettle, goji berries, yucca, etc.) The analytical method including 57 mycotoxins was based on a QuEChERS-like (quick, easy, cheap, effective, rugged, safe) approach and ultrahigh performance liquid chromatography coupled with tandem mass spectrometry. The main mycotoxins determined were Fusarium trichothecenes, zearalenone and enniatins, and Alternaria mycotoxins. Co-occurrence of enniatins, HT-2/T-2 toxins, and Alternaria toxins was observed in many cases.

Bisphenols

Bisphenol A and Three Other Bisphenol Analogues in Canned Fish Products from the Canadian Market 2014

X-L. Cao, S. Popovic

Journal of Food Protection, Vol. 78, No. 7; pp. 1402–1407, 2015

DOI: 10.4315/0362-028X.JFP-15-055

Link to full text: Click here

Significance: The few products with high BPA levels (>100 ng/g) are exclusively from a new brand that has become available on the market only recently.

A sensitive and selective gas chromatography–mass spectrometry method was developed and validated for simultaneous analysis of bisphenol A (BPA), bisphenol B (BPB), bisphenol E (BPE), and bisphenol F (BPF). This method was used to analyze samples of 52 canned fish products to follow up a previous study conducted 5 years ago to investigate any changes in BPA levels since then and levels of other bisphenols due to possible changes in can coating formulations. BPB and BPE were not detected in any of the 52 canned fish products, and BPF was detected in only four products at low levels from 1.8 to 5.7 ng/g. BPA was detected in all 52 canned fish products, but at much lower levels compared with a previous study; levels ranged from 0.96 to 265 ng/g (average, 28 ng/g). Further analysis of canned fish products is planned in the future to capture any changes in BPA levels in these products and to update the exposure assessment of BPA due to consumption of canned fish products.

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The North American branch of the International Life Sciences Institute (ILSI North America) is a public, non-profit scientific foundation that advances the understanding and application of science related to the nutritional quality and safety of the food supply.

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