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Attention: Mr. Dennis Colley

Subject: Microbiological Status of Reusable Plastic Containers – a Follow up Field Study

Fresh produce has been documented by the United States (US) Center for Disease Control and Prevention (CDC) as a leading source of food-borne illness (CDC, 2014). With the recent passage of the US Food Safety Modernization Act (FSMA), the supply chain has become an even greater source of regulatory scrutiny for growers, shippers and even retailers. FSMA now requires US entities take a proactive rather than reactive approach to food safety (US FDA, undated).

Although food-borne illness has not been directly associated with shipping and transport containers, the potential for containers to harbor and transfer microbial loads to the fresh produce placed in those containers has been documented (Danyluk, 2010; Sanders, 2015a; Suslow, 2014; Warriner, 2013 & 2014). To address this, and meet the intent of FSMA, a follow up assessment of the cleanliness of reusable plastic containers (RPCs) was deemed critical as previous field studies indicated a large percentage of containers showed visible contamination and/or microbial contamination that could affect the produce placed within them.

This report summarizes results of recent field tests performed on RPCs. Testing included a visible evaluation, and the microbial assessment of the RPCs. The two goals of the study were to: (1) determine the microbial loads present on RPCs currently in the produce supply chain using standard microbial methods and (2) confirm that these RPCs meet acceptable microbial limits.

Background

Acceptable microbial levels for produce storage and transport containers are not currently defined by any regulatory agencies in the US. A European Union (EU) Commission Decision (2001/471/EC) states that the total viable microorganism count on containers for transport of fresh meat or poultry should not exceed 10 colony forming units (CFU)/cm², while the value of *Enterobacteriaceae* should not exceed 1 CFU/cm² (European Commission, 2011). These limits have been subsequently employed as a benchmark level by the Ireland Food Authority and the New South Wales Food Safety Authority for clean and sanitized food contact surfaces (Ireland Food Authority, 2006; New South Wales Food Safety Authority, 2013). In a publicly available, peer-reviewed study, Cunningham et al. (2011) defined the

acceptable levels of aerobic microorganisms on food contact surfaces as 125 CFU/50 cm² as the upper limit for a clean and sanitized food contact surface (Cunningham et al., 2011).

In a 2013 study of RPCs used for the shipping and transport of fresh produce, Dr. Keith Warriner of the University of Guelph, specified that *Enterobacteriaceae*¹ or thermotolerant coliforms² levels less than 1,000 (log₃) CFU/container would be representative of sanitary conditions and deemed acceptable (Warriner, 2013). The acceptance criteria established by Warriner was used in prior evaluations of container cleanliness (Sanders, 2015b) and is used to evaluate the results from this study.

Project Methodology

RPCs at three different grower/shipper locations across multiple geographical regions were sampled and tested using the protocol that was developed based on a protocol developed and employed by Dr. Trevor Suslow of the University of California, Davis in a previous field study of RPC cleanliness (Suslow, 2014). The current protocol was established to generate data providing an informed view of RPC cleanliness that could be compared to acceptable limits and previous field data. The protocol was reviewed and approved by Dr. Suslow prior to study initiation.

Testing included a visible evaluation of RPCs and the microbial evaluation of RPCs for the presence of two bacterial indicator organisms, *Enterobacteriaceae* and thermotolerant coliforms. Testing was performed on two different container subsets at each location: (1) visibly clean RPCs (random RPCs) and (2) RPCs with visible clues indicating a potential lack of cleanliness (i.e., residual liquid, old product stickers, residual plant material), classified as “for cause” RPCs. A total of 24 random RPCs and 12 “for cause” RPCs were sampled at each of the three locations.

Containers selected for evaluation were chosen from various locations (top, middle and bottom) within each pallet. The entire interior surface of the containers was evaluated using two distinct sponge samples; one of the interior bottom and one of the inside sides and hinges. In addition, areas of visible contamination on the interiors of “for cause” RPCs were also sampled. RPCs were collected and sampled at three unique grower/shipper locations: two California locations (Santa Maria and Temecula) and one location in the Pacific Northwest (Delta, British Columbia, Canada). Sampling and laboratory analysis were performed by Primus Laboratories of Santa Maria, CA.

¹ *Enterobacteriaceae* are often evaluated as an indicator for *Salmonella* spp.

² Thermotolerant coliforms are often evaluated as an indicator organism for *Escherichia coli* (*E.coli*).

Results

VISIBLE OBSERVATIONS

RPCs from pallets wrapped in a green protective wrap were selected for evaluation at each site. IFCO literature indicates that the containers on green wrapped pallets are clean, sanitized, and dry (IFCO, undated).

Image 1: Depiction of IFCO green wrapped RPC pallets



The RPCs were inspected for the presence of visible clues indicating the containers may have not undergone sufficient cleaning, sanitizing, or drying. RPCs that appeared visibly clean and dry were classified as random RPCs, while RPCs with visible moisture, residual plant material, or stickers were classified as “for cause”. Representative photographs taken at the sampling sites of RPCs assessed as “for cause” are presented as Images 2-6.

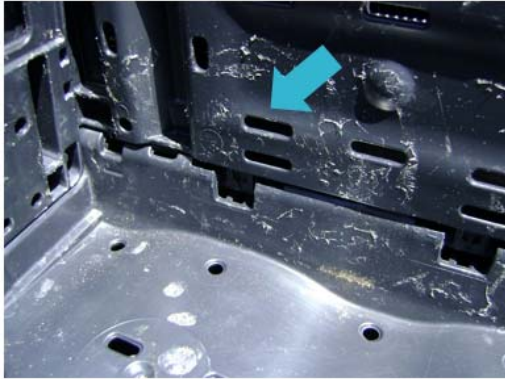


Image 2: Santa Maria, CA - Sample USM16-072088-01B
(residual adhesives and other unspecified residual)



Image 3: Temecula, CA UMS16.102272-01A
(residual label and free moisture)



Image 4: Temecula, CA UMS16.102272-07C
(unknown contamination)



Image 5: Delta, BC Sample USM 104492-01D
(residual label and adhesive)



Image 6: Delta, BC Sample USM 104492-04C
(residual plant material)

STANDARD MICROBIAL TEST RESULTS

The number of viable *Enterobacteriaceae* and thermotolerant coliforms on the interior surfaces of the containers was determined. The distribution of the microbial loads is summarized on both a container and swab/sponge sample basis in Tables 2 and 3. The values presented in Table 2 show the results on an RPC basis, while Table 3 provides a similar analysis on a per swab/sponge sample basis. Over 93 percent of random RPCs (and the associated individual sponge/swab samples) displayed microbial counts below the acceptable limit of 1,000 CFU. All “for cause” RPCs displayed microbial counts below acceptable limits.

Table 2: Organisms per Container (Thermotolerant Coliforms and *Enterobacteriaceae*)³

Sampling location/ container type	# of containers	# with ≤10 CFU	# with >10 - ≤100 CFU	# with >100 - ≤1,000 CFU	# with >1,000 - ≤10,000 CFU	# with >10,000 - ≤100,000 CFU
California-1 Random RPCs	24	16	7	1	0	0
California-2 Random RPCs	24	6	14	3	0	1
British Columbia Random RPCs	24	15	2	3	4	0
All Random RPCs	72	37	23	7	4	1
California-1 “for cause” RPCs	12	4	2	6	0	0
California-2 “for cause” RPCs	12	4	5	3	0	0
British Columbia “for cause” RPCs	12	7	4	1	0	0
All “for cause” RPCs	36	15	11	10	0	0

³ The data used to generate the tables and charts included can be found as Appendices C, D and E.

Table 3: Organisms per Swab/Sponge Samples (Thermotolerant Coliforms and *Enterobacteriaceae*)⁴

Sampling Location/ Container type	# of swab/sponge samples	# with ≤10 CFU	# with >10 - ≤100 CFU	# with >100 - ≤1,000 CFU	# with >1,000 - ≤10,000 CFU	# with >10,000 - ≤100,000 CFU
California-1 Random RPCs	48	38	9	1	0	0
California-2 Random RPCs	48	22	20	5	0	1
British Columbia Random RPCs	48	37	3	4	4	0
All Random RPCs	144	97	32	10	4	1
California-1 "for cause" RPCs	36	25	4	7	0	0
California-2 "for cause" RPCs	36	27	7	2	0	0
British Columbia "for cause" RPCs	36	28	6	2	0	0
All "for cause" RPCs	108	80	17	11	0	0

The data show that a majority of the containers have <1,000 CFU of *Enterobacteriaceae* and thermotolerant coliforms combined.

It is noted, however, that of the five RPCs with microbial loads above 1,000 CFU/container, all appeared visibly clean. The microbial loads from these RPCs were composed either primarily or completely of *Enterobacteriaceae*, rather than thermotolerant coliforms.

Conclusion

RPCs were visibly reviewed and tested to assess the cleanliness of containers currently being used by the fresh produce industry. Previous studies have shown that many RPCs exhibited visible signs that the containers had not undergone adequate cleaning, sanitizing, and drying and that the levels of *Enterobacteriaceae* and thermotolerant coliforms on the interior surface varied greatly from RPC to RPC. Multiple previous field studies, conducted in the US and Canada, showed a large percentage (up to 50%) of RPCs had microbial loads exceeding acceptable limits (1,000 CFU) with some samples having over 1,000,000 organisms per RPC (Suslow, 2014; Warriner, 2013 & 2014).

This most recent work shows that the presence of *Enterobacteriaceae* and thermotolerant coliforms present on the RPCs is much less than identified in previous studies, with seven percent of random RPCs

⁴ The data used to generate the tables and charts included can be found as Appendices A, B, and C.

exceeding acceptable limits. The maximum number of *Enterobacteriaceae* and thermotolerant coliforms present on any RPC was 14,300 CFU. This is likely due to recently implemented improvements in the RPC cleaning and sanitization process, meant to mitigate the presence of visible and microbial contamination as well as residual moisture on the RPCs prior to reuse (IFCO, undated).

The visible inspection performed in this study identified residual product labels (stickers), plant materials and free moisture on clean RPCs. Despite the much improved microbial cleanliness of the RPCs, the identified visible contamination suggest that continued due diligence is still required to manage the cleanliness of RPCs upon reuse. It may be in the RPC Industry's best interest to further refine their processes to identify when these situations might occur and work to eliminate potential food safety issues in the fresh produce industry.

Sincerely yours,
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Attachments:

- Appendix A: California Location 1 Data
- Appendix B: California Location 2 Data
- Appendix C: British Columbia Data

References

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APPENDIX A

California Location 1 Data

**CALIFORNIA LOCATION 1 DATA
RANDOM RPC RESULTS**

Total Organisms per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 but <1000 CFU/Container
24	16	7	1

Total Organisms per Sponge/Swab Sample

Number of Sponge/Swab Samples (two samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample
48	38	9	1

Coliform per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 CFU/Container
24	16	7	1

Coliform per Sponge/Swab Sample

Number of Sponge/Swab Samples (two samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 CFU/Sample
48	38	9	1

***Enterobacteriaceae* and RLU per RPC**

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container
24	22	2

***Enterobacteriaceae* per Sponge/Swab Samples**

Number of Sponge Samples (two sponge samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample
48	46	2

**CALIFORNIA LOCATION 1 DATA
FOR CAUSE RPC RESULTS**

Total organisms per RPCs

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥1 but <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container
12	4	2	6

Total organisms per Sponge Sample

Number of Sponge Samples (two sponge samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥1 but <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample
36	25	4	7

Coliforms per RPCs

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥1 but <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container
12	4	2	6

Coliforms per Sponge Sample

Number of Sponge Samples (two sponge samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥1 but <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample
36	25	4	7

***Enterobacteriaceae* per RPCs**

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container
12	12

***Enterobacteriaceae* per Sponge Sample**

Number of Sponge Samples (two sponge samples/RPC)	Number of Sponge Samples with <10 CFU/Sample
36	36

APPENDIX B

California Location 2 Data

**CALIFORNIA LOCATION 2 DATA
RANDOM RPC RESULTS**

Total Organism per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 but <1000 CFU/Container	Number of RPCs with ≥1000 CFU/Container	Number of RPCs with ≥10,000 CFU/Container
24	6	14	3	0	1

Total Organism per Sponge Sample

Number of Sponge Samples (two samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	Number of Sponge Samples with ≥1000 CFU/Sample	Number of Sponge Samples with ≥10,000 CFU/Sample
48	24	20	3	1	1

Coliforms per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 but <1000 CFU/Container	Number of RPCs with ≥1000 CFU/Container
24	7	14	2	1

Coliforms per Sponge Sample

Number of Sponge Samples (two samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	Number of Sponge Samples with ≥1000 CFU/Sample
48	24	21	2	1

Enterobacteriaceae and RLU per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 but <1000 CFU/Container	Number of RPCs with ≥1000 CFU/Container	Number of RPCs with ≥10,000 CFU/Container
24	16	5	2	0	1

Number of Sponge Samples (two samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	Number of Sponge Samples with ≥1000 but <10000 CFU/Sample	Number of Sponge Samples with ≥10,000 CFU/Sample
48	40	5	2	0	1

**CALIFORNIA LOCATION 2 DATA
FOR CAUSE RPC RESULTS**

Total Organism per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 CFU/Container
12	4	5	3

Total Organism per Sponge Sample

Number of Sponge Samples (two samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample
36	27	7	2

Coliforms per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 CFU/Container
12	4	5	3

Coliforms per Sponge Sample

Number of Sponge Samples (two samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample
36	22	12	2

***Enterobacteriaceae* per RPC**

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 CFU/Container
12	11	1	0

***Enterobacteriaceae* per Sponge Sample**

Number of Sponge Samples (two samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample
36	35	1	0

APPENDIX C

British Columbia Location Data

**BRITISH COLUMBIA DATA
RANDOM RPC RESULTS**

Total Organism per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 but <1000 CFU/Container	Number of RPCs with ≥1000 CFU/Container
24	15	2	3	4

Total Organism Count per Sponge Sample

Number of Sponge Samples (two sponge samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	Number of Sponge Samples with ≥1000 CFU/Sample
48	37	3	4	4

Coliform per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 but <1000 CFU/Container	Number of RPCs with ≥1000 CFU/Container
24	22	2	0	0

Coliform per Sponge Samples

Number of Sponge Samples (two sponge samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	Number of Sponge Samples with ≥1000 CFU/Sample
48	44	4	0	0

Enterobacteriaceae per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 but <1000 CFU/Container	Number of RPCs with ≥1000 CFU/Container
24	17	0	3	4

Enterobacteriaceae per Sponge Sample

Number of Sponge Samples (two sponge samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	Number of Sponge Samples with ≥1000 but <10000 CFU/Sample
48	40	0	4	4

**BRITISH COLUMBIA DATA
FOR CAUSE RPC RESULTS**

Total organisms per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 CFU/Container
12	7	4	1

Total organisms per Sponge/Swab Sample

Number of Samples (three samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample
36	28	6	2

Coliforms per RPC

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 CFU/Container
12	11	1	0

Coliforms per Sponge/Swab Sample

Number of Samples (three samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample
36	34	2	0

***Enterobacteriaceae* per RPC**

Number of RPCs Sampled	Number of RPCs with <10 CFU/Container	Number of RPCs with ≥10 but <100 CFU/Container	Number of RPCs with ≥100 CFU/Container
12	9	2	1

***Enterobacteriaceae* per Sponge/Swab Sample**

Number of Sponge Samples (three samples/RPC)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample
36	31	3	2