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Fibre Box Association
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Attention: Mr. Dennis Colley
President/CEO

Subject: Microbiological Status of Corrugated Containers – First Annual Review Study

The Fibre Box Association (FBA) previously evaluated the microbial cleanliness of corrugated containers from multiple manufacturers across three different regions in the United States (US) in a 2014 study. This current report, sponsored by the FBA, summarizes the results of a recent survey (annual review) that assesses the microbial status of corrugated containers currently being used for the storage and transport of fresh produce across the US and Canada. The goal of the project was to assess the status of the microbial loads on corrugated containers currently being used by the fresh produce industry and confirm that those microbial loads meet acceptable limits.

Fresh produce has been documented by the 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; Warriner, 2013).

Project 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 defined the acceptable levels of aerobic microorganisms on food contact surfaces as 125 CFU/50 cm² (equal to 10^{3.4} CFU/930 cm²) as the upper limit for a clean and sanitized food contact surface (Cunningham et al., 2011). Dr. Keith Warriner of the University of Guelph, in his evaluation of containers used for the transport of fresh produce specified that less than 10³ CFU *Enterobacteriaceae*¹ or thermotolerant coliforms²/container would be representative of sanitary conditions and be deemed acceptable (Warriner, 2013).

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*³ or thermotolerant coliforms⁴ levels less than 10³ CFU/container would be representative of sanitary conditions and deemed acceptable (Warriner, 2013).⁵ This acceptance criteria, established by Warriner, was used to evaluate data from a previous field studies on the cleanliness of both corrugated containers from multiple manufacturers and reusable plastic containers (RPCs) across the US and Canada (Sanders, 2015b). In this review, all corrugated containers tested (N=360) had microbial loads below 1,000 CFU/container (Sanders, 2015a).

Project Methodology

Corrugated containers at three different grower/shipper locations across multiple geographical regions were sampled and tested using the attached protocol (Appendix A: Corrugated Container Sampling and Testing Protocol). This protocol was developed based on the prior sampling protocol with the final protocol reviewed and approved by Dr. Suslow of the University of California, Davis prior to study initiation. Testing included the microbial evaluation of corrugated containers for thermotolerant coliforms and *Enterobacteriaceae*; a total of 96 samples from 48 containers were sampled at each of the three locations.

Containers selected for evaluation were chosen from various locations (top, middle and bottom) from four different pallets. The entire interior surface of the containers was evaluated using two different sponge samples; one of the interior bottom and one of the interior sides and hinges. Sampling and laboratory analysis were performed by Primus Laboratories of Santa Maria, CA.

Corrugated containers were collected and sampled at three unique grower/shipper locations: two California (CA) locations (Santa Maria and Temecula) and one location in the Pacific Northwest (Delta, British Columbia (BC), Canada) were evaluated.

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*).

³ *Enterobacteriaceae* are often evaluated as an indicator for *Salmonella* spp.

⁴ Thermotolerant Coliforms are often evaluated as an indicator organism for *Escherichia coli* (*E.coli*).

⁵ 10³ can also be expressed as 1,000 or log 3.

Results

The results from the microbial sampling of the interior surfaces of the containers were reviewed to assess the presence of microorganisms per container and per sponge sample. The distribution of the microbial load found on the containers is summarized on both a container and sponge sample basis in the Table 1.

Table 1: Organisms per Container (Thermotolerant Coliforms and *Enterobacteriaceae*)⁶

Sampling Location	# of Containers	Containers w/≤10 CFU	Containers w/>10-≤100 CFU	Containers w/>100-≤1000 CFU	Containers w/>1000-≤10,000 CFU
California 1	48	8	36	3	1*
California 2	48	28	20	0	0
British Columbia	48	33	10	3	2**
All Locations	144	69	66	6	3

* Represents the total organisms identified (2,110 CFU) on both the bottom (1,190 CFU) and sides (920 CFU) of the container.

** Represents the organisms identified on the sides of the containers only (5,620 and 6,910); the sponge swab from the bottom of these container exhibited no growth.

Of the three containers above acceptable limits, one had a microbial load of 2,110 CFU/container or log 3.2, this result is minimally above the acceptable limit. The two other containers with the largest number of organisms, 5,620 and 6,910 CFU/container, were both from another location. Although transport containers and representative samples collected at the site were monitored and recorded for temperature, the temperature of these three samples with elevated microbial levels were not recorded.

Although no data are available on the temperatures of the specific samples with high microbial loads, representative samples temperatures were as high as 12.3°C (54.4 °F); therefore, it is possible that the individual sponge samples with the elevated CFU levels may have been exposed to temperatures conducive to microbial growth prior to receipt at the laboratory, which may have falsely elevated the results.

⁶ The data used to generate the tables and charts included can be found as Appendices B, C, and D.

Conclusion

This study was performed as a follow up to the industry-wide corrugated container cleanliness study sponsored by the FBA in 2014 where 360 containers from five (5) different corrugated manufacturers across three different regions in the US were evaluated for the presence of two pathogenic indicator organisms (*Enterobacteriaceae* and thermotolerant coliforms). One hundred percent of all containers from that study had levels of the indicator organisms below the acceptable limits of 1,000 CFU/container (Sanders, 2015a).

The results of this current study indicate that 98% of the corrugated containers sampled at three (3) different grower/shipper sites across the US and Canada had *Enterobacteriaceae* and thermotolerant coliform loads below acceptable limits.

When taken as a whole, the data show that the corrugated industry continues to provide clean containers to the fresh produce industry. Continued due diligence on the part of individual manufacturers and the corrugated industry to mitigate potential sources of contamination is recommended so that clean containers can continue to be provided to grower/shippers.

Sincerely yours,
HALEY & ALDRICH, INC.



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Attachments:

- Appendix A: Corrugated Container Sampling and Testing Protocol
- Appendix B: California Location 1 Data
- Appendix C: California Location 2 Data
- Appendix D: British Columbia Data

References

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APPENDIX A

Corrugated Container Sampling and Testing Protocol

Corrugated Container Sampling and Testing Protocol (final)

May 3, 2016

This protocol provides information and methodologies for the follow-up microbial assessment / cleanliness evaluation of corrugated containers used for the storage and transport of fresh produce. Container sampling will occur at multiple field sites across the United States with laboratory analyses being performed at Primus Laboratories Santa Maria, CA location. The protocol was developed by Haley & Aldrich and Primus Laboratories with input from Trevor Suslow and the Fibre Box Association.

1. Sampling sites

Corrugated containers will be sampled at three unique grower/shipper locations in three different geographic regions: Florida, California and the Pacific Northwest.

2. Sampling date

Sampling will occur on a single day per site

3. The container selection and sampling process will be conducted following Good Laboratory Practices (GLP). The containers chosen for sampling as well as actual sponge samples will be handled according to standard GLP chain of custody technique to ensure sample integrity and identity.

4. Container selection:

- a. Containers will be selected for sampling from two different corrugated manufacturers (if available). If not available, pallets from a single manufacturer may be sampled. Any pallet wrappings will be inspected for evidence of substantial soil/dust deposits or other foreign materials. If deposits are observed alternative pallets will be selected. If all pallets have external deposits on pallet wrappings a dry-brush procedure will be used to exclude as much as practical before removing the wrapping. Regardless of final condition, wrappings will be removed by technical staff wearing sterile disposable gloves and pulled outward and down from the top rather than lifting over the palletized stack.

After the pallet wrapping is removed, individual containers will be removed from the pallet for microbial sampling, by technical staff wearing new sterile gloves. Samples will be chosen from the bottom, middle and top of the pallet. Chosen containers will be handled by an exterior surface during unstacking and selection. Gloves will be changed as necessary to mitigate cross-contamination.

- b. Forty eight total containers will be selected for microbial sampling at each site:

- i. Two shipments will be assessed per location.

Note: Where available, the shipments should be from different Corrugated manufacturers. If shipments from two different corrugated manufacturers are not available, samples should be taken from two different shipments from the same corrugated manufacturer.

- ii. Twenty four containers will be sampled per corrugated shipment.

- iii. Four pallets per shipment.
- iv. Six containers per pallet.
- v. Two containers from the top of the pallet, two from the middle and two from the bottom of each pallet.

5. On-site sampling area:

An on-site area to conduct the swabbing of each unfolded corrugated box will be established with effective separation from on-going local operations, de-palletizing and selection activities, and any other potential sources of contamination or sampling interference. The on-site area will be prepared to facilitate proper aseptic technique in sampling/sample handling:

- a. An on-site work bench or table, small folding table, or similar platform will be used for sampling activities
- b. Prior to sampling, the table surface should be sprayed with a hard-surface sanitizing antimicrobial (bleach and/or 70% alcohol), and/or covered with a new sheet of protective lab paper. This activity will be performed between each pallet being tested.

6. Container Identification:

Each container selected for sampling will be labeled with a unique identifier and include:

- a. Pallet specific prefix to include a corrugated manufacture identification number (to be provided) and a pallet-specific identifier.
- b. Container specific information to include:
 - i. Position on the pallet: T (top), M (middle) or B (bottom)
 - ii. Consecutive number: 1-6

7. Microbial Sampling

- a. Sampling of the container will be performed using aseptic techniques, and in accordance with PrimusLabs SOP 14-20 "Environmental (Sponge) Sampling".
- b. Two microbial sponges will be taken per random containers
 - i. One sponge will be used to wipe the entire interior bottom surface.
 - ii. One sponge will be used to wipe the interior side and corner surfaces.
- c. Interior dimensions of the container will also be recorded.

8. Sample Transportation

- a. All individual sample bags containing swabs/sponges will be uniquely labeled with permanent ink or bar-code label and placed in a master container per individual corrugated box, pallet location, pallet and delivery.
- b. A "Sample Log Sheet" will be generated for each sampling event, reflecting transit time and receipt at the laboratory. This Sample Log Sheet will be signed by the Sampler and Laboratory Personnel to verify it's accuracy.
- c. All samples will be placed in a cooler with blue ice, with the temperature of the cooler and three individual sample bags recorded upon receipt at the laboratory.
- d. If samples are not processed immediately upon receipt at the laboratory, they will be placed in a secure area in a walk-in cooler or refrigerator at 2.0 to 4.0°C. Total time from sampling to processing is not to exceed 24 hours.

9. Microbial Sample Identification

Each microbial sample will be labeled with the container identifier and a notation regarding what part of the container was sampled.

- a. Interior bottom – B
- b. Interior side/corners – S

10. Standard Microbial Methods:

- a. All microbial swabs/sponges will be processed in triplicate using standard quantitative microbiological methods for the Enterobacteriaceae and Coliforms. Sponges will be processed in accordance with PrimusLabs SOPs 14-05 (Coliforms) and 14-116 (Enterobacteriaceae), respectively.
- b. The number of colony forming units (CFU) for each of the triplicate samples will be recorded.
- c. The average CFU per swab and per surface area swabbed will be generated and recorded.

11. Laboratory data reporting:

Results of standard microbial analyses including individual sample and the sample averages (per sponge and per surface area) will be compiled and submitted as raw tabular data.

APPENDIX B

California Location 1 Data

**CALIFORNIA LOCATION 1 DATA
CORRUGATED CONTAINER RESULTS**

Total organisms per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	8	36	3	1

Total organisms per Sponge Sample

Number of Sponge Samples (two samples/Container)	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	RLU range for Sponge Samples with ≥1000 but <10,000 CFU/Sample
96	37	55	3	1

Coliforms per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container
48	13	34	1

Coliforms per Sponge Sample

Number of Sponge Samples (two samples/Container)	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
96	41	45	0

Enterobacteriaceae per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	38	7	2	1

Enterobacteriaceae per Sponge Sample

Number of Sponge Samples (two samples/Container)	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 <10,000 CFU/Sample
96	85	7	3	1

APPENDIX C

California Location 2 Data

**CALIFORNIA LOCATION 2 DATA
CORRUGATED CONTAINER RESULTS**

Total Organisms per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	28	20	0	0

Total Organisms per Sponge Sample

Number of Sponge Samples (two sponge samples/Container)	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	RLU range for Sponge Samples with ≥1000 but <10,000 CFU/Sample
96	75	21	0	0

Coliform per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container
48	28	20	0

Coliform per Sponge Sample

Number of Sponge Samples (two sponge samples/Container)	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
96	75	21	0

Enterobacteriaceae per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers ≥1000 but <10,000 CFU/Container
48	43	5	0	0

Enterobacteriaceae per Sponge Sample

Number of Sponge Samples (two sponge samples/Container)	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 <10,000 CFU/Sample
96	91	5	0	0

APPENDIX D

British Columbia Data

**BRITISH COLUMBIA DATA
CORRUGATED CONTAINER RESULTS**

Total Organisms per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	33	10	3	2

Total Organisms per Sponge Sample

Number of Sponge Samples (two sponge samples/Container)	Number of Sponge Samples with <10 CFU/Samples	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	RLU range for Sponge Samples with ≥1000 but <10,000 CFU/Sample
96	79	11	4	2

Coliforms per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 /Container
48	38	8	2

Coliforms per Sponge Sample

Number of Sponge Samples (two sponge samples/Container)	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
96	86	8	2

***Enterobacteriaceae* per Container**

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	38	5	3	2

***Enterobacteriaceae* per Sponge Sample**

Number of Sponge Samples (two sponge samples/Container)	Number of Sponge Samples with <10 CFU/Samples	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 <10,000 CFU/Sample
96	86	5	3	2