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FIELD STUDY TO ASSESS THE MICROBIOLOGICAL STATUS OF CORRUGATED CONTAINERS AND OTHER PRODUCE STORAGE AND SHIPPING CONTAINERS UPON DELIVERY AT THE CUSTOMER LOCATION

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Elk Grove, Illinois

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Attention: Mr. Dennis Colley
Executive Director

Subject: Assessment of the Microbiological Status of Corrugated Containers and Reusable Plastic Containers upon Delivery to the Customer Location

Dear Mr. Colley:

Fresh produce has been documented by the Centers for Disease Control and Prevention (CDC) as a source of contamination leading to food-borne illness (CDC, 2014). Although food-borne illnesses have not been directly associated with shipping and transport containers, recent studies performed on reusable plastic containers (RPCs) in both the United States and Canada have shown their potential for harboring microbial loads above acceptable guideline levels.

The corrugated packaging industry has monitored the microbiological loads of corrugated containers for many years. This report highlights investigative work on the corrugated manufacturing process and finished product testing; it also details the results of the current Corrugated Packaging Alliance (CPA) field study on corrugated containers used by the produce industry (CPA, 2014). This field study evaluated the microbiological loads on corrugated containers from multiple manufacturers at various customer locations (i.e. grower/packer/shipper operations) across the United States. The data from this study provides an informed view of the nature and consistency of the microbiological profile as observed on corrugated containers upon receipt at the customer location.

Background

In support of this current work, information on the potential for corrugated containers to harbor microbial loads was evaluated. This review included an evaluation of: (1) acceptable microbial limits assigned to food packaging containers, (2) available data on the corrugated manufacturing process, and (3) internal manufacturer data on the microbial testing of finished paper and corrugated products from a CPA member company.

1. Acceptable Microbial Limits

There are currently no United States government regulations regarding microbial levels for food transport / storage containers. An 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)/cm^2, while the value of *Enterobacteriaceae* should not exceed 1 CFU/ cm^2 (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 publically available, peer-reviewed study, Cunningham defined the acceptable levels of aerobic microorganisms on food contact surfaces as 125 CFU/50 cm^2 (equal to $10^{3.4}$ CFU/930 cm^2) as the upper limit for a clean and sanitized food contact surface (Cunningham et.al., 2011).

Taking into account the various EU Member States' guidance and the subsets of microorganisms evaluated that could result in food-borne illnesses, Dr. Keith Warriner of the University of Guelph applied the following levels to define sanitary conditions for containers used to ship fresh produce (Warriner 2013, 2014).

- Aerobic Plate Count (APC): ≤10^4 CFU/container
- *Enterobacteriaceae*[1]: ≤10^3 CFU/container
- Thermotolerant Coliforms[2]: ≤10^3 CFU/container
- Absence of Listeria spp.

Using the acceptance criteria specified in the EU Guidance, (≤1 CFU/cm^2), the acceptable levels of *Enterobacteriaceae* and Thermotolerant Coliforms present on a 930 cm^2 area (equivalent to a 12 x 12 inch area) was identified as ≤10^3 CFU/930 cm^2, using the following equation: 1 CFU/cm^2 x 930 cm^2.

When microbial loads on the surface of the packaging container are below these limits, the containers would not be anticipated to transfer a significant microbial load to product placed within them.

2. Corrugated Manufacturing Process

The European Corrugated Packaging Association (FEFCO) developed a report that provides pertinent information on the corrugated manufacturing process across the industry. The process uses heating elements with temperatures between 180° Celsius (C) and 200°C with the material itself reaching temperatures of at least 100°C three times: once during the manufacture of the paper itself, and twice during the process of conversion to corrugated packaging (FEFCO, 2011). When evaluated against the Higher-Heat Short Time (HHST) charts present in the US Food and Drug Administration (FDA) Code of Federal Regulations, Section 21, Subpart 1240.61 and the recommendations of the International Dairy Foods Association (IDFA) for the effective control of microbial diseases, the time/temperatures incorporated into the manufacturing process would likely be sufficient to result in microbial inactivation even when the differences in materials were taken into account. In addition,

[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*).

the temperatures achieved should desiccate the final product (corrugated container), further mitigating the potential for microbial contamination (Sanders, 2011).

3. Historical Test Data on a CPA Member Company's Finished Products

   A CPA member company has evaluated the ability of the paper and corrugated container manufacturing process to control microbial levels through routine microbial testing of their finished product (paper and corrugated containers). Since 2010, using a defined sampling and testing schedule, over 400 swab samples collected over 55 unique sampling events across more than 40 paper and box facilities were evaluated for microbiological contamination. Test results confirmed the absence of *Listeria spp*. and *Salmonella spp*. as well as demonstrated consistently low numbers of aerobic bacteria (Aerobic plate count: APC) and *Enterobacteriaceae*. When the acceptable limits defined by Warriner (2013, 2014) are applied to the data from the routine testing, all results were below established acceptable limits. Specifically:

   - None of the swabs from paper/box samples identified the presence of *Listeria spp*. on the finished products.
   - None of the swabs from paper/box samples identified the presence of *Salmonella spp*. on the finished products.
   - None of the swabs from paper/box samples identified *Enterobacteriaceae* on the finished product.
   - One hundred percent (100%) of the swabs from the paper/box samples had Aerobic Plate Counts below the acceptable limit for those organisms of 10^4 CFU/930 cm^2.

Current Testing

In late 2014, the CPA sponsored a field study to assess the presence of microorganisms on corrugated containers, upon receipt at the customer location.

1. Study Goal:
   The goal of the study was to provide a data-based, informed view of the nature and consistency of the microbiological profile of corrugated containers upon delivery at customer locations.

2. Study Protocol:
   The CPA study protocol, as designed by Dr. Trevor Suslow of the University of California-Davis, was to be similar to a study he designed for the evaluation of the microbial loads on RPCs (Suslow, 2014).

   - In the Suslow RPC field study, random sampling of RPCs across multiple shipments at the customer location were evaluated for the presence of *Enterobacteriaceae* and Thermotolerant Coliforms. Two hundred and forty (240) microbiological swab samples were collected from 120 randomly selected RPCs. The RPC study also included an evaluation of *for cause* RPCs; these RPCs were defined as containers chosen based on visual signs of contamination. Examples of the contamination observed that resulted in a *for cause* designation included decaying plant material, residual moisture, or labels remaining on the interior surface of the RPC. One hundred

and eighty (180) swabs were collected from sixty (60) *for cause* containers. All microbiological samples were collected and tested by Primus Laboratory.[3]

- In the corrugated container study, sampling was conducted on 12 unique corrugated container shipments from multiple CPA member companies at five customer locations in California, Washington and Florida. Over 720 microbiological swab samples from 360 corrugated containers were collected and tested by Primus Laboratory. Thirty (30) random corrugated containers from each shipment were sampled upon receipt. The sampling occurred over a six (6) week sampling period. The complete interior surfaces (bottom and sides) of the containers were sampled using standard microbial testing and evaluated for the presence of *Enterobacteriaceae* and Thermotolerant Coliforms.

  Note: As no corrugated containers showed any visible signs of microbiological contamination, this study did not include any *for cause* sampling.

3. Data Analysis:
   The raw data from the current CPA field study was reviewed and analyzed by Haley & Aldrich against the noted acceptance criteria. A comparative analysis of the study data was then performed against available data from the CPA member company finished product testing and data from the RPC field study (Suslow, 2014). Prior to submitting the data evaluation to the CPA, identifying information (i.e., dates and locations of sampling) was removed and coded to conceal the manufacturer of the corrugated containers sampled.

4. Results:
   a. Corrugated Field Study
      The corrugated field study showed low levels of microbial contamination on the interior surfaces of corrugated containers selected at random at the customer locations. All samples had Thermotolerant Coliforms and *Enterobacteriaceae* levels below the acceptable limits per swab (and container). Table 1 provides an overview of the complete data set.

Table 1
Organisms per swab sample (Thermotolerant Coliforms and *Enterobacteriaceae* combined)

# of sampling events/samples in the CPA Corrugated Field Study (2014)	Colony Forming Units (CFU)/930cm^2 *				
	≤10	≤100	>100 - ≤200	>200 - ≤1000	>10^3
	Meets acceptable sanitation level				Does not meet acceptable sanitation levels
	# of Samples	# of Samples	# of Samples	# of Samples	# of Samples
12/720	689 (96%)	29 (4.0%)	1 (0.1%)	1 (0.1%)	0

* Normalized to 930 cm^2 (12 in x 12 in area) for comparison purposes

[3] Data from one sampling day was not included in the data analysis as the laboratory was unable to provide accurate counts from the sampling. The values included here represent only the results used for the comparative analysis.

b. Comparative Data Analysis

Although the likelihood that any microbiological contamination would be homogeneous across a surface sampled is slim, the number of organisms identified from any given swabbed area were assumed to be equal across the swabbed surface to allow for a comparative analysis. To facilitate the comparison, the data from the corrugated field study, the RPC field study (Suslow, 2014) and the individual CPA member company finished product testing were normalized to a 12 in. x 12 in. area (930 cm^2).[4] The following calculation was performed to assess the number of organisms per 930 cm^2 on the surface swabbed:

$$\frac{\text{CFU}}{\text{Surface Area (SA) swabbed}} \times \frac{\text{SA swabbed}}{930\ cm^2} = \frac{\text{CFU}}{930\ cm^2}$$

Table 2
Thermotolerant Coliforms per swab sample (Normalized to 930 cm^2)[a, b]

Sample Set	# of swab samples	Colony Forming Units (CFU)/930cm^2				
		≤10	>10 - ≤10^2	>10^2 - ≤10^3	>10^3 - ≤10^4	>10^4
		Meets acceptable sanitation level			Does not meet acceptable sanitation level	
CPA Corrugated Field Study (2014)[c]	720	718 (99.8%)	2 (0.2%)	0 (%)	0	0
RPC Field Study (random subset) (Suslow, 2014)[d]	240	166 (69.2 %)	21 (8.8%)	11 (4.6%)	19 (7.9 %)	23 (9.6%)
RPC Field Study (*for cause* subset) (Suslow, 2014)[e]	100	57 (57%)	11 (11.0%)	9 (9%)	9 (9%)	14 (14%)

[a] Values were normalized for comparison purposes

[b] The CPA member company finished product testing did not evaluate for the presence of Thermotolerant Coliforms.

[c] Two swab samples were taken per randomly selected corrugated container: (1) interior bottom and (2) interior sides.

[d] Two swab samples were taken per randomly selected RPC: (1) interior bottom and (2) interior sides.

[e] Three swab samples were taken per RPC selected *for cause:* (1) interior bottom, (2) interior sides and (3) site of visual contamination. As the area swabbed at the site of visual contamination was not available and likely varied based on the contamination present it was not possible to normalize these values; therefore only the samples of the interior bottom and interior sides of *for cause* RPCs are included for comparison purposes.

[4] Three swab samples were taken per *for cause* RPC selected*:* (1) interior bottom, (2) interior sides and (3) site of visual contamination. As the area swabbed at the site of visual contamination was not recorded and likely varied based on the contamination present, it was not possible to normalize these values; therefore, only the samples of the interior bottom and interior sides of *for cause* RPCs are included in the data comparison tables (Tables 2 and 3).

Table 3
***Enterobacteriaceae* per swab sample (Normalized to 930 cm^2)[a]**

Sample Set	# of swab samples	Colony Forming Units (CFU)/930cm^2				
		≤10	>10 - ≤10^2	>10^2 - ≤10^3	>10^3 - ≤10^4	>10^4
		Meets acceptable sanitation level			Does not meet acceptable sanitation level	
CPA Corrugated Field Study (2014)[b]	720	694 (96.4%)	24 (3.3%)	2 (0.3%)	0	0
CPA Member Company Finished Product Testing	89	89 (100%)	0	0	0	0
RPC Field Study (random subset) (Suslow, 2014)[c]	240	218 (90.4%)	15 (6.3%)	3 (1.2%)	4 (1.7%)	0
RPC Field Study (*for cause* subset) (Suslow, 2014)[d]	100	79 (79%)	5 (5%)	5 (5%)	8 (8%)	3 (3%)

[a] Values were normalized for comparison purposes.
[b] Two swab samples were taken per randomly selected corrugated container: (1) interior bottom and (2) interior sides.
[c] Two swab samples were taken per randomly selected RPC: (1) interior bottom and (2) interior sides.
[d] Three swab samples were taken per *for cause* RPC selected: (1) interior bottom, (2) interior sides and (3) site of visual contamination. As the area swabbed at the site of visual contamination was not available and likely varied based on the contamination present it was not possible to normalize these values; therefore only the samples of the interior bottom and interior sides of *for cause* RPCs are included for comparison purposes.

Both sets of field data (RPC and corrugated containers) identified containers with little or no contamination; however, the variability in the microbial loads present on the RPCs was quite extensive with some swab samples exhibiting greater than 10^6 CFU/swab. The swab samples taken from RPCs selected *for cause* had the highest microbial levels. The maximum number of organisms identified from a single swab sample is noted in Table 4.

Table 4
Maximum Microbial Contamination Levels (CFU/swab)

Container type and study	Number of Containers analyzed	Highest *Enterobacteriaceae* Count (CFU/swab)	Highest Coliform Count (CFU/swab)
CPA Corrugated Field Study (2014)	360	142	37
RPC Field Study (random subset) (Suslow, 2014)	120	24,667	500,000
RPC Field Study (*for cause* subset) (Suslow, 2014)	50	1,500,000	4,000,000

When the study data are compared to the acceptable sanitation levels for *Enterobacteriaceae* or Thermotolerant Coliforms, all of the corrugated containers evaluated in the field study met the identified acceptance criteria. Conversely, a high percentage of RPC samples did not meet those same criteria.

Table 5 details the number of RPCs or corrugated containers that met or exceeded the acceptance criteria on any single sample collected from their interior surfaces.

Table 5
Microbial Contamination Loads on Containers in Relation to Acceptable Limits

Container type and study	Number of Containers analyzed	Percentage of Containers with All Swab Samples that Meet Acceptable Sanitation Standards (≤10^3 CFU/swab)	Percentage of Containers with One or More Swab Samples that Do Not Meet Acceptable Sanitation Standards (>10^3 CFU/swab)
CPA Corrugated Field Study (2014)	360	360 (100%)	0 (0%)
RPC Field Study (random subset) (Suslow, 2014)	120	81 (67.5%)	39 (32.5%)
RPC Field Study (*for cause* subset) (Suslow, 2014)	50	25 (50%)	25 (50%)

Figure 1 provides a visual overview of the comparison of the various study results as compared to the acceptable sanitation criteria for *Enterobacteriaceae and* Thermotolerant Coliforms.[5]

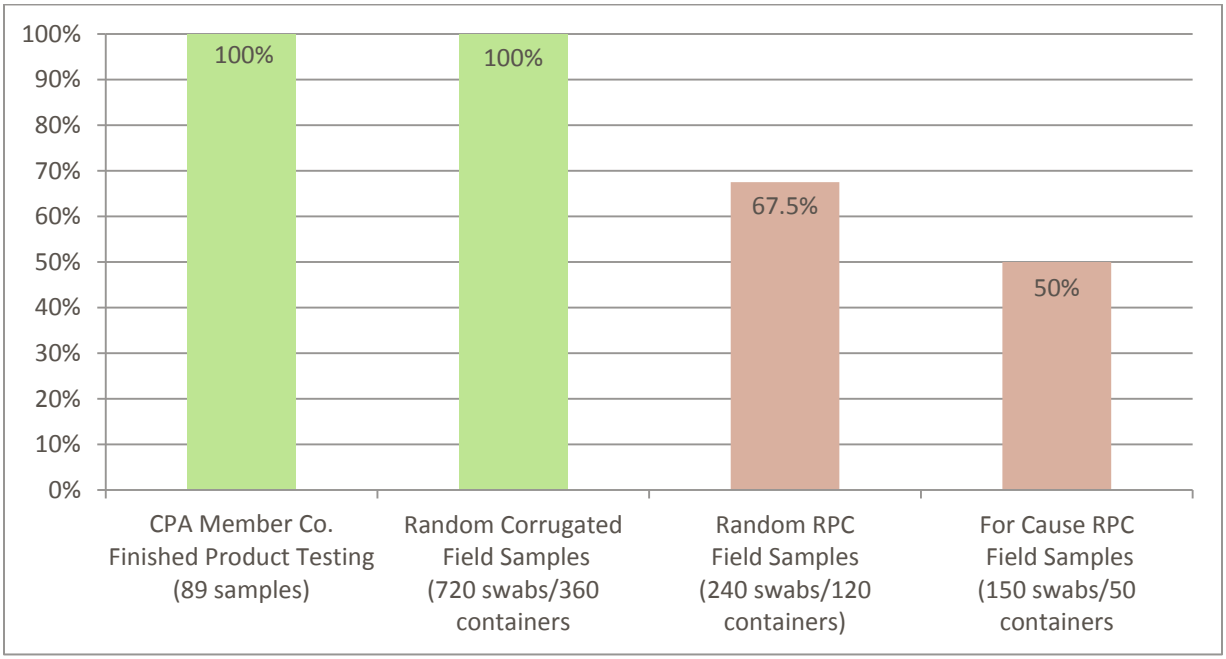

Figure 1. Percentage of Finished Product or Containers with all swab samples meeting Acceptable Sanitation Standards (10^3CFU/swab).[5, 6]

Discussion

The samples taken from all paper and corrugated materials at the point of production and all corrugated containers upon receipt at the customer location had microbial counts below the identified acceptable sanitation levels.[6] As 100% of the paper and corrugated samples from both the CPA Field Study and CPA member company finished product testing met the acceptable sanitation levels, the data suggest that manufacturer finished product testing is representative of corrugated containers as delivered to the customer location and would be sufficient to show that corrugated containers, as supplied to the customer, meet acceptable sanitation criteria.

Conversely, 32.5% of RPCs chosen at random and 50% of RPCs chosen *for cause* at the customer location had microbial levels above the same acceptable limits. In addition, gross contamination in the form of decaying plant material, residual moisture, or product labels from prior use were also present on many RPCs. When this gross contamination, percent of RPCs failing basic sanitation levels, and the high variability in microbial loads (0-4,000,000 CFU-swab) are evaluated as a whole, it is apparent that the cleaning and sanitation process is not well controlled to provide clean containers for reuse.

[5] The CPA member company finished product testing were evaluated for the presence of Aerobic Organisms (APC), *Enterobacteriaceae*, *Listeria spp*. and *Salmonella spp*.; it did not evaluate for the presence of Thermotolerant Coliforms. All finished products tested had microbial loads that met acceptable sanitation levels.

[6] Figure 1 depicts the *Enterobacteriaceae* levels from all datasets and the Thermotolerant Coliforms levels for both RPCs and corrugated container testing in the field as compared to the acceptable sanitation level for these organisms.

Conclusion

Fresh produce has been documented by the CDC as a source of microbial contamination leading to food-borne illness (CDC, 2014). To date, storage/shipping containers used for fresh produce have not been directly linked to food-borne illness and no acceptable microbial limits have been established for these products by a United States regulatory agency. Taking into consideration available food contact sanitation guidelines, Dr. Keith Warriner identified acceptable sanitation levels for food transport containers at less than 10^3 Colony Forming Units (CFU) of either Thermotolerant Coliforms or *Enterobactericeae* (Warriner, 2013 and 2014). These levels of organisms would not be anticipated to result in the container surface transferring organisms to product placed within them and therefore, would not be anticipated to result in a food-borne illness.

To confirm that corrugated containers would have microbial load levels below the identified acceptance criteria, a multi-step process was performed:

1. An evaluation of the corrugated container manufacturing process shows that the use of high heat likely mitigates the potential for the finished corrugated product to harbor microbial loads.

2. A review of a CPA member company's routine microbial test data showed that finished paper and corrugated products do not contain significant microbial loads.

3. A CPA-sponsored microbiological field survey of corrugated containers delivered to multiple produce customer locations across the United States was performed.

   The study showed that the interior container surfaces had microbial loads well below acceptable limits of 10^3 CFU/swab for *Enterobactericeae* or Thermotolerant Coliforms, as detailed by Warriner (2013, 2014). The highest microbial level identified on any of the 720 samples evaluated was 37 CFU/swab and 142 CFU/swab for Thermotolerant Coliforms and *Enterobactericeae*, respectively, with 100% of all containers meeting acceptable sanitation levels.

4. These data were then compared against the results of a CPA member company's finished product testing and a microbial field survey on RPCs (Suslow, 2014).

   - The member company finished product testing correlated well with the corrugated containers sampled in the field; all swab samples from these two datasets met the acceptable sanitation criteria. This similarity suggests that routine microbial testing conducted at the final step in the corrugated manufacturing process would be an appropriate indicator of the cleanliness of the final product as delivered.

   - As noted above, the corrugated container data indicate that minimal microbial contamination is present on corrugated containers sampled in the field with all containers meeting acceptable sanitation levels ($\leq 10^3$ CFU for Thermotolerant Coliforms or *Enterobacteriaceae*). The highest level of Thermotolerant Coliforms and *Enterobacteriaceae* identified on corrugated containers was 37 and 142 CFU/swab, respectively. This is in contrast with similar RPC testing where 32.5% of random RPCs and 50% of *for cause* RPCs did not meet the same acceptable level ($\leq 10^3$ CFU; Coliforms or *Enterobacteriaceae*). The microbial load on individual swab samples from the interior surface of the RPCs was as high as 4,000,000 CFU/swab and 1,500,000 CFU/swab for Coliforms and *Enterobacteriaceae*, respectively.

Under the test conditions of the studies summarized , the microbial load on finished paper, corrugated material, and corrugated containers consistently met acceptable sanitation levels while 32.5% of random RPCs and 50% of *for cause* RPCs did not meet those same standards.

Sincerely yours,
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