ABSTRACT OF THE DISSERTATION

Migration Studies of Chloropropanols from Paperboard Packaging in Contact with Foodstuffs

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The food processing of acid hydrolyzed vegetable protein (HVP) results in the chlorination of residual lipids to form chloropropanols. 3-chloro-1,2-propanediol (3-monochloropropane-1,2-diol; 3-MCPD), and 1,3-dichloro-2-propanol (1,3-DCP), are the most common chloropropanols found in HVP foods and also soy sauces. The manufacturing process of paperboard food packaging may also produce chloropropanols. 3-MCPD and 1,3-DCP can be found in paperboard when wet-strength resins made with epichlorohydrin are used. 1,3-DCP had been determined to be carcinogenic in rats and mice. 3-MCPD was a suspected carcinogen, and has recently been moving towards classification as a carcinogen. The European (EU) Commission and the US Food and Drug Administration (US FDA) have set maximum levels in food and food paperboard packaging for 3-MCPD.
and 1,3-DCP. In October 2010, 3-MCPD and 1,3-DCP were added to the California Proposition 65 list of compounds known to State to cause cancer.

In this investigation, migration studies were conducted to measure 3-MCPD and 1,3-DCP migration into food simulants from the food contact side of polyethylene extrusion-coated paperboard beverage cartons, and also total immersion extractions of both polyethylene extrusion-coated and uncoated paperboard. It is shown that 3-MCPD, found at levels far above the regulatory limits for food packaging, does not migrate at a significant amount through the polyethylene extrusion-coated food contact surface of the paperboard. The aqueous extractions of the entire paperboard and food contact side extractions with aqueous and acidic food simulants were performed using US FDA and EU Commission standard and accelerated migration testing protocols.

In these migration studies, an EU standard method for cold water total immersion extractions was compared to migration cell extractions to measure the chloropropanols migration into food simulant solvents from the entire paperboard and the isolated food contact side of polyethylene extrusion-coated paperboard beverage cartons.

This research demonstrates that polyethylene food contact coated film can function as a barrier to the migration of 3-MCPD into the food packaged in a polyethylene extrusion-coated paperboard engineered for that purpose.
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Migration studies of 3-chloro-1,2-propanediol in polyethylene extrusion-coated paperboard food packaging.

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1. INTRODUCTION

Acid hydrolyzed vegetable protein (HVP) is a widely used flavoring ingredient added to soy and related Asian sauces, and other savory type of broths. Soy sauces are manufactured by two main processes: natural fermentation, and acid hydrolysis of soya vegetable protein. The fermentation process is relatively expensive when compared with acid hydrolysis of vegetable protein. Because of HVP’s lower cost, it is suspected to be used as an adulterant in more expensive “naturally” fermented soy sauces. Hydrolyzed vegetable protein (HVP), soy sauces, bakery goods have been found to contain suspected carcinogenic compounds known as chloropropanols. Paper and paperboard food packaging made with certain types of wet-strength resins are also known to contain chloropropanols. Commercial, store-bought HVP-based foods, soy sauces, toasted cereals and bakery goods have been tested to determine whether the chloropropanols are below levels recognized as safe in foods. As mentioned later in this section, packaging in contact with foodstuffs is also tested to determine the levels of chloropropanols present at established safe levels (Crews 2002, Hamlet 2002, Stadler 2007).

The food processing of acid hydrolyzed vegetable protein results in the chlorination of residual lipids to form chloropropanols. 3-chloro-1,2-propanediol (3-MCPD or 3-monochloropropanediol), and 1,3-dichloro-2-propanol (1,3-DCP),
are the most common chloropropanols found in soy sauces, Asian sauces, and savory broths made with HVP. 1,3-DCP has been determined to be carcinogenic in rats, mice, and in vitro studies. When chloropropanols are found present in food, 3-MCPD and 1,3-DCP are the most abundant. 3-MCPD has been shown to be found at higher levels, and therefore can be used as a good indicator of the possible presence of 1,3-DCP (Crews 2003). As discussed further in this introduction, paperboard food packaging made with epichlorohydrin can generate chloropropanols, with higher levels of 3-MCPD than 1,3-DCP (Boden 1997) also detected. The higher level of 3-MCPD allows for a good marker for development of sensitive analytical methods to accurately quantitate chloropropanols in foods and food packaging. 3-MCPD was used as the main target compound for the paperboard migration studies performed and methods developed in Dr. Thomas G. Hartman’s research group which are reported in this Dissertation (Pace 2010).

Since the mid-1990s, HVP manufacturers have employed process modifications that do not promote the formation of chloropropanols, in order to comply with FDA, EU, and Canadian food regulations to reduce the amounts of chloropropanols in the foods. Studies done in 2000 on the occurrence of chloropropanols in foods have demonstrated a significant decrease in 3-MCPD and 1,3-DCP in HVP containing sauces and broths made in the US, UK, and Canada. However, studies performed on the occurrence of chloropropanols is HVP containing sauces and broths manufactured in China, Hong Kong, and Vietnam
have demonstrated continued high levels of 3-MCPD and 1,3-DCP in those foods (Crews 2003). It speculated by the author that either manufacturing improvements have not been made, or the naturally brewed soy sauces are adulterated with less expensive hydrolyzed vegetable protein.

Although chloropropanols are found predominantly in acid hydrolyzed vegetable protein and Asian sauces, they have also been found to a lesser extent in paperboard beverage cartons. The manufacturing process of paperboard food packaging can also produce small quantities of chloropropanols, in particular, 3-chloro-1,2-propanediol (3-MCPD) and possibly 1,3-dichloro-2-propanol (1,3-DCP). Wet-strength resin additives used in the paperboard which contain residual epichlorohydrin starting material, have been shown to be the source of the 3-MCPD and 1,3,-DCP in the food paperboard packaging (Boden 1997, Pace 2010).

In our research presented in this dissertation, differently designed migration studies were conducted to measure amount of chloropropanol by-products from the epichlorohydrin-based wet-strength resin, and whether the 3-MCPD and 1,3-DCP present will migrate into food simulant solvents through the food contact side of the polyethylene extrusion-coated paperboard beverage cartons. The correlation of mass loading of the Kymene® version of the polyamidoamine-epichlorohydrin wet-strength resin to the amount of chloropropanols generated in the paperboard is presented.
We demonstrate that high levels of 3-MCPD detected in paperboard food packaging does not migrate through the polyethylene food contact surface into food simulants at significantly high levels. It is also shown that no significant amount of 3-MCPD migrates from the unskived (uncoated) edges of the sealed inside seam of the paperboard carton structure (Pace 2010). Accelerated migration testing methods were adapted, for the various experimental designs discussed in the experimental section, from US Food and Drug Administration (FDA) and European Commission (EU) guidelines for food contact substances and materials.

Two different types of migration studies were performed with the experimental variables: Total Immersion extraction of the entire paperboard, based on the cold water paper and paperboard method (EU Standard 1993); and Migration cell extractions of the isolated polyethylene coated food contact side of the paperboard (US FDA 2007, EU Comm 1985). Figure 1 shows the total immersion extraction entails complete immersion of the paperboard sample cut into 1 cm² pieces and placed in the in the water or food simulant. The inner food contact side, the outside printed side and the edges of each cut piece are extracted in the solvent. Figure 2 and Figure 2 show how the migration cell extractions isolate only the inner food contact side of the paperboard sample, which is not cut into small pieces, and with only the food contact side extracted by the food simulant. Full descriptions and figures are detailed in the Experimental section.
Paperboard carton sample was cut into 1×1 cm pieces

Figure 1: Example of paperboard packaging total immersion extraction sample cut into 1cm² pieces in jar
Figure 2: Migration cell assemblies used for Food Contact side extractions of polyethylene surface of the paperboard (shown).

Figure 3: The unprinted polyethylene coated food contact side is placed in the migration cell assemblies and extracted. The opposite printed side is shown for illustration purposes.
Working in Dr. Hartman’s applied research laboratory frequently requires the conversion of GC-MS methods to a GC-FID method, as was done in the Research presented in 2006 (Pace 2006). This conversion has a commercially practical significance, it allows manufacturers, who commonly have a GC-FID in the Quality Control (QC) laboratory, to utilize our methods in QC compliance testing during production runs. This new GC-FID method was developed to analyze for the chloropropanols in our laboratories, and also benefiting those manufacturers without the specialized expertise needed to run and maintain a GC-MS. The GC-MS and GC-FID methodologies presented in the Experimental section were statistically validated.

The European standard paper and paperboard in contact with Foodstuffs extraction method uses cold water extraction of 1 cm² pieces cut from a 10 g sample of the paperboard carton. We desired to test whether this methodology to ensure exhaustive extraction of the chloropropanols in the packaging. The total immersion extraction condition variables made were made more aggressive. We explored whether increasing the exposed surface area of the paper fibers by pulping the carton pieces in a blender with stronger extracting food simulants would result in a higher maximum extractable level of 3-MCPD.

The research presented here, presents evidence that a manufacturer’s design combination of Kymene® wet-strength resin loading, polyethylene extrusion-coated film, and paperboard construction provides an effective
functional barrier to the migration of chloropropanols into the foodstuffs packaged therein.
2. LITERATURE REVIEW

2.1. Chloropropanols in Foods

The most predominant source of chloropropanols in foods are those containing hydrolyzed vegetable proteins and soy sauce (Nyman 2003). Chloropropanols are also found in other foods processed under high heat, such as; cereals, bakery goods, and processed meats, but at much less prevalence and lower levels than hydrolyzed vegetable proteins (HVP) and soy sauces (Crews 2002, Hamlet 2002). Hence, research in the occurrence of chloropropanols in foods has focused on HVP containing foods and sauces, and soy and related Asian sauces.

The chloropropanols present in the food flavoring and texture ingredient hydrolyzed vegetable protein (HVP) and also soy sauces originate from the processing of soya beans under high heat and hydrochloric acid conditions. The glycerol is hydrolyzed and forms an epoxide intermediate, glycidol. The glycidol further reacts under heat and hydrochloric acidic conditions to form the chloropropanols. Figure 4 shows the reaction formation of the chloropropanols, 3-chloro-1,2-propanediol (3-MCPD). In general, this type of aggressive food processing condition results in the chlorination of glycerol from the triacylglycerols in residual vegetable lipids (Collier 1991).

The similarities in the structures of glycidol and epichlorohydrin, the precursor
which forms chloropropanols, is presented in a figure and discussed in the next section on chloropropanols in food packaging.

![Diagram of the formation of 3-MCPD from chlorination of glycerol](adapted from Hamlet 2002, Collier 1991)

**Figure 4: The formation of 3-MCPD from chlorination of glycerol intermediate in hydrolyzed vegetable protein food processing**
Adapted from Hamlet 2002, Collier 1991

3-chloro-1,2-propanediol (3-MCPD) and 1,3-dichloro-2-propanol (1,3-DCP), are the most researched chloropropanols in foods. Both due to their abundance and toxicological concerns. Figure 5 shows the comparison of their chemical structures.

![Chemical structures of 3-MCPD and 1,3-DCP](adapted from Hamlet 2002, Collier 1991)

**Figure 5: The chemical structures of 3-MCPD and 1,3-DCP, the most researched chloropropanols in foods, due to toxicological concerns**
1,3-DCP has been determined to be carcinogenic in rats, mice, and in-vitro studies, but has not been proven carcinogenic in humans (Cho 2008, El Ramy 2007, EU Comm. 2001). When chloropropanols are present, 3-MCPD is found at a higher level, and therefore a good indicator of the possible presence of 1,3-DCP (Hamlet 2002).

2.2. Paper Chemicals in the Manufacturing of Paper and Paperboard

A discussion of the generation of chloropropanols in paperboard food packaging, and their migration through the paperboard structure, needs to be precluded with some background on the specialty paper chemicals used in the manufacture of paper and paperboard.

2.2.1. Paper and Paperboard Chemicals

Paper and paperboard are manufactured with special chemical additives which serve as functional compounds or processing aids. The functional paper additives which impart properties required for the type of use of the final paper or paperboard product. These specialty chemicals give to paper and paperboard water resistance, wet-strength, and flame retardation. The processing chemicals aid the paper manufacturer in controlling the paper sheet resulting in a quality grade of paper or paperboard (1995 Smith).
The main processing aids for paper and paperboard are:

- **Pitch dispersants**, the most widely used of these absorbents are talc and diatomaceous earth;
- **Defoamers**, which compensate for the use of recycled printed paper, air entrained in the paper stock, and increased production speed throughput;
- **Biocides**, with wide range of target organisms and elements;
- **Cleaners**, compositions include surfactants in biodegradable naturally derived carriers, such as, d-limonene;
- **De-inking**, including surfactant-based dispersants, which are highly efficient and effective in removing difficult printing inks, such as, laser jet, flexographic, and radiation curing inks.
- **The main functional paper and paperboard chemicals are listed below.**
  - **Wet-strength resins**; widely used in tissue and towels, corrugated paperboards, food packaging paperboards, and other specialty, high-end grades of paper.
  - **Sizing agents**; including internal sizing and surface sizing.
    - **Internal sizing chemicals** include acid, alkaline, or neutral pH modified tree rosin, and alkaline sizing with alkyl ketene dimer (AKD) or alkenyl succinic anhydride (ASA). Many of the rosin-based chemicals have patented or trade-secret composition and paper processing application protections.
Surface sizing agents are based on styrene maleic anhydride, often used in conjunction with the internal rosin, AKD, and ASA sizing agents.

- Dry-strength resins; polyacrylamide, guar gum, polyvinylalcohol, carboxymethylcellulose, cationic starches, and starch-polyacrylamide blends.
- Retention aids; used to improve structure retention, drainage, strength, and formation.
- Coatings; used in coated paper and paperboard to impart the attributes of gloss, brightness, smoothness, and printability. The many different types of coatings include; clay, starches, titanium dioxide, calcium carbonate, carboxymethyl cellulose, proteins, pigments, styrene-butadiene, ethylene vinyl acetate and ethylene vinyl chloride.

2.2.2. **Wet-Strength Paper Chemicals**

Wet-strength resins function to adhere to the paper pulp and form a cross-linking network to protect the cellulose fiber from swelling in aqueous conditions. The wet-strength resin is:

- water soluble, allowing even coating on the cellulose fibers;
- cationic, to allow absorption onto anionic pulp fibers;
- polymeric and reactive, to form strong bonds onto the cellulose fiber and
cross-linking networks, which make the paper matrix structure resistant to solubility in an aqueous environment.

Resins which impart wet-strength in paper and paperboard function by a protection and reinforcement mechanisms. The wet-strength resin coats the cellulose fiber, and may also diffuse into the fiber. It then cross-links with other wet-strength resin molecules to form an aqueous insoluble network around the cellulose fiber, protecting the fiber from the effects of a rewetting environment. The wet-strength resin forms covalent bonds with the cellulose molecules, creating linkages between cellulose fibers. The covalent bonds are not broken by water, reinforcing the hydrogen bonding of the dry cellulose fiber sheet. To protect and reinforce the cellulose fibers, it is important that these wet-strength resin bonds are made at the weak links of the cellulose fiber network (Espy 1995).

There are various wet-strength resin chemistries.

- Polyamide-epichlorohydrin
- Urea-formaldehyde
- Melamine-formaldehyde
- Epoxide
- Aldehydes
- Polyethylenimine and Chitosan
2.2.3. **Epichlorohydrin Containing Wet-strength Resins**

The most common polymeric wet-strength resin in use is the polyamide epichlorohydrin resin. It was designed for neutral to alkaline pH, but is now used in acidic pH also, replacing urea-formaldehyde resins due to the environmental regulatory limits on formaldehyde. Kymene® is the commercial name of the polyamide epichlorohydrin wet-strength resins most widely used in the paper and paperboard industry. The full chemical name is polyamidoamine-epichlorohydrin (PAAE or PAE), which is formed by an alkylation reaction of a polyamide with epichlorohydrin to form the cyclized propyl alcohol off of the polyamide backbone (Boden 1997, Espy 1995, Rahmen 1991.) Figure 6 shows the polymeric reaction of the polyamide and epichlorohydrin. Figure 7 shows the final PAAE structure.
Figure 6: Reaction formation of Polyamidoamine-epichlorohydrin polymer (PAAE)
Adapted from Espy 1995 and Fischer 1996

Figure 7: Structure of PAAE
Adapted from Espy 1995 and Fischer 1996
The charged nitrogen azetidinium group on the PAAE backbone, also forms cross-linking reactions. The cross-linking covalent bonding network formed by the PAAE resin which is coated on the cellulose fibers also imparts the dry-strength function to the paper or paperboard product (Smith 1995). Figure 8 shows the potential cross-linking reactions. Figure 9 is a scanning electron photomicrograph of the PAAE covalent bonding polymer network attaching to PAAE coated cellulose fibers.

**Potential crosslinking reactions of azetidinium groups**

![Diagram of potential crosslinking reactions of azetidinium groups]

*Figure 8: PAE cross-linking network potential structures*

*Adapted from Espy 1995*
Figure 9: Wet-strength resin coating and network bonding of cellulose fibers

The precursor which is responsible for the formation of chloropropanols in paper and paperboard food packaging is epichlorohydrin. Poly-coated, multi-walled paperboard may contain the wet-strength additive with the commercial name of Kymene®. This wet-strength resin additive imparts the paperboard carton with high stability in the aqueous media of beverage cartons, such as milk (oil in water emulsion) and aqueous / acidic fruit juices. The wet-strength resin’s function is to maintain the paperboard carton’s packaging structure throughout its shelf-life and consumer use. Kymene® is a commercial wet-strength resin which is the reaction product of epichlorohydrin with an amine
or polyamide. There are also epichlorohydrin side reactions which can produce chloropropanols (Riehle 2005). During the formation of a polyamidoamine-epichlorohydrin (PAAE) resin, the epichlorohydrin can be hydrolyzed to produce 3-MCPD (Boden 1997). Depending on the actual starting materials, they can generate 3-MCPD and 1,3-DCP (Stadler 2007). Figure 10 shows the 3-MCPD and 1,3-DCP products formed from epichlorohydrin under hydrolysis conditions. The 3-MCPD is usually found at about a 6:1 ratio compared to the 1,3-DCP (Crews 2002).

![Chemical Diagram](image)

*adapted from Hamlet 2002, Boden 1997*

**Figure 10: The formation of 3-MCPD and 1,3-DCP from epichlorohydrin in side reactions in PAAE wet-strength resins**

Total immersion cold water extracts of polyethylene extrusion-coated and uncoated paperboard cartons containing wet-strength resin made with epichlorohydrin, were found to contain levels of 3-MCPD, significantly higher than the threshold limit established in Europe for paperboard in contact with foodstuffs (Pace 2010, EU Standard 1993, EU Comm. 2001).
2.3. **Health Concerns of Chloropropanols**

1,3-DCP has been found to be carcinogenic in rats and mice. Although it has not been proven carcinogenic to humans, there is sufficient human health concern that threshold levels have been established for 1,3-DCP and 3-MCPD (Cho 2008, El Ramy 2007, EU Standard 2001). The German Federal Agency for Agriculture and Food set a limit in the cold water extracts of paperboard in contact with foodstuffs of 12 µg/l (12 ppb) for 3-MCPD, and non-detectable (detection limit 2 µg/l) for 1,3-DCP (German BLE 2007). The European Committee for Standardization (CEN) has set the minimum sample size to be 10 grams for the paper and board analyzed for compliance with the European Standards (EU Standard 1993). Based on the sample per volume method used in the aqueous extraction method for this research (Brereton 2001), the German limits would equate to 300 ng/g (300 ppb) for 3-MCPD and 50 ng/g for 1,3-DCP. The German standard, authorized by the European Committee for Standardization states that the other countries in the European Union are bound to comply with this regulation (EU Standard 1993, German BLE 2007). The Brereton method complies with both the German BLE standard and the CEN standard for EN 645:1993.

The U.S. Food and Drug Administration (FDA) Guidance levels is <1 mg/kg (1000 ppb) for 3-MCPD in acid-hydrolyzed vegetable protein and Asian-style sauces. Since 3-MCPD is not listed as generally recognized as safe (GRAS) by the
FDA, it is not an approved food additive (US FDA 2007). Accordingly, the packaging food contact substance must be shown not to extract into an aqueous or acidic food simulant liquid extract more than 1 mg/kg, according to the FDA guideline limit.

In extrusion polyethylene-coated paperboard cartons, the polyethylene surface is in direct contact with the food beverage. Therefore, it must be an approved by the US FDA and EU Commission as food contact substance. As such, it is also expected to function as a barrier to the migration of any components from the paperboard beverage carton structure into the food products packaged in it.
2.4. US FDA and EU Commission Regulatory Compliance of Chloropropanols in Foods and Food Packaging

Since the mid-1990s, hydrolyzed vegetable protein (HVP) manufacturers have employed process modifications that do not promote the formation of chloropropanols. These process improvements were employed by the HVP manufacturing to comply with stricter US FDA and EU Commission food and food packaging regulations which were enacted to reduce the amounts of chloropropanols in the foods (EU Commission 2001, US FDA 2007, Note: latest revisions of earlier releases). The mechanisms employed to control chloropropanol levels in HVP used in foods include:

- Reducing the concentration of residual lipids
- Temperature and pH control
- Controlling the acid hydrolysis step
- Optimizing the chlorine content
- Employing alkali treatment to remove formed chloropropanols
- Conversions from acid hydrolysis to an enzymatic process
- Minimizing enzyme activity by reducing water activity and increasing ionic strength

The alkali treatment is a common method used to reduce chloropropanols levels in foods. 3-Chloro-1,2-propanediol is unstable in aqueous media, and
converts back to glycerol, through the intermediate glycidol. This is the reversal of the 3-MCPD formation reaction shown in Figure 3 (Hasnip 2002, Dolezal 2004).

Studies done on the occurrence of chloropropanols in foods from 2000 have demonstrated a significant decrease in 3-MCPD and 1,3-DCP in HVP containing sauces and broths made in the US, UK, and Canada (Crews 2000). However, studies performed on the occurrence of chloropropanols in HVP containing sauces and broths manufactured in China, Hong Kong, and Vietnam have demonstrated continued high levels of 3-MCPD and 1,3-DCP in those foods (Crews 2003, Hamlet 2002).

The mechanisms to control chloropropanols, in particular 3-MCPD, in paper and paperboard packaging also focus on the intermediate chlorohydrin starting material, as in foods. Figure 11 shows similarity in chemical structure of the precursors compounds mainly responsible for the formation reactions of 3-MCPD in foods and food packaging. Glycidol is the intermediate from the chlorination of glycerol in foods, and residual epichlorohydrin from the polyamidoamine-epichlorohydrin (PAAE) resin reaction in paper and paperboard. Hercules made modifications in the manufacturing process of Kymene® 557H wet-strength resin by reducing the amount of epichlorohydrin by-products, along with post-enzymatic and microbial treatment to reduce the chloropropanols to low ppm levels (Riehle 2005, Hardman 1997).
In our research, we confirm that paperboard food packaging containing wet-strength resin made with epichlorohydrin, can yield levels of 3-monochloro-1,2-propanediol significantly higher than the threshold limit of 12 ng/l (equivalent to 300 ng/g or 300 µg/kg in this aqueous extraction method) for aqueous extracts of paperboard packaging in contact with foodstuffs, made with wet-strength resins cross-linked with epichlorohydrin (EU Standard 1993, EU Comm. 2001). We also employ US FDA and EU Commission migration testing to predict whether high levels of chloropropanols found in the paperboard result in significant indirect food additive levels.

### 2.5. USA State of California Regulatory Listing of Chloropropanols

In June 2010 the California Office of Environmental Health Hazard Assessment (OEHHA) Reproductive and Cancer Assessment Branch released
reports on the evidence of carcinogenicity of 3-Monochlorpropane-1,2-diol (3-MCPD) and 1,3-Dichloro-2-propanol (1,3-DCP). The reports were followed by public input in September 2010 of evidence of the carcinogenicity of 3-MCPD and 1,3-DCP (CA OEHHA 2010). As a result of the evidence, in October 2010, OEHHA added 3-MCPD and 1,3-DCP to the California Proposition 65 list of compounds known to the State of California to cause cancer. 3-MCPD and 1,3-DCP are currently on the First Priority List for the Development of No Significant Risk Levels (NSRL) for Proposition 65 Safe Harbor Levels (CA OEHHA 2010).

A chemical identified as suspected to cause cancer in the State of California is required by Law to be labeled accordingly. The repercussions of this ruling to the food packaging industry increase the relevance and importance of the research presented in this Dissertation.

2.6. Methods for Determination of Chloropropanols

The two main methods (Brereton 2001, Hamlet 1997) selected for our work and to perform the research presented in this dissertation for the migration of chloropropanols from food packaging were selected to conform with the US FDA and European Commission standards and specifications. The European Standard for “Paper and Board Intended to Come into Contact with Foodstuffs” specifically states that the minimum sample to be used for the cold water extraction method is 10 grams (EU Standard 1993). The German standard, authorized by the European
Committee for Standardization (CEN) states that the other countries in the European Union are bound to comply with this regulation (EU Standard 1993, German BLE 2007). The Brereton method complies with both the German BLE standard and the CEN standard for EN 645:1993 (Brereton 2001). That method builds on the deuterated 3-MCPD internal standard method discussed below (Hamlet 1997). Other methods for the determination of chloropropanols are discussed in Section 2.6 and Section 2.7.

In order to isolate the effect of experimental design variables presented in the experimental section, we utilized the same sample preparation methodology throughout this entire timeframe which spanned our research presented in this dissertation.

The methods employed for this research are based on a deuterium-labeled 3-chloro-1,2-propanediol [3-chloro-1,2-propanediol-1,1,2,3,3,3,5-\(d_5\) \(\text{3-MCPD-}d_5\)] internal standard, which is a stable isotope (Hamlet 1997). The 3-MCPD-\(d_5\) internal standard is spiked to the sample extract, and the chloropropanols are separated by a liquid-liquid partitioning from the water to diethyl ether on a solid-phase extraction column packed with EXtrelut\textsuperscript{\textregistered} NT20 sorbent. The extracts are concentrated down to dryness, reconstituted with isoctane, and derivatized with heptaoctfluorobutyrylimidazole, then analyzed by gas chromatography-mass spectrometry. Figure 12 below shows the formation of the volatile 3-MCPD heptaoctfluorobutyrylate derivative. The authors, Hamlet and Sutton, justify the
selection of HFBI due to its formation of stable derivatives with monochloropropanols and efficiency as a volatile GC derivative.

![Chemical reaction diagram]

**Figure 12: Formation of the volatile heptafluorobutyrylate derivative of 3-MCPD for GC analysis.**
*Adapted from Hamlet 1997.*

The HFBI methodology used in our research was also validated with collaborative studies on a range of food products including acid hydrolyzed vegetable protein for the determination of 3-chloro-1,2-propanediol. All 12 of the laboratories participating in the study used slightly polar capillary gas chromatography columns based on 5% phenylmethylsiloxane and mass spectrophotometers in electron impact mode (Brereton 2001). This method was also used in a survey of 3-MCPD and 1,3-dichloropropanol (1,3-DCP) in soy sauces and related products (Nyman 2003).

We adapted the methods published by Brereton 2001 and Hamlet 1997 to the aqueous total immersion water extract and migration cells procedures for our research performed on the migration studies of chloropropanols in paperboard.
food packaging. The detailed description procedure and additional
gas-chromatography-flame ionization detector method developed is presented in
the Experimental Section 4.

The sample processing which utilizes the EXtrelut® NT20 sorbent to solvent
exchange the chloropropanols from the cold water into diethyl ether, followed by
concentration, and derivatization with the heptafluorobutyrylimidazole (HFBI)
reagent may need further review for newer methods which are less laborious and
costly. The EXtrelut® NT manufacturer recommends 3 times the column
volumes for elution, which in this case would be up 60 ml, however, the method
calls for 250 ml of diethyl ether. To run 2 samples in duplicate uses 1 liter of
diethyl ether, 40 g of EXtrelut® NT, 20 g of sodium chloride, and 30 g of sodium
sulfate, and takes about ½ day. The purpose of this step is to isolate the
chloropropanols from the aqueous cold water extract and eluting them off of the
EXtrelut® NT sorbent thereby exchanging the chloropropanols into the lower
boiling organic solvent (Merck 2008). Figure 13 shows the pore-like structure of
the EXtrelut® NT.
The aqueous chloropropanol extract is pipetted onto the EXtrelut® NT sorbent, which flows and distributes onto the clay-coated diatomaceous earth, forming the stationary phase, which does elute off with the organic solvent. The clay absorbs water during the equilibration time, and then the chloropropanols are eluted off with the diethyl ether organic solvent, which is immiscible with water, and does not absorb onto the clay coated diatomaceous earth. The organic phase contains the compounds of interest, but is free from emulsions, and can be evaporated at a lower temperature, which protects the compounds from volatilizing during the concentration step. The liquid-liquid exchange mechanism of the EXtrelut® NT sorbent is presented in Figure 14.
1. Extrelut®

Distribution of aqueous phase on Extrelut®

2. Apply aqueous sample

3. Apply organic solvent (not miscible with water)

4. Elution

Lipophilic substances extracted from the sample

The working principle of Extrelut®

Figure 14: E Xtrelut® NT20 working principle.
Adapted from Merck 2008.
The EXtrelut® NT20 packing is no longer supplied in the United States, and is increasingly difficult to order. Also, the derivatized with HFBI includes a quenching step with water. The samples analyzed for the research presented here were found to have only a slightly pink color, making it difficult to remove the lower 1.0 ml water layer from the 200 µl HFBI derivative / iso-octane upper layer. A method which utilizes silica gel could be an alternative to the EXtrelut® NT20 (Chung 2002). A relatively recent method utilizes heptafluorobutyric anhydride modified with triethylamine to replace the HFBI. They reported increased stability and much less sensitivity to moisture than the HFBI derivatives (Xu 2006).

A study which utilizes aluminum oxide as the column packing claims to be more effective and efficient compared to EXtrelut® or silica gel, with reduction in solvents used (Abu-El-Haj 2007).

One of the cleanest methods that overcomes the potential loss during solvent evaporation of 1,3-dichloropropanol (1,3-DCP), due to volatility, is a headspace-gas chromatography-mass spectrometry (HS-GC-MS) method (Crews 2002). The HS-GC-MS method was developed to analyze 1,3-DCP in soy sauces, and was further validated (Nyman 2003). Nyman makes note of the method limitation is that 1,3-DCP is analyzed on a different GC column than the 3-MCPD, precluding analyzing for both 1,3-DCP and 3-MCPD during the same GC-MS run. Also noted is that the underivatized 1,3-DCP from the headspace method results in less selective lower molecular weight fragments which more susceptible to
interferences from other compounds found in the food extracts. However, this method could be a promising screening tool if adapted for the paper and paperboard extracts.

The method described by Boden applicable to 1,3-DCP and 3-MCPD in papers made with wet-strength resins, may be more amenable to paper than the food product methods (Boden 1997). It employs direct analysis of the paper sample in acetonitrile with silylation of the chloropropanols with $N,O$-bis-(trimethylsilyl)trifluoroacetamide (BSTFA). It would need to be adapted to the aqueous extracts of the German method. This method is detailed further in Section 2.7

### 2.7. Chloropropanols in Paperboard Packaging

Paperboard food packaging is often manufactured with a resin to give the product wet-strength during its shelf-life and consumer usage. The wet-strength resins which are manufactured from epichlorohydrin-based starting materials, are known to produce 3-monochloro-1,2-propanediol (3-MCPD, 3-CPD) (Stadler 2007). The other epichlorohydrin by-product formed is 1,3-dichloropropanol (Boden 1997). The formations of 3-MCPD as the a hydrolysis by-product with epichlorohydrin, and 1,3-DCP is the by-product under alkaline conditions in the presence of chloride ions. These reaction was illustrated in Figure 10 of Section 2.2.3.. The 1,3-DCP may react back to form epichlorohydrin under the alkaline and
neutral pH conditions. 1,3-DCP is also reported to be in equilibrium with the free residual epichlorohydrin present from wet-strength resins used in the paperboard carton manufacturing process (Boden 1997, Hamlet 2002).

The method described by Boden was applied directly to the analysis of 1,3-DCP and 3-MCPD in papers made with polyamidoamine-epichlorohydrin (PAAE) EXtrelut® NT20 wet-strength resins. It employs direct analysis of a the paper without solid phase extraction and diethyl ether elution from an EXtrelut® NT20 column. A 0.5 sample of the paper is place in a 20 ml vial with 2.5 ml of acetonitrile and the silylating agent N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA), along with the internal standard 1-flouronaphthlene. The mixture is heated to 40°C for 20 hours to ensure the extraction of the 3-MCPD and 1,3-DCP from the cellulose-PAAE resin matrix. The author presents evidence that this method reduces the reformation of epichlorohydrin from 1,3-DCP. The derivatives are analyzed by GC-MS in selected ion monitoring (SIM). The method works where the moisture of the paper is <10% and contains calcium carbonate fillers. Other paper fillers used in manufacturing, titanium dioxide and kaolinite gave lower recoveries of 1,3-DCP. The Boden method was shown to be effective with papers containing calcium carbonate, which create the alkaline pH environment. The Boden method is recommended in the Future Work Section of this dissertation for to be studied for applicability a wider range of paper and paperboard food packaging, and to be scaled up to comply with the European Committee of Standardization (CEN), as
discussed in Section 2.6.

The European Standard for “Paper and Board Intended to Come into Contact with Foodstuffs – Preparation of a Cold Water Extract” specifically states that the minimum sample to be used for the cold water extraction method is 10 grams (EU Standard 1993). The German standard, authorized by the European Committee for Standardization (CEN) states that the other countries in the European Union are bound to comply with this regulation (EU Standard 1993, German BLE 2007). The Brereton method complies with both the German BLE standard and the CEN standard for EN 645:1993 (Brereton 2001).

There is extensive published research on the presence of 3-MCPD and related compounds in food products (Calta 2004, Collier 1991, Crews 2003, Dolezal 2004, Fu 2007, Hamlet 2002, Stadler 2007). There are also studies in the literature on the migration of printing ink components from paperboard packaging into food products (Castle 1997, Castle 1997 Part 2, Johns 2000 Part 3). In those studies on Functional Barriers, the authors conclude that polyethylene is not a functional barrier to prevent the migration of printing ink components which diffuse from the outside print through the paperboard and polyethylene, into the food products packaged within.

The migration of chloropropanols from paperboard food packaging has been studied much less. In particular, there has not been any published work on the functional barrier effectiveness of the extrusion-coated polyethylene food contact
substance to the migration of 3-MCPD from the paperboard matrix manufactured with wet-strength resin cross-linked with epichlorohydrin into the packaged food product, which is critical information for toxicologists to assess consumer exposure and dietary health hazards.
2.8. 3-MCPD Migration through Polyethylene Extrusion-Coated Food Contact film into Foods

In this investigation, we prove that 3-MCPD detected up to 9,900 ng/g (9.9 µg/kg) used in polyethylene-coated bleached paperboard does not migrate through the polyethylene food contact surface into an aqueous / acidic food simulant solvent mixtures. We also demonstrate that no significant amount of 3-MCPD migrates from the unskived edges on the inside seam of the paperboard structure. This finding does not agree with published research listed in the bibliography, which states polyethylene is not a functional barrier to the migration of components within the paperboard food packaging (Johns 2000, Feigenbaum, 2005).

The mass spectrum of the 3-MCPD N-Heptafluorobutyrylimidazole (HFBI) derivative was published (Pace 2010), as a correction to the mislabeled MS spectra of 3-MCPD in the main reference used for this method (Brereton 2001, Figures 1 and 2).

The cold water extractions (total immersion) described in method EN 645, represent an area identified as requiring further study (EU Standard CEN 1993). The paperboard is not completely pulped, which may leave unexposed surfaces of the paperboard fibrous matrix. Therefore, the extraction efficiency may not be 100%, with remaining additional chloropropanols, 3-MCPD specifically, entrained
within the paperboard.

Furthermore, the US FDA and EU Commission both recommend that food contact substance migration testing should be carried out using the appropriate food simulant solvent and under the conditions of use (EU Standard 1985, US FDA 2000). 100% Cold water is not a food simulant solvent in either of the guidelines. The US FDA and EU Commission recommended food simulant solvents were used for the experimentation performed in this research for the migration studies (Pace 2010).
3. OBJECTIVES

3.1. Hypothesis

Migration studies conducted under accelerated conditions will predict whether chloropropanols, formed from epichlorohydrin wet-strength resins in paperboard packaging, will migrate through the polyethylene extrusion-coated food contact side and become an indirect food additive in the foodstuffs.

3.2. Research Objectives

- Demonstrate that food contact accelerated migration testing of paperboard food packaging must accompany the Regulatory-mandated cold water extraction method, in order to quantify chloropropanols migrating through a polyethylene extrusion-coated food contact surface into a beverage.

- Validate whether the European Standard cold water extraction method for paper and board in contact with foodstuffs extracts the total amount of chloropropanols present in the wet-strength resin - paperboard matrix.

- Investigate whether polyethylene extrusion-coating of the food contact side of paperboard can function as a barrier to the migration of chloropropanols into the packaged food. This would present an alternative viewpoint to literature which states polyethylene is not a functional barrier to the migration of printing ink components in paperboard food packaging.
3.3. Research Tasks

- Apply US FDA and EU Commission accelerated migration testing to determine whether chloropropanols detected at levels significantly higher than the US FDA and EU commission guideline levels will migrate through the polyethylene extrusion-coated food contact surface into the food and become an indirect food additive.

- Experimentation will be performed on the total immersion extractions’ variables to extract the maximum available chloropropanols from polyethylene coated and uncoated paperboard made with epichlorohydrin-based wet strength resin. The variables include using the appropriate food simulant solvent strength, vigorous pulping of the carton to increase the surface availability of the chloropropanols, and using FDA and EU designated accelerated migration testing condition temperatures of 40°C for 10 days (U.S. FDA/CFSAN. 2007, EU Standard CEN 1985).

- Test the current published literature conclusions which state that polyethylene is not a functional barrier to the migration of compounds found in paperboard packaging.

- Expand on the published literature for the generation of chloropropanols from the use of polyamidoamine-epichlorohydrin wet-strength resins in paperboard packaging.
• Develop a gas chromatography – flame ionization detector (GC-FID) method to analyze for the chloropropanols. The GC-FID method would be a routine complement to the use of the current gas chromatographic – mass spectrometric method. GC-FID is more amenable for use by manufacturing and production quality control laboratories. The automated GC-FID method will also allow for automated replicate sample runs, and improved resolution of the 3-MCPD and the 3-MCPD-\textit{d5} internal standard peaks.
4. EXPERIMENTAL DESIGN

4.1. Reagents, Reference Standards, and Materials

The analytical equipment configurations are detailed in the corresponding sub-sections of this Experimental section.

3-chloro-1,2-propanediol-1,1,2,3,3,-d5 (3-MCPD-d5), Isotec # T82-00603, Sigma-Aldrich # 614661, Batch# TV1641, 99%, 98.1atm% D. Isotec, Miamisburg, OH, www.isotec.com.

3-chloro-1,2-propanediol, 98% (3-MCPD), CAS# 96-24-2, Sigma-Aldrich #107271-25ml, Lot# 02417CE, 99.6%, Sigma-Aldrich, Milwaukee, WI.

1,3-dichloro-2-propanol, 98%, (1,3-DCP), CAS# 96-23-1, Lot# 15903DH, Sigma-Aldrich #184489-5G, 99.3%. Sigma-Aldrich, Milwaukee, WI.

2,3-dichloro-1-propanol, >97%, (2,3-DCP), CAS# 616-23-9, Lot# 1342266, Sigma-Aldrich, Fluka #36295, 99.0%. Sigma-Aldrich, Milwaukee, WI.

Derivatizing reagent, heptafluorobutyrylimidazole, (HFBi), sodium chloride, ACS reagent grade, 99+% Sigma-Aldrich, Milwaukee, WI.

Epichlorhydrin, 99%, Sigma-Aldrich #E1055-56, Milwaukee, WI.

Solvents: Ethyl Acetate, Optima grade, 99%min; Isooctane, Optima grade, 99% min; Ethyl ether, ACS grade; glacial acetic acid, ACS grade; anhydrous sodium sulfate, ACS grade; Fisher Scientific, Springfield, NJ. Ethanol, USP grade, dehydrated 200 proof, Pharmco, Products, Brookfield, CT. Distilled water,
in-house Millipore MIIli-Q purification system.

1% Starch indicator solution; 0.8 g potassium iodide, 0.8 g iodine / ml water.

Merck EXtrelut® NT20 refill packs, EM Separations, Gibbstown, NJ. Empty solid phase extraction reservoirs (60ml sized, Cat.# 5-7022, and column frits for packing with EXtrelut® NT20, Supelco, Bellefonte, PA.

Pierce reacti-therm® and 5.0 ml reacti-vials, Thermo Fisher Scientific, Rockford, IL. Shown in Figure 15.

Figure 15: Pierce reacti-therm® for chloropropanols HFBI derivatization

Waring® single speed commercial blender wit 500 ml borosilicate glass container. Waring® Commercial, 314 Ella T. Grasso Ave., P.O. box 3201, Torrington, CT 06790.
Agilent 100 µl autosampler inserts for use with 1.5 ml autosampler vials with Teflon®- lined caps.

Forced draft oven with temperature control and digital readout. Model # 1350GM, Sheldon Manufacturing Inc., 300 N. 228th St., Cornelius, OR 97113.

Dry-Vap® solvent evaporating system with vacuum and nitrogen purge. The vacuum was set to 10 psi, temperature setting 1 = 35°C, 200 ml borosilicate glass flasks with 1 ml concentrator reservoirs at bottom. Model# 5000, Horizon Technology Inc. 45 Northwestern Drive, Salem, New Hampshire 03079. Shown in Figure 16.

Figure 16: Dry-Vap® solvent evaporating system
Migration cell extraction assembly comprised of 10 x 15 cm stainless steel (SS) plates (top & bottom) with A Teflon® spacer gasket between the plates. The assembly is bolted firmly together with 12 SS cap screws around the perimeter. The top plate contains ¼ inch O.D. SS tube ports for filling and emptying and the ports are sealed with ¼ in. SS Swagelok® caps with Teflon® ferrules. The Teflon® spacer provides 51 cm² (7.9 in²) food contact surface area and 30 ml cavity volume available for extraction with the food simulant solvent. Scientific Instrument Services, Inc., Ringoes, NJ, www.sisweb.com. Figure 17 shows the single side extraction cell for migration testing which was designed by Dr. Thomas G. Hartman (co-author).
Figure 17: The single-side extraction cell design schematic for migration testing of the food contact surface side only. The 30 ml Teflon® spacer was used for this research. Adapted from Scientific Instrument Services, Inc. Design by Dr. Thomas G. Hartman.
4.2. Sample Descriptions Used in this Research

Table 1: Polyethylene Extrusion-Coated Paperboard sample descriptions with wet-strength resin loading

<table>
<thead>
<tr>
<th>Paperboard</th>
<th>Description</th>
<th>Wet-strength Resin</th>
</tr>
</thead>
<tbody>
<tr>
<td>C Grade</td>
<td>Polycoated paperboard flatstock, 18.2 caliper board, Structure: 7.2# G - 7.2# M (lowest gauge polyethylene), no seams, no printing</td>
<td>8.5 lbs / ream Kymene®</td>
</tr>
<tr>
<td>A Grade</td>
<td>Polycoated paperboard flatstock, unfolded carton sample, 18.3 caliper board, Structure: 9# G - 18# M, skived edges on seam, beverage product printing</td>
<td>8.5 lbs / ream Kymene®</td>
</tr>
<tr>
<td>V Grade</td>
<td>Polycoated paperboard flatstock unfolded carton sample, 23.0 caliper board, structure: 16# LD/ Bd/ 5# PA/ 5# Tie/ 26# LD, unskived edges on seams, milk product printing</td>
<td>10 lbs / ream Kymene®</td>
</tr>
</tbody>
</table>

Figure 18 shows the skived inside seam of a paperboard beverage carton which has been coated with polyethylene (PE). The skived edges are additional PE coating applied to the inside seam of the paperboard packaging to seal the cut edge exposed to the foodstuff. The PE coating is applied as a bead and brushed smooth, as can be seen by the reflection.

Figure 19 is a top view of a seamed paperboard beverage carton before it is completely sealed and filled with the food product.
Figure 18: Skived edge with coating of polyethylene on inside seam of Paperboard Packaging

Figure 19: Top view of inside seam in paperboard beverage carton
Figure 20 shows a scanning electron microscopy (SEM) photomicrograph of the cross-sectional view of an unskived edge of a paperboard beverage carton. The voids between the paper fibers and the uncoated paper surface are clearly visible.

Figure 20: Cross Section of unskived edge without coating of polyethylene on inside seam of Paperboard Packaging
### Table 2: Paperboard samples before polyethylene coating with various wet-strength resin loading and manufacturing conditions

<table>
<thead>
<tr>
<th>Paperboard</th>
<th>Description</th>
<th>Wet-strength Resin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control loading</td>
<td>19.5 WS</td>
<td>19.5 Wet Strength</td>
</tr>
<tr>
<td>Sample (2)</td>
<td>Condition #1 / 7% M</td>
<td>15.5 Wet Strength 160</td>
</tr>
<tr>
<td>Sample (5)</td>
<td>Condition #1 / 7% M</td>
<td>15.5 Wet Strength 160</td>
</tr>
<tr>
<td>Sample (7)</td>
<td>Condition #1 / 7% M</td>
<td>15.5 Wet Strength 160</td>
</tr>
<tr>
<td>Sample (2)</td>
<td>Condition #2 / 7% M + IR</td>
<td>15.5 Wet Strength 160</td>
</tr>
<tr>
<td>Sample (5)</td>
<td>Condition #2 / 7% M + IR</td>
<td>15.5 Wet Strength 160</td>
</tr>
<tr>
<td>Sample (7)</td>
<td>Condition #2 / 7% M + IR</td>
<td>15.5 Wet Strength 160</td>
</tr>
<tr>
<td>Sample (2)</td>
<td>Condition #3 / 7% M + IR</td>
<td>19.5 Wet Strength 195</td>
</tr>
<tr>
<td>Sample (5)</td>
<td>Condition #3 / 7% M + IR</td>
<td>19.5 Wet Strength 195</td>
</tr>
<tr>
<td>Sample (7)</td>
<td>Condition #3 / 7% M + IR</td>
<td>19.5 Wet Strength 195</td>
</tr>
<tr>
<td>Sample (2)</td>
<td>Condition #4 / 7% M</td>
<td>19.5 Wet Strength 195</td>
</tr>
<tr>
<td>Sample (5)</td>
<td>Condition #4 / 7% M</td>
<td>19.5 Wet Strength 195</td>
</tr>
<tr>
<td>Sample (7)</td>
<td>Condition #4 / 7% M</td>
<td>19.5 Wet Strength 195</td>
</tr>
<tr>
<td>OJ / TTG</td>
<td>Barrier base paperboard</td>
<td>278 lb grade, 11.5 lb/ton Kymene®</td>
</tr>
</tbody>
</table>

The actual details of the samples and conditions in Table 1, Table 2, Table 3, and Table 4 are proprietary to the Company who supplied these samples.
**Table 3: Paperboard formed carton samples for Total Immersion Extracts with different Food Simulant Solvents: Pulping in blender and No Pulping**

<table>
<thead>
<tr>
<th>Paperboard Description</th>
<th>PE Extrusion Coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprinted and formed Paperboard Carton</td>
<td>No PE Coating</td>
</tr>
<tr>
<td>Unprinted Paperboard from roll</td>
<td>No PE Coating</td>
</tr>
<tr>
<td>Unprinted and formed Paperboard Carton from Stacks</td>
<td>No PE Coating</td>
</tr>
<tr>
<td>Printed and formed Paperboard Carton from Stacks</td>
<td>PE extrusion coating</td>
</tr>
<tr>
<td>Unprinted and formed Paperboard Carton from Stacks</td>
<td>PE extrusion coating</td>
</tr>
</tbody>
</table>

**Table 4: Polyethylene Extrusion-Coated Paperboard formed carton samples for Migration Cell Extractables of Food Contact side with different Food Simulant Solvents**

<table>
<thead>
<tr>
<th>Paperboard Description</th>
<th>Wet-strength Resin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Printed and formed Paperboard Carton from Stacks</td>
<td>PE extrusion coating</td>
</tr>
<tr>
<td>Unprinted and formed Paperboard Carton from Stacks</td>
<td>PE extrusion coating</td>
</tr>
</tbody>
</table>
Figure 21 shows two production paperboard samples, with the interior food beverage contact side facing up, and on top of it, also facing up, the printed outside of a typical orange juice food packaging paperboard carton.

Figure 21: Inside Food contact side, and printed outside of a typical orange juice paperboard packaging carton
4.3. Total Immersion Aqueous Extractions of the Entire Paperboard Cartons

In this work, cold water (room temperature, 23°C) aqueous extracts of paperboard cartons were prepared in accordance with the European Standard Method published by the European Committee for Standardization (EU Standard 1993). The two most pertinent published methods (as discussed in Section 2.6), for the determination of 3-chloro-1,2-propanediol (3-MCPD) in foods using GC-MS, were adapted to perform these studies on chloropropanols in paperboard food packaging (Brereton 2001, Hamlet 1997).

The methodology entails blending the food sample (i.e. HVP, soy sauces, salami, fish, cereals, breads) to homogeneity (after a deuterated 3-MCPD internal standard is added), and the contents are placed on an EXtralut® NT20 solid phase extraction column. The 3-MCPD is eluted with diethyl ether, concentrated, and derivatized with the HFBI reagent. The derivatized 3-MCPD, 3-MCPD-d5, and 1,3-dichloropropanol (1,3-DCP) are analyzed by GC-MS on a slightly polar 5% phenyl methyl silicone capillary column in electron impact (EI) mode from 100-500 atomic mass units (amu) using selected ion monitoring (SIM). The response ratio of the 3-MCPD-d5 internal standard to 3-MCPD is used for calibration and quantitation.

Collaborative studies of the food extraction method reported the limit of
quantitation (LOQ) of 10 \(\mu g/kg\) (10 ng/g) and limit of detection (LOD) of 5 \(\mu g/kg\) (5 ng/g) (Brereton 2001, Hamlet 1997, Dayrit 2004).

The total immersion followed the preparation of the cold water (23 \(\pm\) 2°C) aqueous extracts of paperboard cartons as stated in the European Standard Method published by the European Committee for Standardization (EU Standard 1993). The paperboard or paper sample was cut up and extracted, and the extract analyzed for the chloropropanols. The concentration of 3-MCPD and 1,3-DCP in the extracts were measured following the HFBI derivatization and GCMS method stated above and detailed in Section 4.6 (Brereton 2001, Hamlet 1997). The Brereton method, which was used for food products, was slightly modified in accordance with the paperboard preparation in cold water extracts of the EU 1993 method. Linear calibration curves for 1,3-DCP and 3-MCPD were established.

For this research, polyethylene extrusion-coated paperboard carton samples prepared directly by the manufacturer, representing a “worst case” scenario of higher Kymene® resin loading and lower thickness polyethylene were selected for evaluation (Table 1). These same paperboard carton samples were also analyzed by migration study experiments to determine the extractables level of the 3-MCPD and 1,3-DCP which could diffuse through the polyethylene food contact surface. Paperboard samples with a range of Kymene® resin were also analyzed before they were polyethylene extrusion-coated and formed into cartons.
The water extracts of the paperboard cartons were prepared and analyzed in triplicate. In accordance with the European Standard Method, 10 g samples of the paperboard were cut into 1 cm² pieces and placed into 250 ml borosilicate glass jars with Teflon®-lined lids. 200 ml of distilled water was added, the jars were tightly sealed, and placed in a temperature controlled incubator at 21 ± 2°C for 24 hrs with a low agitation setting on a rotary shaker. The extracts were filtered through a medium (10 to 15 µm) porosity glass crucible frit into 250 ml volumetric flasks. The paperboard pieces were washed twice with 25 ml of distilled water, the washings also filtered and combined with the extracts in the flasks, and brought to volume with distilled water.

Subsequent room temperature water extraction studies were performed on “base” paperboard cartons with varying low to high levels of Kymene® wet-strength resin additive and other non-epichlorohydrin resins. The board with known Kymene® wet-strength loading was also analyzed for residual epichlorohydrin (Table 2). The level of 3-MCPD and 1,3-DCP, was determined, and the data was analyzed for a correlation between wet-strength resin loading and the levels of those contaminants in the paperboard.
4.4. Experimental Design variables for Total Immersion Testing of Paperboard Cartons

The sample preparation, extractions solvents, and temperature used for the total immersion extractions were varied to form the experimental design. Experiments were performed on the samples listed in Table 3 to test whether increasing surface area of paper samples by pulping, extractions with a stronger aqueous modified food simulant solvent mixture, and higher extraction temperature will result in a more efficient extraction of the entrained 3-MCPD in the paper fiber matrix. These food simulant solvents and accelerated extraction temperature are taken from the FDA and EU migration testing guidelines (U.S. FDA/CFSAN 2007, EU Standard CEN 1985).

The total immersion extraction variables in the experimental design methodology include:

- increase the extraction temperature to 40° C;
- changing from 100% water to food simulant liquids and mixtures;
  - 10% ethanol - aqueous / acidic (FDA), alcoholic (EU),
  - 3 % acetic acid - acidic (EU),
  - 10% ethanol + 3 % acetic acid – FDA acidic + EU acidic simulants;
- replace the cut 1 cm² pieces to pulping of the paperboard carton sample in a Waring® blender.
The experimental variables and samples are listed in Table 5 and Table 7.

The water extracts of the paperboard cartons were prepared in duplicate and analyzed in triplicate. 10 g samples of the paperboard were cut into 1 cm² pieces, placed into the 500 ml borosilicate glass blender beaker, and 200 ml of HPLC grade water was added. The sample was pulped for 1 minute in the blender. The pulped sample was transferred with 5 x 2 ml washings into 250 ml borosilicate glass jar with Teflon®-lined lids. The jars were tightly sealed, and placed in a temperature controlled oven at 40 ± 2°C for 24 hrs with occasional shaking. The pulped matrix was quickly poured using a rubber policeman into a 100 mm Buchner funnel. The extracts were filtered using water aspiration through the pulped matrix into 250 ml Erlenmeyer flasks. When most of the food simulant solvent was filtered, the pulped matte was pressed down with a 90 mm watch glass to remove as much of the simulant solvent as possible, reaching about 200 ml. The filtrate was transferred into a 250 ml graduated cylinder. The pressed pulp was washed with 4 x 10 ml washings. A small rotary vacuum pump was used to remove the residual simulant solvent mixture. The washings were combined with the extract in the cylinder, and brought to 250 ml volume with HPLC water. The extract was transferred into the 250 ml jars and capped tightly with the Teflon®-lined lids.

The food simulant solvent extracts were accurately spiked with the internal standard solution for an equivalent concentration of 10 µg / g (10 ppm), based on
the 10 g paper sample. The jars were vigorously swirled by hand and using a vortex mixer (with flat rubber adapter) for dissolution of the internal standard. The spiking was performed before pipetting the aliquots for: column exchange; diethyl ether elution and concentration; reconstitution with isooctane; and derivatized with heptaflourobutyrylimidazole; gas chromatography separation with mass spectrometry or flame ionization detector analysis. The methodology was similar to the aqueous extractions section; except that a Dry-Vap® evaporating system was used for the diethyl ether concentration to 10 ml, allowing processing of up to 6 extractions simultaneously.
Table 5: Uncoated Paperboard formed carton samples for Total Immersion Extracts with different Food Simulant Solvents: Pulping in blender and No Pulping

<table>
<thead>
<tr>
<th>Set</th>
<th>Sample</th>
<th>Preparation</th>
<th>Conditions</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Condition #2 (5)</td>
<td>Pulping in Blender</td>
<td>40°C for 24 hrs</td>
<td>100% Water, 10% Ethanol, 3% Acetic Acid</td>
</tr>
<tr>
<td></td>
<td>No PE Coating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1B</td>
<td>Blank Board 19.5WS</td>
<td>Pulping in Blender</td>
<td>40°C for 24 hrs</td>
<td>100% Water, 10% Ethanol, 3% Acetic Acid</td>
</tr>
<tr>
<td></td>
<td>No PE Coated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1C</td>
<td>OJ / TTG Board</td>
<td>Pulping in Blender</td>
<td>40°C for 24 hrs</td>
<td>100% Water, 10% Ethanol, 3% Acetic Acid</td>
</tr>
<tr>
<td></td>
<td>No PE Coating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A</td>
<td>19.5 WS</td>
<td>Pulping in Blender</td>
<td>23°C for 24 hrs</td>
<td>100% Water, 10% Ethanol</td>
</tr>
<tr>
<td></td>
<td>No PE Coating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B</td>
<td>OJ/TTG</td>
<td>Pulping in Blender</td>
<td>23°C for 24 hrs</td>
<td>100% Water, 10% Ethanol</td>
</tr>
<tr>
<td></td>
<td>No PE Coating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A</td>
<td>19.5 WS</td>
<td>1 cm² pieces</td>
<td>23°C for 24 hrs</td>
<td>100% Water</td>
</tr>
<tr>
<td></td>
<td>No PE Coating</td>
<td>No Pulping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A</td>
<td>19.5 WS</td>
<td>Pulping in Blender</td>
<td>23°C for 24 hrs</td>
<td>100% Water</td>
</tr>
<tr>
<td></td>
<td>No PE Coating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3B</td>
<td>OJ/TTG</td>
<td>1 cm² pieces</td>
<td>23°C for 24 hrs</td>
<td>100% Water</td>
</tr>
<tr>
<td></td>
<td>No PE Coating</td>
<td>No Pulping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3B</td>
<td>OJ/TTG</td>
<td>Pulping in Blender</td>
<td>23°C for 24 hrs</td>
<td>100% Water</td>
</tr>
</tbody>
</table>
Table 6: Polyethylene Extrusion-coated Paperboard formed carton samples for Migration Cell Extractables of Food Contact side with different Food Simulant Solvents and accelerated conditions.

<table>
<thead>
<tr>
<th>Set</th>
<th>Sample</th>
<th>Preparation</th>
<th>Conditions</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>4A</td>
<td>OJ No Pulp Printed and PE Coating</td>
<td>51 cm² sample</td>
<td>40°C for 10 days</td>
<td>100% Water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food Contact side</td>
<td></td>
<td>10% Ethanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95% Ethanol</td>
</tr>
<tr>
<td>4B</td>
<td>Blank OJ Board Unprinted and PE Coated</td>
<td>51 cm² sample</td>
<td>40°C for 10 days</td>
<td>100% Water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food Contact side</td>
<td></td>
<td>10% Ethanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95% Ethanol</td>
</tr>
</tbody>
</table>

Table 7: Polyethylene Extrusion-coated Paperboard formed carton samples for Total Immersion Extracts with Food Simulant Solvents at room temperature and accelerated conditions: Pulping in blender and No Pulping

<table>
<thead>
<tr>
<th>Set</th>
<th>Sample</th>
<th>Preparation</th>
<th>Conditions</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>5A</td>
<td>OJ No Pulp Printed</td>
<td>1 cm² pieces</td>
<td>23°C for 24 hrs</td>
<td>100% Water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No Pulping</td>
<td></td>
<td>10% Ethanol</td>
</tr>
<tr>
<td>5B</td>
<td>Blank OJ Board Unprinted</td>
<td>1 cm² pieces</td>
<td>23°C for 24 hrs</td>
<td>100% Water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No Pulping</td>
<td></td>
<td>10% Ethanol</td>
</tr>
<tr>
<td>6</td>
<td>OJ No Pulp Printed</td>
<td>1 cm² pieces</td>
<td>40°C for 24 hrs</td>
<td>100% Water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No Pulping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Board OJ Board Unprinted</td>
<td>1 cm² pieces</td>
<td>40°C for 24 hrs</td>
<td>100% Water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No Pulping</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.5. Migration Cell Studies of Polyethylene Food Contact Surface of Paperboard Packaging

The European standard cold water extraction testing protocol was adapted to include the US FDA and EU Commission guidelines for migration cell extraction testing of food contact substances. This testing was applied only to the converted polyethylene extrusion-coated paperboard converted and formed into the flat samples that would become beverage cartons. Uncoated paperboard samples are not amenable to this testing, since the food simulant would penetrate through the entire paperboard sample during the time and temperature of the testing. Samples of 10 x 5.1 cm were cut from each of the three paperboards (Table 1) and analyzed in triplicate according to these US and EU guidelines on food simulant mixtures for aqueous and acidic foods (US FDA 2006, EU Standard 1985). Additional food simulant solvent mixtures were adapted from those recommendations. The following food simulant solvents and mixtures for the samples in listed in Table 1 were used: 10% ethanol in water; 3% acetic acid in water; 3% acetic + 10% ethanol in water; and 100% water. The 51 cm² paperboard sample was placed on the bottom plate with the polyethylene food contact surface exposed to the 30 ml food simulant solvent within the Teflon® gasket and top plate. The area taken from the formed, embossed, five-panel paperboard cartons with the skived (A Grade sample) and unskived (V grade
sample) edges on the seam were placed to be in contact with the food simulant solvents. The skived seam on the polyethylene extrusion-coated paperboard are completely coated with a bead of polyethylene, while the paperboard with unskived edges do not have a polyethylene bead coating at the cut edge of the seam where the fifth and first panels are sealed on the to form the inside carton. The skived edges represents < 1% of the surface area exposed to contact with the extracting solvent (See Section 4.1, Figure 18).

The surface area of the board in contact with the extracting simulant solvent mixture within the Teflon® cavity provided for a solvent volume to surface area ratio of 0.59 ml/cm² (3.9 ml/in²). This extraction is threefold as concentrated for the food simulant solvent to paperboard surface area (than the FDA compliance testing in the guidelines), resulting in a lower limit of detection of the final concentrated extract. Figure 22 shows the typical size of the migration cell samples as described in this section.
The migration cell samples from Table 1 were prepared in triplicate and extracted for 10 days at 40°C with gentle agitation on a rotary shaker. Duplicate analysis was performed by GC-MS on the triplicates for each sample. The 10 days extraction simulates 6 to 12 months at room temperature storage (US FDA 2007, EU Comm 2001). An additional cell migration set of 100% water extracts were prepared from the V grade carton sample for the spiking and recovery validation. A blank sample of 100% water was run as a control through the method with the
samples.

Each of the Set 4 samples from Table 6 were prepared in duplicate and extracted for 10 days at 40°C ± 2°C in an oven for each of the three food simulant solvents listed, with duplicate analysis using the newly developed GC-FID method for each duplicate of the same sample. That is, each sample was extracted in duplicate, and each extract was analyzed in duplicate. Therefore, each paperboard sample has at least 4 replicate GC results for standard deviation calculations. The 95% Ethanol replaced the acetic acid, so that the range of food simulant solvents would be for aqueous / acidic, alcoholic and fatty foods. 95% ethanol is a fatty food simulant and represents the strongest food simulant extraction solvent, and a worse case food simulant extraction solvent for polyethylene food contact coatings.

4.6. Analytical Methodology for Total Immersion Extractions and Migration Cell Extractables

Figure 23 is a flow diagram of the overall analytical methodology for the total immersion extractions and migration cell studies. It is shown that the sample preparation steps are different, yet follow the same sample work-up procedure, which is detailed following the Figure.
Total Immersion

10 g of films were cut into 1 cm², the whole pieces were immersed into 250 ml food simulant

24 hrs, 23°C

Filtered and spiked with internal standard

Migration Cell

2.5 g films were cut into 10 cm × 5 cm, only PE layer side contacted with 30 ml food simulant

10 days, 40°C

Transferred to test tubes and spiked with internal standard

Pipette onto Extrelut® NT

Elute with diethyl ether

Concentrate to dryness

Reconstitute and derivitize

Analyze by GC-MS or GC-FID

Figure 23: Methodology Flow of Total Immersion Extractions and Migration Cell Studies
The total Immersion paperboard extracts were spiked with 100 µl of a 1.0 µg / µl solution of 3-MCPD-d₅ internal standard. This amount of 100 µg I.S. per 10 g of paperboard sample is equivalent to 10 µg/g or 10 mg/kg (ppm) w/w. 30 ml aliquots of the extracts were transferred into 50 ml borosilicate glass test tubes and sealed with Teflon®-lined screw caps.

To the 50 ml test tubes containing the 30 ml aqueous extract aliquots or the 30 ml cell migration extractables, was added 10 g of sodium chloride, they were capped and mixed thoroughly. 20 ml aliquots transferred onto the EXtrelut™ 20 packed solid phase column fitted with a 3 inch stainless steel Luer-lock pipetting needle on the tip. The sample was allowed to absorb into the bed for 15 minutes, then eluted with 250 ml of anhydrous ethyl ether at a rate of 10 ml / min.

Residual moisture was removed from the ether eluents by mixing in 15 g of anhydrous sodium sulfate. These were concentrated to 10 ml on a rotary evaporator under slight vacuum, using a water aspirator, at 35°C. An additional 200 mg of sodium sulfate was added to the concentrated extracts. The extracts were quantitatively transferred into a 5.0 ml Pierce reacti-vial, slowly concentrated to dryness under a gentle stream of nitrogen at room temperature.

The dried extract was immediately reconstituted with 1.0 ml of iso-octane, 50 µl of HFBI was immediately added, and the vials were mixed on a vortex mixer. The 3-MCPD, 3-MCPD-d₅, and 1,3-DCP were derivatized at 75°C for 20 minutes, with periodic mixing on a vortex mixer. The vials were allowed to cool to room
temperature, 1.0 ml distilled water was added, capped, and vortex mixed for 30 seconds. The lower aqueous layer was removed with a Pasteur pipette, after the vials stood sufficiently to allow for phase separation. This 1.0 ml water added for the reaction quench and washing was repeated. 10 mg of anhydrous sodium sulfate was added to the vial and gently mixed.

The migration cell extractables from the polyethylene food contact surface of the paperboard cartons were transferred into 50 ml borosilicate test tubes. The entire 30 ml of the food simulant extractables was spiked with 10.0 µl of a 100.0 ng / µl solution of 3-MCPD-d₅ internal standard (I.S.). This amount of 1000 ng I.S. per the weight (g) of paperboard sample is equivalent to 1000 ng/g as w/w. The surface area of the cell migration samples is 51 cm². The exact sample weight is dependent on the basis weight of the different paperboards. The exact I.S. equivalent was calculated based on the actual paperboard sample weight. For example, in a 2 g sample, the amount of 1000 ng of I.S per 2 g would be equivalent to 500 ng / g (500 ppb). Exact concentrations were determined for each sample, and used in the quantitation calculations.

HFBI derivatives from Table 1 and Table 2 of the filtered aqueous extracts of the paperboard cartons and polyethylene surface migration extractables were both analyzed by direct injection into the GC-MS in Electron impact mode. A published quantitative analytical procedure was adapted to our GC-MS instrumentation (Brereton 2001).
Furthermore, these and all of the sample extracts from Sets 1, 2, 3, 4, 5, and 6, which are listed in Tables, 5, 6, and 7, were analyzed by a newly developed GC-FID method. The GC-MS method was improved upon, in which, the resolution of 3-MCPD and MCPD-$d_5$ internal standard peaks were optimized to provide for baseline separation and retention time identification using only the FID, without a mass spectrometer. In place of the rotary evaporator, the diethyl ether eluents of those samples were concentrated using the Dry-Vap® solvent evaporating system with vacuum and nitrogen purge. The vacuum was set to 10 psi, temperature setting 1 = 35°C, 200 ml borosilicate glass flasks with 1 ml concentrator reservoirs at bottom.

4.7. Analytical Method for Extracted Chloropropanols using Gas Chromatography - Mass Spectrometry (GC-MS)

A 30 m x 0.32mm i.d. x 0.25µm film thickness MD5-N capillary column was installed in a Varian 3400 GC, with 2 mm i.d. freshly silanized (deactivated) injection port liner. The injector temperature was set at 270°C with a split ratio of 100:1, using helium carrier gas set at 10 psi. The column separation parameters were 50°C for 5 min, ramp at 10° min-1 up to 100°C then 20°C min-1 to 280°C with a 1 min hold. The GC-MS transfer line was set at 280°C and interfaced into a Finnegan MAT 8230 double-focusing magnetic sector MS in Electron impact
ionization mode at 70eV. The MS ion source temperature was set at 250°C with a resolution of 1000, as scan range of 35-550 m/z, a scan rate of 0.6 sec/decade with 0.8 sec interscan time, and electron multiplier gain of 1.8 KV. The Finnegan MAT SS300 and VG MassLynx software were used as the operating and data analysis system.

4.7.1. Gas Chromatography - Mass Spectrometry Calibration

Figure 6 shows the GC-MS calibration curves which were prepared for 3-MCPD (Figure 6a) with standards ranging from 1.0 to 20.0 mg/kg, and for 1,3-DCP (Figure 6b) ranging from 0.1 to 2.0 mg/kg. A five point calibration was performed with duplicate standards and linear regression analysis. The area/amount ratio of the standards to the 3-MCPD-$d_5$ internal standard was used for each calibration point. This regression calibration was used for the quantitation of the 3-MCPD and 1,3-DCP in the samples of Table 1 and Table 2.
Figure 24: (a) 3-MCPD GC-MS calibration curve; (b) 1,3-DCP GC-MS calibration curve.
4.7.2. Validation of GC-MS Migration Cell Methodology for Chloropropanols

The limit of detection for the migration cell extraction was determined by spiking 100% water extract of 30 ml for a 3.5 g x 51 cm² paperboard sample with: 1000 ng of 3-MCPD, equivalent to 285 ng/g (1000 ng/3.5 g); and 175 ng of 1,3-DCP, equivalent to 50 ng/g (175 ng/3.5 g). Figure 5a shows the GC-MS Total Ion Chromatogram (TIC) of the 100% water extract, spiked with 1,3-DCP (peak at scan 298) and 3-MCPD (peak at scan 356), and containing 319 ng/g of the 3-MCPD-$d_5$ internal standard (peak at scan 353). Figure 5b shows the mass spectrum of the 1,3-DCP peak (298). Figure 5c shows the mass spectrum of the 3-MCPD-$d_5$ internal standard peak (353). Figure 5d shows the mass spectrum of the 3-MCPD peak (356).
Figure 25: (a) TIC of 100% water extract, spiked with 175 ng/g of 1,3-DCP (peak at scan 298) and 285 ng/g of 3-MCPD (peak at scan 356), and containing 319 ng/g of the 3-MCPD-\textit{d}_5 internal standard (peak at scan 353); (b) mass spectrum of the 1,3-DCP peak at scan 298; (c) mass spectrum of the 3-MCPD-\textit{d}_5 internal standard peak at scan 353; (d) mass spectrum of the 3-MCPD peak at scan 356.

4.7.3.
**Analysis for epichlorohydrin in the paperboard samples**

The GC-MS conditions needed to be modified for the epichlorohydrin analysis. The GC temperature was 35°C for 5 min, 10°C / min to 80°C (0 min. hold), 25°C/min up to 320°C. The MS ions of 57 (base peak), 49 and 62 were used for identification. The 57 base peak ion is from the loss of the chlorine from the molecular epichlorohydrin molecule.


A 30 m x 0.25 mm i.d. x 1.0 µm film thickness HP-5MS (DB-5MS) from J&W Scientific (cat.No.190915-233) capillary column was installed in a Varian 3400 GC, with 2 mm i.d. freshly silanized (deactivated) injection port liner. A thicker film than the GC-MS method was chosen for improved resolution of chloropropanol peaks of interest for accurate quantification. The injector temperature was set at 300°C with a split ratio of 10:1, using helium carrier gas set at 19.0 psi for a linear carrier velocity of 32 cm/sec at 100°C isothermal. The detector temperature was set at 320°C, using air / hydrogen at 400/40 ml/min rate, with helium as the make-up gas. The final column separation parameters for baseline resolution of the 3-MCPD and 3-MCPD-$d_5$ internal standard were finalized at 80°C for 1 min, ramp at 2°C/min up to 120°C then 25°C/min to 300°C with an 11.8 min hold, for a 40 minute total run time. The initial GC data presented in Table 4 was acquired
and integrated with PeakSimple© GC datastation software.

Subsequently, the new GC calibrations for 3-MCPD and 1,3-DCP shown in Table 3a and Table 3b) were carried out on an Agilent 5890A Series II (without electronic pressure programming feature) with a 2 mm i.d. freshly silanized (deactivated) split injection port liner. The injector temperature was set at 300°C with a split ratio of 10:1, using helium carrier gas set at 19.0 psi for a linear carrier velocity of 32 cm/sec at 100°C isothermal. The detector temperature was set at 320°C, using air / hydrogen at 400/40 ml/min rate, with nitrogen as the make-up gas. The column conditions were 80°C for 1 min, ramp at 2°C/min up to 120°C then 25°C/min to 300°C with an 11.8 min hold, for a 40 minute total run time. The GC data station was Agilent Technologies ChemStation© Rev.B.04.01 version.

4.8.1. Gas Chromatography-Flame Ionization Calibration

Stock standards of 1.0 mg 3-MCPD / ml ethyl acetate, and 1.0 ml of the stock was diluted into 10 ml of ethyl acetate, for a working standard solution of 0.1 ml 3-MCPD / ml ethyl acetate. The GC calibration curve standards were prepared by taking aliquots using Hamilton volumetric syringes with 90° stainless steel needle tip (Tables 3a and 3b). A 250 µl syringe was used for the 20, 15, 12.5, and 10 µg/g (ppm) standards (GP59-1, 2, 3, 4A). A 100 µl syringe was used for the 75, 50, and 25 µg/g (ppm) standards (GP59-5; 61-2, 3A). A 25 µl syringe was used for the 1.0 µg/g (ppm) standards (GP61-4). A 10 µl syringe was used for the 0.5
µg/g (ppm) standard. The 1,3-DCP and 3-MCPD-$d_5$ standards were prepared in a similar manner corresponding to the syringe needed to deliver volume necessary.

Each compound of the standard was pipetted in triplicate into Pierce Reati-vials®, followed by the 1.0 ml of isoctane and 50 µl HFBI, and derivatized as described in Analysis of Aqueous extractions section above.

The GC calibration curve standards were prepared with freshly obtained reagent standards as presented in the Materials and Methods section. Table 8a shows the corresponding notebook numbers, the working standard concentrations, and volumes taken of 3-MCPD, 1,3-DCP, and 3-MCPD-$d_5$. Table 8b shows the actual amount of each calibration standards, along with the GC peak area responses.

The 9 point calibration curve was performed by analyzing each standard in triplicate and performing a linear regression analysis (not forced through zero). The area/amount ratio of the standards to the 3-MCPD-$d_5$ internal standard was used for each calibration point.

4.9. **Structural Evaluations of Selected Paperboard structures**

Polyethylene (PE) coated commercial board samples and uncoated paperboard samples were evaluated using Fourier Transform-Infrared Spectroscopy with attenuated total reflectance (FT-IR-ATR) using a Thermo Nicolet Magna 550 FT-IR, equipped with a Golden Gate diamond crystal micro ATR
attachment and Omnic 6.1a software. Scanning Electron Microscopy (SEM) using a Topcon SM-300 SEM with SM300 Application Version 1.40 software: Setting; KEV= 10, Spot size= 9, WD = 15, Tilt = 0° (for cross sections) or 30° (flat surfaces). Magnifications and scales are on. Optical microscopy an Olympus BX-51 Research Grade Light Microscope with Olympus D. E. (Discover Essentials) software: 10X objective; in photos, field of view (fov) = 1400 x 1050 microns (1.4 x 1.05 mm).

The SEM samples were cut sharply with a razor equipped microtome to give a clean edge cut for cross-sectional analysis. The surfaces of these samples were also observed using the SEM. A separate sample was cut sharply with a razor blade and the edges stained with a potassium iodide stain indicator for starch for optical microscopy observations. The surfaces of uncut samples of the paperboard were directly placed under the ATR attachment on the FT-IR and the infrared spectroscopic absorbances were read.

The samples analyzed by SEM were also tested for starch on the cross-section edge cuts. The cross-section would allow to compare the inside of the paperboards for starch sizing of the paperboard fibers, and allow evaluation of whether there is the starch presence could effect the migration of the chloropropanols from the paperboard matrix. In particular, from the polyamidoamine-epichlorohydrin wet-strength resin coating on the cellulosic paperboard fibers.

Figure 26 and Figure 27 show scanning electron microscopy cross-sections of the two main type of paperboard food packaging used in our research presented in
this dissertation. Figure 26 is the SEM and illustration of a paperboard without polyethylene coating. Figure 27 shows the SEM photomicrograph and illustration of a polyethylene extrusion-coated paperboard carton.

**Figure 26: SEM cross-section of Paperboard without PE Coating from Set 1**

**Figure 27: SEM cross-section of PE Coated Paperboard from Set 6**
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<th>Volume</th>
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<th>Volume</th>
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Table 9: Calibration standards concentrations, GC-FID peak area responses, and Peak area ratios

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Figure 28 shows the nine point GC-FID calibration curves which were prepared for 3-MCPD (Figure 7a) with standards ranging from 0.5 to 20.0 ppm (µg/g), and for 1,3-DCP (Figure 7b) ranging from 0.1 to 2.0 ppm (µg/g). The linear regression calibration was used for the quantitation of the 3-MCPD and 1,3-DCP in all of the paperboard samples listed in Table 5 and Table 7, which were subjected to the optimized extraction and food simulant liquid conditions described in the Food Simulant Solvent Extractions section.
Figure 28: (a) 3-MCPD GC-FID calibration curve; (b) 1,3-DCP GC-FID calibration curve.
5. RESULTS AND DISCUSSIONS

5.1. Experimental Design Variables for the Total Immersion Migration Studies of Chloropropanols in Paperboard

The Control Total Immersion sample preparation follows the European Standard Method published by the European Committee for Standardization. The concentration of the chloropropanols, 3-MCPD and 1,3-DCP, in the extracts were measured following the HFBI derivatization and GCMS method. The sample preparation and extract analyses were detailed in the Experimental Section.

The migration studies we conducted contained two main parts. The first part (Part 1) followed the European standard cold water total immersion extraction method without changes. Migration cell testing of the food contact side was also performed on these same samples, which are listed in Table 1 and Table 2. The second part (Part 2) of the research involved varying the parameters of the standard cold water total immersion extraction methods to form the experimental design on the paperboard samples listed in Table 3. Those samples represent different commercial and manufacturing design tests that were kept proprietary by the manufacturer. Therefore, we are limited by a the Confidentiality Agreement with the manufacturer to discuss proprietary information about the paperboard structure and preparation in the results that are presented in this dissertation. The paperboard samples in Table 3 were tested to determine whether increasing
surface area of paper samples by pulping, extractions with a stronger aqueous modified food simulant solvent mixture, and higher extraction temperature will result in a more efficient extraction of the entrained 3-MCPD in the paper fiber matrix. Figure 29 is the experimental design flow of both Part 1 and Part 2 of our research. The European standard cold water extraction protocol is designated as the Control conditions, which were varied for the experimental design testing.

The European cold water protocol is actually 23°C, and represents room temperature. Cold water for these juice and dairy beverage cartons could be considered to be refrigeration temperature of 4°C. The term of “cold” water could have been used to emphasized extractions without heating.
Figure 29: Experimental Design of Total Immersion Migration Studies for Chloropropanols in Paperboard Food Packaging. Control is the European Standard cold water extraction conditions.
5.2. Migration Cell Studies of Polyethylene Coating as a Barrier to Chloropropanols migration from the Paperboard Carton

The migration cell shown in Figure 2 of Section 4.1 was used to determine if the chloropropanols detected by total immersion extractions would migrate through the polyethylene extrusion-coated food contact surface into the food beverage. In Figure 22 of Section 4.5 it shows the polyethylene food contact side which is the only side exposed to the food simulant in the migration cell. This food contact side migration testing was performed on samples, listed in Table 1 and Table 4, from both Part 1 and Part 2 of this research.

The migration cell test conditions were designed to be accelerated and more aggressive than required by the US FDA and EU Commission for the typical use of refrigerated storage for these paperboard gable top cartons. The food simulant solvents and mixtures ranging from aqueous / acidic to alcoholic food beverages were selected to exacerbate the potential for migration of 3-MCPD and 1,3-DCP from the paperboard carton and through the polyethylene polymer matrix. The design of the experiment utilizes a threefold more concentrated food simulant solvent to food contact surface area of 0.59 ml/cm², providing a more concentrated extract for analysis. This enabled a lower limit of detection than would have been provided if the actual beverage volume to surface area was used.
A typical 0.5 gallon (1.89 L) gable-top beverage carton has a volume to surface area ratio of approximately 2.1 ml/cm²

5.3. Analytical Testing using Gas Chromatography-Mass Spectrometry in Electron Impact Mode

The samples listed in Table 1 and Table 2, were analyzed by the gas chromatography-mass spectrometry (GC-MS) method. These constitute the samples from Part 1 of our research. The description of the GC-MS reaction formation of 3-chloro-1,2-propanediol (3-MCPD) with heptafluorobutyrylimidazole (HFBI) to form the volatile heptafluorobutyryl derivative was shown in Section 2.6. Figure 30 shows the structure of the 3-MCPD-HFBI compound which, when analyzed by gas chromatography-mass spectrometry (GC-MS) in electron impact (EI) mode, give the characteristic EI mass spectra ions used to confirm the presence of the HFBI derivatives, which are; m/z 169, 275, 453, 456; for the HFBI derivatives of 3-MCPD (169,453), 1,3-DCP (169,275) and 3-MCPD-d$_5$ (169,456). Figure 31A to 31C displays the EI spectra of the 1,3-DCP, 3-MCPD-d$_5$, and 3-MCPD HFBI derivatives, respectively. The HFBI derivatization of 3-MCPD and 3-MCPD-d$_5$ produces a heptafluorobutyryl ester the two hydroxyl groups the propanediol backbone, forming di-heptafluorobutyrylate ester derivative. The compounds formed have molecular weights of 502 for 3-MCPD-(2-OC4F7) and 507 for 3-MCPD-d$_5$(2-OC4F7). The electron impact voltage fragments the molecular ions
of 502 and 507, resulting in their absence from the mass spectrum. The mass spectra of all 3 chloropropanols-heptafluorobutyryl esters exhibit a base peak (largest abundance) at m/z 169. The 3-MCPD-(2-OC4F7) spectrum also exhibits a characteristic fragment of m/z 453, due to the loss of 49 CH2-Cl (m/z 502 - 49). The 3-MCPD-\(d_5\)-(2-OC4F7) exhibits a characteristic fragment of m/z 456, due to the loss of CD2-Cl (m/z 507 - 51).

The samples listed in Table 1 and Table 2 were analyzed by GC-MS.

![Chemical structure](image)

**Figure 30:** The volatile 3-MCPD-HFBI derivative formed for GC-MS and GC-FID analysis. Adapted from Divanova 2004

Figure 31c displays the correctly labeled spectrum of the 3-MCPD-(2-OC4F7) [HFBI] derivative. Figures 31a, 31b, and 31c, illustrate the correct mass designation for the 169 peak, which was incorrectly labeled as 100 in Figure 4 & 2, pp 462, 463 in Brereton 2001.
Figure 31: (a) Mass spectra of HFBI derivative for 1,3-DCP; (b) mass spectra of HFBI derivative for 3-MCPD-\textit{d}_5; (c) mass spectra of HFBI derivative for 3-MCPD, with correct label for mass/charge (m/z) ion of 169.
5.4. Analytical Testing using Gas Chromatography-Flame Ionization Detector

All samples listed in Table 3, Table 4, Table 5, Table 6, and Table 7 were analyzed by the new GC-FID methodology. Repeatability runs were performed with a standard solution at the middle of the calibration ranges for 3-MCPD (10.06 µg/g), 1,3-DCP (2.16 µg/g) and 3-MCPD-$d_5$ (10.40 µg/g). The purpose was to check the precision of using the automatic liquid sampler with the 100 µl inserts. In order to accommodate this configuration, it was necessary to adjust the isooctane reconstitution from 100 to 200 µl and from a 1 to 2 µl injection. It was found that the automatic liquid sampler allowed triplicate injections for each vial over a two hour timeframe.

The relative standard deviation of the triplicate runs for each compound was:

3-MCPD = 0.92, 3-MCPD-$d_5$ = 0.85, and 1,3-DCP = 6.61.

Figure 32 shows the resolution improvement for baseline separation of the 3-MCPD-$d_5$ internal standard peak form the 3-MCPD peak.
Figure 32: 3-MCPD-$d_5$ internal standard peak 3-MCPD peak improved baseline resolution separation with New GC-FID method
5.5. Migration Testing of Paperboard with Polyethylene-extrusion coating and without PE Coating: Part 1

The PE-coated and uncoated paperboard samples for the Part 1 migration testing were designed for optimized paperboard structure and wet-strength resin composition by the manufacturer to reduce migration of chloropropanols, enabling conformance and acceptability into the European market for paperboard in contact with foodstuffs. In Section 5.5.1, the results presented are from Part 1 of our research which used only the EU Standard method of cold water total immersion extraction (shown as the Control in Figure 29 of Section 5.1) and was performed on the samples listed in Table 1 and Table 2.

5.5.1. Total Immersion of Polyethylene extrusion-coated commercial paperboard cartons: Part 1

The samples listed in Table 1 were analyzed by the gas chromatography-mass spectrometry (GC-MS) method which is described in Section 4.7. The details of the GC-MS chromatographic peaks and specific mass spectral ions for 3-chloro-1,2-propanediol (3-MCPD) and 1,3-dichloropropanol (1,3-DCP) are presented in Section 5.4.

Table 10 shows the 3-MCPD and 1,3-DCP concentrations found in the total immersion aqueous extracts of the three different polyethylene extrusion-coated
paperboard carton samples with Kymene® wet-strength resin. The 3-MCPD levels found in the paperboard ranged from 4,360 to 9,970 µg/kg (ppb). The results demonstrate that the levels far exceed both the US FDA regulatory limit of 1 mg/kg (equivalent to 1000 µg/kg), and the EU Commission of 300 µg/kg (converting the 12 ng/l for the sample size and extraction volume this cold water extraction method). The regulatory limits were discussed in detail in Section 2.4. There was no trace of 1,3-DCP detected in any of the extract samples, with a limit of detection (LOD) for this method of 2 µg/kg (ppb).

The aqueous extract results shown in Table 10 and Figure 35 also confirm that the higher Kymene® wet-strength resin loading in the V Grade paperboard result in higher amounts of 3-MCPD concentrations in the wet-strength resin coated paperboard.

These samples shown in Table 10 are commercially manufactured and printed with the product graphics on the outside of these polyethylene-extrusion coated paperboard food beverage packaging as shown in Figure 33. Figure 34 shows a commercially manufactured and printed carton cut up for total immersion extractions without pulping.
Figure 33: Example of Commercial Paperboard Food Beverage carton for Total Immersion Extractions. Printed outside showing.

Figure 34: Paperboard Food Beverage carton sample cut up for Total Immersion Extractions.
Table 10: Aqueous Extracts of PE Coated Commercial Paperboard Carton Samples: GC-MS Results

<table>
<thead>
<tr>
<th>Carton Sample</th>
<th>Concentration in µg/kg w/w (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3-MCPD</td>
</tr>
<tr>
<td><strong>C Grade</strong></td>
<td></td>
</tr>
<tr>
<td>8.5 lbs / ream Kymene®</td>
<td>4,360 ± 270¹</td>
</tr>
<tr>
<td><strong>A Grade</strong></td>
<td></td>
</tr>
<tr>
<td>8.5 lbs / ream Kymene®</td>
<td>4,140 ± 780</td>
</tr>
<tr>
<td><strong>V Grade</strong></td>
<td></td>
</tr>
<tr>
<td>10.0 lbs / ream Kymene®</td>
<td>9,870 ± 170</td>
</tr>
</tbody>
</table>

Figure 35: Total Immersion Testing of PE Coated Commercial Paperboard under Control Standard Conditions

1 Results are the mean of ± S.D. (n = 3)
2 n.d. = not detected, validated LOD = 2 µg/kg (ng/g)
Figure 36 shows the MS Ion Profiles of the total immersion 100% water extract of V grade carton sample from Table 1, showing the peaks for the HFBI derivatives of 3-MCPD (453,275), and 1,3-DCP (275) which was not detected in the extracts, confirmed by the absence of an m/z 275 peak at scan 298. The mass spectrum of the 3-MCPD peak at scan 359 is attached below the ion profiles to show all of the ions, for comparison.

Figure 36: V-Grade total immersion 100% water extract; (a) m/z 453 extracted ion for 3-MCPD peak at scan 359, (b) m/z 275 extracted ion for 1,3-DCP peak at scan 298 not detected, the 275 ion is a small ion from the 3-MCPD peak is at scan 359; (c) the entire total ion mass spectrum of 3-MCPD peak at scan 359.
The selection of the test cartons listed in Table 1 represent a worse-case scenario of variables chosen for these experiments. They had high Kymene® content, high paperboard basis weight, low gauge (thinner) polyethylene food contact surface, and unskived edge seams. However, they were also designed and processed by the manufacturer to be a barrier to the migration of chloropropanols.

5.5.2. Migration Cell Extractables of Polyethylene extrusion-coated commercial paperboard cartons: Part 1

Table 11 shows the GC-MS results of the migration cell extractions of the polyethylene extrusion coated paperboard cartons listed in Table 1. 3-MCPD and 1,3-DCP were not found in any of the 4 different food simulant solvents used in the migration cell extractions. Furthermore, the unskived edge on the food contact surface did not provide a migration route for any significant amount of 3-MCPD or 1,3-DCP into the any of the food simulant extractables.
Table 11: Food Contact Migration Testing of the Polyethylene Extrusion-Coated Paperboard Cartons for Part 1: GC-MS Results.

<table>
<thead>
<tr>
<th>Carton Sample</th>
<th>100% Water</th>
<th>10% Ethanol in Water</th>
<th>3% Acetic Acid in Water</th>
<th>10% Ethanol + 3% Acetic Acid in Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3-MCPD</td>
<td>1,3-DCP</td>
<td>3-MCPD</td>
<td>1,3-DCP</td>
</tr>
</tbody>
</table>

These experiments demonstrate that these gable-top beverage cartons manufactured with polyethylene extrusion-coated paperboard, designed and processed properly, did not result in the migration of chloropropanols through the polyethylene food contact coating. That is, even though high ppb levels of 3-chloro-1,2-propanediol was found present in the paperboard 3-MCPD it will not migrate through the polyethylene food contact layer and become an indirect food additive, during the normal shelf life of these packaged food beverage products. Therefore, they will be in compliance with both US FDA and EU commission guidelines for paperboard in contact with foodstuffs.

3 n.d. = not detected, validated LOD = 0.30 mg/kg
4 n.d. = not detected, validated LOD = 0.05 mg/kg
5.5.3. **Total Immersion of Uncoated Paperboard With Varied Wet-Strength and Manufacturing Conditions: Part 1**

These other samples from Part 1 of our research were tested by the total immersion extraction method, and not Migration Cell method, because they have not yet been converted into polyethylene extrusion-coated cartons. The samples listed in Table 2 were also analyzed by GC-MS. Table 12, Figure 37, and Figure 38 show the 3-MCPD and 1,3-DCP concentrations found in the aqueous extracts of the four different uncoated paperboard stock carton samples with Kymene® wet-strength resin. The 3-MCPD content ranged from 115 to 426 µg/kg (ppb). There was no trace of 1,3-DCP detected in any of the extract samples, with a limit of detection (LOD) of 2 µg/kg (ppb). The control loading sample had 3-MCPD levels of 3170 µg/kg (ppb), much higher than the test condition samples.

Table 12 also shows the results of paperboard samples without PE coating, the Control 19.5 Wet Strength and the commercial OJ / TTG paperboard. It is shown that the OJ / TTG paperboard was found to contain the highest level of 3-MCPD at 6187 µg/kg (ppb). That sample was also analyzed for epichlorohydrin, to check whether detectable levels of 3-MCPD and also no detectable 1,3-DCP will result in detectable amounts epichlorohydrin (ECH) in the wet-strength resin paperboard. Table 12 includes the ECH results with the GC-MS results for 3-MCPD and 1,3-DCP for the OJ/TTG uncoated paperboard.
The GC-MS method had to be modified as described in experimental section 4.7.3. Since these GC-MS analysis conditions were different than the chloropropanols analysis, we did not perform comprehensive epichlorohydrin studies. This is suggested in the Future Work Section.

**Table 12: Total Immersion Testing of Paperboard Samples without PE coating for Part 1: GC-MS Results.**

<table>
<thead>
<tr>
<th>Carton Sample</th>
<th>3-MCPD (\mu g/kg) (ppb)</th>
<th>1,3-DCP (\mu g/kg) (ppb)</th>
<th>ECH (\mu g/kg) (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.5 Wet Strength (Control)</td>
<td>3170</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #1 / 7% M (2)</td>
<td>248</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #1 / 7% M (5)</td>
<td>222</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #1 / 7% M (7)</td>
<td>190</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #2 / 7% M + IR (2)</td>
<td>228</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #2 / 7% M + IR (5)</td>
<td>426</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #2 / 7% M + IR (7)</td>
<td>411</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #3 / 7% M + IR (2)</td>
<td>151</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #3 / 7% M + IR (5)</td>
<td>115</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #3 / 7% M + IR (7)</td>
<td>135</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #4 / 7% M (2)</td>
<td>169</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #4 / 7% M (5)</td>
<td>222</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Condition #4 / 7% M (7)</td>
<td>226</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>OJ /TTG Barrier baseboard</td>
<td>6187</td>
<td>n.d.</td>
<td>78</td>
</tr>
</tbody>
</table>

5 n.d. = not detected, validated LOD = 2 \(\mu g/kg\) (ppb)

6 n.a. = not analyzed for epichlorohydrin
3-MCPD Levels from Total Immersion Testing of Paperboard without PE Coating for Part 1: GC-MS

![Bar chart showing 3-MCPD concentrations for three samples under four conditions.]

**Figure 37:** Total Immersion Testing Results of Paperboard without PE coating from Part 1: Samples (2), (5), (7) for Conditions #1, #2, #3, #4.
Figure 38: Total Immersion Testing Results of Paperboard without PE Coating from Part 1: Conditions #1, #2, #3, #4 for Samples (2), (5), (7).

Figure 39 illustrates that the OJ / TTG paperboard was found to contain the highest level of 3-MCPD at 6187 µg/kg (ppb). Figure 39 also contains the results of the Control 19.5 wet-strength and the sample with highest level of 3-MCPD from the four conditions, Condition #2, Sample (5).
These three paperboard samples shown in Figure 39, which are without polyethylene coating, were use for the Total Immersion experimental design variable testing in Set 1 of Part 2 in Section 5.6.1.
The data presented in Table 10 and Table 12 demonstrate the manufacturer’s ability to reduce the chloropropanol levels in the paperboard by optimizing the Kymene® wet-strength resin loading, paperboard additives, and other manufacturing parameters. It also confirms that paperboard with high levels of 3-MCPD will have detectable levels of free epichlorohydrin. It also infers that this low level of ECH is not due to equilibrium conversion of 1,3-DCP from ECH, as stated in Boden 1997. Otherwise, some level above 10 ppb of 1,3-DCP should have been detected, and the levels of ECH should have been found a levels above 500 ppb.

The reason the 1,3-DCP was not detected in any of the samples shown in Table 10 and Table 12 could be due to the evolution of the Kymene® wet-strength resin manufacturing improvements over the years. The Kymene wet-strength resin made by the polyamidoamine-epichlorohydrin (PAAE) reaction has gone through at least three different generations of processing improvements since it’s first use in paper and paperboard products. This is also an area that needs further work, as discussed in the Future Work, Section 7.
5.6. Migration Testing of Polyethylene extrusion Coated Paperboard and Uncoated Paperboard with Experimental Design Variables: Part 2

The results presented in this section are from the second part of our research in which the parameters of the European Standard method of cold water total immersion extraction was varied to form the experimental design on the paperboard samples listed in Table 3.

5.6.1. Total Immersion Migration Testing of Paperboard Cartons with Experimental Variables of Temperature, Pulping, and Food Simulant Solvents

In Figure 29 of Section 5.1 is illustrated the experimental design of the total immersion testing for the migration studies 3-chloro-1,2-propanediol (3-MCPD) from the paperboard made with Kymene® PAAE wet-strength resin. The samples listed in Table 3, designated as Set 1A-C, Set 2, Set 3A-B, Set 5A-B, and Set 6 were analyzed by the newly developed GC-FID method. In Table 5 and Table 7 of Section 4.4, were listed the experimental design variables for:

- the food simulant solvent mixtures;
- pulping in blender or no pulping;
- room temperature and accelerated temperature;
for the total immersion extractions of the samples for 24 hours.

Since the samples listed were found to contain 3-MCPD and no 1,3-DCP, this work of the research was performed focused on 3-MCPD levels in the total immersion extractions. The carton samples with higher 3-MCPD content and representative of the various carton samples were selected. Below is a description of the experimental purpose of design for total immersion for Set 1, Set 2, Set 3, Set 5, and Set 6 listed in Table 5 and Table 7 of Section 4.4.

Set 1 designed as the initial combination ran to test the research objective of the impact of stronger extracting solvent, higher temperature, and pulping to extract the maximum available amount of 3-MCPD in the paperboard. The GC-FID quantitative results are present in Table 13 and Figure 40.
**Table 13: Total Immersion Testing of Set 1 with Accelerated Conditions of 40 °C for 24 hour and Pulped: GC-FID results.**

<table>
<thead>
<tr>
<th>Set</th>
<th>Description</th>
<th>Food Simulant</th>
<th>Extraction Conditions</th>
<th>3-MCPD µg/kg (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Condition #2 (5) - No PE</td>
<td>100% H₂O</td>
<td>Pulping at 40°C for 24hr</td>
<td>1209 ± 114</td>
</tr>
<tr>
<td></td>
<td>Condition #2 (5) - No PE</td>
<td>10% EtOH</td>
<td>Pulping at 40°C for 24hr</td>
<td>891 ± 149</td>
</tr>
<tr>
<td></td>
<td>Condition #2 (5) - No PE</td>
<td>3% HOAc</td>
<td>Pulping at 40°C for 24hr</td>
<td>803 ± 130</td>
</tr>
<tr>
<td>1B</td>
<td>Blank Board 19.5WS - No PE</td>
<td>100% H₂O</td>
<td>Pulping at 40°C for 24hr</td>
<td>1170 ± 62</td>
</tr>
<tr>
<td></td>
<td>Blank Board 19.5WS - No PE</td>
<td>10% EtOH</td>
<td>Pulping at 40°C for 24hr</td>
<td>1658 ± 306</td>
</tr>
<tr>
<td></td>
<td>Blank Board 19.5WS - No PE</td>
<td>3% HOAc</td>
<td>Pulping at 40°C for 24hr</td>
<td>1250 ± 113</td>
</tr>
<tr>
<td>1C</td>
<td>OJ / TTG Board - No PE</td>
<td>100% H₂O</td>
<td>Pulping at 40°C for 24hr</td>
<td>4131 ± 7</td>
</tr>
<tr>
<td></td>
<td>OJ / TTG Board - No PE</td>
<td>10% EtOH</td>
<td>Pulping at 40°C for 24hr</td>
<td>3922 ± 295</td>
</tr>
<tr>
<td></td>
<td>OJ / TTG Board - No PE</td>
<td>3% HOAc</td>
<td>Pulping at 40°C for 24hr</td>
<td>3815 ± 14</td>
</tr>
</tbody>
</table>
However, these results were acquired two years after the first total immersion extractions were done from Part 1. A comparison of Table 11 and Table 13 for the 3-MCPD level extracted with 100% water, shows an increase for Condition #2 (5) from 426 to 1209 ppb. While the 3-MCPD levels decreased for the Blank Board 19.5WS (3170 down to 1170 ppb) and OJ / TTG Board (6180 down to 4137 ppb). The reasons for the changes could be due to the residual epichlorohydrin forming more 3-MCPD in the Condition 2 board, and the diffusion of the 3-MCPD from the other two boards. This is an area that requires further research, as discussed in the
Future Work section. Discussion, Figures, and diagrams on factors affecting chloropropanol migration in polyethylene coated and uncoated paperboards are present in Section 5.7.

The findings of the Set 1 results, did not clearly demonstrate a significant impact of stronger extracting solvent, higher temperature, and pulping to extract the maximum available amount of 3-MCPD in the paperboard. Therefore, the experimental design was modified. Set 2 was designed to give the indication on the effect of pulping to extraction efficiency at room temperature, with 100 % water and 10 % Ethanol. Table 14 and Figure 41 shows the GC-FID results of the 3-MCPD levels. Figure 41 shows a comparison of those levels from Set 1 and Set 2.
Table 14: Total Immersion Testing of Set 2 at 23 °C for 24 hour of Pulped Boards: GC-FID results.

<table>
<thead>
<tr>
<th>Set</th>
<th>Description</th>
<th>Food Simulant</th>
<th>Extraction Conditions</th>
<th>3-MCPD µg/kg (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A</td>
<td>Blank Board 19.5WS - No PE</td>
<td>100% H₂O</td>
<td>Pulping at 23°C for 24hr</td>
<td>1564 ± 88</td>
</tr>
<tr>
<td></td>
<td>Blank Board 19.5WS - No PE</td>
<td>10% EtOH</td>
<td>Pulping at 23°C for 24hr</td>
<td>1332 ± 112</td>
</tr>
<tr>
<td>2B</td>
<td>OJ / TTG Board - No PE</td>
<td>100% H₂O</td>
<td>Pulping at 23°C for 24hr</td>
<td>3763 ± 8</td>
</tr>
<tr>
<td></td>
<td>OJ / TTG Board - No PE</td>
<td>10% EtOH</td>
<td>Pulping at 23°C for 24hr</td>
<td>3760 ± 40</td>
</tr>
</tbody>
</table>

Figure 41: Total Immersion Testing of Set 2 at 23 °C for 24 hour of Pulped Paperboard without PE Coating for Part 2: GC-FID
Sets 1 & 2: 3-MCPD from Total Immersion 40°C & 23°C for 24 hour of Pulped Paperboard without PE Coating for Part 2

Figure 42: Total Immersion Testing Comparison of Set 1 at 40 ºC and Set 2 at 23 ºC for 24 hour of Pulped Boards: GC-FID results.

The Figure 42 comparisons of Set 1 and Set 2 show that for the OJ / TTG Board, higher temperature did have a significant effect on increasing the 3-MCPD level extracted by approximately 10%. The Blank Board 19.5WS showed the same increase effect for 10% ethanol, but the opposite for 100% water.

Based on the results of Set 1 and Set 2, Set 3 was designed to isolate the effect of pulping and no pulping with 100% water at room temperature.
Table 15: Total Immersion of Set 3 at 23 °C for 24 hour using 100% H₂O of Pulped and No Pulped Board: GC-FID Results.

<table>
<thead>
<tr>
<th>Set</th>
<th>Description</th>
<th>Food Simulant</th>
<th>Extraction Conditions</th>
<th>3-MCPD µg/kg (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A</td>
<td>Blank Board 19.5WS - No PE</td>
<td>100% H₂O</td>
<td>Pulping at 23°C for 24hr</td>
<td>1067 ± 2</td>
</tr>
<tr>
<td></td>
<td>Blank Board 19.5WS - No PE</td>
<td>100% H₂O</td>
<td>No pulping at 23°C for 24hr</td>
<td>1086 ± 54</td>
</tr>
<tr>
<td>3B</td>
<td>OJ / TTG Board - No PE</td>
<td>100% H₂O</td>
<td>Pulping at 23°C for 24hr</td>
<td>3952 ± 45</td>
</tr>
<tr>
<td></td>
<td>OJ / TTG Board - No PE</td>
<td>100% H₂O</td>
<td>No pulping at 23°C for 24hr</td>
<td>4148 ± 43</td>
</tr>
</tbody>
</table>

Figure 43 is a graphical representation of the data in Table 15 for Set 3. The level of 3-MCPD did not increase with pulping. It actually decreased by about 8% for the OJ./ TTG Board. The data shows that pulping has little effect on the extractable level of 3-MCPD for paperboard packaging that is not polyethylene extrusion-coated. Further theoretical discussions are presented in Section 5.7.
Set 5 was performed with the Control method conditions (23°C for 24 hr without pulping), and to add the 10% Ethanol simulant solvent as in Set 2. Also, in Set 5 we used commercial polyethylene extrusion-coated printed and unprinted formed paperboard cartons. Similar to the work done in Part 1 of our research presented in Section 5.5. Another purpose was to compare the results of these same samples to the migration cell extractions of only the polyethylene extrusion-coating layer, to determine whether the 3-MCPD would be predicted to migrate into the food from the paperboard matrix Section 5.6.2. The total immersion testing results for Set 5 are presented in Table 16 and Figure 44.
Table 16: Total Immersion of set 5 at 23 °C for 24 hour of Unpulped Polyethylene Extrusion-Coated Paperboard: GC-FID Results.

<table>
<thead>
<tr>
<th>Set</th>
<th>Description</th>
<th>Food Simulant</th>
<th>Extraction Conditions</th>
<th>3-MCPD µg/kg (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5A</td>
<td>OJ NoPulp - PE Coated Board</td>
<td>100% H₂O</td>
<td>No Pulping at 23°C for 24hr</td>
<td>3145 ± 89</td>
</tr>
<tr>
<td></td>
<td>OJ NoPulp - PE Coated Board</td>
<td>10% EtOH</td>
<td>No Pulping at 23°C for 24hr</td>
<td>4273 ± 121</td>
</tr>
<tr>
<td>5B</td>
<td>Blank OJ - PE Coated Board</td>
<td>100% H₂O</td>
<td>No Pulping at 23°C for 24hr</td>
<td>1081 ± 79</td>
</tr>
<tr>
<td></td>
<td>Blank OJ - PE Coated Board</td>
<td>10% EtOH</td>
<td>No Pulping at 23°C for 24hr</td>
<td>1287 ± 171</td>
</tr>
</tbody>
</table>

Figure 44: Total Immersion of Set 5 at 23 °C for 24 hour in 100% H₂O and 10% EtOH of PE-Coated Paperboard without Pulping: GC-FID.
Table 17: Total Immersion Testing of Set 6 at 40 ºC for 24 hour of Pulped PE-Coated Board: GC-FID Results.

<table>
<thead>
<tr>
<th>Set</th>
<th>Description</th>
<th>Food Simulant</th>
<th>Extraction Conditions</th>
<th>3-MCPD µg/kg (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6A</td>
<td>Blank OJ - PE Coated Board</td>
<td>100% H₂O</td>
<td>Pulping at 40°C for 24hr</td>
<td>978 ± 99</td>
</tr>
<tr>
<td>6B</td>
<td>OJ NoPulp - PE Coated Board</td>
<td>100% H₂O</td>
<td>Pulping at 40°C for 24hr</td>
<td>4727 ± 71</td>
</tr>
</tbody>
</table>

Sets 5 & 6: 3-MCPD from Total Immersion with 100% H₂O at 40ºC of Pulped & 23°C of Unpulped PE Coated Board Cartons

Figure 45: Total Immersion Testing of Set 5 at 23 ºC of Unpulped, and Set 6 at 40 ºC of Pulped, PE-Coated Boards: GC-FID Results.
The results of Set 5 and Set 6 presented in Table 16 and Table 17, show that stronger solvent (10% ethanol), pulping, and higher temperature did significantly increase the amount of 3-MCPD extracted from the polyethylene extrusion-coated paperboards. As opposed to uncoated paperboard, in which these variables had little significant effect.

It should be noted that Set 5 and Set 6 are the polyethylene extrusion-coated paperboard, and demonstrate a greater impact of the 10% Ethanol food simulant solvent on the total amount of MCPD detected. The polyethylene film is a hydrophobic interface and would be expected to impede the extraction of the chloropropanols, as compared to the uncoated paperboard. It seems this effect happens even with the small 1 cm² samples, where the PE side represents about ½ the total immersion extraction surface area. It is interesting to note that increasing the surface area by pulping did not have an effect on the PE-coated boards, similar to the uncoated boards.


This set of migration cell extraction experiments from Part 2 of our research, were designed to determine if the commercial polyethylene (PE) extrusion-coated paperboard formed cartons (printed and unprinted) listed in Table 6. These are commercial polyethylene extrusion-coated printed and unprinted formed
paperboard cartons, not part of the manufacturer’s project designed to optimized the polyamidoamine-epichlorohydrin wet-strength resin for low migration of chloropropanols through the PE-coating. Similar to the work done in Part 1 of our research presented in Section 5.5.2.

These commercial PE-coated paperboard cartons were tested in Set 5 for total immersion extractions and Set 4 for migration through the PE food contact coating. The data presented in Section 5.6.1 of the total immersion extractions of the Set 5 samples indicate that 10% ethanol does extract higher amounts of 3-MCPD from the paperboard matrix. Therefore, 95% Ethanol was added to the list of migration cell food simulant solvents in the Set 4 samples. The 10% ethanol is the US FDA and EU Commission recommended food simulant for aqueous / acidic and alcoholic food beverages. The 95% ethanol is the recommended fatty food simulant, and represents the strongest food simulant extraction solvent. The 95% food simulant would represent a worse-case scenario for the testing of migration of 3-MCPD and 1,3-DCP from the paperboard carton, and through the polyethylene polymer coating matrix, under these accelerated conditions.

The design of the migration cell extractions were set up as stated in Section 5.2. The quantitation of the 3-MCPD is based on the amount detected in µg and the weight of the 51 cm² sample used in the migration cell. The results are expressed as µg / kg (ppb). Table 18 and Figure 46 show results for Set 4 sample, analyzed by the new gas chromatography-flame ionization (GC-FID) method. It
should be noted that 100% H₂O is the cold water extraction solvent for the extraction of paperboard according to the EU Standard method, not an FDA or EU Food Contact Simulant Solvent.

Table 18: Food Contact Migration Testing of the Polyethylene Extrusion-Coated Paperboard Cartons of Set 4: GC-FID Results.

<table>
<thead>
<tr>
<th>Set</th>
<th>Description</th>
<th>Food Simulant Solvent</th>
<th>3-MCPD Conc. µg/kg (ppb)</th>
<th>STD DEV</th>
<th>% RSD</th>
<th>Number, n=</th>
</tr>
</thead>
<tbody>
<tr>
<td>4A</td>
<td>OJ NoPulp – PE Coated Carton</td>
<td>100% H₂O</td>
<td>144</td>
<td>16.4</td>
<td>11.4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>OJ NoPulp – PE Coated Carton</td>
<td>10% EtOH</td>
<td>210</td>
<td>20.4</td>
<td>9.7</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>OJ NoPulp – PE Coated Carton</td>
<td>95% EtOH</td>
<td>185</td>
<td>40.9</td>
<td>22.1</td>
<td>4</td>
</tr>
<tr>
<td>4B</td>
<td>Blank OJ – PE Coated Carton</td>
<td>100% H₂O</td>
<td>62.2</td>
<td>3.3</td>
<td>5.4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Blank OJ – PE Coated Carton</td>
<td>10% EtOH</td>
<td>104</td>
<td>20.9</td>
<td>20.1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Blank OJ – PE Coated Carton</td>
<td>95% EtOH</td>
<td>84.1</td>
<td>22.4</td>
<td>26.6</td>
<td>4</td>
</tr>
</tbody>
</table>
The results of the Set 4 migration cell testing demonstrate that 5 to 10% of the 3-MCPD detected by the total immersion extractions in Section 5.6.1 migrated through the polyethylene extrusion-coating food contact layer of these commercial sets of paperboard beverage cartons. The levels of 210 ppb and 104 ppb are higher than the US FDA and EU Commission guidelines of 50 ppb. The levels are also higher than the 10 ppb limit that are assigned for indirect food additive compounds that have limited toxicological studies published in the literature. As of
the writing of this dissertation, the US State of California has released an opinion on the toxicity of 3-MCPD, discussed in Section 2.5. No further decisions beyond those discussed in 2.4 were found for the US FDA or EU Commission.

The comparison of the migration cell extraction results of the food contact inside surface presented in Table 19 of this Section 5.6.2 and Table 11 of Section 5.5.2, indicate that chloropropanol migration through the polyethylene-extrusion coating food contact layer is dependent on; optimization of the wet-strength resin loading, the manufacturing design, and processing of the paperboard carton structure, in conjunction with polyethylene plastic polymer formulation and application.

The migration studies presented in this research demonstrate that accelerated migration cell testing with the appropriate food simulant should be performed to predict whether the chloropropanols, formed from epichlorohydrin wet-strength resins in paperboard packaging, will migrate through the polyethylene extrusion-coated food contact side and become an indirect food additive in the foodstuffs.
5.7. Relationship of Migration Study Testing Results of the Polyethylene Extrusion-Coated and Uncoated Paperboards to Their Structure

The two polyethylene (PE) coated commercial board samples and one of the uncoated paperboard samples analyzed in Section 5.6 were evaluated using Fourier Transform-Infrared Spectroscopy with attenuated total reflectance (FT-IR-ATR), scanning electron microscopy (SEM) of cross-sectional cuts, and optical microscopy with a starch stain indicator. The SEM samples were cut sharply with a razor equipped microtome to give a clean edge cut for cross-sectional analysis. The surfaces of these samples were also observed using the SEM. A separate sample was cut sharply with a razor blade and the edges stained with a potassium iodide stain indicator for starch. The surfaces of uncut samples of the paperboard were directly placed under the ATR attachment on the FT-IR and the infrared spectroscopic absorbances were read. The instruments were described in the Experimental Design section.

5.7.1. Comparison of Polyethylene Coated Food Contact Side: Cross-Section and Surface Structure

Figure 47, Figure 48, Figure 49, and Figure 50 show the polyethylene extrusion-coated food contact surface structure and chemistry of the Part 2, Set 4
samples from Table 19 in Section 5.6.2, which had levels of 3-MCPD at 209 and 104 ppb which migrated through the polyethylene (PE) extrusion-coated paperboards. The PE surface chemistries were evaluated using FT-IR-ATR, and the surfaces and cross-section using SEM, and optical microscopy with a starch stain indicator.

In Figure 47 the FT-IR-ATR spectra clearly show the different in the chemical composition of the Polyethylene (PE) extrusion-coating. The Blank OJ PE coating contains a calcium carbonate filler. The PE absorption peaks are clearly visible in the 2900 to 2800 cm\(^{-1}\) region of both spectra.

![Figure 47: Surface Chemistry of PE-Coated OJ NoPulp and Blank OJ Paperboard Cartons by FT-IR-ATR](image-url)
Figure 48 also clearly show the SEM (500x) cross-sectional different coating thickness of the layers in the polyethylene (PE) extrusion-coatings. The Blank OJ PE coating is ½ as thick at 20 microns (µm), and the calcium carbonate particles are also visible. The OJ NoPulp PE coating is much thicker at 40 µm.

Figure 49 is the low magnification SEM (100x), and Figure 50 is the higher magnification SEM (1000x) of the surface coating layers of the polyethylene (PE) extrusion-coatings. The low magnification shows the Blank OJ PE coating is to be more continuous than the OJ NoPulp PE coating. The higher surface magnification confirms that the Blank OJ PE coating is also denser.

Figure 51 shows the optical microscopy of iodine indicator staining of the edges of the OJ NoPulp and Blank OJ paperboard cartons. Observations under the microscope indicated that the starch sizing is applied before PE coating and ink printing to both sides of the PE coated boards. The OJ NoPulp did show some indication of the starch present farther into the paperboard matrix. Lower magnification showed a more uniform application.

These results presented in Section 5.6 show that the OJ NoPulp commercial paperboard carton had close to 5 times the level of 3-MCPD than the Blank OJ paperboard carton. The levels of 3-MCPD migration through the polyethylene food contact layer were only about twice as high. The reasoning for hindered migration of the 3-MCPD is may be explained by the structural evidence presented in these
Figure 48: Cross-Section SEM photomicrographs (500x) of PE Coated OJ NoPulp and Blank OJ Paperboard Cartons
Figure 49: Surface SEM photomicrographs (100x) of PE Coated OJ NoPulp and Blank OJ Paperboard Cartons
Figure 50: Surface SEM photomicrographs (1000x) of both PE Coated OJ NoPulp and Blank Paperboard samples
Figure 51: SEM photomicrographs of PE Coated OJ No Pulp and Blank Paperboard samples from Section 5.6
The above Figures 48 through 51 illustrations help to explain the results presented in Section 5.6. Those results indicated that the OJ No Pulp commercial paperboard carton had close to 5 times the level of 3-MCPD than the Blank OJ paperboard carton. The levels of 3-MCPD migration through the polyethylene food contact layer were only about twice as high. The reasoning for hindered migration of the 3-MCPD may be explained by the thicker PE coating without calcium carbonate filler, smoother more cohesive PE film coating. The presence of starch was detected on both sides of the OJ No Pulp (printed) and the Blank (unprinted) OJ commercial PE coated paperboard samples. However, the starch was not detected throughout the cross-section cuts of the paperboard, and therefore it does not seem that starch would have a significant impact on the migration of 3-MCPD in the PE-coated OJ No Pulp paperboard carton.

The polyethylene extrusion-coated paperboard samples from Part 1, presented in Table 10 of Section 5.5.1, and Table 11 of Section 5.5.2 were analyzed two years prior to the Part 2 work, and were no longer available at the time of the Part 2 research. It is suggested in the Future Work section that these differences in polyethylene extrusion-coating properties of coating thickness, starch levels, surface morphology, and polyamidoamine-epichlorohydrin wet strength resin loading be investigated further with paperboard selected to cover the experimental design boundaries.
5.7.2. **Comparison of Paperboard with Polyethylene Extrusion-Coating and without PE coating**

Figure 52 shows the FT-IR-ATR spectra of the Set 1B and Set 1C uncoated paperboard samples analyzed in Table 13. The IR spectra are consistent with cellulose paperboard without polyethylene extrusion-coating. Figure 53 shows the FT-IR-ATR overlays from Figure 52 for the uncoated paperboard and Figure 47 of the PE Coated commercial paperboard structures. The cellulose is not detected on the surface of the PE coated paperboards.

Figure 54 shows the SEM photomicrographs of the surface of the OJ/TTG paperboard without PE coating, which had the highest levels of 3-MCPD, found at 4727 ppb, from those sets of uncoated boards in the total immersion testing (see Table 16). The SEM photomicrographs show the voids between the wet-strength resin coated cellulosic fibers.

Comparison of the SEM photomicrographs of the OJ/TTG paperboard without PE coating in Figure 54 with the OJ No Pulp and OJ Blank PE coated paperboard cartons in Figure 49 and Figure 50 show the drastic surface differences.

These differences could explain why the 3-MCPD is readily extracted from the uncoated paperboard regardless of the temperature, solvent, or pulping variables. This also may explain why over the 2 year period from the analysis of the OJ /TTG uncoated paperboard the levels of 3-MCPD went from 6187 ppb (Table 12) to 4148...
ppb (Set 3B, Table 15). The free 3-MCPD can move through the voids between fibers and diffuse into the outside environment of the uncoated paperboards.
Figure 52: Surface Chemistry by FT-IR-ATR of Blank Board 19.5WS and OJ/TTG Board without PE Coating samples Presented in Section 5.6

Figure 53: Surface Chemistry by FT-IR-ATR of Blank Board 19.5WS and OJ/TTG Board without PE Coating samples; and PE Coated OJ No Pulp and Blank Paperboard and samples presented in Section 5.6
Figure 54: SEM (100x and 1000x) photomicrographs of OJ/TTG Board without PE Coating presented in Section 5.6
5.7.3. **Theoretical Diagrams for the Migration of Chloropropanols from Polyethylene Coated Paperboard in the Total Immersion and Migration Cell Studies**

The results of the migration studies and preceding discussions demonstrate that the migration of the chloropropanols into the foods is dependent on the polyethylene (PE) extrusion-coating. The PE does form a barrier that has been shown to function in prohibiting the chloropropanols from migrating out of the paperboard into the food simulant above low ppb levels. We have also shown that commercial PE extrusion-coated paperboard cartons can have migration above the generally acceptable levels for indirect food additives of 50 ppb. Figure 55 illustrates the diffusion of the chloropropanols through the PE food contact layer in the migration cell. The chloropropanols diffuse through the voids between the wet-strength resin coated cellulose fibers and slowly diffuse into the PE layer. Use of the food simulant under accelerated conditions penetrates the polymer to allow the chloropropanols to migrate into the food simulant. We have shown that if the 3-MCPD is prone to migrate through the PE food contact layer, it will do even migrate with 100% water simulant. This demonstrates that the 3-MCPD which is highly hydrophilic water soluble, while the PE is highly hydrophobic may have sufficient micro-voids which allow the 3-MCPD to move through and be carried out by the solvent. This is supported by the results which show higher levels of
3-MCPD migrate into the 10% ethanol aqueous / acidic food simulant. The ethanol helps swell the PE polymer to open up the voids and drive faster diffusion with time.

**Figure 55: Diagram of Chloropropanol Migration through the PE Food Contact layer into the Food Simulant in the Migration Cell**

Figure 56 illustrates the diffusion of the chloropropanols through the both sides of the paperboard: the PE food contact layer and the outside of the paperboard, which would have printed ink and coating on the commercial carton. In this case, the chloropropanols diffuse through the voids between the
wet-strength resin coated cellulose fibers, slowly diffuse into the PE layer, but
diffuse much faster through the thinner ink coating. The PE food contact layer is 20
to 40 microns thick, while the ink coating layer is about 2 microns. The ease of
diffusion of the chloropropanols through the ink coating is shown as they move
from Step 1 to Step 2. Figure 56 further illustrates why the accelerated conditions
for the Total Immersion migration studies of temperature, solvent strength and
increased surface area did not have significant effect on blank paperboard without
a PE extrusion-coating layer.
Figure 56: Diagram of Chloropropanol Migration through both the PE Food Contact layer and outside printed (or unprinted) side in the Total Immersion Extractions of the Entire Paperboard
6. CONCLUSIONS

Manufacturers of paperboard used for food packaging are concerned with the chloropropanols content due to potential human health hazards and regulatory concerns. They are required by the US FDA and European (EU) Commission to prove that chloropropanols; in particular, 3-chloro-1,2-propanediol (3-MCPD) and 1,3-dichloropropanol (1,3-DCP) found present in the paperboard structure will not migrate into the food product packaged therein above acceptable levels. The European standard cold water extraction method for paperboard in contact with foodstuffs does not determine the amount of 3-MCPD or 1,3-DCP which will migrate from the paperboard, through the food contact side which is comprised of a polyethylene extrusion-coated film.

This research proves that migration studies conducted under accelerated conditions will predict whether chloropropanols, formed from epichlorohydrin wet-strength resins in paperboard packaging, will migrate through the polyethylene extrusion-coated food contact side and become an indirect food additive in the foodstuffs.

This work demonstrates that the total immersion cold water extraction methodology sanctioned in the EU Standard, while capable of accurately quantifying the residual chloropropanols in the paperboard, must be accompanied by food contact migration cell extractions with the appropriate food simulant
solvent. This will provide the accurate determination of whether chloropropanols detected within the paperboard food packaging will migrate through the polyethylene extrusion-coating barrier into the foodstuffs at significant levels.

These experiments demonstrate that these gable-top beverage paperboard cartons manufactured with polyamidoamine-epichlorohydrin wet strength resin and polyethylene extrusion-coating, designed and processed properly, did not result in the migration of significant amounts of chloropropanols through the polyethylene food contact-side film. That is, even though part per million (ppm) levels of 3-chloro-1,2-propanediol (3-MCPD) were found present in the paperboard, low part per billion (ppb) amounts of 3-MCPD did not migrate through the polyethylene food contact layer and become an indirect food additive, during the normal shelf life of these packaged food beverage products. Therefore, they will be in compliance with both US FDA and EU commission guidelines for paperboard in contact with foodstuffs.

However, we also show that random sampled commercial polyethylene (PE) extrusion-coated paperboard packaging made with epichlorohydrin wet-strength resin, which also had ppm levels of 3-MCPD, did have ppb levels of 3-MCPD migrating into the food simulant. Those PE films were demonstrated to have different additive and film thickness structures. This investigation demonstrates that the construction of the paperboard and polyethylene extrusion-coated food contact film is critical to impart the functional properties needed to perform as a
barrier to migration of chloropropanols into the foodstuffs.

It has been demonstrated here that although the EU Standard required cold water extractions from paperboard cartons containing wet-strength resins made with epichlorohydrin, can yield levels of 3-MCPD significantly higher than specified by the EU commission; this high amount of 3-MCPD can be prevented from migrating through a polyethylene extrusion-coating food contact substance into the food, if properly designed as a functional barrier to chloropropanols migration.

The results from the testing of the variables of the EU Standard total immersion cold water extractions indicate that using a stronger solvent (10% ethanol), pulping to increase the exposed paperboard fiber surface area, and higher extractions temperatures did increase the amount of 3-MCPD extracted from the polyethylene extrusion-coated paperboards.

The results were different for uncoated paperboard, in which modifying extraction variables of the EU Standard total immersion cold water method did not result in a significantly more efficient and effective extraction of chloropropanols from the uncoated paperboard samples. The variables were based on accelerated migration testing conditions of higher temperature; increased paper fiber surface area by pulping in a blender; more aggressive food simulant solvents mixtures for aqueous, acidic, and fatty foods, based on FDA and EU guidelines.

A new GC-FID method was developed for 3-MCPD and 1,3-DCP which would enable routine quality control compliance testing without the expense or expertise
required by a GC-MS. The GC-MS method is more suitable for research, industrial, and contract laboratories of highly technical skill. The GC-FID method validation was demonstrated for reproducibility; repeatability; linearity; system suitability, and derivative reaction product stability during analysis time.

An article was published based on this research in Food Additives and Contaminants June 2010 issue. This would be one of the first which proves exceptions to the rule stated by the searched literature which states that polyethylene is not a functional barrier in some paperboard constructions.
7. FUTURE WORK

The two main methods (Brereton 2001, Hamlet 1997) selected for our work and to perform the research presented in this dissertation for the migration of chloropropanols from food packaging were selected to conform with the US FDA and European Commission standards and specifications. The European Standard for “Paper and Board Intended to Come into Contact with Foodstuffs specifically states that the minimum sample to be used for the cold water extraction method is 10 grams (EU Standard 1993). The German standard, authorized by the European Committee for Standardization (CEN) states that the other countries in the European Union are bound to comply with this regulation (EU Standard 1993, German BLE 2007). The Brereton method complies with both the German BLE standard and the CEN standard for EN 645:1993 (Brereton 2001). That method builds on the deuterated 3-MCPD internal standard method published by Hamlet (1997). We adapted these methods for this research, since they were developed to analyze for chloropropanols in foods. Further work could be performed to scale down and fully adapt the methodology to the much cleaner matrix of the paper and paperboard.

The Part 2 runs were all by the new GC-FID method. Repeatability runs were performed with standard solutions of 3-MCPD, 1,3-DCP, and 3-MCPD-\textit{d}_5. We adjusted the iso-octane reconstitution from 100 to 200 µl and from a 1 to 2 µl
injection. It was found that the automatic liquid sampler allowed triplicate injections for each vial over a two hour timeframe. This was primarily due to the high volatility of the HFBI 3-MCPD derivative. Cooling of the autosampler trays could be explored. Further work could also be done to adapt other published methods (Xu 2006, Boden 1997) which utilize \( N,O\)-bis-(trimethylsilyl)triflouroacetamide (BSTFA) or heptaflourobutyric anhydride derivatization of 3-MCPD and 1,3-DCP may result in more stable derivatives than the HFBI (heptaflourobutyrylimidazole) derivatives, to allow for autosampler runs of large sets of samples using GC-MS or GC-FID.

The EU standard cold water protocol is actually 23°C, and represents room temperature. Cold water for these juice and dairy beverage cartons could be considered to be refrigeration temperature of 4°C. The term of “cold” water could have been used to emphasized extractions without heating. In our research presented here, we varied the standard cold water total immersion extraction method temperature of 23°C to the accelerated temperature of 40°C. The FDA and EU migration cell accelerated testing conditions are 40°C for 10 days. Further experimentation could be done with temperature and time. This along with stronger solvent (10% ethanol) and pulping may result in a higher significant increase in the amount of 3-MCPD extracted from polyethylene extrusion-coated paperboards.

Results were presented that indicated the decreased levels of 3-MCPD in
uncoated paperboard over a two year period. Accelerated stability studies could also be done to confirm whether the mechanism we proposed, the 3-MCPD moves through the voids between fibers and diffuse into the outside environment, is supported by data. The polyethylene extrusion-coating properties of coating thickness, starch presences, surface morphology, and polyamidoamine-epichlorohydrin wet strength resin can also be investigated further.

Select samples of commercial polyethylene extrusion-coated printed and formed paperboard cartons that were found to contain the high levels of 3-MCPD, were also analyzed for epichlorohydrin (ECH) in the wet-strength resin paperboard. We show that high levels of 3-MCPD will have detectable levels of free epichlorohydrin. It also infers that this low level of ECH is not due to equilibrium conversion of 1,3-DCP from ECH, as stated by Boden et al (Boden 1997). Otherwise, some level above 10 ppb of 1,3-DCP should have been detected, and the levels of ECH should have been found a levels above 500 ppb. Further experiments with fully known samples, need to be performed analyzing for the levels of ECH, along with 3-MCPD and 1,3-DCP to further confirm these testing results of the equilibrium phenomenon. The epichlorohydrin work will need to be done by modification of the GC-MS method parameters for analysis.


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