A BIOASSAY-GUIDED APPROACH TO THE ISOLATION AND IDENTIFICATION OF POTENTIALLY-MUTAGENIC COMPOUNDS IN MARINE LIPIDS

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A BIOGEOCHEMICAL APPROACH TO THE ISOLATION AND
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by

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Abstract

A significant amount of the total organically bound chlorine found in the marine environment has not been identified. A large portion of this unidentified organochlorine has been found to be associated with the lipids of marine organisms. If these unknown compounds are biologically active, then their presence may have negative consequences for the animal. Extracts from the blubber of seals from Newfoundland and from the blubber of seals and beluga whales from St. Lawrence River were tested for mutagenic activity using the Ames test before and after GPC fractionation in order to attempt to identify fractions containing mutagenic activity. However, no significant mutagenic activity was present in either case, although the whole lipid extracts were able to cause a higher number of revertant colonies than the fractionated samples. The presence of known organochlorine compounds were confirmed in the beluga whale samples by acid hydrolysis followed by GC/ EI MS analysis. Details of methodologies and results of this work will be presented.

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Abbreviations and Symbols Used

2-AF	2-aminofluorene
BA	bromoacetic acid
BF	bromoform
CA	chloroacetic acid
CF	chloroform
CI	chemical ionization
CPRG	chlorophenol red-β-galactopyranose
DBA	dibromoacetic acid
DCA	dichloroacetic acid
DDE	dichlorodiphenyl dichloroethylene
DDT	dichlorodiphenyl trichloroethane
DFO	Department of Fisheries and Oceans Canada
DMSO	dimethyl sulfoxide
EI	electron impact ionization
EOCl	extractable organochlorine
EOX	extractable organically bound halogen
g	gram
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
GC/EI MS	gas chromatography/electron impact mass spectrometry
GPC	gel permeation chromatography

HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
K_{ow}	water-octanol partition coefficient
mg	milligram
min	minute
mL	milliliter
MX	3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone
m/z	mass to charge ratio
NADP	nicotinamide adenine dinucleotide phosphate
ng/g	nanogram/ gram
NIST	National Institute of Standards and Technology
PCBs	polychlorinated biphenyls
PICI	positive ion chemical ionization
SA	sodium azide
TBA	tribromoacetic acid
TCA	trichloroacetic acid
TIC	total ion chromatogram

1 Introduction

1.1 Anthropogenic Organohalogens

Organohalogens, that is, any organic compound containing a carbon-chlorine, fluorine, bromine or iodine bond, are a major concern in the marine environment. Organohalogens have the ability to bioaccumulate in marine organisms because of their lipophilic properties. These compounds are taken in by the organism either through the food chain or from exposure in water. Blubber samples from large marine organisms (i.e. dolphins, seals, whales etc.) contain the highest concentration of organochlorine compounds when compared with other internal tissue samples. Not only is this because of the lipophilic nature of organochlorines, but also in part because of the lack of enzymatic activity in the storage lipids of the blubber [1-4].

With the extensive use of chlorine by man, it is not surprising that organochlorine compounds are of interest. There are a wide variety of organochlorine compounds that have been made by man for a variety of purposes. Polychlorinated biphenyls (PCBs) were made in the 1920's for certain industrial processes. Hexachlorocyclohexane (HCH), and other organochlorines, have been used as pesticides since the 1930's. Organochlorines are also used as solvents and in plastics [5].

Non-commercial use of chlorine is also a problem. Chlorine is used in the pulp and paper industry as a bleaching reagent and by many municipalities for water disinfection [6].

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The high usage of organochlorine compounds is not the only reason to study them. Some organochlorines have been found to have toxic properties. For example, certain chlorinated pesticides were found to cause fatalities in some species of birds.

Long-term exposure to organochlorines is also a health concern. It has been estimated that at least 5% of anthropogenic compounds in any ecosystem may be mutagenic. These mutagenic compounds are usually carcinogenic and cause tumor formation in animals and humans. Mutagenic activity can be found in drinking water samples that were disinfected with chlorine [7], chlorinated pulp and paper effluent [8], tissues from marine plants and animals [9], as well as other sources.

Estrogenicity is another health problem linked to organochlorine compounds. The estrogenic activity of these contaminants has been linked to certain cancers, damage to reproductive systems and development problems in humans and animals [10, 11].

1.2 Natural Organohalogens

It is largely believed that all organochlorine compounds found in the environment are anthropogenic (man-made) compounds and extremely dangerous. However, this is not the case. A wide variety of organo-chlorine, bromine and iodine compounds are made in nature and must be considered when studying organohalogens in the environment. Currently, over 2,000 naturally occurring organohalogen compounds have been identified [12]. The majority of naturally occurring organohalogens is found in the marine environment and is produced by different types of algae [13].

1.3 Lipid and Organochlorine Extraction

The method of lipid extraction was chosen based on a few criteria: overall speed of the method, efficiency for extracting lipids and organochlorine compounds and the solvents used. Commonly used methods such as Soxhlet extraction and Bligh-Dyer extraction did not fit these criteria. Soxhlet extraction is very time consuming and Bligh-Dyer extraction uses chloroform as a solvent. Since the focus of this research was to study organochlorine compounds in marine lipids, introducing an outside source of chlorine, such as chloroform, was thought to be inappropriate. To overcome the time limitation of the Soxhlet extraction method, a polytron apparatus was used. The polytron homogenizes and sonicates the sample for quick and easy extraction. Various solvent systems for lipid and organochlorine extraction have been studied using shrimp tissues [14]. A mixture of 1:1 hexane/acetone was found to have a high efficiency for lipid and organochlorine extraction while keeping the procedural blank level of organohalogens low.

1.4 Unidentified Extractable Organochlorine

Many anthropogenic organochlorine compounds have been identified in marine samples. However, these identified compounds account for only 10-15% of the total extractable chlorine (EOCl) found in these samples. Wesen *et al.* [15] used gel permeation chromatography (GPC) to study the molecular weight of the EOCl. Approximately 60% of the EOCl was associated with matter having a molecular weight greater than 300 and was found to co-elute with triacylglycerols from rainbow trout [16].

Therefore, it is possible that chlorinated fatty acids bound in acylglycerols may contribute to the unknown component of EOCl in marine organism and sediment samples.

The biological activity (toxicity, mutagenicity, estrogenicity etc.) of the identified organochlorine compounds has been well documented, while the possibility of biological activity of the unidentified components has been neglected. Therefore, the testing for biological activity of lipid samples after the removal of the identified contaminants may provide insight in the possible health and environmental effects the unidentified EOCl may have. Gel permeation chromatography can be used for this separation. Cleanup of chlorinated pesticides and PCBs from fish lipids has been accomplished using GPC [17]. The resulting fractions associated with the lipids of the sample can be tested for biological activity using a variety of bioassays and compared to the biological activity of the whole lipid.

1.5 Lipid Hydrolysis

Another way of separating persistent organic pollutants from lipids is to use a concentrated acid (i.e. concentrated sulfuric acid) to hydrolyze the lipids. Adding concentrated sulfuric acid to the lipid sample will break the triacylglycerols of the lipid into the protonated form of its free fatty acids. When extracted with hexane, the protonated free fatty acids will remain in the polar phase while the nonpolar phase extracted will contain any nonpolar contaminants present. PCBs and various chlordanes are a couple examples of contaminants that have been identified using this procedure by gas chromatography/mass spectrometry (GC/MS).

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After the nonpolar solvent extraction, the polar phase is then neutralized to a pH of ~7, which reverts the fatty acids back to their nonpolar form. Another nonpolar extraction will yield the fatty acids of the triacylglycerols and any nonpolar contaminants that may be present. This fraction undergoes one extra step before GC/MS analysis, unlike the first extraction. The fatty acids must be derivatized to a more volatile form. The most common derivative used is the methyl esters of the fatty acids.

1.6 Lipid Derivitization

There are several different methods of converting fatty acids to their methyl ester derivatives, many reviewed by William Christie [18]. Christie states that using methanolic hydrogen chloride (5%) is probably the best all-purpose esterifying agent. However, as mentioned before, keeping the addition of chlorinated compounds to a minimum was a goal of this research and so an alternative method was used. Christie also mentions that a 1-2% (v/v) of concentrated sulfuric acid with methanol can be used to transesterify lipids in the same manner as the methanolic hydrogen chloride solution. Another advantage the 1-2% sulfuric acid/methanol solution has is its ease of preparation when compared to the preparation of the methanolic hydrogen chloride. However, when using the 1-2% sulfuric acid/methanol solution care must be used to insure that no polyunsaturated fatty acids are decomposed. To protect against this decomposition, the mixture is heated overnight at 50°C instead of being refluxed for a few hours.

$$RCOOR' + CH_3OH \xrightarrow{\quad H^+ \quad} RCOOCH_3 + R'OH$$

Figure 1.1 Formation of Methyl Esters Under Acidic Conditions

1.7 Sample Selection

Seal from several areas off the coast of Newfoundland and seal and beluga whale from the St. Lawrence River were used in this research. There were several reasons for these choices. First, marine mammals have been used as biomonitors all over the world. Second, both species are at high trophic levels of the food chain, allowing them to bioaccumulate organohalogens at high levels. Third, they have long life spans, which also enable them to accumulate high levels of organohalogens. Finally, due to the extensive previous studies of both species, samples were easy to obtain from other researchers.

Seal samples from the St. Lawrence River were used to compare with samples obtained from the Newfoundland areas. The samples from the St. Lawrence River area were thought to have a higher potential for mutagenic activity because of the higher level of pollution associated with the St. Lawrence River. Beluga whale samples were used for testing because, although extensive research has been conducted concerning the levels of organochlorines present in beluga whales, not much work has been done on the possible biological activity these samples may elicit.[4]

1.8 Ames Test

The Ames test is a bacterial assay used to test for mutagenic activity. The Ames test has been used to test a wide variety of chemicals and samples for mutagenicity. In the environment alone it has been used to test for mutagenicity in drinking water samples, animal and plant samples, and soil and sediment samples just to name a few. With proper

training and experience, the Ames test can be used as a quick and effective screening method for mutagenic activity.

The assay uses a series of genetically modified *Salmonella typhimurium* bacteria strains. The bacteria strains are modified so that they require histidine to grow. However, each strain contains a different modification in the histidine operon, which allows the different strains to detect different types of mutations.

Test compounds are combined with the bacteria strain in a melted agar solution and poured onto minimal glucose agar plates. These plates are incubated for 48h at 37°C and individual colonies are counted. Mutagenic compounds have the ability to revert the modified bacteria back to its wild form thus allowing the bacteria to grow without the presence of histidine. The number of colonies is then compared to the number of spontaneous revertants that have grown on the control plates. A sample is considered to be mutagenic when the number of colonies on its plate is at least double the number of spontaneous revertants on the control plate.

Some mutagens require enzymatic activity to induce their mutagenic effect. This hurdle is overcome by adding rat liver homogenate to the top agar mixture of bacteria and sample. For example, benzo(a)pyrene is not a mutagenic compound but after enzymatic activity, it is converted to several diolepoxides which are strong mutagens that can be detected by the Ames test.

1.9   Summary of Objectives

A large portion of the unidentified organochlorine in marine samples is associated with the lipid content of each type of organism. Although the toxicity and persistence of

known organochlorine compounds is well documented, the biological activity of the unknown fraction has not been studied. Therefore, one of the goals of this work was to compare the biological activity of the whole lipid sample to that of lipid samples that have been cleaned up using GPC. The Ames test was the assay chosen to test for a mutagenic activity.

The second goal of this research was to identify any compounds responsible for a positive mutagenic response. Any samples that produced a positive response in the Ames test were then subjected to further analysis using GC/MS.

Further work was conducted using a variety of solvent/solvent extractions on hydrolyzed lipid samples. The goal of this procedure was to remove the lipids from any nonpolar organochlorine compounds that may be present. These compounds are mainly anthropogenic in nature and belong to the small percentage of identified organochlorine compounds, but were identified using GC/MS as evidence that the samples studied were contaminated.

2 Literature Review

2.1 Organochlorines

The study of organochlorine compounds has been a high priority since their discovery in the environment in the 1950's. Classifying organochlorines can be useful when discussing the different types of compounds. One useful classification is anthropogenic organochlorines *versus* naturally occurring organochlorines.

2.1.1 Anthropogenic Organochlorines

There are many types of anthropogenic organochlorines that are, or have been, used for various purposes. The organochlorine compounds of most interest to lipid chemists are insecticides and PCBs, and these are the ones for which the most information exists [5]. Organochlorines have been used as insecticides since the insecticidal property of 1,2,3,4,5,6-hexachlorocyclohexane (HCH) was discovered in 1933 [19].

Since then, many other organochlorines have been used as pesticides. One compound in particular, DDT (dichlorodiphenyl trichloroethane) had been widely used. DDT was first prepared in the late 19th century but remained under the radar of most chemists until the 1940's when it was found to also have insecticidal properties which led to its widespread use. By the 1960's DDT was linked to fatalities in birds of prey which, in turn, drew attention to other health concerns over the use of these and other organochlorine compounds. Since then, numerous organochlorines have been linked to

various negative responses in the environment. For example, another group of organochlorine compounds, dioxins, has been linked to cancer in humans and are highly toxic to certain animals.

The number of anthropogenic organochlorines studied is staggering. Just in the case of PCBs alone, there are hundreds of possible congeners and, while only some of them are toxic, they are still an important persistent organochlorine pollutant due to the large production volumes, continued widespread release, and their stability against biological and physical degradation [20]. PCBs were introduced in the 1920's for industrial uses that required compounds that were very stable chemically over a wide range of physical properties. Some examples of their applications include uses as plasticizers, hydraulic fluids and heat transfer agents. The large number of PCB congeners and their presence as highly complex mixtures of these congeners present many problems for analytical chemistry and toxicology [5].

Adverse effects from exposure to PCBs and chlorinated pesticides are still being studied today. In 2003, Muscat *et al.* [21] found a link between breast cancer recurrence and the concentration of PCBs in adipose tissue of women with non-metastatic breast cancer in New York.

Although the occurrence of organochlorines in marine and other lipid systems is widespread, it is mainly accidental. Organochlorine insecticides were intentionally introduced into the environment, but their uptake and accumulation by non-target organisms, especially in the marine environment, was not expected. Industrial organochlorines, such as PCBs, were not intentionally spread into the environment and

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their occurrence in it is mainly attributed to the lack of control over their use and disposal [5].

2.1.2 Natural Organochlorines

The idea that natural organochlorines are a major constituent of organochlorine levels is a relatively new one. Naturally occurring organochlorine compounds were identified in the early 1960's, but, as of late, the numbers of identified natural organochlorines has been increasing. In 1968, more than thirty naturally occurring organochlorines were reported. That number grew to more than 1,500 by 1992 [13] and climbed again to 2,400 by 1999 [12]

Naturally occurring organochlorines are usually classified depending on their source, either terrestrial or marine. The main marine source is red algae, but also includes sponges, tunicates, marine worms and some microbes [12]. The terrestrial compounds are usually produced in soils by bacteria, terrestrial plants and by volcanic activity. The majority of naturally occurring organohalogens identified are found in marine organisms.

It has also been found that natural enzymatic, thermal and other processes occurring in the oceans, soils and atmosphere are constantly producing halogenated compounds, such as chlorinated and brominated phenols, and dioxin, *etc.* Before this evidence was reported, many of these chemicals were thought to be of an anthropogenic origin only [13].

Research suggests that natural processes have been responsible for the production of halogenated compounds and have been important part of our world for at least

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thousands of years. Organohalogen compounds associated with fulvic acids were isolated from groundwater samples that date back 1,300, 4,600 and 5,200 years. Also, organohalogens have been found in sediments that date back to the 13th century. A piece of evidence that may date organohalogen compounds even farther back than this has to do with microfossils found in Precambrian rocks. These microfossils are identical to the blue-algae *Nostoc*, which is a present day source of organohalogen compounds, and it could be speculated that it was also a source of organohalogens, dating back billions of years [13].

2.1.3 Unidentified Organochlorines

Studies of known organohalogens, and the measurement of the total extractable organically bound halogen (EOX), have shown that identified compounds make up a small portion of the total EOX [22]. Using neutron activation analysis, the total EOCl can be determined. Comparing these measurements with the total chlorine measurements due to known compounds, such as PCBs, it has been found that the identified pollutants attribute for only 10-15% of the total EOCl. For example, PCBs and chlorinated pesticides accounted for <1% of the total extractable organochlorines in mussel tissues from the Kentucky Lake and Kentucky Dam tailwater [23].

Evidence has shown that approximately 60% of EOCl was found to have a molecular weight greater than 300 Da, and, using GPC, was found to elute together with triacyglycerols of fish lipids. It was also found that 60-80% of organically-bound bromine in marine fish was associated with brominated fatty acids [15]. It is thought that chlorinated and brominated compounds may have similar fates in the marine environment

and that the majority of unidentified organochlorine compounds may be chlorinated fatty acids and bound in acylglycerols.

2.1.4   Formation of Chlorinated Fatty Acids through Chlorine Bleaching

Chlorinated fatty acids can be formed in a variety of ways. Bleaching processes, either of wood pulp or flour, have been known to produce chlorinated fatty acids. Also, disinfection of drinking-water by chlorination is also a known source of chlorinated fatty acids. Photochemical degradation of chlorinated pollutants can result in the chlorination of unsaturated fatty acids. Man-made sources are not the only possible way to form chlorinated fatty acids; natural enzymatic processes in certain marine organisms can also chlorinate unsaturated fatty acids to produce chlorinated fatty acids [6].

2.1.4.1   Bleaching of Wood Pulp

During the production of bleached paper, chlorine has been used to oxidize and remove lignins from the wood pulp. If the wood is not washed sufficiently before this step, unsaturated fatty acids present in the wood pulp can be carried over and exposed to chlorine. The most common unsaturated fatty acids found in wood pulp are oleic acid and linoleic acid. The chlorination of these unsaturated acids can form dichlorostearic acid, which has been found in bleached pulp mill effluents. Also, tetrachlorostearic acid and chlorohydrin derivatives of stearic acid have been found, which implies that a variety of chlorinated fatty acids can be formed during the pulp bleaching processes [6].

2.1.4.2 Bleaching of Flour

Chlorine is used to bleach flour to improve the baking quality of some products. Chlorine gas is used to bleach the flour and it has been found that 98% of the chlorine used is consumed by oxidation or chlorination reactions with the constituents of the flour. When radioactive chlorine was used to study the chlorination of flour; 34% of the ^{36}Cl was found to be associated with the lipids of the flour. The major chlorinated fatty acids found in flour are dichlorooctadecenoic and chlorohydroxyoctadecenoic acids. Others have been found though, such as dichlorostearic acid, tetrachlorostearic acid and trichlorohydroxystearic acid [6].

2.1.4.3 Water Disinfection by Chlorine

Drinking-water has been disinfected since the 19th century. The need to disinfect drinking-water is obvious and one of the main methods of disinfection is the use of chlorine. Chlorine used in disinfection reacts quickly with many organic compounds found in water. The study of this class of compounds, known as disinfection by-products (DBPs), has grown recently. Studies have revealed a relationship between exposure to the chlorinated disinfection by-products, in particular trihalomethanes (THMs), and a rise in human cancer mortality [7].

Unsaturated fatty acids found in the water are quickly consumed in chlorination processes to form a variety of chlorinated fatty acids. Chlorine addition to the lipids is directly proportional to the number of double bonds contained by the lipids; though typically, free fatty acids more rapidly incorporate chlorine than their respective methyl esters or triacylglycerols [6].

2.1.5 Analysis of Chlorinated Fatty Acids

Gas chromatography with a variety of detectors has been used extensively for the analysis of chlorinated fatty acids. Wesen *et al.* [24] used an electrolytic conductivity detector for a large portion of their work. Using reference chlorinated compounds they were able to account for, but not identify, 70-90% of the total EOCl.

Sudin *et al.* [25] used an electron capture detector to analyze chlorinated and non-chlorinated fatty acid methyl esters. The detector was able to respond to dichlorinated samples but not monochlorinated ones. They also used a flame ionization detector to compare the retention times of the chlorinated fatty acid methyl esters to the non-chlorinated fatty acid methyl esters. They found that, when using a nonpolar column such as DB5, the addition of one chlorine atom increased the retention time of the compound to the same extent as the addition of two methylene units. This difference in retention time allowed them to tell the difference between monochlorinated compounds and non-chlorinated compounds [25].

Mass spectrometers have been used with varying success to identify chlorinated fatty acids. White *et al.* used a GC/EI MS to assist with identification of the fatty acid chlorohydrins in jellyfish. No molecular ion was observed but the extensive fragmentation pattern, coupled with the comparison of retention times of standards, aided in the identification of chlorohydroxypalmitic acid isomers and chlorohydroxystearic acid isomers [26].

Electron impact ionization is not an ideal method of ionization for the analysis of chlorinated compounds. The high energy ionization causes extensive fragmentation.

Also, EI usually results in immediate loss of any chlorine atoms on the chlorinated fatty acids. The resulting spectra look very similar to that of their respective fatty acid methyl esters.

Sundin *et al* [25]. decided to investigate chemical ionization, a softer ionization method, to see if it would provide more useful information for the analysis of chlorinated fatty acids. Using ammonia gas as the reagent gas in positive ion chemical ionization (PICI), they were able to observe molecular adduct ions $[MH + NH_3]^+$ with the correct isotope ratio for chlorine [27].

2.2   Toxicology of Organochlorines

The toxicology of organochlorines is an important area of study as organochlorines are found throughout our environment and are accumulated by many animals. Acute toxic effects are responses that occur within a few days of exposure to the organochlorine, while chronic effects deal with the consequences of long term exposure to the organochlorines.

The majority of the studies done have focused on acute effects even though the concentrations of organochlorines present in the environment are much lower than is required to cause them. The problem studying long-term effects is that the indicators of exposure are only revealed after a long time after an initial single incident of exposure or after chronic low-level exposure to the organochlorines. Another problem with predicting or quantifying long term effects is the high level of unidentified organochlorine compounds in the environment. It is impossible to test the long term effects a compound may have if the compound has not been identified [30].

In marine studies, the chronic effects may be more important than the acute effects since most organisms in the marine environment are exposed for a long time to low levels of organochlorines, too low to elicit any acute effects. Two of the main chronic effects that organochlorines may elicit in marine organisms are mutagenicity and estrogenicity. [9, 28-30]

2.2.1 Mutagenicity

The mutagenic capacity of many organochlorines has been identified in various environments. For example, chlorinated compounds from the disinfection of drinking-water and organochlorine compounds accumulated in the tissues of marine animals and plants can all give rise to mutagenic activity.

2.2.1.1 Mutagenicity of Drinking-water

The mutagenic effects of organochlorines in drinking-water have been studied extensively. It was first thought that mutagenic compounds in drinking-water were mainly due to the contamination of the drinking-water sources. The sources of the contamination of the drinking-water were not known but it is assumed that the majority of this contamination is from industrial discharges, municipal discharges, municipal wastewater treatment plant effluents and surface or agricultural run-offs [31-33].

The concerns of drinking-water source contamination are still alive today, but the majority of studies in recent years have focused on drinking-water treated with chlorine. Many of the halogenated compounds found in drinking-water are formed as a result of the reaction of naturally-occurring organic material in the water and the chlorine used for disinfection of pathogenic (disease causing) organisms [31].

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Zhang *et al.* [34] studied the mutagenic activity of river water before and after chlorination. Four samples were taken from the river and the corresponding chlorinated samples were taken from four waterworks and then tested for mutagenicity using two mutagenicity assays. Both the chlorinated and non-chlorinated samples were mutagenic. However, using a dose-relationship curve, it was found that the mutagenic response was higher in the chlorinated samples.

In a study undertaken in southwestern Greece, the mutagenic activity of 30 drinking-water samples from 30 small communities and seven samples from the city of Patras (population 153,000) were tested [7]. It was found that 84% of the samples tested were considered mutagenic and 39% of the samples were considered to show a strong mutagenic response. As might be expected, the mutagenic response was shown to be higher in chlorinated water samples than it was in the untreated water samples.

Kargalioglu *et al.* [28] studied the mutagenic activity of 10 disinfection by-products commonly found in drinking-water samples. These compounds were bromoform (BF), bromoacetic acid (BA), dibromoacetic acid (DBA), tribromoacetic acid (TBA), chloroform (CF), chloroacetic acid (CA), dichloroacetic acid (DCA), trichloroacetic acid (TCA), 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX) and potassium bromate ($KBrO_3$). The mutagenic activity of this set of compounds was ranked as follows MX > BA > DBA > DCA > CA with TBA, TCA, BF and CF not producing a mutagenic response.

2.2.1.2 Mutagenicity in Marine Organisms

The mutagenic organochlorines found in drinking-water as well as other organochlorines, are known to bioaccumulate in marine organisms. Not only is this a problem for the organisms contaminated with these compounds, but can also cause health problems in humans who consume these organisms. It has been estimated that at least 5% of anthropogenic pollutants in the environment are mutagens. As stated previously, the levels of these pollutants in the environment are too low to elicit acute toxicity effects but prolonged exposure is assumed to be a serious problem. Mutagenic compounds in the environment are especially dangerous because their effects can be seen over several generations. Also, many mutagenic compounds are carcinogenic and cause tumor growth in animals and humans. Recent studies have found an increase in the occurence of tumors in fish tissues, and while some of these may be naturally occurring, many are considered a direct result of the pollution of the world's water with mutagenic and carcinogenic compounds [9].

One difficulty in trying to determine the effect of mutagenic compounds in marine organisms is the fact that they can produce a mutagenic response even when present in very low concentrations. There may also be synergistic effects that are challenging to quantify. This makes the identification of individual mutagenic compounds, particularly novel compounds, very difficult. However, even if identification were possible, it is impossible to determine if a compound is mutagenic solely using chemical means. Therefore, a variety of bacterial *in vitro* assays have been created to determine mutagenic

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activity. The Ames test is one such bacterial assay and is the assay used for the majority of environmental samples. It is discussed in detail in section 2.3 [9, 31].

White *et al.* studied the mutagenic activity of dichloromethane extracts from bivalve mollusks in the Saguenay Fjord of Canada. The samples were taken from areas downstream of several aluminum refineries and forestry product industries that were known to release mutagenic compounds into the area. The extracts from the mollusks found downstream of these industrial sites were found to produce a mutagenic response using the SOS Chromotest (a short bacterial mutagenicity assay) [35].

In 1998, White *et al.* again studied the mutagenic activity of the Saguenay Fjord, but also extended it to include samples from the St. Lawrence River system, another highly contaminated water system in Canada. In this study, extracts of fish and macro invertebrates were tested for mutagenic activity again using the SOS Chromotest. Of the 152 samples (120 fish samples and 32 invertebrates) that were tested, approximately 70% of the fish extracts and 88% of the invertebrate samples yielded a positive, marginal or erratic response [36].

Extracts from a wide range of organisms from the Lake Baikal, Russia, and Hornoya Island, Norway, ecosystems were tested for mutagenic activity using the Ames test by Stepanova *et al.* [9]. The organisms tested were plants, plankton, decapods, mollusks, fish, Baikal seals and fish eating birds and their eggs. The majority of extracts from plants, planktons, decapods, mollusks and fish did not produce a mutagenic response. However, the majority of seal samples from the Lake Baikal region did produce a positive mutagenic response. In particular, the majority of the positive

responses were in the female seals of the region. In the fish, bird and bird egg samples, some species produced a mutagenic response, while others didn't. This can be attributed to different areas of migration, different diets and different activity of the detoxification enzyme systems of the species [9].

2.2.1.3 Mutagenicity in Other Environmental Samples

Mutagenic compounds in the environment are not just limited to drinking water and marine organisms. A number of strong mutagens have been identified after the chlorination of softwood kraft pulp. These include 2-chloropropenal, 1,3-dichloracetone and MX. These spent liquors contain a risk to the rivers, lakes and oceans they are released into [8].

Contaminated sediment and soil samples can also be tested for mutagenicity. During the Gulf War, there were major oil spills in the Kuwaiti area. Sato *et al.* [37] tested the oil found in soil samples taken from the contaminated oil fields and oil found in sediment samples from the Kuwaiti coastal environment for mutagenic activity. The Ames test results showed that the mutagenicity of oils in the Kuwaiti environment was not a significant problem.

2.2.2 Estrogenicity

Another rising health concern related to organochlorine pollutants is the estrogenic activity of certain endocrine disrupting compounds. These compounds have been linked to increased incidence of breast, testicular and prostate cancers [10], damage to reproductive systems and development problems in humans and wildlife. Exposure of

male fish to endocrine disrupting compounds can result in testis abnormalities, inhibited testicular growth and the growth of intersex gonads [11, 38-40].

These health problems can be caused by exposure to natural and synthetic hormones and other xenobiotic chemicals found in the environment. Natural estrogens include the female hormones estradiol, estrone and estriol. Synthetic estrogens are pharmaceutical products that are designed to mimic the activity of natural female hormones. Two examples of these are found in birth control pills, ethinylestradiol and diethylstilbestrol. Xenobiotic chemicals are environmental and industrial pollutants that are designed for other purposes but are also able to mimic estrogenic activity [38]. These xenobiotic compounds include, but are not limited to, organochlorine pesticides, PCBs, bisphenol-A and alkylphenolic chemicals [10].

Chlorination can also affect the estrogenicity of a compound. The chlorinated products of 4-nonylphenol [41] and bisphenol-A [30] were tested for estrogenicity using a yeast two-hybrid assay. The binding affinity of the chlorinated bisphenol-A was found to be much greater than it was before chlorination. The opposite was found with the chlorinated products of 4-nonylphenol. After chlorination, estrogenic activity was reduced compared to the estrogenic activity of the non-chlorinated 4-nonylphenol.

This illustrates how difficult it is to determine the estrogenicity of a compound using chemical and structural based methods. Therefore, short-term bioassays are used to determine if a compound exhibits any estrogenic activity. The majority of estrogenic activity testing is performed using a recombinant yeast screen called the yeast estrogen screen (YES) [42]. A brief explanation of the assay follows. The DNA sequence for the

human estrogen receptor is integrated into the yeast *Saccharomyces cerevisae*. When a chemical binds to the human estrogen receptor site, gene transcription takes place and the enzyme β-galactosidase is produced. This enzyme metabolizes a chromogenic substrate, chlorophenol red-β-galactopyranose (CPRG), and it changes color, from yellow to red. This difference in color can be measured by absorbance [10]. Routledge and Sumpter [11] give a detailed description of yeast strain maintenance, preparation of medium components and the assay procedure.

2.3   Ames Test

As mentioned before, the Ames test has been used on the majority of environmental samples tested for mutagenicity [28]. Between the years of its first publication in 1975 [43] and the publication of the revised methods for the Ames test in 1983, over 5000 chemicals had been tested [44]. The correlation between mutagenic and carcinogenic compounds is quite high. At first the correlation was thought to be as high as 90%, but is now believed to be approximately 80%.

The assay uses a series of genetically modified *Salmonella typhimurium* bacteria strains. All strains are histidine dependent (they require histidine to grow), but each strain contains a different mutation in the histidine operon in order to detect different types of mutagens. For example, frame-shift mutations and base-pair substitution mutations are two types of mutations that can be detected using the Ames test. The bacteria also contain other mutations that make them more sensitive to mutagens. The *rfa* mutation causes partial loss of the lipopolysaccharide barrier of the bacteria, allowing larger molecules to penetrate the cell wall. The *uvrB* mutation is a deletion of a gene that codes

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for the DNA excision repair system, increasing the sensitivity of the bacteria to detect many mutagens. The last mutation, pKM101, is an R-factor plasmid and has two purposes. First, the pKM101 mutation increases the chemical and spontaneous mutagenesis of the bacteria by enhancing an error-prone DNA repair system that is normally present. This mutation provides increased sensitivity to a large number of mutagens. The second purpose is that it provides the bacteria with a resistance to ampicillin. This resistance to ampicillin aids in the re-isolation of the bacteria strains [44].

The assay is based on the ability of mutagenic compounds to revert the genetically modified bacteria strains back to their wild-type. If a compound is mutagenic and able to cause this reversion, the bacteria will be able to grow without histidine. The test compound is mixed with each bacteria strain using an agar medium and is then poured onto minimal glucose agar plates. These plates are incubated at 37°C for 48h and the individual colonies are counted. The assay also incorporates control plates and positive diagnostic plates. The positive diagnostic plates insure that the bacteria strains are able to detect known mutagens. The control plates are used to count the number of spontaneous revertants that are associated with the bacteria strain. During incubation, some bacteria are able to revert back to their wild-type, without the presence of a mutagen. These colonies are also counted and compared to the test compound plates. A compound is considered to have a positive mutagenic response when the colony count of its plates is at least double the colony count of the control plate.

Some indirect mutagens require metabolic activation to produce a mutagenic effect. The Ames test uses a rat liver homogenate S9 mix to overcome this limitation. Plates containing the test compound, bacteria strain and the S9 mixture are also poured and counted the same way the non-S9 plates are. Again, after incubation, the colonies are counted and compared to the S9 control plate. For example, benzo(a)pyrene is not mutagenic but is converted by liver enzymes to diolepoxides, which are very strong mutagens. Without the S9 mixture, the Ames test would not have detected this class of potential mutagens.

2.4 Bioaccumulation

Organochlorine compounds have the ability to accumulate in the lipids of marine organisms because of their lipophilic properties. These compounds can be accumulated over a period of time, either through the food chain or exposure to chemicals in the water. Bioconcentration is the term applied to the uptake, with little or no elimination, of these chemicals by an organism exposed to contaminated water or food, such that the organism contains a higher concentration of the contaminant than the surrounding environment. Biomagnification is defined as the accumulation of these compounds via the food chain; bioaccumulation encompasses all routes of exposure.

One important way of predicting how a compound will be retained is the water-octanol partition coefficient (K_{ow}). The higher the K_{ow} value, the more likely it will be retained in the lipid. However, if the value is too high the compound may be insoluble in water and not available for aqueous intake. These compounds can be absorbed by particulate matter and be taken in by the organism in this fashion. Other models for

retention prediction take into account the animal growth and uptake and elimination rates along with the K_{ow} factor [45].

As might be expected, concentrations of bioaccumulated compounds are also related to the position of a species in the food chain. In comparing values of PCBs in species from Lake Michigan separated by two trophic levels, i.e. macroinvertebrates to salmon, the Coho and Chinook salmon were found to biomagnify PCBs by 20-30 times [46].

2.5   Marine Organisms as Biomonitors for Organochlorines

Animals that live in areas contaminated with organochlorines are very likely to contain measurable amounts of these same compounds. Therefore, marine animals can be used as biomonitors for contaminants present in a given area.

Mussels have been used to monitor contaminants in many areas. They are useful biomonitors because they are known to accumulate organic contaminants very well. Furthermore, they are static, making them good indicators for specific areas, and their filter-feeding system allows for uptake of contaminants from the water, sediment and particulate matter [47]. Industrial contaminants and pesticides from the St. Lawrence River and the Ottawa River have been monitored by mussels. Mussels from the Ottawa River had concentrations of organochlorines that were 50-75% lower than those of the mussels found in the St. Lawrence River. The high concentrations can be attributed to the pollution the St. Lawrence River receives from the Great Lakes, from tributaries draining a highly industrialized area of New York State, and from municipal and

industrial effluents that are discharged directly into the river from both the Canadian and American shores [47].

In Kentucky Lake and Kentucky Dam tailwater areas, high levels of mortalities of mussels have occurred over the past two decades. These areas also receive industrial wastes like that of the St. Lawrence River region. It was found that the organochlorine levels of the mussels in the Kentucky Dam tailwater area were extremely high and may be the cause of the high mortality rate of the mussels [23].

In California, USA, the mussel is used as a long-term monitor of total DDTs, total PCBs and total chlordanes. The California State Mussel Watch program collected mussel samples from 378 stations along the coast of California for 15 years, from 1977-1992. This program was instituted by the State Water Resources Control Board and the Department of Fish and Game to measure long-term trends of these particular organochlorines. Approximately half of the stations sampled showed declines in the concentration of DDTs and chlordanes, while a decline in PCB concentration was noticed in approximately 27% of the stations [48, 49].

Organochlorine pesticide levels in fish of the Ganges-Brahmaputra-Meghna Estuary in Bangladesh is a major concern. Each year, one third of Bangladesh is submerged underwater causing pesticides to make their way into ponds, streams, rivers and lakes of Bangladesh. These lakes have high potential as possible sites for fishery, but fish populations have been found to be declining due to the environmental contamination and disruption of the ecosystem. The Ganges Perch, *Lates carcarifer*, was selected as a bio-indicator for the region as it is a carnivorous fish with a high commercial value.

The concentration of organochlorines in the perch was found to be related to the lipid content of the fish. Higher levels of residue were found during the dry season of the year due to the higher lipid content in the fish. The pesticides found in the lipids can also be mobilized to the eggs and transferred to the fingerlings after spawning. This burden is possibly too much for the fingerling to survive and may be the cause of the documented decline in fish stocks [50].

This relationship between lipid content and organochlorine pesticide concentrations was also noted in the San Francisco Bay area. Sport fish were collected from the area to determine organochlorine levels. Not surprisingly, of the 13 locations sampled, the greatest concentration of contaminants was found in tissue samples taken from fish near San Francisco's industrial areas. It was also found that fish with high lipid content (croaker and surfperch) in their muscle tissue generally contained higher levels of organic contaminants compared to fish with relatively low lipid levels (halibut and shark) [51].

Marine mammals are also used as monitors of xenobiotic compounds. Their positions at the top of the food chain and long life spans make them important tools for the monitoring of contaminants in the marine environment. Organochlorine compounds are likely to pose a threat to offspring of marine mammals as well. Female marine mammals depurate some of their body burden of organochlorines to their offspring by prenatal transplacental transfer and postnatal transfer by lactation. This is a very significant problem because there is no way to predict the long term-effects these compounds may have on the marine mammal. As a result, marine mammals can be used

as models for long-term effects of environmental pollutants as well as biomonitors of organochlorines [45].

Between June 1987 and March 1988, over 740 bottlenose dolphins of the eastern US coastal migratory stock were found dead along the coastline, apparently overcome by the morbillivirus and/or bacterial infections. Tissue samples taken from these dolphins were found to have higher concentrations of PCBs and chlorinated pesticides than other dolphin species measured at the same time [1].

Weisbrod *et al.*[1] studied the bioaccumulation of white-sided dolphins, pilot whales and their prey in the northwestern Atlantic Ocean region. The white-sided dolphins inhabit waters that are used as a major shipping corridor and are close to the populated and industrialized areas of Boston and New York City. They also have a smaller geographical range than the pilot whales and therefore, may contain higher concentrations of anthropogenic pollutants than the pilot whales that migrate over greater distances. There were three main purposes of the study: to measure the concentrations of 30 PCBs and 20 chlorinated pesticides in seven tissues of stranded white-sided dolphins, pilot whales and their prey; to identify any patterns that may be present between organochlorine bioaccumulation and species, tissues, life stages and genders; and to interpret the bioaccumulation trends into trophic transfer trends for the dolphin and whale species.

As might be expected, due to their restricted geographical range in a highly polluted area, the dolphins were found to have a higher concentration of PCBs, almost

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twice as high, than those found in the pilot whales. However, the concentration of chlorinated pesticides was similar between species.

The same trends were found in the seven tissue samples taken from each species. Of the seven tissue samples taken from the white-sided dolphins, the skin samples generally had the highest concentration of pollutants. However, the highest concentration of organochlorines was found in the blubber of the white-sided dolphins. In pilot whales, the concentration found in skin samples was again lower than the concentration found in blubber samples, but was higher than the six internal organs sampled.

Gender related differences in organochlorine concentration were found in the white-sided dolphins, but not the pilot whales. Male dolphins had higher levels of PCBs and hexachlorocyclohexane isomers.

Although the pilot whale and white-sided dolphin are at the same trophic level and store a similar profile of organochlorine contaminants, the dolphins accumulated higher levels of p,p'-dichlorodiphenyl dichloroethylene (p,p'-DDE) and PCBs, which may be harmful to the animals. These higher concentrations not only arise from exposure due their geographically restricted migration area, but also because of the high level of pollutants their prey is exposed to in the region [1].

Elevated levels of organochlorine contaminants have been linked to reproduction problems and population decreases in the beluga whale of the St. Lawrence River area. The whale population of the St. Lawrence River is isolated from other whale populations of eastern Canada. Studies conducted in the 1980's [52] showed that levels of PCBs in

the belugas of the St. Lawrence River were 25 times higher than those of their eastern Arctic counterparts and the level of mirex to be about 100 times higher.

In 1996, Muir *et al.* [53] studied a wider range of organochlorines in beluga whales and, using reports on the level of organochlorine contaminants in fish of the area, were able to determine the biomagnification factor of the organochlorine compounds for the beluga whales in the region.

As was expected, the whales of the St. Lawrence River region had elevated levels of organochlorines when compared to their Arctic counterparts. One interesting conclusion Muir found was that the biomagnification factors for the Beluga whales of the St. Lawrence River and the Arctic region were very similar. Therefore, the elevated levels of pollutants in the St. Lawrence belugas is from exposure to water with higher levels of contaminants and from the relatively high levels of contaminants found in their prey [53].

Seals have also been used as global indicators for organochlorines. In northeastern Europe, three subspecies of ringed seals were used to compare the pollution levels in three different areas, Lake Saimaa, Finland, Lake Ladoga and the White Sea in Russia. In previous studies, Lake Saimma had the highest level of contaminants, while the White Sea was reported to only have background levels of organochlorines. The same trend was found in the blubber samples of the seals analyzed. The highest concentration of organochlorines was found in the ringed seals of the Lake Saimma region, with the lowest concentration in the seals of the White Sea region [54].

Female seals transfer some of their organochlorines to their offspring. In the 15-20 days it takes to raise and wean a single pup, female seals can reduce their organochlorine loads by 15-30%. Also, approximately 98% of the pup's accumulated organochlorines come from maternal milk that is extremely lipid rich. This almost immediate exposure makes it important to study organochlorine levels in seal pups.

Levels of organochlorines were studied in different organ tissues from seal pups in Norway. Jenssen *et al*[3]. set out to determine the distribution and accumulation of organochlorines in different tissues and organs. It was found that concentration of organochlorines in the liver was about 75% of that found in blubber samples. Only two PCB congeners were found in the brain tissue studied and had a concentration that was only 1% of that found in blubber. The PCB pattern of blood and blubber differed significantly from that found in liver and brain samples. The distribution pattern is dependent on the physio-chemical properties of the different PCB congeners and the lipid content of the different tissues [3].

This distribution was also found in seals sampled from the southern Labrador area. Blubber, liver, kidney and muscle samples from ten harp seals were analyzed for levels of organochlorine and polycyclic aromatic compounds. Again, blubber samples had the highest level of contaminants, almost 100 times higher than that found in muscle tissues. Liver and kidney tissues had intermediate levels of contaminants. [4]

As in dolphins, there were differences in contamination levels related to gender. The level of PCBs and pesticides in the muscle, liver and kidney were 2-3x higher in male seals than they were in the female seals. The concentration of contaminants in the

blubber samples were found to be similar, even though the blubber of the female seals was 30% thicker than that of the males. The concentration of PCB congeners found in the different tissue samples are presented in Table 2.1 and the concentration of other organochlorine compounds are found in Table 2.2 [4].

Table 2-1 PCB Congener Concentrations in Harp Seal Organs (ng/g wet wt.) [4]

No.	Congener	Muscle		Kidney		Liver		Blubber	
		Female	Male	Female	Male	Female	Male	Female	Male
1	8/5	0.00	0.00	0.00	0.00	0.00	0.08	3.10	3.90
2	18	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.38
3	26	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.11
4	31/28	0.03	0.06	0.05	0.07	0.10	0.20	7.25	6.00
6	52	0.15	0.32	0.24	0.45	0.73	1.65	29.00	30.00
7	49	0.04	0.08	0.00	0.12	0.18	0.44	8.80	9.00
8	47/48	0.09	0.15	0.04	0.21	0.33	0.63	15.00	12.00
9	44	0.00	0.03	0.00	0.00	0.00	0.16	3.05	3.40
10	42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17
11	41/71/64	0.00	0.00	0.00	0.00	0.00	0.09	2.65	1.90
12	74	0.14	0.23	0.25	0.46	0.44	1.25	34.00	35.00
13	70/76	0.07	0.12	0.11	0.14	0.34	0.60	13.50	12.00
14	58	0.01	0.02	0.00	0.00	0.06	0.16	3.50	2.90
15	56/60	0.00	0.00	0.00	0.00	0.00	0.08	1.45	1.10
16	95	0.05	0.13	0.03	0.20	0.31	0.75	9.95	12.00
17	91	0.00	0.00	0.00	0.00	0.00	0.10	1.25	1.10
18	84/89	0.04	0.07	0.06	0.11	0.15	0.39	6.95	5.70
19	90/101	0.21	0.47	0.43	0.88	0.98	2.70	39.00	32.00
20	99	0.38	0.84	0.56	1.45	1.60	4.95	67.50	59.00
22	97	0.00	0.00	0.00	0.00	0.00	0.00	0.84	0.93
23	87	0.08	0.14	0.11	0.28	0.35	1.05	15.00	13.00
24	85	0.12	0.28	0.26	0.50	0.63	1.75	25.00	22.00
25	110	0.04	0.11	0.07	0.15	0.24	0.68	10.10	8.00
26	107	0.02	0.04	0.00	0.03	0.07	0.21	3.65	3.00
27	118	0.29	0.60	0.57	1.25	1.20	4.05	48.50	44.00
28	114	0.00	0.00	0.00	0.00	0.00	0.07	0.99	0.75
29	105	0.07	0.11	0.12	0.21	0.27	0.81	10.60	8.80
30	136	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.75
31	151	0.03	0.06	0.00	0.09	0.32	0.94	5.95	3.90
32	144/135	0.00	0.02	0.00	0.00	0.00	0.19	2.60	2.40
33	149	0.04	0.12	0.05	0.22	0.26	0.97	8.65	8.70
35	148	0.06	0.12	0.05	0.18	0.23	0.84	7.85	5.00
36	153	0.91	2.50	1.50	5.25	4.50	19.00	180.00	190.00
37	141	0.02	0.03	0.00	0.00	0.00	0.23	3.45	3.20
38	137	0.02	0.05	0.00	0.06	0.09	0.33	3.65	4.40
39	138/163/164	0.70	1.90	1.20	3.55	3.60	14.00	155.00	160.00
40	158	0.02	0.07	0.00	0.13	0.24	0.40	4.75	3.70
41	129	0.00	0.03	0.00	0.00	0.00	0.12	0.00	0.00
42	128	0.10	0.28	0.00	0.44	0.55	1.85	23.00	25.00
43	156	0.02	0.05	0.00	0.06	0.00	0.39	4.45	5.20
44	157	0.00	0.00	0.00	0.00	0.00	0.15	1.30	0.93
45	179	0.00	0.00	0.00	0.00	0.00	0.00	0.47	0.41
46	178	0.02	0.06	0.00	0.09	0.25	1.01	5.15	5.30
48	187/182	0.13	0.32	0.27	0.66	2.20	9.60	22.50	20.00
49	183	0.04	0.13	0.06	0.27	0.38	1.40	8.60	7.70

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Table 2-2 PCB Congener Concentrations in Harp Seal Organs (Cont.)

50	174	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.47
51	177	0.04	0.08	0.00	0.10	0.45	1.35	6.55	5.20
52	171	0.00	0.00	0.00	0.00	0.00	0.25	1.60	1.50
53	172	0.00	0.00	0.00	0.00	0.00	0.20	1.75	1.20
54	180	0.27	0.54	0.33	1.10	0.95	4.45	32.00	25.00
55	193	0.02	0.04	0.00	0.00	0.00	0.38	2.35	1.80
57	170/190	0.09	0.17	0.00	0.37	0.33	1.55	12.60	9.60
59	199	0.03	0.07	0.00	0.00	0.38	1.39	3.90	3.00
60	196/203	0.00	0.04	0.00	0.00	0.00	0.46	2.30	1.10
61	195	0.00	0.00	0.00	0.00	0.00	0.05	0.33	0.00
62	194	0.00	0.00	0.00	0.00	0.00	0.48	3.25	0.00
63	208	0.00	0.00	0.00	0.00	0.00	0.12	0.23	0.00
Total		4.39	10.48	6.25	19.17	22.71	84.95	866.75	823.60

Table 2-3 Organochlorine Concentrations in Harp Seal Organs (ng/g wet wt.) [4]

No.	Compound	Muscle		Kidney		Liver		Blubber	
		Female	Male	Female	Male	Female	Male	Female	Male
1	HCB	0.86	2.10	1.60	5.30	4.05	11.00	65.00	110.00
2	α-HCH	1.00	2.20	1.70	3.45	3.15	6.60	190.00	230.00
3	β-HCH	0.00	0.00	0.00	0.39	0.27	0.84	26.00	27.00
4	γ-HCH	0.00	0.00	0.00	0.39	0.23	0.55	9.55	10.00
5	Heptachlor	0.00	0.00	0.00	0.00	0.14	0.12	0.53	0.65
6	Aldrin	0.00	0.00	0.00	0.00	0.06	0.05	0.16	0.19
7	OxyChlordane	0.41	0.85	1.90	3.45	5.30	16.00	300.00	390.00
8	trans-Chlordane	0.00	0.00	0.00	0.00	0.05	0.07	1.60	2.50
9	cis-Chlordane	0.00	0.12	0.00	0.00	0.05	0.28	9.60	18.00
10	op'-DDE	0.00	0.00	0.00	0.00	0.02	0.07	0.65	1.10
11	pp'-DDE	3.00	7.20	4.20	11.50	11.00	37.50	460.00	460.00
12	t-Nonachlor	0.86	3.40	2.00	5.30	5.55	21.50	415.00	530.00
13	c-Nonachlor	0.08	0.28	0.12	0.38	0.28	1.15	17.50	27.00
14	op'-DDD	0.00	0.00	0.00	0.00	0.14	0.40	0.81	0.83
15	pp'-DDD	0.12	0.43	0.28	0.75	1.02	3.55	19.50	24.00
16	op'-DDT	0.00	0.00	0.00	0.00	0.53	0.72	4.20	6.90
17	pp'-DDT	0.00	0.08	0.00	0.00	0.04	0.13	38.50	35.00
18	Mirex	0.00	0.00	0.00	0.20	0.20	0.63	1.95	1.75
19	Heptachlor epoxide	0.44	1.50	1.40	2.65	4.60	9.80	61.50	69.00
20	α-Endosulfan	0.00	0.00	0.00	0.00	0.01	0.01	0.04	0.04
21	Dieldrin	0.26	0.92	1.60	4.00	2.55	7.40	52.00	90.00
22	Endrin	0.00	0.00	0.00	0.00	0.02	0.03	1.20	2.60
23	Methoxychlor	0.00	0.00	0.00	0.00	0.07	0.10	0.41	0.68
24	Aroclor 1242	0.00	0.00	0.00	0.00	0.32	1.01	30.50	26.00
25	Aroclor1254	3.80	8.20	8.60	21.50	23.00	73.00	945.00	890.00
26	Aroclor 1260	2.00	4.20	3.50	9.85	9.55	46.00	295.00	240.00
	Total	12.83	31.48	26.90	69.11	72.20	238.51	2946.20	3193.24

In addition to beluga whales, the St. Lawrence River is home to a variety of seal species that have been studied to determine if there were any differences between the ways seal species accumulate organochlorines. Blubber samples from male harbour seals from the St. Lawrence estuary, and from male grey, harp and hooded seals from the Gulf of St. Lawrence were analyzed for PCBs and other organochlorine compounds. There were many differences found between seal species and contaminant levels. PCB and mirex concentrations were found to be higher in harbour seals and DDT concentrations were found to be higher in hooded seals. Harbour and hooded seals had a similar concentration of chlordanes that were higher than that found in gray and harp seals, while harp and grey seals had a similar concentration of HCB that was higher than that in harbour and hooded seals. PCB patterns between the four species were also quite different.

There are a few reasons that can be attributed to these differences in contaminant levels. These species have different migratory patterns, proximity to industrial and agricultural pollution, and diets. The different seal species may also have different abilities to metabolize and eliminate the different compounds [55, 56].

The role of seals and beluga whales in global monitoring of organochlorine contaminants supports the use of both of these organisms for any work concerning organochlorine contaminants in the marine environment.

3   Materials and Method

3.1   Materials

All chemicals used in the preparation of solutions for the Ames test, except where noted, were purchased from Sigma (Sigma-Aldrich Canada, Oakville, Ontario). These chemicals were ampicillin, magnesium sulfate heptahydrate, anhydrous dibasic potassium phosphate, citric acid monohydrate, sodium ammonium phosphate, D-biotin, L-histidine·HCl, sodium chloride, potassium chloride, potassium nitrate, magnesium chloride hexahydrate, sodium dihydrogen phosphate, disodium hydrogen phosphate, anhydrous sodium sulfate, nicotine adenine dinucleotide phosphate, glucose-6-phosphate and glucose. Two mutagenic compounds were used to produce a positive result in the Ames test; these were sodium azide and 2-aminofluorene. The solvents used in this work, all ACS grade, included hexanes, acetone, dimethylsulfoxide, and methanol. Concentrated sulfuric acid and concentrated ammonium hydroxide were ACS grade from Sigma. The fatty acid standards used were myristic acid, stearic acid, and palmitic acid.

Specialized chemicals used in the Ames test were purchased from a variety of sources. Both types of agar for the culture plates, Difco agar and Difco-Bacto, were purchased from Voigt Global (Kansas City, USA). Oxoid Nutrient Broth #2 was obtained from Oxoid (Nepean, Ontario). The rat liver S9 in 0.154M KCl used in metabolic activation of the Ames test was supplied by Moltox (Boone, NC, USA). The genetically altered *Salmonella typhimurium* bacteria strains, TA98 and TA100, came from the Salmonella Genetic Stock Center located at the University of Calgary.

3.2 Sample Collection and Preparation

Muscle and blubber samples from five male seals were provided by Dr. Garry Stenson of Fisheries and Oceans Canada (DFO) in St. John's, NL. Data for the collected seal samples is given in Table 3.1. Location 342 corresponds to an area near St. Anthony, NL, and locations 340 and 339 correspond to the Notre Dame Bay area of Newfoundland. Also, three seal samples and three Beluga whale samples from regions of the St. Lawrence were provided by Dr. Michel Lebeauf from the Institut Maurice-Lamontagne (DFO), Mont Joli, Quebec. Data for the samples can be found in Table 3.2 and 3.3 respectively.

The blubber and muscle was divided into 5g and 10g individual samples that were labeled and stored at −80°C until lipid extraction and further analysis.

Table 3-1 Data of Individual Seals Collected from Newfoundland Region

Sex	ID No.	Year	Month	Day	Location	Weight(kg)	Length(cm)	Girth(cm)	Blubber(cm)	Age
M	19973291	1997	1	28	342	122	169.5	124	3.5	22
M	19973306	1997	2	19	340	112	154.5	124.5	5.8	21
M	19973325	1997	1	28	339	167	183	141	5	31
M	19973338	1997	2	2	342	122	175	131	4.1	10
M	19973362	1997	1	18	342	80	141	110	4	3

Table 3-2 Data of Seal Samples Collected from the St. Lawrence River

Sex	ID No.	Year	Month	Day	Location	Weight(kg)	Length(cm)	Girth(cm)	Blubber(cm)	Age
M	PvB02-01	2002	5	13	Bic	50	125	86	2.5	-
M	PvB02-02	2002	5	13	Bic	63	140	92	2.6	-
M	PvB02-05	2002	10	11	Bic	60	137	93	2.9	-

Table 3-3 Data of Beluga Whale Samples Collected from the St. Lawrence River

Sex	ID No.	Year	Month	Day	Location	Weight(kg)	Length(cm)	Girth(cm)	Blubber(cm)	Age
M	DL-004-2002	2002	7	24	Sainte-Flavie	-	411	-	-	22++
M	DL-007-2002	2002	8	24	Ile Saint-Barnabé	-	408	-	-	23+
M	DL-009-2002	2002	11	8	Baie-des-Sables	-	420	-	-	20+

3.3 Lipid Extraction Methodology

Lipid extractions were performed using a Brinkman polytron, which homogenizes the blubber samples, by ultra high speed mechanical cutting and sonic means, aiding the liquid-solid extraction. Approximately 10g of blubber were extracted with 40mL of a 1:1 hexane/acetone mixture in a cylinder shaped glass tube. The mixture was then homogenized for 5 minutes with the polytron, after which, the solid matter was allowed to settle and the solvent was transferred to a separatory funnel. The extraction procedure was repeated twice more and the extracts were combined in a separatory funnel.

The extracts were then washed using a "nitrate wash" and "acid wash" of sulfuric acid at pH 2 as described by Martinsen *et al.* [57] to remove inorganic halides. This is required if the samples were to be analyzed for total organochlorine at a later time. The "nitrate wash" was prepared by dissolving 0.5g of dibasic ammonium phosphate and 4.5g of potassium nitrate in DDW to 500mL. The "acid wash" was prepared using sulfuric acid diluted to pH 2.

The extracts were washed three times with the "nitrate wash" (30, 20, 20mL). If an emulsion formed, a centrifuge was used to separate the polar and nonpolar phases. The nonpolar extracts were combined and then washed three times with the acid wash

(30, 20, 20mL). Again, the extracts were centrifuged if an emulsion was formed. The nonpolar extracts were combined in an Erlenmeyer flask. These extracts were dried over anhydrous sodium sulfate for 3 hours. After drying, the extract was stored at $-80^{\circ}C$ until further analysis.

The concentration of lipid was determined gravimetrically. One mL of the hexane/acetone extract was transferred to a tared Erlenmeyer flask and allowed to evaporate overnight. The sample was left for no longer than 24 hours to reduce the effects of oxidation on any unsaturated lipids that could be present. Evidence from methylation-GC analyses confirmed that little or no oxidation of the lipids took place under these conditions.

3.4 GPC

GPC fractionation was performed using a Hewlett Packard Series 1050 quaternary pump with a Spectra Chrom CF-1 Fraction Collector. A Perkin Elmer LC-25 Refractive Index detector with a Varien Model 9176 Strip Recorder was used for detection. The column used was a preparative Phenomonex Phenogel Column with a particle size of 5μm, a porosity of 50Å and dimensions of 300mm x 21.2mm. The molecular weight separation range for this column was 100-3000Da. A Phenogel guard column with a particle size of 5μm, a porosity of 50Å and dimensions 50mm x 7.8 mm was employed to protect the preparative column. The packing material for both columns is polystyrene-divinylbenzene.

The lipid extracts were fractionated on this system using a solvent mixture of hexane:acetone 1:1 at a flow rate of 4mL/min. The fraction collector collected forty-one

20mL fractions into tared vials that were then rotary evaporated to remove the hexane and weighed. Any vials containing a residue were then stored at −80°C for further analysis. The fractions collected using this method provided insufficient separation, as the majority of the mass residue was collected in only two vials.

The procedure was then changed so that the fraction collector collected 4mL fractions instead of 20mL fractions. This spread the lipid fractions out into five or six vials, making analysis much easier. Also, instead of rotary evaporating off the solvent, the smaller volume made it possible to use nitrogen blow-down to evaporate the organic solvent. Once the solvent is removed, 0.5mL of dimethyl sulfoxide was added to those vials that were to be carried over to an Ames test. DMSO was used as the solvent for the Ames test because it is considered "bacteria-friendly" to the bacteria strains used. Vials that were to be analyzed further by GC/MS were derivatized into their methyl esters and stored in hexanes at a concentration of 1mg/mL. All vials were then stored at −80°C until needed.

3.5 Ames Test

The Ames test requires the preparation of many sterile solutions before an assay can be performed. The procedure for preparing these solutions will be listed here and a detailed preparation method is provided in Appendix A. All solutions and procedures in the following sections are done according to Ames *et al* [44].

3.5.1 Preparation of Solutions

Vogel-Bonner medium E (50x) is required for the minimal glucose plates. The compounds were added in the following order to 670mL of warm distilled water: 10g of

magnesium sulfate hexahydrate, 100g of citric acid monohydrate, 500g of dibasic anhydrous potassium phosphate and 175g of sodium ammonium phosphate. Each salt was allowed to dissolve before the next was added and the final volume was adjusted to 1L with DDW. The mixture was then poured into two 1L glass bottles and autoclaved, with loose caps, for 20 minutes at 121°C. Once the solutions were cooled, the caps were tightened and the solutions were stored until needed.

A further component required for the preparation of the plates for the Ames test is 0.5mM histidine/biotin which is added to the top agar before use in the mutagenicity assay. To 250mL of distilled water, 30.9mg of D-biotin (MW 247.3Da) and 24.0mg of L-histidine·HCl was added. The water was heated to boiling to help dissolve the biotin. The solution was then autoclaved for 20 minutes at 121°C, then stored in a glass bottle at 4°C.

The top agar is used in the mutagenicity assay as a means to combine the sample, bacteria and S9 mixture together. To make the top agar, 6g of Difco agar and 5g of sodium chloride were dissolved in 1L of distilled water. The agar was dissolved using either a hot plate to warm the DDW. Once the agar was dissolved, the solution was mixed thoroughly and 100mL aliquots were transferred to 250mL glass bottles. These bottles containing 100mL of the agar solution were autoclaved for 20 minutes at 121°C with loose caps. Once the agar cools, the caps were tightened and the bottles were stored until needed.

A salt solution of 1.65M KCl and 0.4M MgCl$_2$ was required for the S9 mix. Distilled water was added to 61.5g of potassium chloride and 40.7g of magnesium

chloride hexahydrate. Once the salts were dissolved, the solution was diluted to 500mL. The salt solution was autoclaved for 20 minutes at 121°C and was stored at room temperature until needed.

A phosphate buffer at pH 7.4 was made from 60mL of a 0.2M sodium dihydrogen phosphate (13.8g per 500mL) and 440mL of 0.2M disodium hydrogen phosphate (14.2g per 500mL). The volumes for these solutions are approximate and the pH should be checked; if it is too low more 0.2M disodium hydrogen phosphate was added until the pH is 7.4. This phosphate buffer was then autoclaved for 20 minutes at 121°C.

The S9 mixture also required a 0.1M nicotinamide adenine dinucleotide phosphate (NADP) solution. Five mL of sterile distilled water was used to dissolve 383mg of NADP. The water was sterilized beforehand since the NADP cannot be autoclaved at the high temperature required. The NADP solution was stored in a conventional freezer and is stable for up to 6 months.

The last solution needed for the S9 mix is a 1M glucose-6-phosphate solution consisting of 2.82g of glucose-6-phosphate dissolved in 10mL of sterile distilled water, sterile water being used for the same reason as cited above. Again, the solution was stored in the freezer and it will remain stable for up to 6 months.

The S9 mix was prepared from rat liver S9 induced by Aroclor-1254 and was made fresh the day of the assay. The solutions were added in the following order so that the rat liver S9 was added to a buffered solution. To 19.75mL of sterile, distilled water, 25mL of 0.2M phosphate buffer, 2mL 0.1M NADP, 0.25mL 1M glucose-6-phosphate, 1mL magnesium chloride-potassium chloride salts and 2mL of the rat liver S9 were

added. This mixture produces a S9 concentration of 4%. A higher concentration of 10% can also be used with two minor changes made to the preparation of the mixture: the water volume is reduced to 16.75mL and the amount of rat liver S9 is increased to 5.0mL. The freshly made S9 mix was stored on ice and must be used that day. Any left over S9 or S9 mix is discarded since it cannot be refrozen and used again.

The genetically altered bacteria strains, TA98 and TA100, are modified to be ampicillin resistant and thus can be isolated on ampicillin plates. Preparation of an ampicillin solution is necessary before making these plates. The solution consists of 0.8g of ampicillin dissolved in 100mL 0.02M sodium hydroxide and it can be stored at 4°C until needed. To make the ampicillin plates, 15g of Difco-Bacto agar was dissolved in 910mL of distilled water and autoclaved for 20 minutes at 121°C. Then, 20mL of 50x Vogel-Bonner solution, 50mL of 40% aqueous glucose solution and 10mL of L-histidine·HCl solution (2g/400mL of distilled water) were added to the hot agar. After the solution is mixed thoroughly and allowed to cool to approximately 50°C, 6mL sterile 0.5mM biotin solution and 3.15mL of the ampicillin solution were added. 30mL of the resulting mixture was poured into Petri plates and the plates were stored at 4°C. These plates should be stable for 2 months; after this time period, the plates were tested for ampicillin activity or discarded and fresh ones made.

Minimal glucose plates are used in the mutagenicity assay. Again, 15g of Difco-Bacto agar was dissolved in 930mL of distilled water and autoclaved for 20 minutes at 121°C. When the solution had cooled slightly, 20mL of the 50x Vogel-Bonner solution

and 50mL of 40% glucose solution were added and mixed well. Aliquots of 30mL of the solution were poured into Petri dishes and stored in a refrigerator.

3.5.2 Procedure for Growing Cultures

Bacteria tester strains are grown in Oxoid nutrient broth No.2. The culture broth was prepared by dissolving 2.5g of the Oxoid nutrient broth in 100mL of distilled water and autoclaving for 20 minutes at 121°C. The volume and mass was scaled depending on the volume required for each strain. The 100mL of nutrient broth stock solution was divided into 50mL aliquots and were transferred to culture flasks. Cultures were inoculated from frozen permanents, which are described in section 3.5.3. The cultures were then incubated in an Innova 4230 Refrigerated/Incubator Shaker at 37°C. To insure the cultures were aerated adequately during incubation, they were shaken at 120rpm. A growth period of 16h was first used but was lowered to a maximum of 12h so that the viability of the culture broth did not decrease. Once the growth period is over, the cultures were stored in the incubator at room temperature until needed.

3.5.3 Storage of the Bacteria Strains

The bacteria tester strains were stored in a −80°C freezer to be used as frozen permanents. A cryoprotective agent, dimethyl sulfoxide, was added to fresh overnight cultures that were grown in Oxoid nutrient broth. To the fresh culture, approximately 0.3-0.4mL of dimethyl sulfoxide was added per 1mL of culture. The flask was swirled gently to dissolve the dimethyl sulfoxide and aliquots were distributed to sterile 1.8mL Nunc cryotubes. The tubes were filled nearly to the top, allowing a little space for

expansion of the solution due to freezing. This reduced the amount of space left vacant at the top of the tube, minimizing oxidative damage. The tubes were then transferred to a −80°C freezer for storage. For each strain, one of these tubes was placed aside and only opened when more frozen permanents were needed.

When re-isolating the strains; the frozen permanent should not be allowed to thaw. Bacteria was scraped off the top of the frozen bacteria solution by a sterile applicator stick and then streaked on the ampicillin plates. It is very important to make sure the tubes do not become contaminated when opened. After 5-10 uses, a tube should be discarded and a new one used.

The frequent use of the same tube can present a problem for contamination, especially if the freezer is located in an area where air contaminants may be high. This problem was overcome by preparing a large number of frozen permanents a few times a year. With this procedure, one frozen permanent was sacrificed for each assay. The frozen permanent was allowed to thaw and a measured volume is used for inoculating the broth. The recommended volume is 20μL/5mL of broth to be inoculated.

3.5.4 Re-isolation of Tester Strains

The two strains used in this work, TA98 and TA100, both contain an R-factor that makes them resistant to ampicillin. To ensure that the cultures inoculated from the streaked plates are the correct bacteria and not airborne contaminants, the strains were streaked on minimal glucose plates enriched with histidine and biotin and containing ampicillin made to the correct concentration as described in section 3.5.1. This ensures that only the strains required were grown on the plates.

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In order to obtain well-isolated colonies on the plate, a method known as the quadrant streak technique was used. This method allowed sequential dilution of the culture broth over the entire surface of the plate. The inoculating loop was sterilized using a Bunsen burner. The loop was placed in the flame until it was red hot and then allowed to cool. The loop was then used to remove a small amount of the culture broth or frozen permanent. It was then immediately streaked with a back and forth motion over a quarter of the plate. After, the loop was flamed again and allowed to cool. Then the loop was taken to the edge of the area that was just streaked and the streaks were extended into a second quarter of the plate. This procedure was repeated twice more, bringing the final streaks into the middle of the plate. The fourth streak should yield single colonies after the dilution. Figure 3.1 illustrates the procedure.
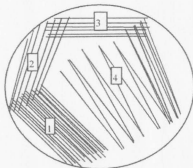
Inoculation of a streak plate
1. Area of initial inoculation and first streaks yields heavy growth.
2. Area of second streaks from area 1 yields less dense growth
3. Area of third streaks from area 2 yields weak growth
4. Area of fourth streaks from area 3 yields single colonies.

Figure 3.1 The Quadrant Streak Method

The plates were then incubated at 37°C for 48h. A well-isolated colony was then picked for overnight growth in the Oxoid nutrient broth.

3.5.5 Top Agar

The top agar (refer to section 3.5.1 for top agar preparation) is the medium in which the test compound, bacteria and S9 mix are all mixed in and then poured on the minimal glucose plates. Prior to the mutagenicity assay, 10mL of the 0.5mM histidine/biotin solution was added to 100mL of the top agar. This trace of histidine allows the histidine dependent bacteria to undergo a few divisions to produce a bacterial background lawn, but is low enough to limit normal colony growth unless the required mutation occurs in the bacteria. Some mutagens require DNA replication for mutagenic activity to be detected and the trace histidine present allows the bacteria to grow and meet this requirement.

3.5.6 The Plate Incorporation Mutagenicity Test

The plate incorporation method mixes the test sample, bacteria strain and S9 mix in a soft agar which was poured onto a minimal glucose plate as the top agar layer and allowed to harden. The plates were then placed in an incubator for 48h at 37°C. After incubation, the number of revertant colonies were counted on each plate and compared to control plates of spontaneous revertants. Spontaneous revertants are bacteria colonies that were able to revert back to their wild form and grow in the absence of a mutagen.

Before the assay is started, all glassware and media was sterilized and a fresh batch of S9 mix prepared. The test samples collected after GPC fractionation dissolved in 0.5mL of dimethylsulfoxide and frozen were thawed out and prepared in the following manner for assay.

The top agar was gently melted in a microwave oven and then placed in a water bath at 45°C. The histidine and biotin are added as described in section 3.5.5 after the agar had cooled. To a sterile tube, 2mL of the agar, 0.1mL of the bacteria culture, 0.1mL of the sample were added in a sterile culture tube and mixed by vortexing gently for 3s. When a sample was to be tested with metabolic activation, 0.5mL of S9 mix was also added to the top agar mixture. The agar was then poured onto a minimal glucose plate and the plate was rotated on an angle to ensure the plate was covered evenly. The mixing, pouring and distribution should take less than 20s, then, the top agar was allowed to harden on a level surface for a few minutes. These guidelines were put in place to make sure that all plates were poured in a consistent manner and that the top agar does not harden midway through the distribution. Once the agar plates were ready, they were placed in the incubator at 37°C for 48h. After the incubation period, the numbers of colonies on each plate was counted.

Control plates were prepared by pouring the top agar containing only dimethyl sulfoxide as the test compound. These plates were used to count the number of spontaneous revertants arising from each strain and used as a reference for that particular assay. A compound is considered to have a positive mutagenic response when the number of colonies on its plate is at least double the number of spontaneous revertants on the control plates. Control plates with dimethyl sulfoxide and S9 are also poured because the presence of the S9 mix can change the number of spontaneous revertants slightly.

Two positive diagnostic chemicals were used to confirm the ability of the bacteria strains to detect mutagens. Sodium azide (SA) was used to confirm the reversion

capability of the TA100 strain and 2-aminofluorene (2-AF) was used to confirm both strains, TA98 and TA100, with the addition of the S9 mix.

When using two bacteria strains, the final number of plates should be; 4 control plates, 4 positive diagnostic plates and 4 plates for each of the test samples. After the 48h, pictures of the plates were taken with a digital camera. The choice to use digital photography was partially driven by the challenge of counting large numbers of colonies by simple visual inspection. Using a digital image of each plate, the image can be enlarged so that the colonies can be counted more easily. An added advantage of this technique is that the images are a permanent a record of the experiments.

3.6 Lipid Derivatization

Before analysis of lipid samples, either whole or fractionated, by GC/MS, they must be saponified, then derivatized to methyl esters. Christie [18] has described many methods for the preparation of these esters; the method used in this work was an acid catalyzed esterification known as a Fischer esterification. In the case of intact acylglcerols, the method is *trans*-esterification, owing to the fact that the fatty acids are already bound to glycerol through an ester bond. [18].

The acid catalyzed *trans*-esterification for the derivatization of lipids uses a solution of 1-2% sulfuric acid in methanol. Ten mL of the solution was added to the lipids and placed in the incubator at $50^{\circ}C$ overnight. Water can hamper the reaction considerably and, thus, a small amount of anhydrous sodium sulfate was added to the vial. After heating, the fatty acid methyl esters were extracted with 5mL of hexane three times. Again, anhydrous sodium sulfate was used to dry the hexane extracts. The hexane

extract was then transferred to a tared vial and the hexane evaporated off under a stream of nitrogen. The fatty acid methyl esters were then re-dissolved in hexane at a concentration of 1mg mL^{-1} as is typical for analysis by GC/MS. The methyl ester derivatives were made from the lipids in each of the fractions produced by GPC.

3.7   GC/EI MS

Analysis of the fatty acid methyl esters of each vial was performed on a HP 5970 GC/MS with a DB5 column. The column had an inner diameter of 0.25mm, was 30m in length and had a film thickness of 0.25µm. One µL of the sample was injected. The temperature program for the GC was as follows: an initial temperature of 50°C which was rapidly increased at a rate of 40°C/min to 150°C and then increased to a final temperature of 230°C at a rate of 2°C/min and then was held at 230°C for 5min. The total run time was 51.50min.

A mass range of 35-500 *m/z* was used with an EI voltage of 70eV. To identify the FAMEs, the mass spectra obtained were compared to the mass spectra available on the internet at the Scottish Crop Research Institute Lipid Library homepage. These pages were produced by William Christie for the use of lipid analysts and can be found at http://www.lipidlibrary.co.uk. To identify any chlorinated compounds that were present, the NIST mass spectra library was used.

3.8   Lipid Hydrolysis – Acid Treatment

Treatment with concentrated sulfuric acid was used to hydrolyze the lipids and in conjunction with an extraction with hexane, can be a means to isolate neutral persistent

organic pollutants from matrix lipids. The addition of the sulfuric acid to the triacyclglycerols hydrolyzed the ester linkage to yield the protonated, positively charged form of their constituent fatty acids. For every 200mg of lipid, 5mL of concentrated sulfuric acid was added. Immediately after the addition of the acid, the persistent nonpolar compounds (i.e. no lipids) were extracted with three 5ml portions of hexane. The combined extract was analyzed by GC/EI MS and should contain any chlorinated pesticides, PCBs, *etc*.

Following the nonpolar extraction, the polar acidic phase was neutralized with dilute ammonium hydroxide. This converted the free fatty acids back to their non-protonated, nonpolar form allowing extraction into a neutral solution. A hexane extraction was done again (5mLx3) to remove the fatty acids. This extract was then derivatized as in section 3.6 and analyzed by GC/EI MS. This analysis was performed as in section 3.7. A flow chart of the whole acid treatment procedure is illustrated in Figure 3.2.
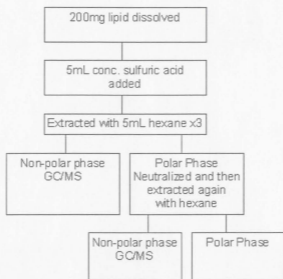
Figure 3.2 Flow chart of Acid Treatment Procedure

4   Results and Discussion

4.1   Lipid Extraction

The percentage of lipid extracted was determined gravimetrically for each sample. These percentages are found in Table 4.1. The lipid extracted from these samples appears to be lower than what is expected when compared to literature values. For example, Zitko et al. [4] reported mean lipid contents between 80-90% for their blubber samples. However, these discrepancies can be attributed to differences in extraction procedure, time of year the samples were taken, and in the ages of the seals sampled. Samples labeled BC3-BC5 correspond to sub-samples taken from Newfoundland seal # 19973325. The seals sampled by Zitko et al. were all approximately 9 months old, which is a significant difference between the ages of the seals sampled for this work (~31 months). Also, the time of year of sampling was much different. The Newfoundland seal 19973325, containing sub-samples BC3-BC5, was collected in January and the seals collected by Zitko et al. were collected in November.

Table 4-1 % Lipid Extracted

Sample Number	% Lipid Extracted
BC3	68.2%
BC4	62.5%
BC5	67.0%
PvB02-01	65.1%
PvB02-02	60.0%
DL-04-2002	67.1%

4.2   GPC

Gel permeation chromatography was used to separate the extracted lipids from any anthropogenic contaminants that may be present in the sample. Previous work has

shown that a high percentage of unidentified chlorinated compounds has been associated with lipid extracts using GPC [14,15]. Therefore, the lipid fractions collected from the GPC procedure were analyzed by GC/MS and tested for mutagenicity using the Ames test. Sample BC3 was the first extract to undergo fractionation. The fraction collector was set to collect 20mL fractions and the lipid peak was found to elute in vials 3 and 4. Figure 4.1 shows the results of one of these separations. The results are shown as a percentage of the total sample injected.

Due to the large size of the fractions collected in the two vials, the fraction collector was later set to collect 1mL fractions. This allowed the lipids to be collected in five vials instead of two, making GC/MS analysis easier. Also, this separation is expected to give more specific information when the Ames test is employed. Lipid extracts from sample BC4 from the Newfoundland area and PvB02-01 from the St. Lawrence River area were separated this way and their results are presented in Figure 4.2 and Figure 4.3 respectively. As can be seen in the figures, for the most part the lipid profiles for the samples were still very similar to that of BC3 despite the smaller fraction sizes. This similarity was expected since both samples were taken from the same Newfoundland seal. The St. Lawrence River seal shows a slightly different lipid profile than the Newfoundland seal samples. This can be attributed to various reasons such as, differences in diet, habitat environment and time of year the sample was taken. Again, the results are shown as a percentage of the total lipid injected.

Fractions that were analyzed by GC/MS were diluted with hexanes to concentrations of 1mg mL^{-1} and fractions to be tested for mutagenicity were dissolved in 0.5mL of DMSO. All fractions were then stored at -80°C.
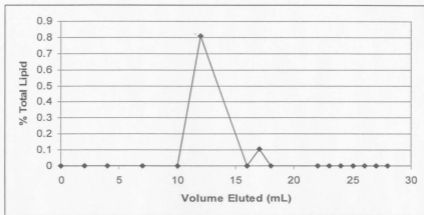
Figure 4-1 GPC Results for Newfoundland Sample BC3
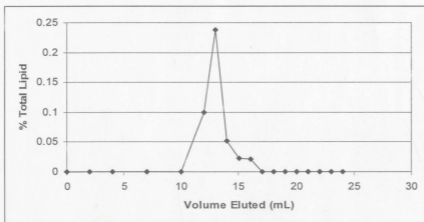
Figure 4-2 GPC Results for Newfoundland Sample BC4
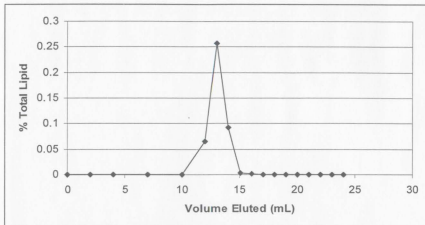
Figure 4-3 GPC Results for St. Lawrence River Sample PvB02-01

4.3 Ames Test

Confirmation of mutagenic activity in the Ames test is based upon the number of bacteria colonies that grow on a series of plates. The test indicates if there are any mutagenic substances in the sample being analyzed but does not indicate that mutagenic events have taken place in the organism sampled. Thus, it is only an indicator of the mutagenic potential of compounds present in a given sample. The assay compares the number of bacteria colonies growing on the test plates to those found on the control plates. A positive mutagenic effect is said to have occurred when the number of bacteria colonies on the test plates is at least double that of spontaneous revertants on the control plates. Results can be expressed as bacteria counts per plate and as a ratio between the number of bacteria colonies on the test plate to the number of spontaneous revertants on the control plate, the convention used in this work.

The fractionated BC3 sample was first tested for mutagenic activity. Vials 12-17, corresponding to the lipid peaks of the GPC separation, were tested. The assay includes plates with known mutagens for the strains TA100, TA98 + S9 and TA100 + S9. Sodium azide was used as the mutagen for TA100, and 2-aminoflourene was used as the mutagen for both strains when testing with metabolic activation by S9. The bacteria counts for each fraction are found in Table 4.2 as an average of all tests performed on the vials and the ratios of each test plate to the control plates are presented in Table 4.3. There was no mutagenic activity detected in the fractionated lipids. There were some elevated bacteria counts, but none were higher than double the control plates. These elevated counts may have arisen from trace quantities of mutagenic compounds in the samples, but they were not present in a concentration high enough to elicit a positive response in the Ames test.

Table 4-2 Average of Revertant Bacteria Colonies from the Ames Test Analysis of the Fractionated BC3 Sample with Metabolic Activation (+S9) and Without (-S9)

	TA 98 - S9	TA 100 - S9	TA 98 + S9	TA100 + S9
Control	15 ± 2	75 ± 3	34 ± 1	102 ± 1
Positive	NA	223 ± 2	818 ± 5	849 ± 6
Fraction 12	15 ± 5	102 ± 3	43 ± 4	111 ± 1
Fraction 13	16 ± 5	108 ±7	44 ± 7	123 ± 6
Fraction 14	10 ± 5	67 ± 1	31 ± 1	119 ± 4
Fraction 15	11 ± 2	77 ± 2	43 ± 2	110 ± 2
Fraction 16	13 ± 1	75 ± 1	35 ± 4	107 ± 3
Fraction 17	11 ± 2	104 ± 4	33 ± 1	103 ± 5

Table 4-3 Ratios of Revertant Colonies on Test Plate to Number of Revertant Colonies on the Control Plates for the Fractionated BC3 Sample

	TA 98 - S9	TA 100 - S9	TA 98 + S9	TA100 + S9
Control	1.0	1.0	1.0	1.0
Positive	NA	3.0	24.1	8.4
Fraction 12	1.0	1.4	1.3	1.1
Fraction 13	1.1	1.4	1.3	1.2
Fraction 14	0.7	0.9	0.9	1.2
Fraction 15	0.8	1.0	1.3	1.1
Fraction 16	0.9	1.0	1.0	1.0
Fraction 17	0.8	1.4	1.0	1.0

After these results, the whole lipids of the Newfoundland seal, St. Lawrence seal and the St. Lawrence beluga whale were tested for mutagenic activity. The GPC fractionation of the lipids may remove any mutagenic compounds present in the lipid samples, reducing the likelihood of eliciting a positive response in the Ames test. Also, removal of any anthropogenic compounds by GPC may remove any synergistic effects that may be present in the lipid samples.

The whole lipids of the Newfoundland seal sample BC4, St. Lawrence seal sample PvB02-01 and St. Lawrence beluga whale sample DL-04-02 were tested. Again, the colony counts for the plates of each sample can be found in Table 4.4 and the ratios of the bacteria colony counts for each plate to the control plate calculated and tabulated in Table 4.5. While some of the bacteria counts were not quite double that of the control plates, some were close, with ratio values of 1.6.

Table 4-4 Average of Revertant Bacteria Colonies from the Ames Test Analysis of the BC4, PvB02-01 and DL-04-02 Whole Lipid Samples with Metabolic Activation (+S9) and Without (-S9)

	TA 98 - S9	TA 100 - S9	TA 98 + S9	TA100 + S9
Control	44 ± 2	35 ± 3	47 ± 3	58 ± 3
Positive	NA	460 ± 7	863 ± 10	819 ± 3
BC4	45 ± 2	40 ± 3	66 ± 4	60 ± 7
PvB02-01	45 ± 4	45 ± 1	74 ± 3	61 ± 1
DL-04-02	68 ± 3	38 ± 2	73 ± 1	50 ± 10

Table 4-5 Ratios of Revertant Colonies on Test Plate to Number of Revertant Colonies on the Control Plates for the BC4, PvB02-01 and DL-04-02 Samples

	TA 98 - S9	TA 100 - S9	TA 98 + S9	TA100 + S9
Control	1.0	1.0	1.0	1.0
Positive	NA	13.3	18.5	14.1
BC4	1.0	1.1	1.4	1.0
PvB02-01	1.0	1.3	1.6	1.0
DL-04-02	1.6	1.1	1.6	0.9

The higher values of the whole lipid tests were expected. Stepanova *et al.* [9] reported that the whole lipid of seals and other organisms was mutagenic in the Ames test. The majority of the seal samples from Lake Baikal were mutagenic to the TA98 strain with metabolic activation which is in agreement to the higher number of revertant colonies of the whole lipid PvB02-01 and DL-0402 samples from the St. Lawrence River. However, in the Lake Baikal region, samples from female seals had more mutagenic

activity than samples from male seals. This difference may account for the lack of mutagenic activity in the samples tested from the St. Lawrence River and Newfoundland regions since all samples tested were males. The results for the seal samples from Lake Baikal are shown in Table 4.6 as a ratio of the bacteria colonies on the test plates to the number of revertant colonies on the control plates. These results also show that in the absence of metabolic activation, the seal lipids have a possible toxic effect or an antimutagenic effect on the TA100 strain, causing the number of revertant colonies on the samples plates to be lower than the spontaneous revertants counted on the control plates. The higher colony counts for the whole lipid samples agree with the theory that GPC fractionation removes any mutagenic compounds present and any synergistic effects present in the lipids.

Table 4-6 Ratios of Revertant Colonies on Test Plate to Number of Revertant Colonies on the Control Plates for the Lake Baikal Seal Samples [9]

Sex	Age	TA 98 - S9	TA 100 - S9	TA 98 + S9	TA100 + S9
Male	3 months	2.6	0.3	2.2	1.1
Male	1 year	1.6	0.5	1.9	1.2
Male	5 years	1.0	0.2	1.1	0.4
Male	5 years	1.8	1.0	1.7	1.2
Male	7 years	3.1	0.4	2.0	1.3
Male	9 years	1.3	0.8	1.5	0.9
Male	10 years	0.9	0.3	1.4	0.9
Female	3 months	1.2	1.0	1.3	1.1
Female	3 months	2.0	0.7	2.2	1.1
Female	3 months	0.9	0.5	2.0	1.1
Female	1 year	1.1	0.3	1.3	1.0
Female	1 year	2.9	0.2	2.4	0.7
Female	2 years	1.1	0.4	1.5	0.9
Female	2 years	2.1	0.4	2.2	1.1
Female	6 years	2.0	0.6	1.9	1.2
Female	7 years	1.3	1.2	1.9	1.0

4.4 GC/ EI MS of Fractionated Samples

After GPC fractionation, the methyl esters of the fractions were prepared for analysis by GC/EI MS. These samples were analyzed by GC/MS to better understand the fractions collected and to compare the lipids extracted with the lipids expected to be found in the samples. The seal lipids analyzed were found to contain saturated and unsaturated fatty acids with an even number of carbon atoms, ranging from 14-22 carbon atoms. This result agrees with the literature. Christie wrote, "The common fatty acids of plant and animal origins contain even numbers of carbon atoms…and may be fully saturated or contain one, two or more (up to 6) double bonds, which generally, but not always, have a *cis*-configuration." [18].

Vials 13-15 contained the most data since they collected the majority of the lipids after fractionation. The total ion chromatograms (TIC) for each were almost identical. As an example, the TIC for fraction 15 is presented in Figure 4.4. The major peaks were identified as their fatty acid methyl esters by their mass spectra. A list of the fatty acid methyl esters identified, along with the ions used for identification, can be found in Table 4-7.

Analysis of the fractions by GC/EI MS did not aid in the identification of any chlorinated compounds that may be present.

Table 4-7 Fatty Acid Methyl Esters Identified and their Corresponding Ions Identified by GC/ EI MS

Compound	Chain Length:Unsaturation	Identifying Ions m/z
Methyl Myristate	14:0	242, 74
Methyl Hexadecanoate	16:1	236, 69
Methyl Palmitate	16:0	270, 74
Methyl Octadecadienoate	18:2	294, 67
Methyl Octadecanoate	18:0	298, 74
Methyl Eicosatetraenoate	20:4	247, 79
Methyl Eicosenoate	20:1	292, 69
Methyl Docosenoate	22:1	320, 69
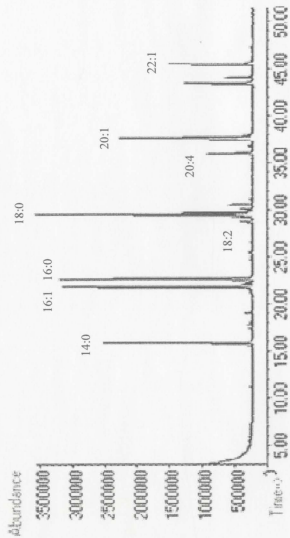
Figure 4-4 Total Ion Chromatogram of Fraction 15

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4.5 GC/EI MS of Acid Treated Samples

In an effort to confirm the presence of any persistent organochlorine pollutants that may be present, the samples from the Newfoundland and the St. Lawrence River regions were subjected to an acid treatment procedure followed by non-polar extraction with hexanes (Section 3.8). This extract should contain any persistent organic pollutants found in the lipids. The samples extracted in this way were the Newfoundland sample BC4, St. Lawrence seal sample PvB01-2 and St. Lawrence beluga whale sample DL-04-2002.

The residue left after the solvent was blown-off with nitrogen was dissolved in hexanes at an initial concentration of 1mg mL^{-1} for the GC/MS analysis. These three analyses (Newfoundland seal and St. Lawrence River seal and beluga whale) yielded a series of peaks between 7 and 9 minutes, but nothing afterwards. The samples were then brought to a higher concentration to see if the residue was a mixture of compounds found in low concentrations. For the Newfoundland seal sample, the same peaks were found between 7 and 9 minutes, but some new peaks were found in the TIC (Figure 4.5) at times of 22.5 minutes, 26.8 minutes, 36.2 minutes and 39.9 minutes. For the more concentrated St. Lawrence seal sample, there was only one new peak, at a retention time of 20.8min, along with those between 7 and 9 minutes (Fig. 4.6). In the St. Lawrence beluga whale sample however, there was a significant difference. Many new peaks were found throughout the TIC (Fig 4.7).

Using the NIST mass spectra library, an attempt to identify these peaks from their mass spectra was made. The mass spectra of the peaks between 7 and 9 minutes of all

the samples could not be identified with any confidence using the NIST library. The mass spectrum for this region of the BC4 sample TIC is shown in Fig. 4.8.

The mass spectra of the new peaks (Figs. 4.9-4.13) for sample BC4 also posed problems for identification using the NIST library. However, these mass spectra do have some interesting characteristics. The peak at a retention time of 22.4min (Fig. 4.10) has a mass spectrum that looks similar to that of a fatty acid. The base peak of saturated fatty acids is found at an m/z of 74, while this one has a base peak at an m/z of 73, with a similar fragmentation pattern found in fatty acids.

The same can be said for the major peak found at 26.87min (Fig. 4.11). Again, the base peak is found at an m/z of 73 instead of 74, but contains a fragmentation pattern similar to that of a fatty acid.

The peak at 36.25min (Fig. 4.12) does have a base peak at 74, but without a molecular ion clearly visible, it was difficult to identify. It is possible that a small amount of fatty acid may have been extracted by the hexanes and could be the cause of this peak. Since no lipids should be present in this sample, a more likely reason may be that there are non-polar compounds present that have fragmentation patterns similar to those of lipids without the characteristic polar head group.

The last peak found in the TIC, at a retention time of 39.92min (Fig 4.13), has an unusual mass spectrum. It contains a very large base peak at an m/z of 149. There is a possible molecular ion at m/z 279, but the lack of a fragmentation pattern made identification difficult again. Due to the large base peak at an m/z of 149, it is likely this peak could be identified as a phthalate ester.

Again, the Pv-B01-02 sample contained the usual peaks found between 7 and 9 minutes for all the samples. The mass spectrum, generally the same throughout this region, is presented in Fig. 4.14.

The new peak in the TIC is found at a retention time at 20.80min. The mass spectrum (Fig. 4.15) is very interesting though. It looks very similar to the spectrum of a fatty acid with one degree of unsaturation. The difference though is that all the ions are 2 m/z units higher than those of unsaturated fatty acids. For example, in Fig. 4.8, the mass spectrum of methyl docosenoate (22:1) shows peaks at m/z's of 55, 69, 83, 97, 111 and 137. The spectrum in Fig. 4.15 has peaks at m/z's of 57, 71, 85, 99, 113 and 139 all 2 units higher than that of the unsaturated fatty acid methyl ester until it reaches an m/z of 183, which occurs in both mass spectra. Also, the ions m/z's of 55, 69, 83, 97 and 111 are all separated by 14 amu's which is very characteristic of an aliphatic hydrocarbon.

As with the BC4 and Pv-B01-02, the DL-040-02 TIC contained the same peaks between 7 and 9 minutes with the same mass spectra (Fig. 4.16). The mass spectrum of the peak at 20.83min (Fig. 4.17) is very similar to the mass spectrum of the peak found at 20.80min of the Pv-B01-02 sample and has a similar retention time. This could be indicative of a compound present in the St. Lawrence River area that is not present or could not be detected in the sample from the Newfoundland area. Again, many of the ions are separated by 14 amu's, possibly indicating the presence of an aliphatic hydrocarbon.

The mass spectrum of the peak at 22.56min (Fig. 4.18) does not show any significant fragmentation pattern and could not be identified using the NIST library.

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However, some of the other new peaks were identified as persistent organochlorine pollutants.

The identified organochlorine pollutants belong to a few major families of pollutants. One of these families is the polychlorinated biphenyls (PCBs). PCBs can exist as one of a possible 209 congeners. PCBs have been used in industrial settings for a variety of purposes such as coolants and lubricators. However, manufacture of PCBs was stopped in the US in 1977 because of evidence that they persist in the environment and can cause harmful health effects.

PCBs may have entered the environment during their manufacturing, use or disposal. Since they do not easily break down, they can remain for long periods of time. Also, they can be transported by air, allowing them to be transported over long distances from their areas of use. In the marine environment, PCBs mainly stick to organic particles and are taken up by small organisms and fish. Animals that use these organisms as a food source accumulate PCBs to levels that are much higher than that in the water. [58]

Another group of compounds identified belonged to the dichlorodiphenyltrichloroethane (DDT) family of pesticides. DDT was used as a pesticide until it was banned in the US in 1972. However, it is still used in some countries today. DDT breaks down in the environment to dichlorodiphenyldichloroethylene (DDE) and is the major component of DDT found in the environment. DDT found in the environment is either left over from its use as a

pesticide in the past or due to long range transport from regions that still use DDT as a pesticide. [59]

PCBs and organochlorine pesticides are widespread in surface waters of North America, especially in the Great Lakes and the St. Lawrence River. The sources of contamination of the St. Lawrence River are the Great Lakes, the Ottawa River and industrial and municipal activities along the St. Lawrence River and its tributaries. [60]

A very small peak with a retention time of 26.06min (Fig 4.19) was the first organochlorine compound identified. It was identified as tetrachlorobiphenyl, a PCB isomer. Its structure can be found in Fig. 4.30. Identification was made possible by the isotope ratio present at an m/z of 292.

The peak at 26.7min gave a mass spectrum (Fig. 4.20) that contained a molecular ion at an $m/z's$ of 256, the same as a C:16 fatty acid methyl ester, but the rest of the fragmentation pattern does not fit that of a fatty acid.

The peaks found at 28.64min and 29.79min give the same mass spectra (Figs. 4.21 and 4.22) and were identified as belonging to another PCB, this one with five chlorine atoms. The differences in retention times may arise from the possibility that the chlorine atoms may be arranged differently around the two phenyl rings. A general structure for pentachlorbiphenyl is found in Fig. 4.30. Again by the isotope ratio of the ion cluster, this time at an m/z of 326, was a positive indicator for identification.

The next peak, found at 29.97min, was identified as another organochlorine compound, *trans*-nonachlor. The mass spectrum of this compound is shown in Fig. 4.23 and its structure can be found in Fig. 4.30.

The large peak at 30.74min had a mass spectrum (Fig. 4.24) that identified it as p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) a metabolite of DDT. Its structure can also be found in Fig. 4.30.

Another PCB, this one containing six chlorine atoms was found to be associated with peaks at 31.93min, 32.89min and 33.94min. Their mass spectra are shown in Figs. 4.25-4.27. Again, the differences in retention times and the slight differences in the mass spectra may be a result of the chlorine atoms being arranged in different positions around the phenyl rings. A general structure for hexachlorobiphenyl is shown in Fig. 4.30. The ion cluster indicative of hexachlorobiphenyl can be found at the m/z 360. The ion cluster has the isotope pattern that is characteristic of a compound containing six chlorine atoms.

Finally, the peaks at 34.50min and 36.50min were identified as belonging to another PCB, this time with seven chlorine atoms. Their mass spectrums are shown in Fig. 4.28 and Fig. 4.29 respectively. Once again, the difference in retention time is possibly due to the different way the seven chlorine atoms may be arranged around the phenyl rings. A general structure of a heptachlorobiphenyl molecule can be seen in Fig. 4.30. Heptachlorobyphenyl was identified by the characteristic ion cluster for seven chlorine atoms found at an m/z of 394.

These results show that persistent organochlorine pollutants can be isolated and identified from contaminated lipid samples using an acid treatment procedure. The higher contamination levels of the beluga whale samples may be the reason why GC/MS analysis could be used to identify specific organochlorine contaminants.
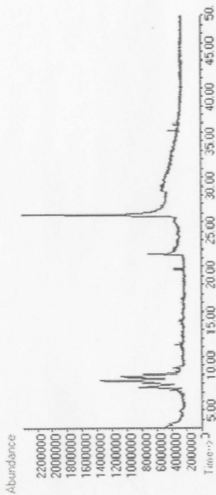
Figure 4-5 Total Ion Chromatogram of Hexane Extract of Sample BC4

71
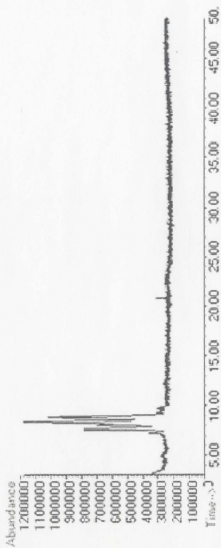
Figure 4-6 Total Ion Chromatogram of Hexane Extract of Sample Pv-B01-02

72
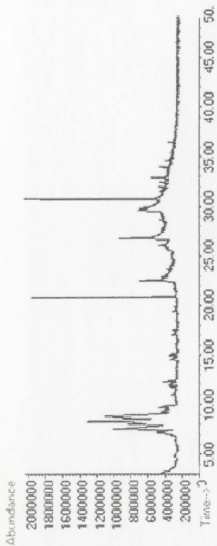
Figure 4-7 Total Ion Chromatogram of Hexane Extract of Sample DL-04-02

73
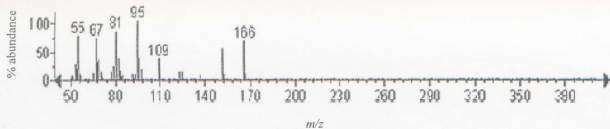
Figure 4-8 Mass Spectrum of Peak at RT: 8.34min from BC4 Sample
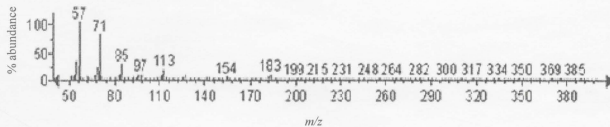
Figure 4-9 Mass Spectrum of Peak at 20.79min of BC4 Sample
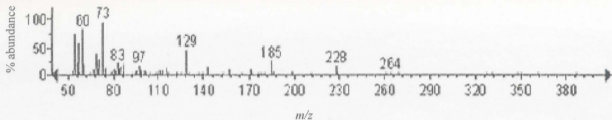
Figure 4-10 Mass Spectrum of Peak at 22.48min of BC4 Sample
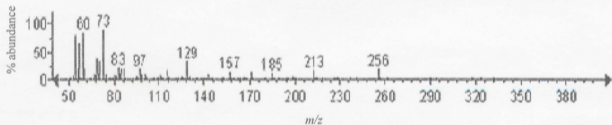
Figure 4-11 Mass Spectrum of Peak at 26.87min of BC4 Sample
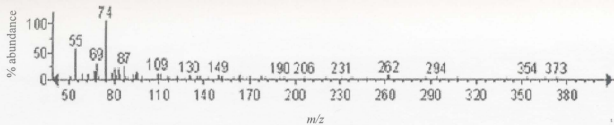
Figure 4-12 Mass Spectrum of Peak at 36.25min of BC4 Sample
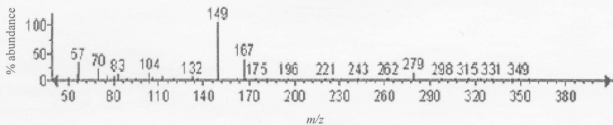
Figure 4-13 Mass Spectrum of Peak at 39.92min of BC4 Sample
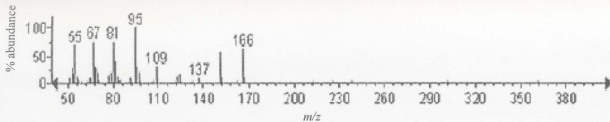
Figure 4-14 Mass Spectrum of Peak at 8.37min of PvB01-02 Sample
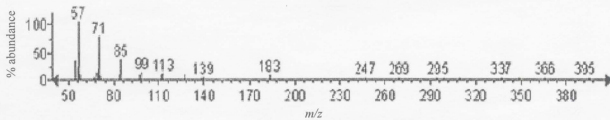
Figure 4-15 Mass Spectrum of Peak at 20.80min of PvB01-02 Sample
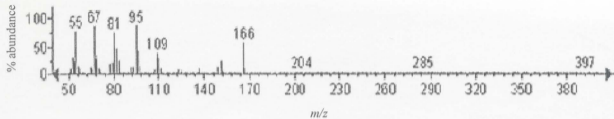
Figure 4-16 Mass Spectrum of Peak at 8.86min of DL-04-02 Sample
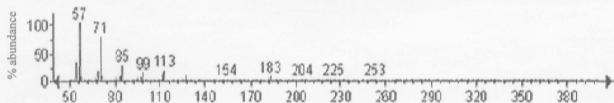
Figure 4-17 Mass Spectrum of Peak at 20.83min of DL-04-02 Sample
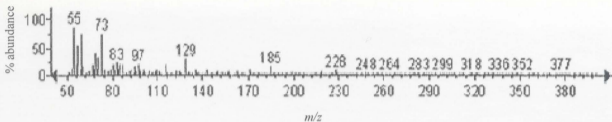
Figure 4-18 Mass Spectrum of Peak at 22.56min of DL-04-02 Sample
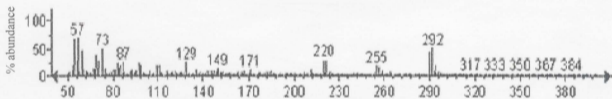
Figure 4-19 Mass Spectrum of Peak at 22.56min of DL-04-02 Sample Identified as Tetrachlorobiphenyl
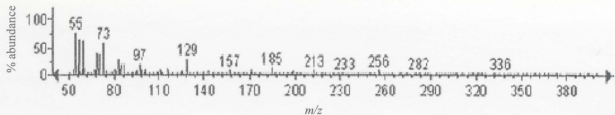
Figure 4-20 Mass Spectrum of Peak at 26.80min of DL-04-02 Sample
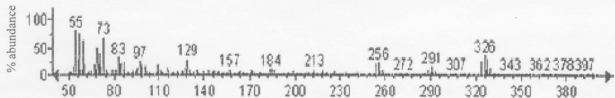
Figure 4-21 Mass Spectrum of Peak at 28.64min of DL-04-02 Sample Identified as Pentachlorobiphenyl
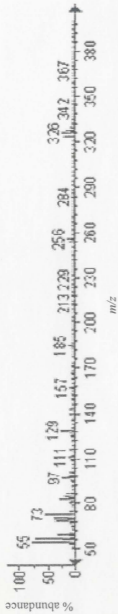
Figure 4-22 Mass Spectrum of Peak at 29.79min of DL-04-02 Sample Identified as Pentachlorobiphenyl
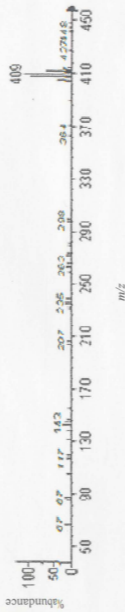
Figure 4-23 Mass Spectrum of Peak at 29.97min of DL-04-02 Sample Identified as *trans*-Nonachlor
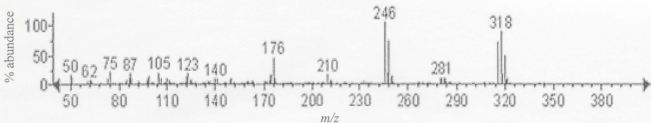
Figure 4-24 Mass Spectrum of Peak at 30.74min of DL-04-02 Sample Identified as p,p'-DDE
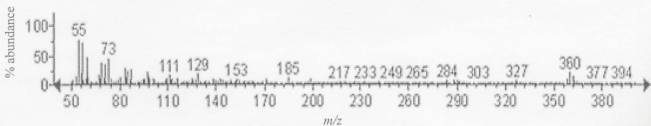
Figure 4-25 Mass Spectrum of Peak at 31.93min of DL-04-02 Sample Identified as Hexachlorobiphenyl
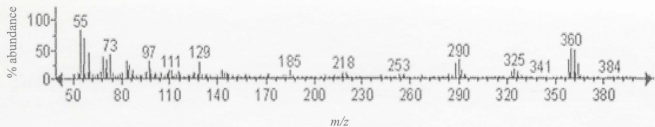
Figure 4-26 Mass Spectrum of Peak at 32.89min of DL-04-02 Sample Identified as Hexachlorobiphenyl
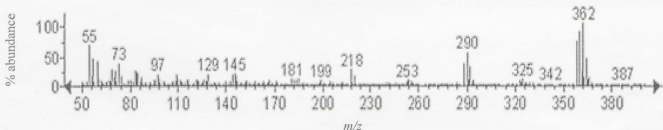
Figure 4-27 Mass Spectrum of Peak at 33.94min of DL-04-02 Sample Identified as Hexachlorobiphenyl
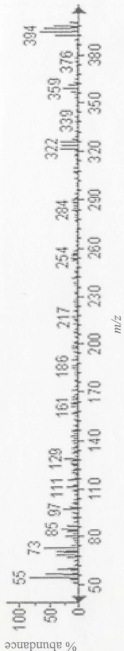
Figure 4-28 Mass Spectrum of Peak at 34.50min of DL-04-02 Sample Identified as Heptachlorobiphenyl
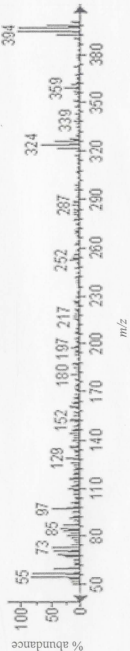
Figure 4-29 Mass Spectrum of Peak at 36.50min of DL-04-02 Sample Identified as Heptachlorobiphenyl

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Tetrachlorobiphenyl

Pentachlorobiphenyl

Hexachlorobiphenyl

Heptachlorobiphenyl

p,p'-DDE

Nonachlor
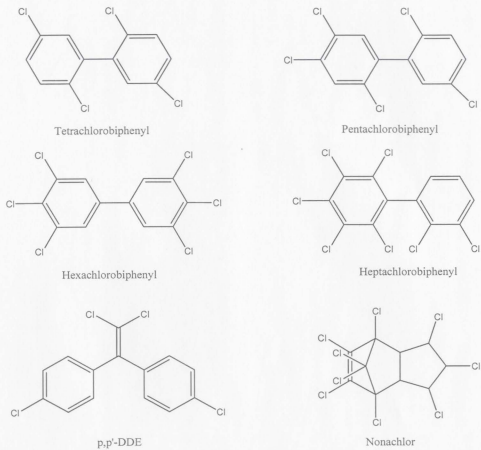
Figure 4-30 Structures of Identified Persistent Organochlorine Pollutants in DL-04-02 Sample

5 Conclusions and Future Work

Unidentified organochlorine compounds in the environment still pose problems for identification by traditional analytical methods. The use of bioassays as guides to locate biologically active compounds in fractionated samples could still lead to the discovery of novel organochlorine compounds, even though this was not achieved during the course of this research.

As mentioned previously, the Ames test has been used to test whole lipid samples of marine organisms with much success. However, after fractionation the mutagenic potential of the samples were lowered. There may be a variety of reasons for this. First, if there are any mutagenic compounds in the sample, they may be present in a low concentration and be forced to compete with the lipid for access to the bacteria. Second, the strong mutagens found in the whole lipid samples may be separated from the lipid during the GPC fractionation. Third, compounds in the lipid fractions may require compounds separated by the GPC fractionation for a synergistic mutagenic effect. Finally, the lipids may have an inherent antimutagenic effect, lowering the chance of reversion by the bacteria.

There is a variety of biological activities that can be tested for that might be of use in the discovery of biologically active organochlorine compounds found in the environment. Organochlorine compounds have been linked to defects in reproductive systems of male fish and other estrogenic activities. Thus, testing for estrogenicity using bioassays could be used in much the same way as the Ames test was used in this research.

One bioassay that could be used for this type of estrogenicity screening is the recombinant yeast assay developed by Routledge *et al.* [11]. This assay uses a recombinant yeast strain to test for compounds that can interact with the human estrogen receptor (hER). It has been used to test for estrogenic activity in a variety of environmental samples and could be used to test lipid samples from organisms as well [10].

Other approaches may also aid in making progress in this work, for example, employing techniques to either concentrate the chlorinated compounds in the sample or to reduce the non-chlorinated compounds found in the lipid matrix. One method for removing polyunsaturated fatty acid methyl esters is silver ion complexation. The silver ions form a complex with polyunsaturated fatty acid methyl esters so that they can be removed in the aqueous phase, leaving the saturated fatty acids and chlorinated fatty acid methyl esters in the organic phase.

Another method of separation is urea complexation. Unsubstituted, straight-chained, saturated fatty acid methyl esters are caught in hexagonal inclusion complexes when urea crystallizes. Due to their bulkiness, other fatty acid methyl esters, such as chlorinated or polyunsaturated fatty acids, do not form these types of complexes.

Modifications to the GPC method used could aid in the separation of lipid fractions. A column with a smaller molecular weight range would provide better separation if the target masses are known.

The acid treatment of lipids was shown to be an effective way to confirm the presence of persistent organic pollutants when they are present in high enough

concentrations in the sample. Using this method, known organochlorine compounds were identified in the beluga whale sample from the St. Lawrence River. It would be interesting to see if any biological activity is present in the extracts separated in this procedure.

A significant problem was encountered when trying to identify chlorinated compounds using GC/EI MS analysis. Electron impact ionization (EI) on chlorinated compounds effectively removes the chlorine atoms from many chlorinated compounds. Therefore it is difficult to see any molecular ions with the chlorine atoms still attached and containing the proper isotope ratio pattern. The use of a softer ionization technique, such as chemical ionization (CI) could be helpful in the identification of chlorinated compounds by producing less fragmentation and a more recognizable molecular ion.

Besides CI, other detectors could be used to help identify novel organochlorine compounds. Electron capture detectors are very sensitive to halogenated compounds and could be used to confirm the presence of molecules containing chlorine. Also, tandem mass spectrometry might be useful in identifying compounds containing chlorine by performing neutral loss experiments. Using this scan mode, compounds containing chlorine can be identified by the loss of HCl. This method could be used for the rapid screening of any molecules that contain chlorine.

While no novel organochlorine compounds were identified, the groundwork for using bioassays as guides for identification has been laid. There is still much work that can be done to aid in the identification of the unknown chlorine found in the environment.

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Appendix A

Vogel-Bonner medium E (50x)
Use: Minimal agar

Warm distilled water	670mL
Magnesium Sulfate Heptahydrate	10g
Citric Acid Monohydrate	100g
Potassium Phosphate, dibasic (anhydrous)	500g
Sodium Ammonium Phosphate	175g

Add salts in the order indicated to warm water in a 2L beaker placed on a magnetic stirring hot plate. Allow each salt to dissolve completely before adding the next. Adjust volume to 1L. Distribute into two 1L glass bottles. Autoclave, loosely capped, for 20 min. at 121°C. When the solutions have cooled, tighten the caps.

0.5mM Histidine/Biotin Solution
Use: Mutagenicity assay

D-Biotin	30.9mg
L-Histidine· HCl	24.0mg
Distilled Water	250mL

Dissolve the biotin by heating the water to the boiling point. This can be done in a microwave oven. Sterilize by filtration through a 0.22µm membrane filter or autoclave for 20 min. at 121°C. Store in a glass bottle at 4°C.

Top Agar
Use: Mutagenicity assay

Agar	6g
Sodium Chloride	5g
Distilled Water	1000mL

The agar may be dissolved in a steam bath, a microwave oven or by autoclaving briefly. Mix thoroughly and transfer 100mL aliquots to 250mL glass bottles with screw caps. Autoclave for 20 min. with loosened caps. Slow exhaust. Cool the agar and tighten caps.

Salt Solution
Use: S9 mix for mutagenicity assay

Potassium Chloride	61.5g
Magnesium Chloride Hexahydrate	40.7g
Distilled Water	To final volume of 500mL

Dissolve ingredients in water. Autoclave for 20 min. at 121°C. Store in glass bottles at in a refrigerator or at room temperature.

0.2M Sodium Phosphate Buffer, pH 7.4
Use: S9 mix for mutagenicity assay

0.2M sodium dihydrogen phosphate (13.8g/500mL)	60mL
0.2M disodium hydrogen phosphate (14.2g/500mL)	440mL

These are approximate values. Test the pH. If it is too low, add more 0.2M disodium hydrogen phosphate to pH 7.4. Sterilize by autoclaving for 20 min. at 121 °C.

0.1M NADP Solution (nicotine adenine dinucleotide phosphate)
Use: S9 mix for mutagenicity assay

NADP	383mg
Sterile Distilled Water	5mL

Add NADP to pre-weighed glass tubes with screw caps. Do not add water. It is convenient to prepare a dozen or more of these dry aliquots at one time. Wrap the tubes with metal foil to protect them against light and label each tube with the correct weight. It is not necessary to weigh out 383mg exactly as long as the weight is indicated on the label along with the calculated volume of water to give a 0.1M solution. Place all the tubes of weighed NADP in a jar with a tight fitting lid. Silica gel or other desiccant should be placed in the bottom of the jar. Store in a -20°C freezer. When needed for making S9 mix, remove one tube from the jar, add the specified amount of water and mix by vortexing until the NADP is dissolved. Place the tube in an ice bath. It is not necessary to filter sterilize NADP solutions prepared this way but it can be done using a 0.22μm filter. Replace the left over solution in the storage jar and return to the freezer for further use. Solutions of NADP stored in the freezer are stable for at least 6 months.

1M Glucose-6-Phosphate
Use: S9 Mix for mutagenicity assay

Glucose-6-Phosphate	2.82g
Sterile Disttiled Water	10mL

Pre-weighed aliquots of glucose-6-phosphate are prepared as described for NADP and stored in desiccated jars in the freezer. Solutions of G-6-P can also be stored in the freezer and are stable for up to 6 months. If necessary, the solutions may be filter-sterilized using a 0.22µm filter.

S9 Mix (rat liver microsomal enzymes + cofactors)
Use: Mutagenicity assay

Rat liver S9 (Aroclor-1254 induced)	2.0mL (4%)	5.0mL (10%)
MgCl$_2$-KCl salts	1.0mL	1.0mL
1M G-6-P	0.25mL	0.25mL
0.1M NADP	2.0mL	2.0mL
0.2M Phosphate buffer, pH 7.4	25.0mL	25.0mL
Sterile Distilled water	19.75mL	16.75mL

Liver from other mammalian species such as hamster or mouse may be used. Other tissues may be used. The ingredients should be added in the reverse order indicated above so that the liver will be added to a buffered solution. The solution must be prepared fresh and kept on ice. All ingredients should be chilled. Any left over S9 or S9 mix should be discarded. *Never refreeze S9.*

Ampicillin Solution
Use: Tests for ampicillin resistance, master plates for R-factor tester strains

Ampicillin Trihydrate	0.8g
0.2M Sodium Hydroxide	100mL

It is not necessary to sterilize ampicillin solutions but they can be filtered through a 0.22µm membrane filter. Store in a glass bottle at 4°C.

Minimal Glucose Plates
Use: Mutagenicity assay

Agar (Bacto-Difco)	15g
Distilled Water	930mL
50x VB Medium	20mL
40% Glucose	50mL

Add 15g of agar to 930mL of distilled water in a 2L flask. Autoclave for 20 min using slow exhaust. When the solution has cooled slightly, add 20mL of sterile 50x VB medium and 50mL of sterile 40% glucose. For mixing, a magnetic stir bar can be added to the flask before autoclaving. After all the ingredients have been added the solution should be stirred thoroughly. Pour 30mL into each Petri dish.
Note: The 50x VB medium and the 40% glucose should be autoclaved separately.

Ampicillin Plates and Ampicillin/Tetracycline Plates
Use: Master plates for strains carrying the plasmids, pKM101 and pKM101+pAQ1
Tests for ampicillin/tetracycline resistance

Agar (Bacto-Difco)	15g
Distilled Water	910mL
50xVB Medium	20mL
40% Glucose	50mL
Sterile Histidine·HCl·2 H$_2$O (2g/400mL H$_2$O)	10mL
Sterile 0.5mM Biotin	6mL
Sterile Ampicillin Solution	3.15mL
Sterile Tetracycline Solution*	0.25mL

Autoclave agar and water for 20 min. Add sterile glucose, 50x VB Medium and histidine to the hot solution. Mix. Cool to approximately 50°C. Add sterile biotin and ampicillin solutions aseptically (it is not necessary to sterilize antibiotic solutions but this can be done if necessary, using a 0.2μm filter).
Tetracycline is added only for use with the TA102 strain which is tetracycline resistant.
The 50x VB medium and 40% glucose solutions are sterilized separately by autoclaving for 20min. Histidine and biotin solutions can be autoclaved or filter-sterilized.
Plates to be used for tests of tetracycline and/or ampicillin resistance can be stored for approximately 2 months at 4°C. After 2 months they should be tested for ampicillin/tetracycline activity with a non R-factor strain such as TA1535. Plates should be discarded if the non R-factor strain grows.
Master plates should be prepared within a few days after the agar is poured.

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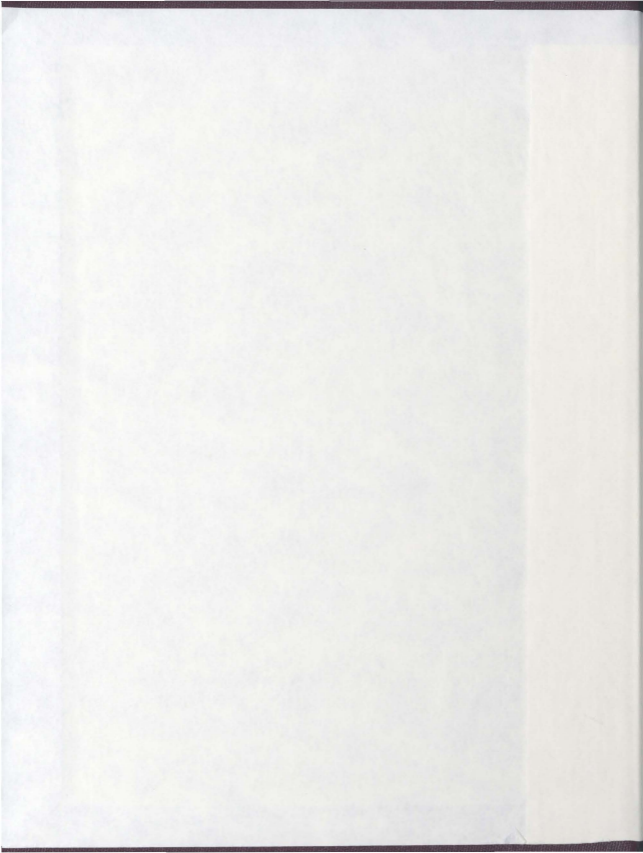
JOANNE N. HODDER

A biomechanical assessment of selected patient transfers: The effects of
instruction and experience for improving thoracolumbar motions and
electromyographic muscle activities

by

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ABSTRACT

Billions of dollars are being spent each year in North America alone on the treatment and compensation of low back disorders (LBD) related to work processes. Nursing characterizes one of those professions at high risk for the development of LBD. Previous research has identified that patient handling has been the leading cause of occupationally-related LBD for nurses. In response to a need for ergonomic intervention, the Back Injury Prevention Program (BIPP) was introduced in Newfoundland in 1989. While there was some anecdotal evidence that this macro-ergonomic approach was successful in reducing risk of injury, there has been no reported biomechanical evidence that the patient transfer techniques prescribed by BIPP were related to these reductions. The purpose of this study was to examine whether instruction in BIPP transfer techniques is related to beneficial changes in biomechanics metrics thought to be associated with risk for developing LPB. Two comparisons were considered in this model. First, novice subjects were compared prior to and following a standardized BIPP training session, specific to three patient transfer techniques. Secondly, an experienced group of active institutional nurses were measured while performing the same tasks. These transfers were selected based on their history of high incidence of injury and included repositioning a patient to the head of the bed from a side-on position and a position superior to the patient's head and a transfer from a sitting bed position to a wheelchair. Bilateral electromyography (EMG) and a Lumbar Motion Monitor (LMM) were employed to monitor each subject during the execution of a task. Results suggest that BIPP principles for patient transfers reflect sound biomechanical principle, as participant experience increased, the biomechanical demands decreased. Further investigations should consider the administrative controls involved in the implementation of this program in the workplace.

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CHAPTER 1: INTRODUCTION

1.1 Introduction

Manual material handling (MMH) has been identified as a major etiological factor for musculoskeletal disorders (Marras, 2000; Marras, Lavender, Leugrans, Fathallah, Ferguson, Allread and Rajulu, 1995). The most common musculoskeletal injury that occurs as a result of manual materials handling activities are low back disorders (Marras, 2000; Marras, 1999; Marras et al., 1995). Professions that sustain the majority of manual materials handling injuries are construction, forestry and laborer as loads are often either heavy or frequently lifted (Orr, 1997; Guo, 2002). It is estimated that the cost of low back injuries is approximately \$25.6 billion per year in Canada (Kim, Hayden and Moir, 2004).

The high cost of back injury has lead ergonomists and researchers alike to assess what aspects of manual materials handling tasks are related to musculoskeletal injuries. Low back disorders (LBD) have a complex and mulitfactorial etiology comprised of physical, organizational, psychosocial and individual factors (Karwowski, Jang, Rodrick, Quesada and Cronin, 2005). In order to determine how to address and prevent injuries from happening, ergonomists and researchers have begun to analyze how each factor relates to the overall risk of developing LBD.

Physical and organizational factors possess an obvious relationship to LBD and have been found to be the easiest to mediate towards reducing the overall risk of injury. The physical factors include: static work, posture and technique, load characteristics, handles and coupling, frequency and repetitive handling, asymmetrical handling and non-uniform loads, space confinement and restraints (Jones and Kumar, 2001). Work organization factors include availability of necessary staff and equipment and synchronicity of the tasks required for the job. These factors are related to the task efficiency as well as the physical demands involved in performing tasks. Physical and organizational factors can be optimized to reduce the risk of injury to an operator.

Psychosocial factors are best described as the mental and social stresses of a worker. How psychosocial stress impacts upon an injury is not well understood. There are two theories postulated for potential pathways of how psychosocial stress can manifest into physical injury. The first theory suggests that psychosocial stress will cause an increase in tension in the muscles and therefore increase their susceptibility to injury (Yip, 2001). In combination with this theory is that increased psychosocial stress could cause the worker to be mentally distracted. Lack of attention and focus would inhibit an operator from being able to identify a hazardous situation which otherwise could have been avoided (Yip, 2001).

The second theory regarding the impact of psychosocial factors in injury is that these stresses amplify the sensation of pain. Therefore, an injury that may have already existed with only minor symptoms may become more symptomatic and exaggerated when psychosocial stress is added to the situation (Yip, 2004). Therefore stresses such as job dissatisfaction, monotony of work, limited job control, and lack of social support can be addressed to reduce the risk of injury (Cartledge, 2001; Yip, 2001; Bewick and Gardner, 2000, Retsas and Pinikahana, 2000; Lagerstrom et al., 1998; Cohen-Manfield et al., 1996).

Individual factors have also been reported as impacting the susceptibility of an individual to injury (Smedley et al., 1997). These factors are inherently not easily changed to create a reduction in risk of injury. These factors include age, previous history of back injury, stature and obesity (Smedley et al., 1997). There are but few professions which permit consideration of physical characteristics in the employee selection process. For example, before entering the profession of firefighting an individual must meet a physical fitness standard. However, it is constitutionally unacceptable to consider such criteria in a profession such as nursing.

The profession of nursing is an unexpected profession seen to be ranked next to forestry and construction professions for its high incidence of back injury due to manual materials handling (Yip, 2004, Retsas and Pinikahana, 2000; Marras, Davis, Kirkling and Bertsche, 1999; Smedley, Egger, Cooper and Coggon, 1997; Cohen-Mansfield, Culpepper and Cooper, 1996; Owen and Garg 1991). In Newfoundland and Labrador,

health care is ranked the 4th highest sector for workplace injuries, with most of the injuries being musculoskeletal injuries to the low back (WHSCC, 2004). Research has indicated that patient handling is a major contributor to the prevalence of back pain in nurses and other health care professionals (Venning, 1988; Owen & Garg, 1991; Cohen-Mansfield et al., 1996; Hignett, 1996; Orr, 1997; Engkvist, Hagberg, Hjelm, Menckel and Ekenvall, 1998; Marras et al., 1999; Lagerstrom et al., 1998; Bewick & Gardner, 2000; Yip, 2001; Skotte et al., 2002). Nursing has a unique set of challenges with regards to manipulating physical characteristics of manual materials handling tasks. Nurses are faced with the difficulty that they are in fact handling patients and not inanimate objects.

In other professions, ergonomic interventions generally focus on manipulating the load, either changing the dimensions, the handles or the weight of the load and adjusting the positions and distances in which they are moved. However, in the health care setting these loads can not be altered and vary from shift to shift. Workload will depend on the number of transfers and state of well-being of patients being handled. The frequency of lifting and the amount of weight lifted on any particular shift can not be standardized or always controlled. Unfortunately this has left the profession with a limited ability to make ergonomic accommodations to patient handling tasks.

Factors other than patient handling demands also contribute to the nursing profession being at a high risk for injury. The emotional stress associated with nursing, the lack of control that nurses have over their patients and the stress of shiftwork all increase the risk of injury (Cartledge, 2001; Retsas and Pinikahana, 2000; Lagerstrom et al., 1998). The aging workforce in the nursing profession also impacts upon the high risk of injury (Buchan, 2002). Administrative factors such as lack of adequate staff and lack of necessary equipment will also increase a nurse's exposure to risk (Engvist et al., 1998).

Interventions that have been used with some success to reduce the risk of injury attributed to MMH include: mechanical aides, back education, job redesign and employee selection (Veening, 1988). In Newfoundland and Labrador, the Back Injury Prevention Program (BIPP) was developed in 1989 to educate practicing and student nurses on proper transfer and patient handling techniques. The BIPP patient handling techniques were designed on basic biomechanical principles and attempted to reduce the loads acting

on the body, particularly the upper extremities. Stinsen (2000) reported that there was, in fact, a decrease in the number of back injuries since the introduction of the BIPP program. Since its introduction, however, the transfers considered in the BIPP have never been assessed for their biomechanical efficacy. In consideration of the predicted shortage of nursing staff by the year 2020 and its obvious impact upon the health care system (Janiszewski, 2003) any program that reduces risk of injury to a person performing a patient handling task will be timely.

The purpose of this study was to investigate whether the BIPP provides sufficient user knowledge regarding body mechanics and movement technique during selected patient transfers to reduce the risk of musculoskeletal injury.

1.2 Hypotheses

This study had two hypotheses. The first hypothesis proposes that the training from the back injury prevention program (BIPP) would not have an effect on thoracolumbar kinematics and selected muscle electromyographical activities between pre- and post-trained novice participants for selected patient transfers.

The second hypothesis proposes there would be no significant difference between the thoraco-lumbar kinematics and selected muscle electromyographical activities recorded from novice participants pre- and post-training results and experienced nurses when performing selected patient transfer tasks.

1.3 Assumptions

Assumptions made in this study include: the effectiveness of BIPP instruction, the reliability of the LMM as a surrogate metric to measure thoracolumbar kinematics and their relation to low back stress. This study also assumed that laboratory atmosphere of the BIPP room of the Miller Center provided sufficient ecological validity for the methodological design. A healthy patient was used in the study in order to protect the health of participants and as well, guard the participants from any unexpected behavior

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that could occur with a real patient. It is assumed that the model patient behaves in a consistent and realistic manner.

1.4 Limitations

Construct limitations include the possibility for the Hawthorne effect to influence the results. The Hawthorne effect explains the changes to human behavior that occurs as a result of a subject knowingly being observed and consequently modifying the behaviour to more closely satisfy what the subject perceives to be the expectations of the researchers (Muchinsky, 2000).

There are limitations to using EMG activity and LMM values to reasonably predict musculoskeletal loading and therefore risk of low back disorder (LBD). The use of maximum voluntary contractions (MVC) to normalize the EMG data is also a limitation to the accurate description of the participants' muscle activity.

The ethical concerns that human investigation studies must consider often result in methodological limitations. In this study the novice participants are never truly unaware of the proper techniques employed in patient transfers as each viewed a BIPP training video prior to the pre-training testing protocol. This was done in consideration of participant safety as patient handling can be considered a heavy handling task.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Introduction

There is considerable concern regarding the continual increase of reported low back disorders (LBD) in industrial populations (Burton et al., 1997; McCoy et al., 1997). Employers are reported to be spending upwards of $100 billion annually in compensation costs for LBD (Guo, 2002). The high rates of LBD and the resulting economic impact upon society requires further investigation. Research has focused on the multifactoral etiology of LBD and what components of a task or profession contribute to the incidence of pain and discomfort (Karwowski, Jang, Quesada and Cronin, 2005). Studies have also identified nurses amongst the top professions for risk of developing occupational-related LBD (Karwowski et al., 2005). This profession includes a unique number of characteristics unlike other professionals typically examined. This chapter will review the research conducted in the areas of the cost of back injuries, etiology of LBD, and workplace contributors and interventions specific to the nursing profession.

2.2 Cost of Occupational Injury

It has been estimated that 80% of the American population will experience low back pain during their lifetime (Marras, 2000; McCoy et al., 1997). The economic costs of LBD in the workplace can be staggering. According to Marras (1999) LBD have increased by 2700% from 1980 to 1993, with compensation authorities worldwide reporting expenditures somewhere in the range of $5000US to over $60 000US per LBD claim. Even though LBD only account for 26% of all workplace injuries, they represent approximately 30-40% of all injury compensation costs (Marras, 1999; McCoy et al., 1997; Smith, 1997). In Canada alone, costs of musculoskeletal disorders were reported at $25.6 Billion (Kim, Hayden and Moir, 2004).

The cost of a low back disorder claim can be broken down into two accounting factors: direct and indirect costs. Direct costs include the costs associated with medical

care, compensation for lost income due to a disability, the management of disability and the potential increases in insurance premiums (Marras, 1999; Cohen-Mansfield, Culpepper and Cooper, 1996). In Canada, the direct costs were reported to be $7.5 billion (Kim et al., 2004).

Each claim will also have related indirect costs. These costs can include, for example, expenses due to absenteeism, loss of production due to overtime, the recruitment of a new hire to replace an injured worker, decreased production while training the new hire and cost of mistakes by new employees (Marras, 1999; Cohen-Mansfield et al., 1996). The indirect costs in Canada were reported by Kim et al. (2004) to be $18.1 Billion. In the United States costs were calculated to be approximately $16 Billion US in 1984 (McCoy et al., 1997). By 1990 the direct costs rose to just over $23.5 Billion US (McCoy et al., 1997). More recent annual estimates reveal that approximately one fifth of all work-related injuries are related to the spine with upwards of US$100 Billion being spent on LBD annually (Marras, 2000; Cohen-Mansfield et al., 1996). Considering that Marras (1999) reports 47% of the population performs physically demanding jobs that place workers at risk for developing LBD, it becomes clear that this issue presents itself as a significant economic and social problem.

2.3 Etiology of Low Back Disorders

2.3.1 Introduction

Investigation of how the LBD occurred is vital when attempting to prevent and control occurrences of future injury. Due to the complex anatomy and physiology of the spine and psychosocial variance of humans, there are many factors that combine to create a situation for an injury to occur. Karwowski et al. (2005) stated that LBD have a mulitfactorial etiology consisting of physical, work organizational, psychosocial, individual and socio-cultural factors.

2.3.2 Physical loads

The most obvious and most researched factors regarding LBD are the characteristics of the load and body posture during the manual materials handling (MMH) event (Marras, 2000; Marras, 1999; Marras, Lavender, Leugrans, Fathallah, Ferguson, Allread and Rajulu , 1995). Spinal loading is related to both internal and external forces acting on the system. An external load is a force that acts on the body (Marras, 2000). An internal load is described as the magnitude of forces created by the muscles, tendons, ligaments and skeletal structures. The mass of the external load, the distance at which this load is located from the articulation and the velocity at which this load must travel will impact the amount of internal load that is required to effect the task (Marras, 2000; Marras, 1999).

2.3.3 Types of loading

Occupational-related back injury occurs when the biophysical tolerance of the soft tissues is exceeded and can be related to the compression, torsion and shear forces acting upon the system. Much research has been conducted to determine the magnitude of forces in each plane that will result in a musculoskeletal injury (Marras, 2000; Marras, 1999; Waters, Putz-Anderson and Garg, 1994).

Compression forces acting on the trunk due to a MMH activity can result in trauma to muscles, ligaments, tendons, discs and endplates located in the spinal column. An injury will occur if the absolute or cumulative forces exceed the soft tissue tolerance. Previous research from the National Institute of Occupational Safety and Health (NIOSH, 1981) identified maximal compression forces that can be tolerated by the spine. An average limit of 3400 N was determined to be tolerable before vertebral endplate damage is likely to occur (NIOSH, 1981). Since the vertebral endplate is responsible for facilitating nutrient flow to the disc, trauma to these structures can result in atrophy and degeneration of the disc (Marras, 1999). The NIOSH compression limit became widely accepted as the action limit, or the allowable limit of disc compression for a job to be considered safe. The maximum permissible limit indicated was 6400 N of compression, at which jobs requiring exertions above this limit would be considered hazardous

(Marras et al., 1995; Waters et al., 1994). Subsequent research has revealed that repetitive loading appears to reduce the loading tolerance of involved tissues. While static models may provide a means of initial evaluation of a task, it is not a valid approach to understanding the etiology of chronic LBD due to more dynamic MMH activities (Marras, 2000).

2.3.4 Injury pathway

Injuries may be classified as either acute or chronic. An acute injury is generally a result of a single accident or trauma (Pengal, Herbert, Maher and Refshauge, 2003). Clinical evidence in the UK has concluded that approximately 90% of persons suffering from acute back injuries make a medical recovery within 6 weeks (Pengel et al., 2003). Acute back injury cases, with no previous history of injury, will have less scar tissue and patients can be rehabilitated more quickly than a chronic injury case. Chronic injury is typically a result of untreated acute injuries, cumulative loading and/or long-term exposure (Marras, 2000; Marras, 1999).

Recently, Marras (2000) postulated a pathway that is believed to represent the sequence of events that precede the occurrence of LBD. Excessive or repetitive loading as a result of the internal and external forces cause microfractures to the vertebral end plates. When these microfractures occur the individual will typically feel no pain as few pain receptors are located in the disc fibers. Of greater concern are end plate microfractures, as scar tissue begins to form. End plates are responsible for transporting nutrients to the disc fibers. Since scar tissue is denser than normal tissue it will impede the nutrient flow to the disc. When this occurs the discs will atrophy and weaken, resulting in degeneration. This is the mechanism that has been thought to be responsible for the lowered tolerance of the spine after cumulative loading (Marras, 2000).

Kumar and Jones (2001) indicated that repeated exposure to stress creates hysteresis in the collagenous materials due to its viscoelastic properties. Hysteresis is a tissue property described as the difference in the amount of relaxation that occurs in tendon and ligament tissue during the loading phase compared to the unloading phase. Even repeated exposures to minimal loads can lead to permanent elongation of the

collagen structures of ligaments and tendons, otherwise known as creep. As a result of the stretch, capillaries in the muscle may become compressed and this may cause ischemia, tearing of muscle fibers and inflammation. To compound the issue further the body needs to compensate for the lack of stability sustained from lax ligaments and tendons and therefore will need to use active mechanisms not normally required to perform a movement. The fatigue of the active mechanisms will eventually result in instability, thus creating higher risk for overexertion injuries to occur (Jones and Kumar, 2001).

Since it is evident that research has not yet agreed on the exact pathway that leads to the development of a LBD, the ability to provide post trauma care is limited at best. Research must be focused on the factors that precede injuries, and of those factors, what can be controlled to prevent the injury from even occurring.

2.4 Factors Related to Occupational LBD

2.4.1 Introduction

Research on workplace prevention strategies has focused on identifying task- and operator-related factors most associated with risk of LBD. It has become clear to ergonomists that some professions are at higher risk for developing LBD than others. From these professions, the physical demands of the job have been analyzed and conclusions drawn on which physical factors likely contribute to the incidence of LBD. Personal characteristics and psychosocial stresses have also been identified as being contributors to LBD. Thus, a holistic approach to studying the problem may be necessary (Yip, 2004; Carledge, 2001; Skotte, Essendrop, Hansen and Schibye, 2002; Yip, 2001; Bewick and Gardner, 2000; Caboor, Verlinden, Zinzen, Van Roy, Van Riel and Clarys, 2000; Marras, 2000; Owen and Garg, 1991; Owen, 2000; Retsas and Pinikahana, 2000; Marras, Davis, Kirkling and Bertsche, 1999; Josephson and Vingard, 1998; Lagerstrom, Hansson and Hagberg, 1998; Burton et al.,1997; Smedley, Egger, Cooper and Coggon, 1997; McCoy et al., 1997; Cohen-Mansfield et al., 1996).

2.4.2 Personal Factors

Personal physical factors involved in the risk of developing a LBD are variables such as age, previous history of back injury, stature and obesity (Smedley et al., 1997). Other variables that have been reported to have potential correlation with LBD are smoking and low income status (Marras, 2000). Ferguson and Marras (1997) reviewed 57 industry-base surveillance studies that indicated that personal factors were the most frequently investigated risk factor of LBD. This review identified several personal factors that had a significant relationship to LBD (Ferguson and Marras, 1997). These factors were previous history of LBD (87%), household income/unemployment (66%), smoking (44%) and intelligence/education level (40%). This study also reported an interaction between age and gender with regards to prevalence of LBD. The risk for men reportedly peaks at approximately 40 years of age, whereas risk for women has been reported to be between 50-60 years of age (Ferguson and Marras, 1997).

Anthropometric measures were related to LBD (Ferguson and Marras, 1997). This study indicated that sitting height and obesity were the only measures that have been associated with greater risk of LBD (Ferguson and Marras, 1997). However, Smedley et al.. (1997) indicated in a review of the prevalence of back pain for nurses, that neither weight nor height could be considered to have a strong enough correlation to LBD for these factors to be considered as exclusion criteria for employment.

Another associated factor that has been studied is strength, in particular isometric strength (Marras, 2000). Although no evidence has directly linked isometric strength with LBD, it has been suggested that when matched with job requirements, it is an indicator of risk (Marras, 2000). Similar considerations were made for muscular endurance (Marras, 2000).

There is evidence that mood and lack of physical activity are risk factors for psychosocial stress and therefore LBD (Yip, 2001; Marras, 2000). By increasing the amount of physical activity an individual engages, typically morale and perception of the external environment becomes more positive. With a more positive outlook, the

psychosocial stresses may seem less daunting to an individual and will not create as much internal stress and tension (Yip 2004). Despite the efforts of previous research, Marras (2000) concluded that personal factors only have a modest correlation with the incidence of LBD and therefore can only be considered as compounding factors involved in the development of LBD.

2.4.3 Occupation-Related Factors

The major factor for the development of a LBD is often acknowledged as task-related (Guo, 2002; Marras, 2000; Marras et al., 1995, Marras et al., 1993). In particular, professions with heavy MMH tasks tend to be strongly correlated with the incidence of low back pain. MMH occupations include heavy and/or repetitive lifting, pushing, pulling, whole body vibration, awkward postures or movements, and prolonged static postures. All of these factors have been correlated with the presence of LBD (Yip, 2004; Marras, 2000; Karwowski and Marras, 1999; Marras et al., 1999; Smith, Ayoub and McDaniel, 1992). According to Marras (2000) critical reviews of the literature have strongly supported the association of LBD risk with lifting and forceful movements, bending and twisting movements and whole body vibrations. However, the magnitude of force and at what frequency that force is applied for an injury to occur still requires further study (Marras, 2000).

Jones and Kumar (2001) categorized risk factors for LBD injury into four categories: genetic traits, morphological traits, psychosocial traits and biomechanical aspects of the job. Genetic and morphological characteristics are fixed and employee morphology may not fit the physical demands of the job. However, employee selection based on these traits is only marginally acceptable for certain occupations (eg. firefighters). Psychosocial aspects of an occupation, for example the level of emotional stress, relationships with coworkers/supervisors, are difficult to adjust and tend not to be addressed by ergonomists. However, the biomechanical aspects of the job are able to be altered and have been the primary focus of ergonomic intervention strategies (Jones and Kumar, 2001).

The following is a list of the occupational risk factors as reviewed by Jones and Kumar (2001): static work, posture and technique, load characteristics, handles and coupling, frequency and repetitive handling, asymmetrical handling and non-uniform loads, space confinement and restraints, work environment, work duration, work organization. These are the biomechanical factors that can be reviewed in the workplace to provide a safe and healthy work environment for all employees.

Lifting postures and technique for safe lifting have been generally misunderstood according to Jones and Kumar (2001). Physical restrictions imposed by the work space will generally dictate the posture adopted by the worker. Therefore, the concept of the "safest technique" for lifting needed to be considered (Jones and Kumar, 2001).

Mital (1997) provides guidelines regarding lifting techniques in a variety of situations. Moderate weight should be handled between the knees and lifting infrequently, a squat posture is appropriate. When the load cannot fit between the knees and requires frequent handling, the load should only be lifted with the help of another person or a mechanical aid. When two people are required to lift an object, they should be matched for height and coordinate the lift through verbal communication. Mital (1997) also recommended avoiding the following when lifting: movement to the extreme of ranges of motion, twisting and jerky motions and statically loaded postures. Lifting above shoulder height or below the knees should be avoided. Twisting alone can reduce the tensile strength of the muscle by approximately 50% due to the alignment of the fibers of the annulus fibrosus within which each vertebral disc lies (Mital, 1997).

The characteristics of the load should be controlled to allow for safer lifting. The load should be rigid and uniform in shape and no larger than 50 cm in depth. If the load is required to be carried for any distance, then the line of sight should be unobstructed by the dimension of the load. Depending on the frequency, load size and the complexity of the lift, the magnitude of the mass should be adjusted. Frequency should also be limited to a maximum of 10 lifts per minute in an 8 hour shift, and should not exceed 12 lifts per minute for the durations greater than 2 hours (Mital, 1997).

The coupling of the load is another characteristic that must be considered to avoid risk of injury due to lifting. Mital (1997) specifies that handles should be 11.5 cm long

and 2.5-3.8 cm wide and if the handles are cylindrical, 3-5 cm of clearance around the entire handle. These handles should be placed at diagonally opposite ends providing stability about the horizontal and vertical planes. If the load does not have handles then the mass of the load should be reduced by 15% to avoid injury. The symmetry of the load will impact the magnitude of shear forces acting on the spine. If a load is asymmetrical the shear forces about the spine will increase. A reduction in load by 15% is recommended if the load is require to be lifted 90° in the saggital plane from its original position. The space in which the lift must occur may also become a risk factor if it restricts the operator from adopting a safe lifting posture (Mital, 1997). Although these recommendations alleviate the biomechanical risk factors involved with lifting, they may be too ideal to be prescribed for all workplaces.

2.4.4 Psychosocial Occupation- Related Factors

Psychosocial factors are the mental and social stresses involved with work such as job dissatisfaction, monotony of work, limited job control, and lack of social support (Cartledge, 2001; Yip, 2001; Bewick and Gardner, 2000, Retsas and Pinikahana, 2000; Lagerstrom et al., 1998; Cohen-Manfield et al., 1996) and have been associated with the incidence of LBD. Carayon and Lim (1999) identified eight psychosocial factors that may contribute to stress. Job demands comprise the quantitative workload, the monotony of the workload, the pressure to work and the cognitive demands. All of these factors can influence the amount of psychosocial stress that an individual will experience at the workplace. Job content was the second factor to be considered, which included issues regarding the amount of repetition the job requires, the challenge of the job and whether the skill set of the individual is being used. The third factor was job control. This involves the level of instrumentation of the task, the control the individual has over the task, involvement in decision making and organization of work. The control over the physical environment, pace of work and resources available will also impact the perceptions of psychosocial stress of a worker (Carayon and Lim, 1999). The level of social interaction was the fourth factor considered. This factor includes social support

from supervisors and colleagues and having to deal with difficult clients. The fifth factor was regarding roles, specifically if the role of the individual is clearly defined, and therefore no ambiguity or conflict will arise. Job security and future job prospects comprised the sixth factor (Carayon and Lim, 1999).

Carayon and Lim (1999) list work organization and management issues as a contributor to psychosocial stresses. Management style and the amount of input the employee is allowed will dictate perceptions of the workplace. If the employee perceives the workplace to be repressive and lacks support, psychosocial stress may contribute to an increase risk of injury.

All of these factors can have an impact on an employee's overall perception of their workplace and their own position within that workplace. When support systems are not available to reduce that amount of psychosocial stress that an individual will encounter, then employee perceptions of work become negative. Psychosocial stresses are thought to interact with the physical demands to create a biomechanical situation in which an injury will occur, such as lack of awareness of an appropriate lifting posture. Psychological stress at work or at home can also increase musculoskeletal tension, increasing the potential of an injury occurring (Yip, 2001). Increased psychosocial stresses may also affect a person's attention to work detail, reducing a person's ability to recognize hazardous situations or influencing judgment about how tasks should be completed.

Yip (2004) suggested that injury will result in psychosocial stress which in turn may even intensify the sensation of pain , exasperating or perhaps exaggerating the seriousness of the injury (Yip, 2004). Regardless of whether the stress came first or last, it is still impacting the duration and cost of the rehabilitation of the injury, and therefore cannot be ignored. While some may perceive the relationship of psychosocial factors to LBD as a statistical association rather than a clinical one, there is a growing consensus that these factors have a more direct effect on how a person physically performs a task and thus psychophysical factors must be considered in every ergonomics intervention strategy.

2.4.5 Assessment and Determining Occupational Guidelines

Given what is known about the mechanisms of LBD, the physical environment of the workplace and the physical demands upon the operator should be modified by ergonomists to provide a safer and healthier workplace. Workstations should be designed to ensure that force, repetition, awkward and static postures of physical tasks are within safe and allowable limits relative for the population performing the task (Yip 2004; Marras, 2000; Smedley et al., 1997).

A variety of models have been applied to assess physical demands, including biomechanical, physiological, psychophysical and epidemiological approaches. Due to the diversity of these approaches and of the tasks they are analyzing, no universal guidelines have emerged (Kumar and Mital, 1992). Laboratory experimentation to accurately determine the safe and allowable limits of occupational physical work are limited. The attempt to extrapolate participant responses from short experimental collection periods to a whole work day has obvious limitations.

Dempsey (1998) reported that psychophysical data established from short collection periods, less than 25 minute, are valid for low frequency lifting tasks, but may not be appropriate for tasks that employ lighter loads handled more frequently.

Buckle et al. (1992) reported that they seldom found direct application of published guidelines to be satisfactory. They recommended that each specific task should be systematically examined to assess the interaction of all relevant variables. Snook and Ciriello (1991) expressed the opinion that the best protective guidelines would be for the operator to understand his or her physical, physiological and psychological limits and to work within them. However, the reality remains that employees are not likely to be able to select what loads and at what frequencies to handle in an occupational setting. This is particularly true in a health care setting.

2.5 Occupational Factors Specific to the Nursing Profession

2.5.1 Introduction

Nurses have been reported to be among the highest at risk professions for incidence for LBD (Yip, 2004, Retsas and Pinikahana, 2000; Marras et al., 1999; Smedley et al., 1997; Cohen-Mansfield et al., 1996; Owen and Garg 1991). Cohen-Mansfield et al.. (1996) reported that nursing was the third leading profession for LBD and workplace injury. Typically, nurses have a number of personal, physical and psychosocial characteristics that place them in the high risk category for developing LBD. However, patient handling is the most frequently reported factor related to LBD (Carteledge, 2001; Yip, 2001; Retsas and Pinikahana, 2000; Marras et al., 1999; Engvist et al., 1998; Josephson et al., 1998; Lagerstrom et al., 1998; Cohen-Mansfield, 1997; Owen and Garg, 1991).

2.5.2 Nursing Shortage

Worldwide, the labour market is experiencing the effects of a cohort of retiring baby boomers. This vast number of retirees has left many occupational sectors, particularly the nursing profession, with a lack of experienced workers (Buchan, 2002). Vacancy rates, staff turnover rates, extended use of temporary staff and the amount of overtime only compounds the physical and mental demands placed upon health care workers. New diseases and infections such as SARS and HIV/AIDS are also related to the increased work demands of health professionals (Buchan, 2002).

Since the 1980's nurses have been changing orientations within or have left the profession altogether as a result of overexertion injuries (Owen, 2000). In a study conducted by Hemsley-Brown and Foskett (1999), youth are generally "aiming higher" than nursing, viewing the pay, conditions and required personality characteristics as undesirable. The attention on the nursing shortage has only fueled the continuing need

for injury prevention in order to maintain the health and wellness of the nurses that are currently in the workforce.

2.5.3 Nursing and Personal Factors

A cohort study conducted by Smedley et al. (1997) reported that there was no relation between age, mass or body mass index (BMI) and reported musculoskeletal injury. Yip (2004) investigated whether level of leisure activity outside the workplace would have an impact on incidence a low back injury. The suggestion was that the effects exercise can have on mood, anxiety, spine mobility and relaxation would positively impact the tolerance of the nurse to perform work duties with reduced risk for injury (Yip, 2004). However, this study reported that factors such as being comparatively new on the ward, bending frequently during work and having poor relationships with colleagues were independent predictors of LBD; while the level of exercise outside the workplace was not (Yip, 2004).

O'Brien-Pallas et al. (2004) investigated the contributing factors leading to injury claims by Canadian nurses. The study investigated workload and staffing data, the nurses' lost time injury claims data, organizational factors and individual nursing characteristics. One of the findings of this study was that even though Canadian nurses response to questions regarding their level of health was overwhelmingly positive (97% reporting excellent, very good or good health), other experimental results suggested otherwise (O'Brien-Pallas, 2004). When data regarding frequency of health related absenteeism, pain in the back, buttock, neck and shoulder and burnout were considered, 44% reported having one or more occasions of being absent due to these reasons in the previous three months leading to the questionnaire collection (O'Brien-Pallas et al., 2004). Sixteen percent reported back or buttock pain and 17% reported neck or shoulder pain in the previous week. Twenty five percent reported working with musculoskeletal pain for most of or all of the time (O'Brien-Pallas et al., 2004). O'Brien-Pallas et al. (2004) mentioned that nurses may compare their level of health to the patients which they care for, therefore resulting in a more positive outlook on their own health. However, it

seems clear that nurses therefore may not recognize the importance of their perceptions of health as precursors to injury.

2.5.4 Nursing and Physical Loads

The most commonly reported physical demands of nursing that likely contribute to the development of a LBD are lifting, pushing/pulling, transferring patients (Skotte, et al. 2002; Retsas and Pinikahana, 2000; Marras et al., 1999; Engvist et al.,1998; Lagerstrom et al., 1998; Cohen-Mansfield et al., 1997; Smedley et al., 1997), repetition of patient handling tasks (Yip 2004, Cohen-Mansfield et al., 1996), inadequate space to perform patient handling tasks (Bewick and Gardener, 2000; Cohen-Mansfield et al., 1996), poor work posture (Yip 2004; Engvist et al., 1998; Lagerstrom et al., 1998; Smedley et al., 1997; Cohen- Mansfield et al., 1996), lack of proper technique during patient handling (Bewick and Gardner, 2000; Engvist et al., 1998; Cohen-Mansfield et al., 1996), inadequate staff (Engvist et al., 1998; Cohen-Mansfield et al.,1996) and inadequate rest (Beynon et al., 2000).

According to Engvist et al. (1998) there are twenty-two identified factors related to the risk of injury and that these factors can be grouped into five categories. These factors have been summarized in the Table 2.1.

Table 2.1: Summary of the twenty-two factors involved in the injury process according to Engvist et al. (1998).

Category	Risks
Work Organization	lack of adequate staff
	lack of information on the patients condition that day
	nurses felt stresses or rushed
	nurses performing transfer alone
	lack of training regarding technique or lost grip during transfer
Workplace factors	risks inherent in the environment
	no proper transfer devices
	nurses assuming awkward postures due to space constraint
Nurses	assuming an awkward posture on own accord
	lack of communication between nurse and patient regarding transfer
	Nurse making a sudden movement
Patients	Mass greater than 80kg
	sudden loss of balance or resistive to being moved
Specific Transfers	moving a patient in the bed
	moving the patient to or from the bed
	moving the patient to or from the toilet
	moving the patient from floor level
	moving patient from a stretcher or x-ray table

Yip (2001) supports Engvist et al.. (1998) with similar findings regarding risk involved in patient transfers between beds and chairs, onto trolleys, repositioning in bed, and assistance during ambulation or walking exercises resulting in the highest reports of injury. Another major contributor that Yip (2001) recognized is the relationship of repetition of patient handling tasks and risk of injury.

Studies have identified specific units within the hospital that most injuries have occurred and include: geriatric (Cohen-Mansfield, 1997), surgical and medical wards (Engvist et al., 1998). The Health Care Corporation of St. John's, NL reported in 2000 that the continuing care unit, followed by the critical care unit as the location of the most injuries (Stinsen, 2000). These are units that involve a high volume of patient handling.

2.5.5 Patients as a risk factor

The potential to automate a task involving the transfer of a live human being can be limited. Referring to Mital (1997), the load that nurses lift does not comply with any of the traditional manual materials handling guidelines. Patients that nurses are responsible for transferring are not rigid bodies, are asymmetrical with respect to load distribution and impose awkward coupling. Nurses do not have the ability to control the magnitude or the shape of the load handled, nor the ability to predict the movement of the load or the pattern of lifting that will be present during a shift. The number, mass and dependency level of the patients that will be under a nurse's care for any particular shift cannot always be predicted or standardized. Furthermore, the patient themselves can be unpredictable. The patient may or may not be compliant when being transferred. Depending on the condition of the patient, the nurse may become unexpectedly loaded if the patient suddenly loses balance or consciousness. The end result being that the patients themselves can not be administratively controlled to the benefit of the nurse. Thus, related research has focused more on the adaptations the nurses can make to transfer techniques and the surrounding environment.

Kjellberg, Lagerstrom and Hagberg (2004) assessed the comfort of the patient during handling and transfer tasks. These authors found a moderate correlation between

the comfort of the patient (r=0.23) and the self reported evaluation of the nurses' own work technique for both tasks analyzed (r=0.21), concluding that safe transfers with regards to the nurses' musculoskeletal health are also the transfers that are most comfortable and secure for the patient (Kjelberg et al., 2001). Mannion, Adams and Dolan (2000) reported that sudden and unexpected loading can substantially increase the compressive load on the spine and that eliminating such events are recommended. If the patient is more confident that the transfer will be performed in a safe and secure manner, it could be postulated that safe transfer methods could reduce the factors of resistance to transferring, such as combative behavior, thus reducing the chances of unexpected loading events. The inherent issues involved with manipulating the patient in a safe and efficient manner has lead to important research regarding safe transfer skills and other intervention strategies to prevent injury for nurses.

2.5.6 Patient handling

According to research conducted over the past ten or so years, the following are considered the most prevalent patient handling maneuvers that lead to injury: repositioning/ adjusting patient in bed (Skotte, Essendrop, Hansen and Schibye, 2002; Yip, 2001; Marras et al., 1999; Engvist et al., 1998), transferring patient from bed to chair and chair to bed (Marras et al., 1999; Smedley et al., 1997; Owen and Garg, 1991), transferring patient from chair to commode and commode to chair (Smedley et al., 1997; Owen and Garg, 1991; Marras et al., 1991), assisting a patient while walking (Yip 2001; Engvist et al., 1998) and assisting patient stand from a sitting position (Skotte et al., 2002). A prevalent theme emerging from this body of research was that there was not adequate training in proper technique to prevent over-exertion injuries (Bewick and Gardner, 2000; Engvist et al., 1998; Cohen-Mansfield et al., 1996). Nurses are now being provided with education programs, mechanical lifts, and other tools to aid in transferring and handling patients. However, Hignett (1996) believes that back education alone will not solve the high injury incidence of nurses.

2.5.7 Psychosocial stresses of nursing

Psychosocial stress has been identified as a contributing factor related to the occurrence of LBD. The stresses in the workplace that nurses have to compete with are mainly the stress of being responsible for ill or dying people (Cartledge, 2001; Retsas and Pinikahana, 2000), lack of authority (Lagerstrom et al., 1998), lack of social support (Cartledge, 2001; Retsas and Pinikahana, 2000; Lagerstrom et al., 1998), lack of job desirability (Cohen-Mansfield et al., 1996), and the effects of shiftwork (Cartledge, 2001; Retsas and Pinikahana, 2000; Josephson et al., 1998; Lagerstrom et al., 1998). According to Josephson et al. (1998) the most influential psychosocial stress for nurses is shift work. The sleep disturbances that often accompany shift work can lead to "low control" in planning a patient transfer task, easily leading to mistake and injury (Josephson, 1998).

The lack of job desirability has also become a recent issue. With the retirement of the baby boomers and the tendency for high turnover of nurses in particularly heavy patient handling units, there is now a shortage of nurses able to work.

2.6 Ergonomic Interventions Related to the Nursing Profession

2.6.1 Introduction

A number of interventions have been employed in attempt to decrease the threats to job-related injuries. These have included tools to aide transfers, mechanical lifts, back education and practical application of specific transfer training (Collins, Wolf, Bell and Evanoff, 2004; Finch, Guthrie, Westphal, Dahlman, Berg, Behnam and Ferrell, 2004; Keir and MacDonell, 2004; Li, Wolf and Evanoff, 2004; McCannon, Miller and Elfessi, 2004; Johnsson, Carlsson and Lagerstrom, 2002; Caboor et al., 2000; Zhuang, Stobbe, Hsiao, Collins and Hobbs, 1999; Burton, Symonds, Zinzen, Tillotson, Caboor , Van Roy and Clarys, 1997;Goodridg and Laurila, 1997; Charney, Zimmerman and Walara, 1991;

Owen and Garg, 1991; Venning, 1988). Each intervention has been reviewed to assess which are the most effective for reducing overexertion injuries for nurses.

2.6.2 Back education and transfer instruction

Guthrie et al. (2004) reported that back education alone has not been effective in changing work related injuries for nurses. The primary reason for the lack of success with solely implementing a back education program is that most back education programs are not directly applicable to specific patient handling tasks, therefore most of the standard rules for body mechanics under load cannot be employed by nurses (Guthrie et al., 2004). Ergonomic interventions for nurses combining back education with practical training of safe transfer methods and /or the designation of lifting teams have been more successful.

Practical training in transfer techniques have been recognized to reduce poor postures and other risk factors that typically result in injury. McCannon et al. (2003) compared the differences between a control group (had no previous patient handling experience), a basic body mechanics training group and a job site training group. Two transfers were assessed, the one person pivot transfer and repositioning a patient up in bed. The results of this study indicated that job site training (ie specific patient transfer training) is the most effective intervention in producing safe postures as opposed to back education alone (McCannon et al., 2003).

Another concept that has been introduced as an intervention strategy against injury is for nurses to lift in teams. Charney et al. (1991) found that a lifting team approach was able to reduce lost time injuries by 95%. The lifting team are professional patient handlers that have had specialized training on the unique characteristics of patient transferring and understand the system's complexity (Guthrie et al., 2004). By having a lift team that will be screened, trained, equipped and then dispatched to perform the patient transfer tasks, the nurses are relieved from this work and can focus on patient care (Guthrie et al., 2004). Charney (1992) found that the implementation of a lifting team reduced the number of total injuries related directly to patient handling, those with and

without lost time by 69%. Not only will a lifting team provide a reduction in the physical related risks for back injury of nurses, Charney et al. (1991) also reported greater nursing morale, recruiting nurses was much easier when lifting teams existed. Therefore, there are psychosocial benefits to a lifting team approach. The team lifting approach does have some negative aspects which have therefore prevented it from becoming a universally accepted approach. The primary reason for lack of adaptation of this approach by other countries is that the increased cost of employing a specialized team to perform only these duties. The return on investment however will offset these costs, as injury rates has shown to dramatically decrease as a result of this approach.

2.6.3 Tools and mechanical aides

Owen (1988) and Bell (1984) reported that employing mechanical devices during patient transfers could take up to 17 times longer compared to a more traditional manual transfer technique. The majority of this time is taken by having to position the patient in an appropriate manner in order to secure them within the device. Owen and Garg (1991) found that two out of the three mechanical devices that they used for patient transfer were perceived by those using the device to have as high or higher physical stress level than a manual transfer. Although previous literature indicates that transfer devices, if used, would provide a reduction in injury, nurses still are not using them as much as expected (Owen and Garg, 1991). The reasons that nurses often provide for not using the transfer aides is that too much time is involved in using the device, that the staff lacked experience in the usage or there was lack in availability of the device at the time of need (Owen and Garg, 1991).

Owen (2000) commented on the lack of use of assistive transfer devices. Nurses indicated that the reasons they were not using them was because the device required was unavailable; the device took too much time, the device was unstable or otherwise unsafe and that the patients were not comfortable with the devices being used. Nurses' perceptions of the assistive devices in this study were changed when they were properly trained on how to use then, when there were appropriate numbers of devices and were

supported by management to use the device (Owen, 2000). A more recent study conducted by Zhunag et al. (2004) reported on the use of a basket sling or an overhead lift used to aid a resident into a standing position and/ or transferring into a chair. Zhunag et al. (2004) found that the manual transfer without employing a mechanical aid would result in 3454 N of compression, a value exceeding the 3400N NIOSH recommendation, in comparison to the 2698 to 2951 N range of compression predicted when using an overhead or basket sling lift. The range was a result of the differences between those nurses who pushed the patient to their side (2698 N) rather than pulling to roll the patient to their side in bed (2951 N). Li et al.. (2004) also reported similar findings, stating that the implementation of the mechanical lifts reduced the reported musculoskeletal symptoms, injury rates and lost day injury rates. Li et al. (2004) noted that mechanical lift usage compliance gradually decreased during the six month trial period, thus strategies must be developed to encourage future usage of transfer devices.

Since nurses perceived mechanical lifts as more stressful then manual transfers, Keir and MacDonell (2004) assessed the electromyography (EMG) activities of primary movers (bilateral upper and lower erector spinae, bilateral latissimus dorsi, bilateral trapezius) in experienced nurses. They hypothesized that experience has an impact on the reduction of EMG measured during the use of mechanical lifts and manual transfers. Initially, it appeared that the manual transfer would result in the greatest amount of muscle activation. However, when the EMG of each manual and mechanical transfer method were integrated over the time it took to complete the transfer, they found that the manual transfer took much less time than the mechanical lifts and therefore had the lowest level of integrated EMG (Keir and MacDonell, 2004). With empirical evidence such as this, the anecdotal reports from nurses that manual transfers are more efficient and less stressful than a mechanical lift may seem valid.

In summary, transfer aides can be effective but often are unavailable or underutilized (Finch Guthrie et al., 2004). Furthermore, empirical evidence supports the allocation of resources to develop manual transfer methods and educational approaches to reduce the risk of LBD in nurses.

2.6.4 Back Injury Prevention Program (BIPP)

A focus on manual transfers led the Occupational Health Services Department of the Health Science Center in St. John's, Newfoundland and Labrador to develop the Back Injury Prevention Program (BIPP) (Bouchier, 1996). The BIPP was designed to teach professional and student nurses patient transfer techniques which adhere to proper biomechanical principles. Those principles are out lined as "the ME rules". These rules are a used as a reminder to the nurses to protect themselves ("ME"), when performing patient handling tasks. Those rules are:

"(1) Test the weight of the object. Be certain I can lift without injury risk. (2) Hold object/person close to my body and in front of me. (3) Feet wide apart to lower my center of gravity and to weight-shift. (4) Trunk upright and stabilized on the pelvis. (5) Shoulders stabilized, elbows close to sides, wrists straight. (6) Don't twist, move symmetrically. (7) Use my legs to make any movements not my trunk. (8) Move smoothly, do not jerk."

The program provides instruction on how to perform various patient handling tasks while applying these biomechanical principles. The nurses are taught what questions to ask themselves to aid in selecting an appropriate transfer for the patient and their condition. In other words, nurses are conditioned to perform a risk analysis prior to attempting a patient transfer. The nurses that continue to work with the Health Care Corporation of St. John's, NL are retrained every few years to ensure they are still applying the principles of BIPP. Since the implementation of the BIPP the Health Care Corporation of St. John's, NL has seen a decrease in the number of back injuries in most departments (Stinsen, 2000).

Jensen (1990) reported that back education programs provide little evidence to support their efficacy for reduction in overexertion injuries. However, Jenson (1990) does state that back education in conjunction with other ergonomics interventions, such as identifying the tasks with greatest amount of risk of injury and modifying these tasks to incorporate mechanical aides should negate some of these risks. While this approach may have high elements of content validity, not all hospitals have the financial resources to

install a mechanical lift in needed rooms. The BIPP requires little equipment, therefore if its instruction can provide reasonable protection against injury, then its implementation can be a cost effective method hospitals can employ to protect their employees.

CHAPTER 3: METHODOLOGY

3.1 Introduction

There were two data collection periods for this study. The first data collection period, Phase 1, considered novice participants who had no previous experience with patient handling. Volunteers who were not female, reported previous back injury, were pregnant or were not of consenting age were excluded from this study. This collection period occurred over two consecutive days. The first day was to examine the muscle activity and lumbar motions of participants before being taught the BIPP (Back Injury Prevention Program) method of patient handling. The second day consisted of instruction according to the BIPP methods and subsequent re-examination of the novice subjects performing the same patient handling tasks.

The second data collection period, Phase 2, assessed experienced, active nurses performing the same patient handling tasks as Phase 1. No instruction was provided regarding handling techniques to the experienced nurses prior to data collection. Thus, subjects in Phase 2 were only sampled once. The study protocol was approved by the Human Investigations Committee of Memorial University.

3.2 Dependent Measures

3.2.1 Electromyography

Five muscles, measured bilaterally, were selected for analysis during the patient transfers. These included the trapezius, posterior deltoid, external oblique, erector spinae and rectus femoris muscles. Prior to electrode placement, the skin was shaved, abraded and cleaned with rubbing alcohol to help increase the transfer of the signal from the muscle to the electrode. Surface electrodes (Kendall ® Medi-trace 100 series) were used in a bipolar configuration and were placed at the midbelly of each muscle. A ground electrode for each bipolar arrangement was placed within 10cm of the recording site. The

ME3000P (Mega Electronics Ltd. Kuopio, Finland) unit was used to collect the EMG profiles. The external unit was connected to the communications port of a personal computer via an optical cable to collect the data in real-time. Each of the eight channels was sampled at 1000 Hz, band-pass filter between 20 Hz and 500 Hz amplified (differential amplifier , common mode rejection ratio $\geq$ 130 dB, gain x 1000, noise $\leq$ 1μV) and analogue to digitally converted (12-bit) and stored on a personal computer for later analysis. Table 3.1 outlines the muscles that were assessed for each patient handling task.

Table 3.1: Selected EMG Collection Sites Based on Patient Transfer

Patient Handling Task	Muscles Assessed				
	Erector Spinae	Trapezius	Deltoid	External Obliques	Rectus Femoris
Repositioning up in bed: head	X	X	X	X	
Repositioning up in bed: side	X	X	X	X	
Bed to Chair	X	X		X	X

3.2.2 Lumbar Motion Monitor

A Lumbar Motion Monitor (LMM) was employed to measure the subject's thoraco-lumbar displacements in three dimensions. The data from the LMM were collected at 60 Hz, converted to a digital record and stored on a computer for further reduction and analyses. The LMM was attached to the subject by a backpack harness system using a chest and pelvis straps (Figure 3.1).

Figure 3.1: Placement of LMM harness and backpack on participant.

3.3 Patient Handling Tasks:

Three patient handling tasks were considered in this study:

(1) The patient was reposition towards the head of the bed using a draw sheet. This is a two person transfer, both positioned at the **side** of the bed (Figure 3.2, 3.3. In reference to this study, this will be called "Transfer 1".

(2) The patient was reposition towards the head of the bed using a draw sheet. This is a two person transfer, both positioned at the **head** of the bed (Figure 3.4, 3.5). In reference to this study, this will be called "Transfer 2".

(3) The patient was transferred from a bed to a wheelchair. This is a one person transfer and employs a transfer belt (Figure 3.6. In reference to this study, this will be called "Transfer 3".

3.3.1 *Repositioning up in bed using a draw sheet while positioned at side of the bed (Transfer 1)*

For reposition up in bed positioned at the side of the bed the participants begin the transfer at the side of the bed with their center of mass in line with the pelvis of the patient. With feet and arms parallel, palms facing upward and arms bent between 90-100° of flexion, the participant grasped the draw sheet tight between their hands and the patient's body. The participant will start with all of their weight on the leg closest to the foot of the bed. The patient will be displaced up in bed as the participant transfers their weight from this leg to the other leg, while keeping their elbows braced at their sides and knuckles staying in contact with the bed (Figure 3.2a &b).

Figure 3.2 a: Repositioning up in bed from side: position at the start of the transfer.

Figure 3.2 b: Repositioning up in bed from side: position at the end of the transfer.

3.3.2 Repositioning up in bed using a draw sheet while positioned at head of bed (Transfer 2)

When repositioning a patient up in bed, positioned at the head of the bed the participant would begin the movement by kneeling on the corner of the bed with their navel in line with the corner of the footboard diagonal from them (Figure 3.3 a &b), The participant, ensured that their knee was positioned such that the patient's shoulder would clear from obstruction when repositioned up in bed. The participant would stand tall on the kneeling knee, grasping the draw sheet tight with palms facing up. While bracing their elbows at their sides, the participant transferred weight by sitting down on their heel, effectively transferring the patient to the head of the bed.

Figure 3.3 a: Repositioning up in bed from head: position at the start of the transfer.

Figure 3.3 b: Repositioning up in bed from the head: position at the end of the transfer.

3.3.3 Bed to Chair using one person pivot method (Transfer 3)

For assisting the patient from bed to chair using the one person pivot technique (refer to Figure 3.4), the participant began the lift by applying the transfer belt to the patient's waist and then grabbed the handles located on the back of the belt with the participant's hips square with those of the patient. To clear the patient from the bed three rocking motions were used to gain enough momentum to clear the patient from the bed. The participant's legs were positioned on either side of the patient's legs to ensure control over the patient. The wheelchair was located at a 45 degree angle to the bed. Once the patient was clear from the bed the participant pivoted at the feet, not at the trunk, and lowered the patient slowly by bending the legs.

Figure 3.4: Transferring from bed to chair using the one person pivot technique: sequence of movement events.

3.3.4 Successful Transfer Criteria

A criterion was established to identify a successfully completed transfer. For Transfers 1 and 2 (reposition up in bed), successful completion required the patient to be repositioned to at least 6 cm from the head of the bed. For Transfer 3 (transferring patient from bed to chair), successful completion required that the patient be effectively relocated to the chair.

3.3.5 Patient Level of Dependency

For each of these patient handling tasks, a healthy surrogate patient was used. This "patient" was recruited to participate in all the patient handling tasks for the entirety of the experiment. The patient was requested to maintain a consistent level of dependency throughout the experiment. During Transfers 1 and 2 (reposition up in bed) the patient was completely passive. According to the BIPP requirements to employ a one person pivot from bed to chair, a patient must be able to follow verbal instructions and able to bear his own weight, but not necessarily be able to go from a sitting to standing position unassisted. Thus, the surrogate patient was asked to act in this manner. To ensure inter-trial consistency by the surrogate patient a familiarization session with a qualified BIPP instructor was implemented. The one person pivot transfer was performed until the instructor was confident that the patient was bearing an equal amount of weight for each practice transfer.

3.3.6 The Second Patient Handler

Two people were required to complete Transfers 1 and 2 according to BIPP protocol. The same person performed this task consistently throughout all trials. To ensure this consistency the person had completed two BIPP training sessions and BIPP instructors confirmed that this aide completed the transfers properly.

3.4 Experimental Protocol

3.4.1 Phase 1: Novice Participant Pre and Post- training

Subjects

Twelve female participants (stature of 1661 ± 6 mm; mass of 65 ± 12.2 kg; age of 23.7 ± 1.4 years) volunteered to participate in the study. These subjects had no previous training in patient handling procedures.

Session 1:

Participants were required to attend three experimental sessions for Phase 1 of the study. The experimental protocol was explained and written and verbal consent obtained during the first session. The participant's mass and stature were recorded. Surface EMG electrodes were placed on the left and right trapezius (TRAP), left and right deltoid (DELT), left and right erector spinae (ES), left and right external oblique (EO) and left and right rectus femoris (RF). Maximum voluntary contractions (MVC's) were then collected. For collection of the MVC's the participant was positioned and resistance was provided while verbal encouragement was given to the participant to exert a maximal voluntary effort. For the trapezius MVC's the participant's hand was held and gripped at the wrist by a research assistant to provide enough resistance against a isometric shoulder elevation activation. Right and left trapezius were collected separately. For the deltoid MVC's, the participant was positioned next to a wall with her elbow flexed at a 90 degree angle and performed and isometric shoulder abduction activation. Right and left deltoids were collected separately. Both left and right erector spinae MVC's were collected simultaneously while the participant flexed forward at the hip onto a bed while two research assistants were positioned to provide resistance against the right and left scapula while the participant performed an isometric lumbar extension activation. For the external oblique MVC's, the participant stood with their back against a wall and were instructed to rotate medially about the longitudinal axis. A research assistant provided enough resistance against the anterior aspect of the participant's shoulder to ensure an isometric activation was performed. The rectus femoris MVC's were collected while

participant sat in a chair with her knee flexed at 90 degrees. A cuff was secured to the back two legs of a chair and the subject's ankle to provide the resistance against an isometric knee extension activation. Right and left legs were collected separately. All activations were held for 5 seconds and two repeated measures for each muscle were collected. The largest activation for each muscle was selected for normalization purposes.

The participants then viewed a 3 minute video of the three transfers considered in this study. No further instruction was give to participants. The LMM was then calibrated and mounted on the subject employing manufacturer's suggested guidelines. At this time, the subject was ready to begin data collection. The three transfers were randomly assigned to each subject. Data integrity was monitored during collection and the recorded raw data were verified before proceeding to the next trial. Each transfer was repeated three consecutive times.

Session 2:

The participants attended a 2 hour BIPP instruction session. During this session the participant was provided standardized instruction of the biomechanical principles of the BIPP and practiced the transfers. The technique was critiqued and corrected by a BIPP instructor during this session.

Session 3:

The participant then repeated the first session protocol, only without viewing the video. Again, the transfers were assigned in a random order to the subjects. Refer to Figure 3.5 for chronological sequence of events for Phase 1 of the study.
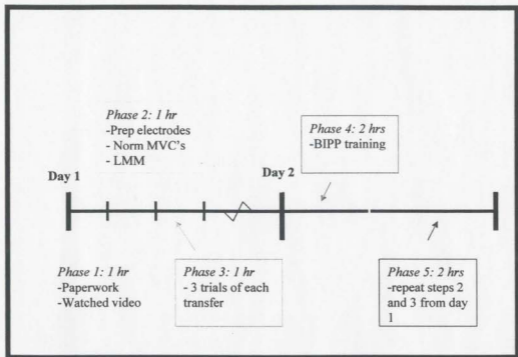
Figure 3.5: Chronological sequence of three sessions in Phase 1 completed by novice participants.

3.4.2 Phase 2: Experienced Nurses

Subjects

Ten female participants (stature of 1599 ± 59.9 mm; mass of 73 ± 19.5 kg; age of 41.6 ± 10.2 years) volunteered to participate in this study. These participants were experienced nurses (14.3 ± 9.5 years of employment) from the Health Care Corporation of Newfoundland. The dependent measures and patient handling tasks were similar to those described in Phase 1 of the methodology.

The experienced nurses were required to attend one experimental session as their requirement to participate in the study. The experiment was explained and written and

verbal consent obtained at this time. The participant's mass and stature were recorded. The same procedures were employed as Phase 1 to collect MVC's for the experienced nurse participants. The LMM was then calibrated and mounted on the subject employing manufacturer's suggested guidelines. At this time, the subject was ready to begin data collection.

The experienced nurse was then informed of what transfers she was required to complete. The three transfers were randomly assigned to each subject. Data integrity was monitored during collection and the recorded data were verified before proceeding to the next trial. Each transfer was repeated three consecutive times.

3.5 Statistical Analysis

In Phase 1 a paired t-test was used to compare the pre- and post-training values calculated from the EMG- and LMM-time histories. Significant differences were identified using a 95% confidence interval. In Phase 2 an analysis of variance (ANOVA) test was used to compare the pre- and post-training novice participants to the experienced nurse participants. Significant differences were identified using a 95% confidence interval. A Least Squared Difference (LSD) Post Hoc test was employed in those cases where the ANOVA identified significant inter-group differences.

CHAPTER 4: RESULTS

4.1 Introduction

The EMG and LMM data were collected, reduced and analyzed in a similar manner for both Phases 1 and 2 of the study. The EMG and LMM data were synchronized in time using the proprietary software purchased with these devices. The start of each transfer movement was clearly indicated on both the EMG- and LMM-time histories which allowed for easy synchronization of the data sets.

The EMG data were normalized in magnitude in reference to the largest mean MVC value sampled during the middle three seconds of the isometric activation. Normalization values were always collected prior to the collection of patient transfer tasks and are experimental session specific. For each trial the maximum and average EMG value were determined for each muscle. The means of these three repeated measures data were then calculated and used in subsequent statistical analyses.

The LMM collected displacement-time series data in the sagittal, lateral and twisting planes. The LMM software (Ballet 2.1, BIOMEC Inc.) was employed to derive the velocity -time histories in all three planes of movement using a finite differences approach. For each trial the mean displaced position in both directions of the three movement planes were determined and then a mean was calculated across the three trials. For each trial the maximum and average LMM velocity values were determined for each movement plane. The mean of these data were then calculated and used in subsequent statistical analyses.

4.2 Comparative analysis of pre- and post-training novice participant's results

Repeated measures t-tests were employed to analyze the pre- and post- training results from the novice participants. The following section will describe the results of this comparison.

4.2.1 Transfer 1: Repositioning up in bed, positioned at the side of the bed for novice participants pre- and post-training

The mean EMG values for the repositioning up in bed, while positioned at the side of the bed demonstrated significant decreases in the left trapezius ($p < 0.005$) and left ($p < 0.005$) and right ($p < 0.025$) erector spinae (Figure 4.1). The maximum EMG values showed similar decreases in activities for these muscles during the post-training session (Figure 4.2). While not statistically significant, there were also decreasing trends in both the mean and maximum EMG data for the right trapezius, as well as the left and right deltoid.

Figure 4.1: Mean EMG data for novice participants during transfer 1: reposition up in bed, positioned at the side of the bed.

Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005

Figure 4.2: Maximum EMG data for novice participants during transfer 1: reposition up in bed, positioned at the side of the bed.

*Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.*

The LMM ranges and velocities in three planes of motion (lateral, sagittal and twist) were compared between the pre- and post-training test sessions. The repositioning up in bed when positioned at the side of the bed resulted in two significant differences in the LMM data. There was a reduction in the maximum amount of extension recorded ($p<0.025$) and the maximum amount of flexion recorded ($p<0.005$) for the novice participants (Figure 4.3). There were not significant differences between the pre and post-training novice results with regards to thoracolumbar velocity (Figure 4.4).

Figure 4.3: Mean LMM displacement in novice participants performing transfer 1: reposition up in bed, positioned at the side of the bed.

Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.

Figure 4.4: Mean and maximum LMM velocity in novice participants performing transfer 1: reposition up in bed, positioned at the side of the bed.

Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.

Significant decreases were found in the mean muscle activity for the left ($p<0.05$) and right ($p<0.025$) trapezius, the left erector spinae ($p<0.005$) and the left ($p<0.025$) and right ($p<0.05$) external obliques in the post-training data (Figure 4.5). Similar results were observed for the maximum EMG results, with only the right trapezius being significantly different ($p<0.05$) (Figure 4.6). The right deltoid also showed a trend to decrease its mean and maximum activity during post-training.
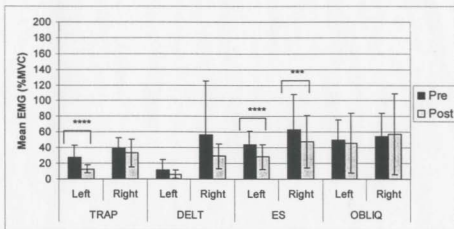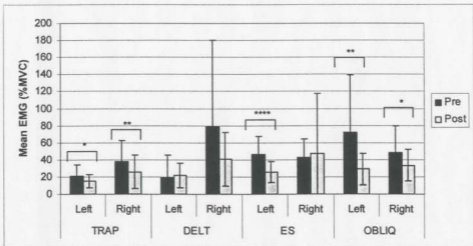

Figure 4.5: Mean EMG data for novice participants during transfer 2: repositioning up in bed, positioned at the head of the bed.

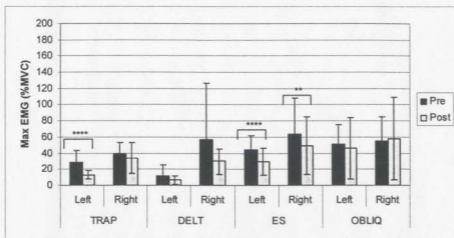*Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.*
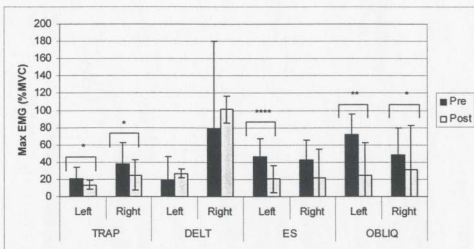
Figure 4.6: Maximum EMG data for novice participants during transfer 2: repositioning up in bed, positioned at the head of the bed.

*Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.*

The mean LMM displacements had significant decreases in post training compared to the pre-training session for the following measures: maximum right bend (p<0.001), maximum extension (p<0.044), and flexion (p<0.05); maximum lateral range and mean lateral velocity (p<0.01); maximum lateral velocity (p<0.005) (Figures 4.7 and 4.8). A significant increase was also identified for mean results during post training test in maximum left twist (p<0.01).
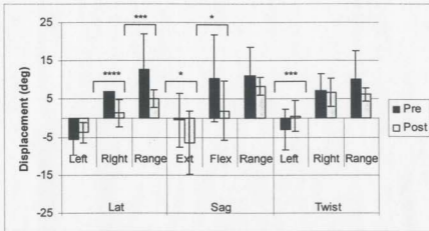
Figure 4.7: Mean LMM displacement in novice participants performing transfer 2: reposition up in bed, positioned at the head of the bed

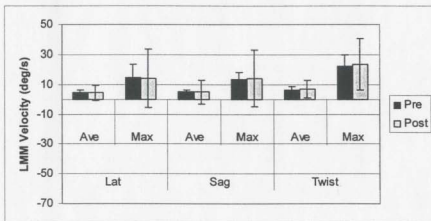*Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.*

Figure 4.8: Mean and maximum LMM velocity in novice participants performing transfer 2: reposition up in bed positioned at the head of the bed.

*Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.*

4.2.3 Transfer 3: Bed to Chair, one person pivot technique

In light of the limited number of EMG data collection channels, it was decided that it was more important to measure the rectus femoris musculature than the deltoid muscles for the one person pivot transfer (refer to Table 3.1). Significant decreases in the mean EMG activities were identified for the left ($p<0.01$) and right ($p<0.005$) trapezius, left ($p<0.005$) and right ($p<0.005$) erector spinae and left external obliques ($p<0.005$) (Figure 4.9). A significant increase in right rectus femoris ($p<0.025$) activity was observed. Similar results were found for the maximum EMG values. There were decreases in the left ($p<0.01$) and right ($p<0.05$) trapezius, left ($p<0.025$) and right ($p<0.01$) erector spinae, left external obliques ($p<0.01$) and an increase in right rectus femoris ($p<0.005$) activity (Figure 4.10).
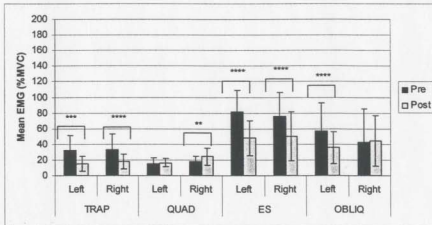
Figure 4.9: Mean EMG data for novice participants during transfer 3: bed to chair using one person pivot technique.

*Note: *$p< 0.05$, **$p< 0.025$, ***$p< 0.01$, ****$p< 0.005$.*

Figure 4.10: Maximum EMG data for novice participants during transfer 3: bed to chair using one person pivot technique.

Note: $*p < 0.05$, $**p < 0.025$, $***p < 0.01$, $****p < 0.005$.

A significant increase was identified for the maximum right bend displacement ($p<0.025$) and maximum lateral range ($p<0.01$) in the post training test (Figure 4.11). The one person pivot method of transferring from bed to chair revealed a significant increase maximum lateral range ($p<0.025$) (Figure 4.11). A significant reduction in mean sagittal ($p<0.05$) and twist ($p<0.005$) velocities was observed (Figure 4.12). The results for novice participants indicated a significant decrease in mean twist velocity ($p<0.005$) and mean sagittal velocity ($p<0.005$) (Figure 4.12).
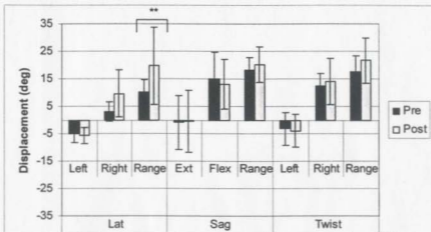
Figure 4.11: Mean LMM displacement in novice participants performing transfer 3: bed to chair using one person pivot technique.

Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.
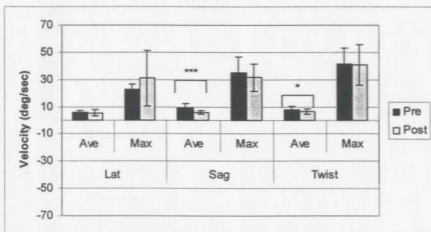
Figure 4.12: Mean LMM velocity in novice participants performing transfer 3: bed to chair using one person pivot technique.
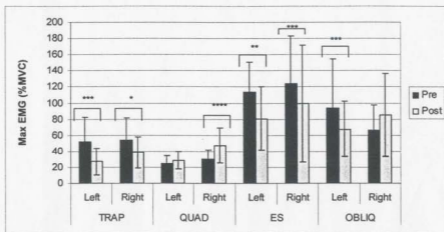
Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.

4.3 Comparison of Novice Participants and Experienced Nurses

This section compares the pre- and post- training measures of the novice participants with the data collected for the same patient transfers performed by the experienced nurses. An independent measures ANOVA was employed in recognition that two different subject pools were being considered in these analyses, although it is acknowledged that two of the three sets of grouped data come from the pre- and post-novice subjects. In this respect, one could assume that violations of homogeneity of variance are less likely and statistical interpretations using this model strategy would be valid, in light of all limitations and assumptions identified in this study.

4.3.1 Transfer 1: Repositioning up in Bed, positioned at the side of the bed for experienced nurses compared to novice participants pre- and post-training

An analysis of variance was employed to examine the difference between the novice participant results and experienced nurses' results. A significant difference identified from the ANOVA was for the left trapezius ($p<0.001$). The post-training novice participants recorded significantly lower mean (Figure 4.13) and maximum (Figure 4.14) left trapezius activation levels then the experienced nurses.

The mean EMG results were compared between groups (Table 4.1. Significant decreases in magnitudes were identified for the left ($p=0.022$) and right ($p=0.028$) trapezius between the pre-training results and the post training results for right ($p<0.001$) and left ($p=0.005$) trapezius activity. There were no significant differences found in the maximum EMG activity data (Table 4.2). In the Tables the "-" sign represents the experienced nurses recorded a significantly smaller value of the indicated measure with respect to the novice participants. A "+" sign represents the experienced nurses recorded a significantly greater value of the indicated measure with respect to the novice participants.
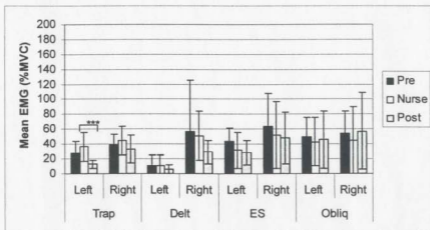
Figure 4.13: Mean EMG data for novice participants and nurses during transfer 1: reposition up in bed, located at the side of the bed.

*Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.*
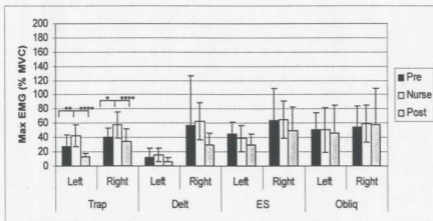
Figure 4.14: Maximum EMG data for novice participants and nurses during transfer 1: reposition up in bed, located at the side of the bed.

*Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.*

Table 4.1: Summary of the significant differences of the mean EMG values (expressed as a percent of MVC) between the experienced nurses and novice participants for transfer 1: reposition up in bed, positioned at the side of the bed.

Note: (-) and (+) denotes the direction of the change relative to the experienced nurses.

Mean	Left TRAPEZIUS	Right TRAPEZIUS	Left DELT	Right DELT
Pre	NS	NS	NS	NS
Post	p<0.001 +	NS	NS	NS
Mean	Left ES	Right ES	Left OBLIQ	Right OBLIQ
Pre	NS	NS	NS	NS
Post	NS	NS	NS	NS

Table 4.2: Summary of the significant differences of the maximum EMG values (expressed as a percent of MVC) between the experienced nurses and novice participants for transfer 1: reposition up in bed, positioned at the side of the bed.

Note: (-) and (+) denotes the direction of the change relative to the experienced nurses.

Max	Left TRAPEZIUS	Right TRAPEZIUS	Left DELT	Right DELT
Pre	NS	p<0.028 +	NS	NS
Post	p<0.001 +	p<0.005 +	NS	NS
Max	Left ES	Right ES	Left OBLIQ	Right OBLIQ
Pre	NS	NS	NS	NS
Post	NS	NS	NS	NS

The pre-training mean results reported significantly greater maximum LMM sagittal range than the experienced nurse results (p<0.031) (Table, 4.3, Figure 4.15). Both the pre- (p< 0.02) and post-training (p<0.028) results showed significantly more maximum twist angle than the experienced nurses (Figure 4.16).

Table 4.3: Summary of the significant differences of the LMM values between the experienced nurses and novice participants for transfer 1: reposition up in bed, positioned at the side of the bed.

Note: (-) and (+) denotes the direction of the change relative to the experienced nurses.

Mean	Max L Bend	Max R Bend	Max Lat Ran
Pre	NS	NS	NS
Post	NS	NS	NS

Mean	Max Ext	Max Flex	Max Sag Ran
Pre	NS	NS	p<0.031 -
Post	NS	NS	NS

Mean	Max L Twist	Max R Twist	Max Angle
Pre	NS	NS	p<0.02 -
Post	NS	NS	p<0.028 -

	Mean Lat Vel	Max Lat Vel	Mean Sag Vel	Max Sag Vel	Mean Twist Vel	Max Twist Vel
Pre	NS	NS	NS	NS	NS	NS
Post	NS	NS	NS	NS	NS	NS
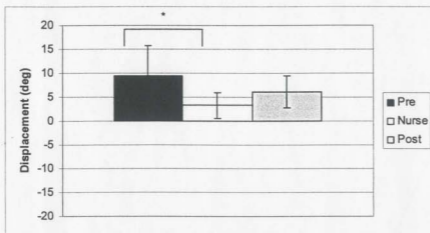
Figure 4.15: Maximum LMM sagittal range for experienced nurses and novice participants for transfer 1: repositioning up in bed, positioned at side of the bed

Note: (*p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005).
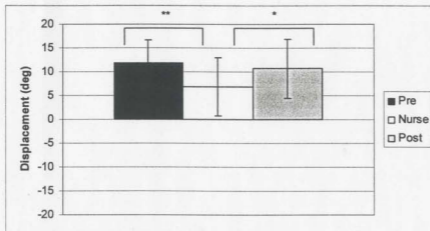
Figure 4.16: Maximum LMM twisting angle for experienced nurses and novice participants for transfer 1: reposition up in bed, positioned at side of bed

Note: p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.

The LMM displacement had significantly greater maximum left bend movement for the pre-training group (p<0.039) when compared to experienced nurse results (Figure 4.17).
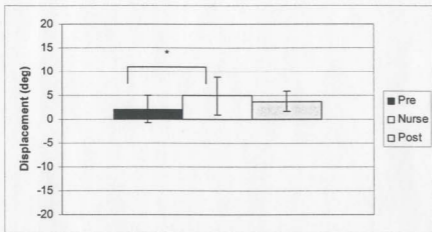
Figure 4.17: Maximum LMM left bend for experienced nurses and novice participants for transfer 1: reposition up in bed, positioned at side of bed.
*Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.*

4.3.2 Transfer 2: Repositioning up in Bed, positioned at the head of the bed for experienced nurses compared to novice participants pre- and post-training

The post-training results for mean left trapezius activity (p<0.023) showed a lower level of muscle activity than that of the experienced nurses (Figure 4.18, Table 4.4). There was also a significant difference between the mean of the pre-training results and the experienced nurses for the left erector spinae. The pre-training results had higher activation (p< 0.016) than that recorded for the mean of the experienced nurses.
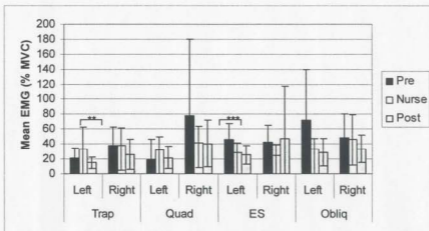
Figure 4.18: Mean EMG data for novice participants and nurses during transfer 2: reposition up in bed, located at the head of the bed.

Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.

The pre and post-training results reported significantly lower levels of maximum muscle activity for the left trapezius (p<0.023 and p<0.007 respectively). The pre-training group had significantly lower maximum levels of left deltoid (p<0.031) activation than the mean of the experienced nurses (Figure 4.18, Figure 4.19, Table 4.5). The mean of the post-training group results showed significantly lower levels of maximum muscle activation for the right trapezius (p<0.033).
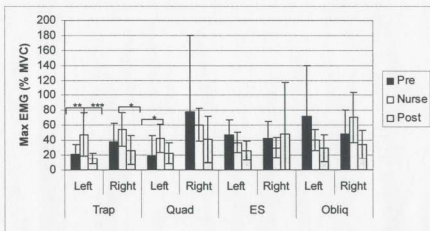
Figure 4.19: Maximum EMG data for novice participants during transfer 2: reposition up in bed, located at the head of the bed.

*Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.*

Table 4.4: Summary of the significant differences of the mean EMG values (expressed as a percent of MVC) between the experienced nurses and novice participants for transfer 2: reposition up in bed positioned at the head of the bed.

Note: (-) and (+) denotes the direction of the change relative to the experienced nurses.

Mean	Left TRAPEZIUS	Right TRAPEZIUS	Left DELT	Right DELT
Pre	NS	NS	NS	NS
Post	p<0.023 +	NS	NS	NS
Mean	Left ES	Right ES	Left OBLIQ	Right OBLIQ
Pre	p<0.016 -	NS	NS	NS
Post	NS	NS	NS	NS

Table 4.5: Summary of the significant differences of the mean EMG values (expressed as a percent of MVC) between the experienced nurses and novice participants for transfer 2: reposition up in bed positioned at the head of the bed.

Note: (-) and (+) denotes the direction of the change relative to the experienced nurses.

Max	Left TRAPEZIUS	Right TRAPEZIUS	Left DELT	Right DELT
Pre	$p<0.023$ +	NS	$p<0.031$ +	NS
Post	$p<0.007$ +	$p<0.033$+	NS	NS
Max	Left ES	Right ES	Left OBLIQ	Right OBLIQ
Pre	NS	NS	NS	NS
Post	NS	NS	NS	NS

The LMM displacement data for transfer 2 revealed many more significant differences between groups than the transfer 1 (Table 4.6). The pre-training results for maximum right bend were significantly greater ($p<0.001$) than the experienced group. The pre-training group revealed significantly greater displacements for maximum lateral range ($p<0.001$) when compared to the experienced nurses. Significantly less maximal extension was measured for the mean of the post training ($p<0.044$) group compared to the mean extension of the experienced nurses. The pre-training results were significantly larger for maximum sagittal range ($p<0.021$), maximum twist angle ($p<0.001$), mean lateral velocity ($p<0.001$), maximum lateral velocity ($p<0.001$), mean twist velocity ($p<0.015$) and maximum twist velocity ($p<0.004$) in comparison to nurses (Figure 4.20).

Table 4.6: Summary of ANOVA post hoc results comparing the pre- and post-training results of novice participants to the experienced nurses for transfer 2: reposition up in bed positioned at the head of the bed.

Note: (-) and (+) denotes the direction of the change relative to the experienced nurses.

Mean	Max L Bend	Max R Bend	Max Lat Ran
Pre	NS	p<0.001 -	p<0.001 -
Post	NS	NS	NS

Mean	Max Ext	Max Flex	Max Sag Ran
Pre	NS	NS	p<0.015 -
Post	NS	NS	NS

Mean	Max L Twist	Max R Twist	Max Angle
Pre	p<0.000 +	NS	p<0.000 +
Post	p<0.006 +	NS	p<0.006 +

	Mean Lat Vel	Max Lat Vel	Mean Sag Vel	Max Sag Vel	Mean Twist Vel	Max Twist Vel
Pre	p<0.001 -	p<0.001	NS	NS	p<0.015 -	p<0.004 -
Post	NS	NS	NS	NS	NS	NS
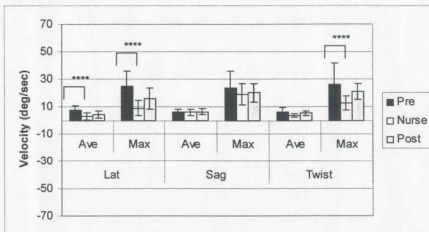
Figure 4.20: Mean LMM velocities for experienced nurses compared to novice participant's pre- and post-training result for transfer 2: repositioning up in bed, positioned at head of bed.

Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.

4.3.3 Transfer 3: Bed to chair, one person pivot technique for experienced nurses compared to novice participants pre- and post-training

A summary of the statistical analyses of the EMG between the experienced nurses and novice participants are summarized in Table 4.7 and Table 4.8. The experienced nurses' results demonstrated significantly less activity for the left ($p<0.005$) and right erector spinae ($p<0.027$) when compared to the values recorded from the pre-training novices. The mean left rectus femoris ($p< 0.027$) activity was significantly higher for the experienced nurses then the novice pre-training trials (Figure 4.21, Table 4.7).
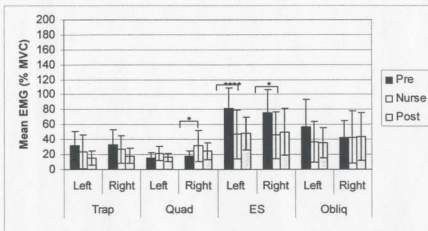
Figure 4.21: Mean EMG data for novice participants and nurses during transfer 3: bed to chair using one person pivot technique.

*Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.*

The maximum values of the experienced nurses were significantly smaller for left erector spinae (p<0.004) activity compared to the pre-training novice group. There were increases in maximum right trapezius activity for the experienced nurse results when compared to the pre (p<0.04) and post-training (p<0.011) results (Figure 4.22, Table 4.8).
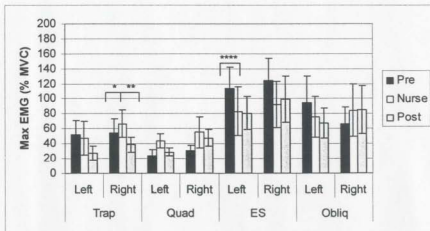
Figure 4.22: Maximum EMG data for novice participants and nurses during transfer 3: bed to chair using one person pivot technique.

Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.

Table 4.7: Summary of the significant differences of the mean EMG values (expressed as a percent of MVC) between the experienced nurses and novice participants for transfer 3: bed to chair using one person pivot technique.

Note: (-) and (+) denotes the direction of the change relative to the experienced nurses.

Mean	Left TRAPEZIUS	Right TRAPEZIUS	Left QUAD	Right QUAD
Pre	NS	NS	NS	p<0.027 +
Post	NS	NS	NS	NS

Mean	Left ES	Right ES	Left OBLIQ	Right OBLIQ
Pre	P<0.005 -	p<0.032 -	NS	NS
Post	NS	NS	NS	NS

Table 4.8 Summary of the significant differences of the maximum EMG values (expressed as a percent of MVC) between the experienced nurses and novice participants for transfer 3: bed to chair using one person pivot technique.

Note: (-) and (+) denotes the direction of the change relative to the experienced nurses.

Max	Left TRAPEZIUS	Right TRAPEZIUS	Left QUAD	Right QUAD
Pre	NS	p<0.04 +	NS	NS
Post	NS	p<0.011 +	NS	NS
Max	Left ES	Right ES	Left OBLIQ	Right OBLIQ
Pre	P<0.004 -	NS	NS	NS
Post	NS	NS	NS	NS

The mean LMM displacements for the experienced nurses for transfer 3 were compared to the novice participants' pre and post-training group (Figure 4.23, Table 4.9). The pre-training results were significantly greater for maximum twist angle ($p<0.028$), mean lateral velocity ($p<0.008$), mean sagittal velocity ($p<0.014$), mean twist velocity ($p<0.000$) and maximum twist velocity ($p<0.015$). In one instance, the mean of maximum right bend displacement, the post-training results were significantly smaller then the experienced nurses. The post-training group however recorded significantly greater results for the maximum lateral range displacement ($p<0.017$), the maximum twist angle ($p<0.027$), mean lateral velocity ($p<0.024$), maximum lateral velocity ($p<0.022$) and mean twist velocity ($p<0.005$) (Table 4.9, Figure 4.24).
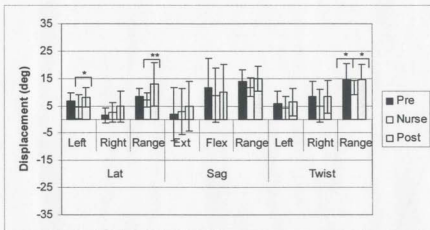
Figure 4.23: Mean LMM displacements for experienced nurses compared to novice participant's pre- and post-training result for transfer 3: bed to chair, one person pivot transfer.

Note: *p< 0.05, **p< 0.025, ***p< 0.01, ****p< 0.005.

Table 4.9: Summary of ANOVA post hoc results comparing the pre- and post-training results of novice participants to the experienced nurses for transfer 3: bed to chair, one person pivot technique.

Note: (-) and (+) denotes the direction of the change relative to the experienced nurses.

Mean	Max L Bend	Max R Bend	Max Lat Ran
Pre	NS	NS	NS
Post	p<0.043 +	NS	p<0.017 -

Mean	Max Ext	Max Flex	Max Sag Ran
Pre	NS	NS	NS
Post	NS	NS	NS

Mean	Max L Twist	Max R Twist	Max Angle
Post	NS	NS	p<0.028 -
Pre	NS	NS	p<0.027 -

|	Mean Lat Vel	Max Lat Vel	Mean Sag Vel	Max Sag Vel	Mean Twist Vel	Max Twist Vel
Pre	p<0.008 -	NS	p<0.014 -	NS	p<0.001 -	p<0.015 -
Post	p<0.024 -	p<0.022 -	NS	NS	p<0.005 -	NS
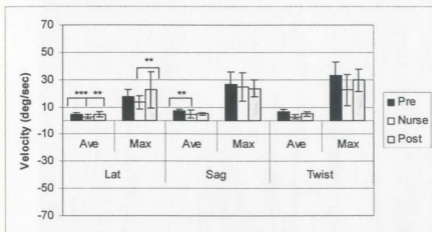
Figure 4.24: Mean LMM velocities for experienced nurses compared to novice participant's pre- and post-training result for transfer 3: bed to chair, one person pivot transfer.

Note: $*p< 0.05$, $**p< 0.025$, $***p< 0.01$, $****p< 0.005$.

CHAPTER 5: DISCUSSION

5.1 Introduction

This study analysed two experimental hypotheses. The acceptance of the first null hypothesis would suggest that the back injury prevention program (BIPP) does not have any positive effect on lumbar motions and upper musculoskeletal electromyographical activities during patient transfer tasks. A repeated measures t-test between pre- and post-training novice participants indicated that the first null hypothesis was rejected, as there was a significant difference between groups in both the EMG and LMM measures. Employing the BIPP transfer protocols seemed to create a reduction in both lumbar motions as measured by a lumbar motion monitor (LMM) and upper musculoskeletal activity as measured by electromyography (EMG) in this study.

Accepting the second stated null hypothesis would signify that there is no significant difference between the pre- and post-training novice participants' lumbar motions and muscle activities compared to that of experienced nurses. An analysis of variance (ANOVA) between the results of the pre- and post-trained novice participants and the experienced nurses supports a rejection of the null hypothesis as there was a significant difference between the novice participant pre-training results and the experienced nurses. Having some training in the BIPP protocols seemed to have reduced lumbar motions as measured by a LMM and upper musculoskeletal activity as measured by EMG in this study.

5.2 Limitations of the Study

Discussion of the results of this study must be done in light of the limitations in this study.

5.2.1 Construct limitations

In any investigations of human performance, especially those conducted outside a typical work setting, there is the possibility for the Hawthorne effect to influence the results. The Hawthorne effect explains the changes to human behaviour that occurs as a result of being observed (Muchinsky, 2000). When knowingly being observed, humans will modify behaviour to more closely satisfy what the subject perceives to be the expectations of the researchers or what they assume is the correct experimental response (Muchinsky, 2000). In this study the experienced nurses may have been more mindful of their posture when performing patient transfers than they would have been during a typical work shift. Therefore, the lumbar motions and muscle activity during testing protocols for the experienced nurses may have been less then what may actually measured when not being tested.

There are limitations to using EMG activity and LMM measures to reasonably discuss musculoskeletal loading and therefore risk of low back disorder (LBD). The EMG unit that was employed in this study had the capability to analyze eight muscles simultaneously. However, the number muscles required to perform the patient transfer tasks would likely exceed those selected to be analyzed. Therefore, the EMG activities recorded is only providing a partial picture of the overall muscle recruitment needed to complete the patient transfer.

To be able to compare results for all participants with varying morphologies and therefore varying muscles masses, the EMG values were normalized using maximal voluntary isometric contractions (MVC) taken at the beginning of each testing protocol. There are limitations to using a MVC for this purpose, in particular considering the repeated measures model employed for the novice participants. In the situation where the participant was not familiar with this protocol, the results of the MVC's taken from their

first experience with MVC's would likely differ when compared to their second day of MVC's. Therefore comparing these data can introduce higher measures of intra- and inter-subject variability, masking changes that may otherwise be found to be statistically different.

Although the LMM is considered a reliable method to estimate thoracolumbar motions it is not a direct measure of lumbar motion (McGill and Kavcic, 2005; Marras, 1999). However, this technology has been widely acknowledged as a surrogate metric for measuring thoracolumbar motions.

5.2.2 Methodological limitations

Ethical concerns must always be considered in human investigations and often result in limitations within the experimental protocol. In this study the novice participants were never truly unaware of the proper techniques employed in patient transfers as each viewed a BIPP training video prior to the pre-training testing protocol. This was done in consideration of participant safety as patient handling can be considered a heavy materials handling task.

The tasks selected for this study were based on previous research that reported certain transfers to be a higher risk for injury to a nurse or allied health professional (Yip, 2001; Engvist, Hagberg, Hjelm, Menckel and Ekenvall, 1998). However, two of the three transfers selected required a second person to assist in the transfer the patient. This second person underwent training in these patient transfer tasks in order to maintain consistency in performance from subject to subject. However, it is possible that the actions of this second experimental personnel influenced how the participant attempted the transfer.

The participant was required to perform the transfers on the right side of the bed. The control of the side of the bed was consistent regardless of the handedness or side preference of the participant. Therefore, the transfer for some participants may have been more awkward than other participants.

In light of these limitations there are statistical trends that emerged from the data collected during this study which suggest that level of experience and training will reduce the magnitude of biomechanical metrics assumed to be related to the risk of overexertion injuries such as LBD.

5.3 Transfer 1: Repositioning up in bed, positioned at the side of the bed

A significant reduction in normalized muscle activity was observed for novice participants in both left and right erector spinae post-training with BIPP (see Figure 4.1). When also considering the LMM results, there was a significant decrease between the pre- and post-training sagittal lumbar extension and flexion (see Figure 4.3). The biomechanical principle important for this BIPP transfer requires the participant to employ a weight shift from side to side to create momentum in a horizontal plane for the transfer (Bouchier, 1995). In the experiment the participants were consistently located to the left of the patient, and therefore the head of the bed and the direction of the transfer would be to the right for the participant. The weight shift for participants in the study would then consist of transferring the body weight from their left leg, located in line with the hip level of the patient, to the right leg. The participant performed this transfer with the mirror volunteer. The participant gave a count of three to commence the movement. Using such a weight shift approach, the participant was able to reduce the amount of hip flexion and extension motions as seen in the decrease in sagittal range and muscle activity in the left and right erector spinae (see Figures 4.1, 4.3).

The results also demonstrated a decrease in lateral lumbar range and right bend displacements in post-training results. After BIPP training, novice participants were able to transfer the patient to their right with weight shifting again while maintaining a more neutral thoraco-lumbar posture (see Figure 4.3).

There was a reduction in maximum left trapezius muscle activity following BIPP training (see Figure 4.1). The BIPP program instructs patient handlers to brace their elbows at their sides, to elevate the bed height such that their upperarms and forearms are

forming a 90 degree angle (see Figures 3.2, 3.3), and while transferring the patient using the drawsheet their knuckles should skim along the bed (Bouchier, 1995). These postures were recommended to minimize the use of the neck, shoulders and arms to transfer the patient towards the head of the bed. The mean and maximum activities of the left trapezius were higher for the pre-training conditions as participants likely tended to try and lift the patient using the drawsheet and, with the aid of the left arm, pull the patient up in bed (Figure 4.1).

When comparing the results from novice participants to that of experienced nurses, the post training group had significantly lower mean left trapezius activity levels than the experienced workers (see Figure 4.13). The maximum EMG values revealed that both the pre- and post-training result for left and right trapezius were significantly less then that recorded from the experienced nurses (see Figure 4.14). These results would suggest that perhaps more emphasis on the posture of the shoulder girdle needs to be given during BIPP instruction. Currently the BIPP instructs the patient handler to maintain a 90 degree orientation at the elbow while they are braced against the sides of the handler and to skim the knuckles along the bed, as not to lift the patient during the transfer. It has been acknowledged that when adjusting postures to minimize risk of low back pain that the loads are transferred to another joint, such as the shoulders, increasing the risk of injury to tissues other than those of the back (Gagnon, Chehade, Kemp and Lortie, 1987, MacKinnon and Vaughan, in press). Keir and MacDonell (2004) noted that there has not been many muscle activity studies during patient transfer tasks, especially for muscles other than the back extensors. This knowledge gap makes it difficult to decide whether the level of upper extremity muscle activity observed in this study relates to levels of exertion sufficient to increase the risk of injury or not. Results from this study are comparable to Keir and MacDonnel (2004) who reported similar trends in normalized EMG values recorded for trapezius activity. In their study, regardless of the side of the bed or the transfer technique used, the experienced nurse consistently had higher trapezius activity levels. Keir and MacDonell (2004) theorized that this increase was due to the experienced nurses activating their trapezius as a protective strategy to stabilize the elbows at their sides. Similar interpretations can be made for this study.

When comparing experienced nurses' LMM values to those collected pre- and post-training for the novice participants, the experienced nurses were not significantly different from the novice participants for most LMM measures recorded. The pre-training novice participant results had significantly higher maximum left bend, sagittal range and twist angle than the nurses (see Figures 4.15, 4.16, 4.17). This would indicate that the post training group and the experienced nurses were able to adopt a more neutral body posture which allowed for less twist and flexion/extension than those who have not received previous training.

These results would indicate that the experienced nurses were not significantly different from the post-training group suggesting that the training from BIPP which has previously been provided to improve biomechanics of patient transferring are still influencing the experienced nurses' transfer techniques. This result suggests that consideration be given to the frequency of re-training in order to optimize learning retention. The experienced nurses that volunteered for this study varied in respect to the time since they were last trained in the BIPP techniques. The standard deviations for the experienced group, however, appear to be no greater than those calculated for the pre- and post-training groups. Previous research has focused on the efficacy of transfer education or the use of lift assisting devices pre- and post-training (Finch Gutherie, Westphal, Dahlman, Berg, Behnam & Ferrell, 2004; Johnsson, Carlsson and Lagerstrom, 2002; Goodridge & Laurila, 1997). To date there is little known research regarding the retention of learning transfer techniques and the appropriate duration before retraining should occur, and perhaps more importantly, if retraining is in fact effective.

When reflecting on the results recorded for thoracolumbar positions and displacements for the post-training group and the experienced nurses, the values obtained are below those Marras et al.. (1995) considered to be correlated with low risk, with the exception of left bend (Figure 5.1). The lumbar velocities recorded for all experimental groups were lower compared to Marras et al..'s (1995) velocities suspected to put operators in a low risk category for overexertion injuries (Figure 5.2). Marras et al.. (1995) examined various occupational "lifting" tasks. While the patient transfers considered in this study have a "lift" component, the BIPP principles attempt to minimize

the motions in the vertical direction and promote motions in the horizontal direction. Furthermore, the average load masses considered in Marras' data set are considerably lower than loads typical of patient transfers. Therefore, comparison between these data and the data set from Marras (1995) may not be appropriate. However, in light of the lack of suitable guidelines for safe transfer techniques, these empirical data may still provide some benchmark comparisons as to whether or not the lumbar motions typical of patient transfer tasks can be considered safe.

Figure 5.1: Comparison of novice participant pre and post-training, experienced nurses and Marras et al. (1995) reported low risk values for maximum displacement for transfer 1: reposition up in bed, positioned at side of bed.
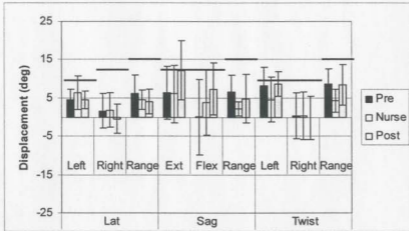
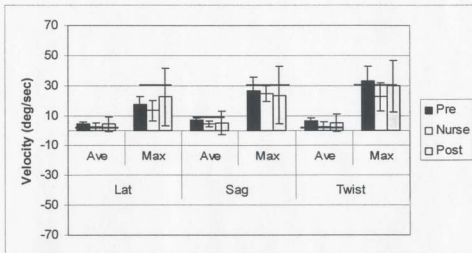*Note: Bold line represents Marras et al.. (1995).*

Figure 5.2 Comparison of novice participant pre and post-training; experienced nurses and Marras et al. (1995) reported low risk values for maximum velocity for transfer 1: reposition up in bed, positioned at side of bed.

Note: Bold line represents Marras et al.. (1995).

5.4 Transfer 2: Repositioning up in bed, positioned at the head of the bed

A decrease in maximum EMG activity was observed between pre- and post-training novice participants for both left and right external oblique, the left and right trapezius, and left erector spinae muscles (see Figure 4.6). The participants also had a reduction in lateral right bend and range, sagittal extension and flexion, left twist and lateral velocities following the BIPP training (see Figure 4.7). From these results it appears that prior to training, the novice participants would use a combination of sagittal extension, lateral velocity and twist to reposition the patient up in bed when the participant initiated the transfer from the head of the bed. BIPP instructors instruct the patient handlers to use a weight shift technique to transfer the patient up in bed. The participant is instructed to stand "tall" on their knee while holding the drawsheet tight, then to transfer body weight by sitting on their back heel. The participant will have been

positioned at an angle such that the midline of their body is inline with the opposite end of the footboard (see Figure 3.5). Since this is a two person transfer, the same position is mirrored by the second patient handler on the opposite side of the bed. The participant would communicate verbally with the other handler when to initiate the transfer. Both would then transfer weight from the knee to sitting back onto the heel (sees Figure 3.4, 3.5). The system mechanics considered in this transfer require that both persons performing the transfer be at equivalent relative angles to the patient when the weight transfer is applied. Thus, an equal effort would create a resultant force vector in the direction towards the head of the bed.

When comparing the normalized EMG for all experimental groups for the repositioning up in bed while at the head of the bed, reveals the post-trained novice participants having a significantly higher left trapezius activity then the nurses (see Figure 4.21). As previously discussed, recruitment of the trapezius muscle can be considered a protective strategy that nurses can employ during such a maneuver (Keir & MacDonnell, 2004; Gagnon et al., 1987). The left deltoid activity was lower for the post-trained novices when compared to the nurses. The position during the transfer may have dictated the reason why the deltoid activity would be higher for nurses. The BIPP program would situate the patient handler at a diagonal to the footboard of the bed, however if the patient handler was not on an exact diagonal to the opposite foot of the bed, it was observed that the left arm was held in a more extended position during the transfer, increasing the involvement of the arms during the transfer (see Figures 3.4, 3.5). A recommendation from this study would be to attempt a more in depth study of the muscle activities of the neck, shoulders and arms during this transfer.

The experienced nurses and the post-training measures had consistently smaller LMM displacements and velocities than the novice participants prior to training. When comparing the results of all experimental groups to the low risk values determined by Marras et al. (1995), all motions were smaller, with exception of left bend (Figures 5.3, 5.4).

Figure 5.3: Comparison of novice participant pre and post-training, experienced nurses and Marras et al.. (1995) reported low risk values for maximum displacement for transfer 2: reposition up in bed, positioned at the head of the bed.

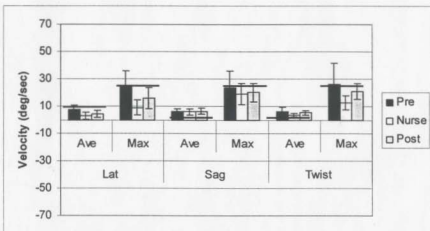*Note: Bold line represents Marras et al. (1995).*

Figure 5.4: Comparison of novice participant pre and post-training, experienced nurses and Marras et al.. (1995) reported low risk values for maximum velocity for transfer 2: reposition up in bed, positioned at the head of the bed.

Note: Bold line represents Marras et al. (1995).

5.6 Transfer 3: Bed to chair, one person pivot technique

During the transfer from bed to chair using the one person pivot technique, following training, the novice participant demonstrated a reduction in normalized EMG activity for left and right trapezius, left and right erector spinae, and left oblique (see Figure 4.9, 4.10). The right quadriceps increased its maximum and mean EMG activity following training. The LMM results indicated a reduction in lateral displacement, as well as sagittal and twisting velocities (see Figure 4.11, 4.12). During this transfer the BIPP instructs patient handlers to begin the lift by gaining momentum by initiating a rocking motion from the front to the back of the feet. The hips of the patient handler are to be square to the patient. The patient will then be rocked until enough momentum is produced to clear the patient's gluteus from the bed. The patient handler then pivots and guides the patient towards the wheelchair which is positioned at an angle of 45 degrees to the bed. The participant was instructed to pivot on the right foot and to follow the patient, remaining in a parallel position to the patient and to squat using the legs to place the patient gently in the chair (see Figure 3.6). The reduction in lateral displacement, twist velocity and oblique normalized EMG activity would indicate that the participants are pivoting with their patients when turning to place them in the wheelchair.

The reduction in trapezius and erector spinae normalized EMG activity and increases in quadriceps activity along with reduction in sagittal LMM velocity would indicate that following training the participants are adhering more consistently with the weight transferring from the front to back foot as opposed to employing a strategy of extending and flexing the trunk in the sagittal plane.

While the one person pivot technique does not require communication between two patients handlers, this transfer appeared to be the most difficult to master of those considered in this study. Higher maximum and mean normalized EMG values were recorded for right trapezius activity for experienced nurses when compared to both pre- and post training values. As previously discussed, Keir and MacDonnel's (2004) indicate that increases in trapezius activity may serve as a protective strategy. The normalized EMG values obtained for both left and right erector spinae of the pre-training novice participants were significantly higher then those recorded for the experienced nurses (see

Figures 4.27, 4.28), suggesting that after BIPP training, using a weight shift from front to back with a staggered stance is effective in reducing thoracolumbar extension and flexion while still generating sufficient momentum to clear the patient fully from the bed. Further support of this interpretation is the significant increase in left quadriceps activity for the experienced nurses compared to the pre-trained novices. Due to standardized equipment set up, the wheelchair in which the patient was required to be placed into was consistently to the right of the participant; thus, the left leg would be lunged forward and the right leg extended backward to have the staggered leg posture necessary to perform the weight shift. When adopting the techniques instructed by BIPP, the left quadriceps would experience more activity as the legs are in a bent position to allow the nurse to lower her trunk to that of the patient without forward flexion at the lumbar spine.

When considering the LMM results, more support can be given for the use of weight shifting by the participants. A reduction in thoracolumbar twisting displacements and velocities, lateral displacement and velocities and sagittal displacements and velocities were recorded for the experienced nurses when compared most often to the pre-training novices (see Table 4.29, Figure 4.32). This indicates that that they are keeping the pelvis more square and reducing motion in the thoracolumbar spine. When comparing all experimental groups' lumbar motions to those reported by Marras et al. (1995), all of the results are below those indicated to be low risk (Figure 5.5, 5.6).

Figure 5.5: Comparison of novice participant pre and post-training, experienced nurses and Marras et al. (1995) reported low risk values for maximum displacement for Transfer 3: Bed to chair, one person pivot technique.
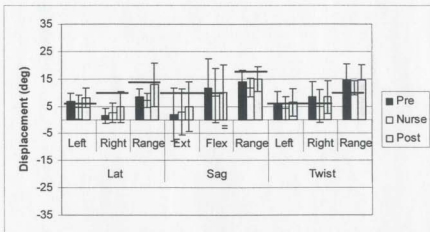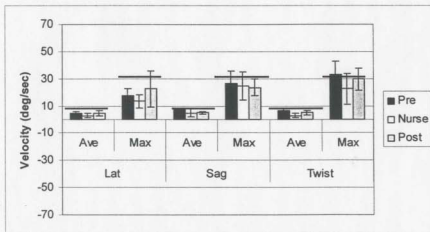

Figure 5.6: Comparison of novice participant pre and post-training, experienced nurses and Marras et al.. (1995) reported low risk values for maximum velocity for Transfer 3: bed to chair, one person pivot technique.

Considering all transfers of the BIPP analyzed in this study, the postures and techniques that the BIPP instructs was able to reduce the degree of motion and amount of muscle activation when compared to those who have not received training. The results of this study indicate that some back education such as that received by the post-training novice participants and the nurses, had more favorable postures and muscle activity levels then not having any training as represented by the pre-training novice participants.

CHAPTER 6: CONCLUSION

6.1 Conclusion

This study rejected both null hypotheses upon reflection of the data collected in this study. The first null hypothesis was rejected suggesting that the back injury prevention program (BIPP) has a positive effect on lumbar motion and upper musculoskeletal electromyographical activity during patient transfer tasks. The results of this study indicated that there is a significant difference in transfer technique between pre and post-training novice participant's techniques.

The second null hypothesis was also rejected inferring that there was a significant difference between the pre- and post-training novice participant's lumbar motions and muscle activity compared to that of experienced nurses. The analysis of variance (ANOVA) employed to compare the data from the novice participants' and the experienced nurses indicated that there is a significant difference in the metrics employed to assess these patient transfers. In all three transfers the post-training results showed more favorable muscle activities and lumbar motions that those before training. Whether the experienced nurses had a better technique with regards to the dependent measures that have been measured during this experiment than the post-training data from the novice participants was variable depending on the measure and the transfer. However, since all the experienced nurses that participated in the study would have been trained with the BIPP program at some point in their career, it can be concluded from this evidence that training from the BIPP program should induce lower muscle activity level in the trunk, neck and shoulders, as well has less lumbar motions compared to no previous training.

A recommendation from this study would be to further research the retention of the BIPP by participants. Another recommendation of this study would be to assess the availability of transfer aids, such as the transfer belt, as well as assess if there is adequate

nurses available during each shift such that two person transfers can be efficiently performed when appropriate. Further research should also investigate the capability of the nurses to employ the BIPP techniques in the workplace when factors such as inadequate space, lack of available staff, and fatigue from shift work are present.

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