ARE OVER-THE-COUNTER FISH OIL SUPPLEMENTS EFFECTIVE AND SAFE FOR TREATING MOOD DISORDERS? STUDIES ON THE TOP 10 FISH OIL SUPPLEMENTS AVAILABLE IN NEW ZEALAND

A thesis submitted in fulfillment of the requirements for the degree of Masters of Science in Psychology at the University of Canterbury by Shelby Hantz

University of Canterbury
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Acknowledgements

I would like to express my deepest gratitude to my supervisors Ian Shaw and Julia Rucklidge, for their unwavering support, guidance, and encouragement throughout my thesis journey.

Finally, a special thank you to my family for their constant love and support throughout my years at university.
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Abstract

Background
Due to the rapid increase in the incidence of major depression and bipolar disorder, these disorders are expected to surpass cardiovascular disease as the leading health concern worldwide over the next decade. The current front line treatment, psychotropic medications, is not having an impact on the rising incidence of mood disorders; as such alternative treatments are required. One treatment that is gaining attention as a potentially effective treatment for mood disorders is omega-3 fatty acids, found in fish oil supplements. However, there are worries about increasing environmental levels of mercury and their implications for human health. Since mercury bioaccumulates in marine species, there is particular concern about mercury levels in fish oil supplements.

Methods

**Efficacy of omega-3 fatty acids:** 22 clinical research trials assessing the efficacy of omega-3 fatty acids in the treatment of mood disorders were reviewed. In addition, the ingredients and doses of over-the-counter fish oil supplements were examined. The amounts of omega-3 fatty acids contained per capsule were determined by an independent laboratory using Gas Chromatography on the 10 most popular over-the-counter fish oil supplements and were compared with amounts stated on product labels. These doses were then compared to the doses used in the reviewed research trials.

**Mercury levels:** the fish oil supplements were analysed for mercury by an independent laboratory using Inductively Coupled Plasma Mass Spectrometry.

Results

**Efficacy of omega-3 fatty acids:** Results from the 22 clinical trials selected revealed that 50% of trials showed omega-3 fatty acids to be more effective than placebo in the treatment of mood disorders, and 50% of trials showed no benefit of omega-3 fatty acids over placebo. Independent laboratory tests indicated that product labels for 50% of the supplements were accurate regarding omega-3 fatty acid content, whereas 50% contained between 48 – 69% of amounts stated on labels. Product labels recommend a minimum of three and maximum of seven fish oil capsules per day for brain health. To determine the potential efficacy of these doses for managing mood disorders, four statistical analyses were performed using a two-tailed, nonparametric Mann-Whitney test. The first and second analyses compared the effective dose in the positive clinical trials to a seven capsule dose (label vs. actual amounts) from over-the-counter supplements. The difference in dose was non-significant in both analyses. The third and fourth analysis compared the effective dose in clinical trials to a three capsule dose (label vs. actual amounts) from the supplements analysed. The third statistical analysis revealed a non-significant difference between the dose used in the clinical trials and a three capsule dose based on product labels. Conversely, the fourth analysis showed a significantly greater (p = .001) dose of omega-3 fatty acids in clinical trials reviewed versus the actual amount of omega-3 fatty acids in over-the-counter fish oil supplements.

**Mercury levels:** mercury was not detected in any sample.

Conclusions
These findings indicate a daily dose between three and seven capsules of the most popular New Zealand over-the-counter supplements may ameliorate mood symptoms. Importantly, the risk of mercury contamination is negligible.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>5-HT</td>
<td>Serotonin</td>
</tr>
<tr>
<td>5-HTT</td>
<td>Serotonin Transporter</td>
</tr>
<tr>
<td>ALA</td>
<td>Alpha Linolenic Acid</td>
</tr>
<tr>
<td>BDI</td>
<td>Beck Depression Inventory</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain-Derived Neurotrophic Factor</td>
</tr>
<tr>
<td>CGI</td>
<td>Clinical Global Impression Scale</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic Acid</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic Acid</td>
</tr>
<tr>
<td>EPDS</td>
<td>Edinburg Postnatal Depression Scale</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>GAS</td>
<td>Global Assessment Scale</td>
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<tr>
<td>GC</td>
<td>Gas Chromatography</td>
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<tr>
<td>GDS</td>
<td>Geriatric Depression Scale</td>
</tr>
<tr>
<td>GLP</td>
<td>Good Laboratory Practice</td>
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<tr>
<td>HAM-D</td>
<td>Hamilton Depression Rating Scale</td>
</tr>
<tr>
<td>IANZ</td>
<td>International Accreditation New Zealand</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductively Coupled Plasma Mass Spectrometry</td>
</tr>
<tr>
<td>IDS</td>
<td>Inventory of Depressive Symptoms</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LA</td>
<td>Linoleic Acid</td>
</tr>
<tr>
<td>LoD</td>
<td>Limit of Detection</td>
</tr>
<tr>
<td>LT</td>
<td>Leukotrienes</td>
</tr>
<tr>
<td>MADRS</td>
<td>Montgomery-Asberg Depression Rating Scale</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>NZTDS</td>
<td>New Zealand Total Diet Survey</td>
</tr>
<tr>
<td>PANSS</td>
<td>Positive and Negative Syndrome Scale</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandins</td>
</tr>
<tr>
<td>PTDI</td>
<td>Provisional Tolerable Daily Intake</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>PTWI</td>
<td>Provisional Tolerable Weekly Intake</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated Fatty Acid</td>
</tr>
<tr>
<td>SAMe</td>
<td>S-adenosyl methionine</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricyclic Antidepressants</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Neurosis Factor</td>
</tr>
<tr>
<td>VMAT2</td>
<td>Vesicular Monoamine Transporter</td>
</tr>
<tr>
<td>YMRS</td>
<td>Young Mania Rating Scale</td>
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1. Introduction
1. Introduction into Fish Oil Supplements and Mood Disorders

1.1. Fish oil supplement use in psychiatric disorders.

Fish oil supplements are among the most popular dietary supplements on the global market, as they are widely believed to contain nutrients imperative to heart and brain health (Albert et al., 2015; Innis, 2007; Kris-Etherton, Harris, & Appel, 2002a; Youdim, Martin, & Joseph, 2000). In fact, research demonstrating the importance of fish oil for mental health has accumulated over the last decade and it has since emerged as a promising therapy for the management and/or treatment of psychiatric disorders (Freeman et al., 2008; Freeman, 2000; Hibbeln & Salem, 1995; Mischoulon et al., 2008; Peet & Stokes, 2005; Schachter et al., 2005; Su, Huang, Chiu & Shen, 2003). The effectiveness of fish oil supplementation has been explored across many psychological domains (Amminger et al., 2007; Dullemeyer et al., 2007; Sonuga-Barke et al., 2013; Mazereeuw, Lanctot, Chau, Swardfager, & Herrmann, 2012), though considerable improvements have been observed for mood disorders (Freeman, 2000; Hallahan, Hibbeln, Davis, & Malcolm, 2007; Hibbeln & Salem, 1995; Lin & Su, 2007; Logan, 2003; Logan, 2004; Morreale, 2012). The consistent amelioration of psychological symptoms has resulted in an increasing number of clinical trials investigating the impact of fish oil supplements on mood disorders and is regarded as a novel approach to the treatment of mental illness (Ross, Seguin, & Seizwerda, 2007). However, despite the momentum gained in recent years, the evidence appears highly controversial and concerns have been raised about the effectiveness of supplementation (Amminger et al., 2010; Costarelli, 2011; Ronzio, 2003). A possible explanation for this concern may be the large differences among between studies, including the patient population, severity of baseline symptoms, psychological measures, presence or absence of concomitant medications, inclusion and exclusion criteria, fish oil dose and composition, trial duration and choice of placebo (Grosso et al., 2014; Stoll, 2008). Determining whether the therapeutic outcomes are comparable between studies is therefore challenging.

The rationale behind concentrating on encapsulated fish oil supplements is based on evidence that demonstrates the Western diet may be deficient in essential nutrients derived from fish oil, while excessive in nutrients that promote inflammatory diseases and thus mood disorders (Simopoulos, 2002). As humans evolved on a diet that contained an abundance of high quality – nutrient and energy dense foods, the highly processed foods
introduced through the western diet have altered the balance between certain nutrients (Cordain et al., 2005; Popkin, Adair & Ng, 2012). Fish oil supplementation may therefore promote and restore equilibrium within the body to prevent the occurrence of psychiatric illness. The purpose of the current study is to establish whether dietary fish oil supplements sold over-the-counter in New Zealand may provide therapeutic benefits to patients with major depression and bipolar disorder. The inquiry begins with omega-3 fatty acids.

1.2. Omega-3 fatty acids.

Fish oil is a substance that contains an abundance of nutrients imperative to human health, in particular, the omega-3 fatty acids – eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)(Din, Newby, & Flapan, 2004; Nettleton, 1991). These are essential nutrients found to enhance brain function and quality of life (Kidd, 2007) and need to be obtained through the diet since the body cannot synthesis these nutrients on its own (Coletta, Bell, & Roman, 2010; Su, 2009). Omega-3 fatty acids are long-chain polyunsaturated fatty acids (PUFAs) that range in length from 20 to 22 carbon atoms and contain many double bonds that begin at the third carbon near the methyl end of the molecule (Figure 1)(Osher & Belmaker, 2009). Research has shown that omega-3 fatty acids play a crucial role in brain development and influence brain structure and function through incorporation in cell membranes (Coletta, Bell, & Roman, 2010; Innis, 2008; Surette, 2008; Valentine & Valentine, 2004). In fact, evidence has demonstrated that cell membranes are dependent on essential fatty acids for normal cell function and neural transmission (Haag, 2003; Simopoulos, 2009). The omega-3 fatty acids also play an important role in cellular and neural inflammation, cell signaling, and gene regulation (Chandola & Tanna, 2014; Deckelbaum, Worgall, & Seo, 2006; Kitajka et al., 2004; Robinson, Ijioma, & Harris, 2010)(refer to section 1.6). Specifically, EPA regulates inflammation.
1.2.1. Eicosapentaenoic acid (EPA).

Interestingly, recent evidence suggests that EPA may be more influential than DHA on behaviour and mood based on the premise that it can reduce inflammatory processes and preserve normal brain function (Kidd, 2007; Martins, 2009; Simopoulos, 2002). In fact, it has been revealed that chronic inflammation may lead to the development of neuropsychiatric disorders, including major depression (Deckelbaum et al., 2006; Miller, Maletic, & Raison, 2009). Inflammation contributes to the destruction of neural pathways and appears to be influenced by the presence of proinflammatory cytokines (Rosenblat, Cha, Mansur, & McIntyre, 2014). These have been found to interact with processes involved in mood disorders, including neurotransmitter metabolism, neuroendocrine function and neural plasticity (Miller et al., 2009). However, evidence shows that the formation of proinflammatory cytokines is inhibited when the diet contains appropriate amounts of omega-3 fatty acids as they prevent the incorporation of arachidonic acid (AA; an omega-6 fatty acid) in cell membranes (Mozurkewich, Berman, & Chillimigras, 2010; Simopoulos, 2002; Verlengia et al., 2004). Further, consuming high quantities of omega-3 fatty acids may lead to oxidative stress within brain regions (Chen, Liu, Ouellet, Calon, & Bazinet, 2009), which could be prevented by a diet rich in antioxidants. Fish oil supplementation may provide amounts of EPA necessary for the prevention and/or treatment of mood disorders. Whereas EPA regulates inflammation, the role of DHA is more specific to brain structure and function.
1.2.2. **Docosahexaenoic acid (DHA).**

In contrast to the role of EPA in mood disorders, evidence shows that DHA is essential for pre- and postnatal brain development (Kidd, 2007) and plays an integral role in the structure and function of the brain (Innis, 2008). In fact, changes in membrane structure, due to less than optimal DHA levels, may induce permanent alterations in brain development (Innis, 2008) by impairing neural processes fundamental to cognitive function. These processes include neurogenesis, myelination (Georgieff & Innis, 2005), synaptogenesis, synaptic pruning, and dendritic arborization (Innis, 2008). Further, membrane function is influenced by the composition of fatty acids incorporated into the lipid bilayer of cell membranes. In other words, DHA is necessary to enhance membrane fluidity, volume and lipid-protein exchanges to improve neural transmission (Campoy, Escolano-Margarit, Anjos, Szajewska, & Uauy, 2012; Uauy & Dangour, 2006; Valentine & Valentine, 2004). Research also demonstrates that reduced amounts of DHA can impair the production of many neurotransmitter systems and their metabolism (Hadders-Algra, 2010). Neurotransmitters (e.g. dopamine, serotonin, norepinephrine, acetylcholine) have been recognised to play an important role in the pathogenesis of mood disorders (Bernardi, de Souza Escobar, Ferreira, & Silveira, 2012; Bertrand, O’Kusky, & Innis, 2006; Innis, 2008). Chronic deprivation of omega-3 fatty acids can cause dopaminergic pathways to function abnormally (Kodas, Vancassel, Lejeune, Guillateau, & Chalon, 2002) and has been implicated in major depression and bipolar disorder (Cousins, Butts, & Young, 2009; Dailly, Chenu, Renard, & Bourin, 2004), thereby demonstrating the importance of EPA and DHA synthesis as omega-3 fatty acids in the diet.

1.2.3. **Synthesis of omega-3 fatty acids.**

The biosynthesis by which the body can synthesis EPA and DHA involves the omega-3 short-chain precursor alpha linolenic acid (ALA) (Doughman, Krupanidhi, & Sanjeevi, 2007). Metabolism of ALA to EPA and then DHA occurs as a result of many desaturation and elongation reactions (Calder, 2012; Koletzko et al., 2008; Swanson, Block, & Mousa, 2012)(Figure 2); however, the conversion of ALA to EPA and DHA within the body has been demonstrated to be inefficient and highly restricted (Plourde & Cunnane, 2007; Swanson et al., 2012). It has since been revealed that the rate of conversion is influenced by the
individual amounts of ALA and linoleic acid (LA) in the diet (Goyens, Spilker, Zock, Katan, & Mensink, 2006), a finding that is in contrast to the widely held belief that EPA and DHA synthesis is influenced by the ratio of ALA and LA (Gerster, 1998). In both instances, the most appropriate way to improve the synthesis of EPA and DHA would be to increase dietary ALA and decrease daily intakes of LA (Goyens et al., 2006). Though to ensure normal brain function, it is recommended that sources of EPA and DHA be obtained through the diet (Swanson et al., 2012). Sources of EPA and DHA include plants and fish.

![ChemDraw Illustration of Omega-3 Fatty Acid Biosynthesis](image)

Figure 2. Biosynthesis of the omega-3 fatty acids (EPA and DHA). Illustration generated by ChemDraw by Author.

1.2.4. Source of omega-3 fatty acids.

Researchers agree that fish oil derived from large predatory fish is the premier source of omega-3 fatty acids and is superior to plant sources; however, the quantity differs
according to the species of fish (Table 1)(Nettleton, 1991). Cold-water fish contain large amounts of omega-3 fatty acids as a consequence of their physiology, environment and diet (University of Michigan, 2015). However, more recent evidence has shown high omega-3 fatty acid content in krill, comparable to that present in fatty fish. Krill are small arthropods that feed on marine algae (i.e. phytoplankton – microscopic organisms that reside in the sunlit layers of fresh water oceans to produce energy through photosynthesis) to accumulate fatty acids, and are predominantly found in the colder ocean waters (Cripps & Atkinson, 2000). As krill are positioned lower in the food chain they provide a vital nutritional source for baleen whales, small fish and seabirds (Bargu et al., 2002). However, research shows a compositional difference between the omega-3 fatty acids derived from krill and cold-water fish. Differences in EPA and DHA composition between krill and fish are based on the premise that krill oil contains high levels of phospholipids, whereas in fish oil the fatty acids are bound to natural triglycerides (Burri, 2014).
<table>
<thead>
<tr>
<th>Sources</th>
<th>Omega-3 Content (g /100 g)</th>
<th>Omega-3 Content</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish Species</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atlantic Salmon</td>
<td>1.8</td>
<td>High</td>
</tr>
<tr>
<td>European Anchovy</td>
<td>1.7</td>
<td>High</td>
</tr>
<tr>
<td>Wild Salmon</td>
<td>1.6</td>
<td>High</td>
</tr>
<tr>
<td>Pacific and Jack Mackerel</td>
<td>1.6</td>
<td>High</td>
</tr>
<tr>
<td>Pacific Sardine</td>
<td>1.4</td>
<td>High</td>
</tr>
<tr>
<td>Atlantic Herring</td>
<td>1.2</td>
<td>High</td>
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<tr>
<td>Atlantic Mackerel</td>
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<tr>
<td>Swordfish</td>
<td>0.7</td>
<td>Moderate</td>
</tr>
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<td>White Tuna</td>
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<td>Halibut</td>
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<td>Yellow Fin Tuna</td>
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<td>Low</td>
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<tr>
<td>Atlantic Cod</td>
<td>0.1</td>
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<tr>
<td><strong>Plant Sources</strong></td>
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</tr>
<tr>
<td>Flax Seeds</td>
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<td>High</td>
</tr>
<tr>
<td>Chia Seeds</td>
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<td>High</td>
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<td>Canola</td>
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<td>Moderate</td>
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</tr>
<tr>
<td>Soybean</td>
<td></td>
<td>Moderate</td>
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</table>

*Note. Data interpretation from European Food Information Council (2003) and University of Michigan (2015).*

### 1.2.5. The forms and bioavailability of omega-3 fatty acids.

While phospholipids and triglycerides are both lipid molecules, they differ in their structures despite being composed of fatty acids and a glycerol molecule (*Figure 3*) (Nelson & Cox, 2013a). Based on these compositional differences, researchers have compared the bioavailability of phospholipids and triglycerides to determine which form is absorbed faster in the body to enhance therapeutic outcomes (Mathews et al., 2002). Although the
evidence is highly controversial, few studies have reported faster absorption rates for fatty acids derived from phospholipids (Schuchardt et al., 2011). A recent study found the highest absorption for fatty acid phospholipids compared to triglyceride forms, although the differences were not significant. However, the researchers postulate that the high bioavailability of phospholipids – 22% total EPA and 21% total DHA – was influenced by the free fatty acid content in the krill oil sample (Schuchardt et al., 2011). Since omega-3 fatty acids in free fatty acid form are not bound to other molecules (Kastelein et al., 2014), they are not reliant on pancreatic enzymes and thus have enhanced bioavailability (Davidson, Johnson, Rooney, Kyle, & Kling, 2012). Based on the unexpected finding of enhanced bioavailability of free fatty acids, researchers propose that the content of free fatty acids is more important for absorption than the structure of phospholipids (Schuchardt et al., 2011), though further investigation is necessary. Differences exist between EPA and DHA found naturally and those processed and encapsulated as dietary fish oil supplements sold over-the-counter.
1.2.6. **Forms and bioavailability of omega-3 fatty acids in fish oil supplements.**

In fish oil supplements, however, the omega-3 fatty acids are either in the form of triglycerides or ethyl esters (Gross & Klein, 2011; Kremer, 2000). Due to the upsurge of
interest in supplementation, ethyl esters have become more prevalent as they provide a
more concentrated compound to increase the EPA and DHA content and are cheap to
produce (Dyerberg, Madsen, Moller, Aardestrup, & Schmidt, 2010). Following the
refinement process, fatty acids in fish oil supplements may be presented as free fatty acids,
ethyl esters or re-esterified triglycerides (Figure 4), which has sparked a highly contentious
debate surrounding the quality and bioavailability of this highly refined and synthetic
product (Dyerberg et al., 2010; Anonymous, 2016).

As depicted, ethyl esters are composed of free fatty acids bound to an ethanol
molecule and are a product of trans-esterification (Armenta, Vinatoru, Burja, Kralovec, &
Barrow, 2007; Breivik, Haraldsson, & Kristinsson, 1997; Doyle et al., 1994). During this
process, the glycerol backbone of triglycerides is removed to enable the free fatty acids to
be attached to an ethanol molecule (Breivik et al., 1997). Following this, ethyl esters are
molecularly distilled to enhance the omega-3 fatty acid content through the removal of
short chain and saturated fatty acids (Dyerberg et al., 2010; Hickman, 1937). Molecular
distillation also refers to a process necessary for the removal of environmental
contaminants in fish oil – heavy metals, dioxins, and polychlorinated biphenyls (PCBs)(Gross
& Klein, 2011; H. Lungu, personal communication, January 29, 2015). However, despite
research demonstrating that ethyl esters cannot be transported in the blood, many fish oil
supplements present omega-3 fatty acids as ethyl esters to reduce production costs
(Dyerberg et al., 2010). In fact, research has shown the absorption process is much less
efficient than in natural forms, as they must be reconverted to triglycerides to enable
intestinal absorption (Beckermann, Beneke, & Seitz, 1990; Dyerberg et al., 2010; Offman et
al., 2013). Poor absorption has important implications for the bioavailability of fatty acids
derived from fish oil and the effectiveness of supplementation for mood disorders.

Figure 4. The conversion process of triglycerides (derived from fish oil) to produce ethyl esters and re-esterified triglycerides present in fish oil supplements, and their compositions. Diagram produced by Author.
Research has consistently demonstrated lower absorption rates for ethyl esters compared to re-esterified triglycerides and natural triglycerides (Davidson et al., 2012; Dyerberg et al., 2010; Lawson & Hughes 1988; Visioli, Rise, Barassi, Marangoni, & Galli, 2003). In fact, a recent study comparing the bioavailability of fatty acid formulations from marine oils found that the absorption of re-esterified triglycerides – 124% – was comparable to natural fish oil, yet superior to ethyl esters – 73% (Dyerberg et al., 2010). This demonstrates that omega-3 fatty acids are absorbed better in the form of triglycerides than ethyl esters, although this may be explained by the fact that ethyl esters require further digestion by intestinal lipases to be absorbed (Davidson et al., 2012; Offman et al., 2013). However, the researchers postulate that the absorption of ethyl esters may be dependent on the fat content in the diet (Lawson & Hughes 1988), based on the premise that a high fat meal would stimulate pancreatic enzymes to encourage intestinal absorption (Davidson et al., 2012). A double-blind placebo-controlled study comparing the bioavailability of different omega-3 preparations revealed that a high-fat meal, containing 44 grams of total fat, significantly enhanced the absorption of fatty acids from ethyl esters to 60%, indicating a three-fold increase (Lawson & Hughes 1988). This is compared to an increase from 69% to 90% for EPA in the form of triglycerides, which appears consistent with the notion that a meal’s fat content aids digestion and subsequent absorption (Davidson et al., 2012). In contrast however, the fat content of the meal did not influence the absorption of DHA from fish oil triglycerides (Lawson & Hughes 1988), which suggests a possible difference in the bioavailability of individual fatty acids.

In another double-blind placebo-controlled trial, the researchers compared the incorporation of fatty acids in red blood cell membranes between the different chemical formulations (Neubronner et al., 2011). From baseline to three months, fatty acid incorporation had increased by 186% for re-esterified triglycerides compared to 161% for ethyl esters, demonstrating a significant difference. At six months, cell membrane incorporation had increased to 197% and 171% for fatty acid triglycerides and ethyl esters, respectively. The researchers concluded that triglycerides provide a much faster and higher increase in absorption (Neubronner et al., 2011), which provides evidence to support their superior bioavailability over ethyl esters. The implication of these findings is that fish oil supplements may differ in their effectiveness to alleviate symptoms of mood disorders.
depending on the form of omega-3 fatty acids—triglycerides, ethyl esters, or free fatty acids. Just as there are differences in the bioavailability of omega-3 fatty acids, there are also differences in the functional roles played by omega-3 fatty acids as phospholipids and triglycerides.

1.2.7. The function of phospholipids and triglycerides.

The functional activity of phospholipids and triglycerides is vast (Alberts et al., 2002; Kidd, 2007; Speake, 2006); however, for the purposes of this research, the study will focus on their roles in the context of the brain. Phospholipids perform essential functions and are an integral part of cell membranes (Carrie, Clement, de Javel, Frances, & Bourre, 2000; Tayebati & Amenta, 2013). In fact, they function primarily via cell membranes through the formation of lipid bilayers (Cooper, 2000). These are stable barriers that separate the two aqueous environments of a cell—a vital function based on evidence that phospholipids comprise two hydrophobic tails (fatty acid chains) that repel water and a hydrophilic head group (Alberts et al., 2002; Cooper, 2000). Since hydrophobic and hydrophilic molecules differ in their interactions with water, phospholipids are arranged within the membrane to ensure the hydrophobic tails are buried in the interior and thus protected from water (Alberts et al., 2002; Cooper, 2000). This means that in an aqueous environment phospholipids form either micelles with their polar head group facing the water matrix, or bilayers (as in cell membranes) (Figure 5). The fluidity of the membrane is determined by the packing efficiency of the phospholipid. Unsaturated phospholipids pack less well and thus increase fluidity. Fluidity is a key factor in biological membrane function (Nelson & Cox, 2013b).
Lipid bilayers containing long-chain unsaturated fatty acids (i.e. phospholipids) play a pertinent role in cell membrane fluidity (Cooper, 2000). This is crucial for the transportation of nutrients by membrane proteins (Lenaz, 1987). Evidence has further shown that membrane proteins (e.g. receptors, ion channels, enzymes) essential for normal brain function are incorporated in phospholipid membranes (Manku & Horrobin, 2003). In fact, signal transduction processes appear reliant on the activation of phospholipases following the release of neurotransmitters that bind to membrane receptors. This is based on the observation that phospholipase activity produces compounds that are central to neuronal function (Manku & Horrobin, 2003), and demonstrates the importance of phospholipids in the brain. These findings have lead researchers to suggest a link between abnormal phospholipid metabolism and the pathogenesis of mood disorders, with particular interest in depression, bipolar disorder and schizophrenia (Kesebir, 2014; Peet, 2003; Peet, 2002)(refer to section 1.6).

In contrast to the role of phospholipids, evidence shows that triglycerides are an important energy source and are involved in a vast range of metabolic processes throughout the body (Berg, Tymoczko, & Stryer, 2002). Compared to glycogen derived from carbohydrates and proteins, triglycerides provide an abundance of energy for human cells.
and have evolved as the primary energy reservoir in the body (Berg et al., 2002). Triglycerides are thus stored in adipose cells in which they are released by hormones to provide energy when necessary (Fain & Shepard, 2013). While they are unable to form lipid micelles in cell membranes, research has revealed that triglycerides are degraded to free fatty acids and monoglycerides by intestinal lipases to enable incorporation into micelles (Grosvenor & Smolin, 2009; Berg et al., 2002; Ewe & Karcab, 2012). Incorporation facilitates the absorption of triglycerides and provides cells with energy to perform vital functions (Cohen, 2008). However, it has been shown that brain cells are an exception, based on the fact that glucose is the sole energy source for the brain (Berg et al., 2002). Irrespective of brain glucose levels, adipose cells are dependent on glucose for the release of fatty acid triglycerides in the blood. Despite playing a central role in human metabolic process, elevated triglyceride levels have been found to increase the risk of coronary artery disease (Ballantyne et al., 2001). However, this may be caused by factors related to the metabolic syndrome (Ballantyne et al., 2001) and thus independent of triglyceride levels derived from fish oil and demonstrates the importance of fatty acid triglycerides for cellular function. A major concern is whether or not fish oil supplements derived from fish contain mercury.

### 1.3. Mercury.

Although fish contain many nutrients (e.g. essential fats, protein, vitamins, minerals), research has demonstrated an increased risk of mercury poisoning, based on the premise that fish consumption is the main source of dietary exposure (Freeman, 2008). Mercury is a toxic heavy metal released to the environment by natural and anthropogenic sources in the form of elemental (Hg⁰), inorganic (Hg⁺, Hg²⁺), and organic mercury (e.g. methylmercury HgCH₃Cl and dimethylmercury CH₃HgCH₃)(Lamborg et al., 2014; Shaw, 2012)(Table 2). All forms of mercury pose a risk to human health; however, organic mercury is far more damaging in a toxicological context because it binds to cysteine to mimic methionine and crosses the blood brain barrier (Figure 6)(Kerper, Ballatori, & Clarkson, 1992; Shaw, 2012). Inorganic mercury appears less detrimental to the central nervous system as it does not mimic methionine and is therefore unable to cross the blood brain barrier (Park & Zheng, 2012; Shaw, 2012). While further research is necessary to better understand the detail involved in the transportation, distribution and transformation of mercury, evidence has
shown that inorganic mercury (Hg$^{2+}$) is converted to organic mercury – particularly methylmercury (HgCH$_3$Cl) – by sediment bacteria in the ocean waters (U.S. Geological Survey, 2000; Shaw, 2012). Since methylmercury is lipid soluble it bioaccumulates in the aquatic food chains (or interactions between chains to form complex food webs – see section 1.3.4.) to reach high levels in large predatory fish, which are at the top of the food chain (e.g. tuna, swordfish, king mackerel)(Shaw, 2012; Trasande, Landrigan, & Schechter, 2005). Bioaccumulation is the process by which organisms are exposed to contaminants much faster than what the body can eliminate, leading to accumulation over time (U.S. Geological Survey, 2000). Consumers of contaminated fish are often exposed to very high levels of methylmercury, which accumulates in human adipose tissue and causes detrimental effects to the developing brain and central nervous system (Pirrone et al., 2010; Shaw, 2012; Tchounwou, Ayensu, Ninashvilli, & Sutton, 2003; Trasande et al., 2005). A well-documented example of methylmercury toxicity is Minamata disease.

Table 2
Sources of Mercury Released in the Atmosphere

<table>
<thead>
<tr>
<th>Natural Sources</th>
<th>Volcanic Eruptions, Geologic Deposits, Volatilization from Ocean, Sediments, Erosion, Weathering Rocks, Soil, Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthropogenic</td>
<td>Gold Mining, Alkali and Metal Processing, Coal Incineration, Dental Amalgams</td>
</tr>
</tbody>
</table>

Figure 6. Methylmercury superimposed on methionine to show how methylmercury crosses the blood brain barrier, as methionine is actively taken up by the brain. Shaw (2012). Diagram adapted from Shaw (2012), with permission.

1.3.1. Minamata disease.

The neurotoxicity of methylmercury was first seen in a large population in the 1950s in Minamata, Japan, where the residents of Minamata Bay were exposed to high concentrations of methylmercury in fish originating from the Chisso Corporation (Trasande et al., 2005). High levels of exposure to methylmercury resulted in the development of a neurological disease – termed ‘itai itai’ – and many cases of cerebral palsy in children born to mothers who ingested contaminated fish while pregnant (Harada, 1995; Shaw, 2012). Authorities discovered that inorganic mercury was released to the environment as wastewater discharge, also from the Chisso Corporation’s chemical factory, and was methylated to form methylmercury by sediment bacteria, and, as a result, was absorbed and concentrated up the food chain in marine species (Harada, 1995; Shaw, 2012). The industrial plant was therefore held accountable for the 1,785 deaths caused by Minamata disease (Shaw, 2012). Methylmercury and its effects on the unborn fetus will be discussed in section 1.3.2.
1.3.2. Methylmercury toxicity to the developing fetus.

Evidence supports that methylmercury crosses the placental barrier attaining high concentrations in the neonatal brain (Yip, Dart, & Sullivan, 2001; Grandjean, & Nielson, 2009). Exposure to methylmercury during prenatal development, therefore, has the potential to cause adverse neurological effects (methylmercury is lipophilic and mimics methionine and so crosses the blood brain barrier)(Gilbert & Grant Webster, 1995; Grandjean & Nielson, 2009; Trasande et al., 2005). In fact, exposures to environmental contaminants during the third trimester influence the formation of nervous system pathways, which indicates an enhanced brain vulnerability to neurotoxins (Grandjean & Herz, 2011). However, researchers postulate the neurotoxic effects are dependent on the level of exposure in utero (Grandjean et al., 1999). High levels of methylmercury have been found to cause diffuse and extensive central nervous system damage, consistent with Minamata disease, in particular, severe mental retardation, deafness, blindness, ataxia and cerebral palsy (Oken & Bellinger, 2008; United States Environmental Protection Agency, 2000). In contrast, chronic low-level exposure to methylmercury leads to decreased birthweight, developmental delays, and poor cognitive function (Gilbert & Grant Webster, 1995; Oken & Bellinger, 2008; Trasande et al., 2005). Since the developing fetus and infants are sensitive to the harmful effects of methylmercury, current recommendations encourage pregnant women and women of childbearing age to restrict their consumption of fish known to contain high levels of methylmercury (i.e. shark, swordfish, king mackerel)(Gilbert & Grant Webster, 1995; Grandjean & Hertz, 2011; Scarmoutzos & Boyd, 2003). Methylmercury accumulates in the body faster than it is released and predominantly stores in adipose tissue.

1.3.3. Methylmercury accumulation in adipose tissue.

There are two forms of methylmercury, methylmercury and dimethylmercury, which differ in their water and lipid solubility. The octanol water partition coefficient, as defined by $K_{ow}$, reflects the extent to which a chemical species is soluble in water and lipids. Since octanol is comparable to biological lipids and human adipose tissue in terms of solubility characteristics, it is used to indicate the potential absorption of mercury compounds. The larger the $K_{ow}$, the higher the lipid solubility; the lower the water solubility. Dimethylmercury is highly lipid soluble ($\log K_{ow} = 2.59$) (Scarmoutzos & Boyd,
2003) indicating that exposure would cause detrimental neurological effects during early development. Fortunately, however, dimethylmercury environmental contamination is considered to be extremely low (Wilken & Hintelmann, 2013). On the contrary, methylmercury (CH$_3$Hg$^+$) is much more soluble in water than in lipids (Log $K_{ow}$ = 0.41) Scarmoutzos & Boyd, 2003) because of its ionic form; therefore, is unlikely to cross the blood brain barrier on polarity grounds. However, methylmercury forms a complex with cysteine (cysteinyl methylmercury complex; Figure 6), which mimics the essential amino acid methionine. Cysteinylmethylmercury complex is taken across the blood brain barrier by the methionine carrier system and thus accumulates in the brain to cause havoc. Therefore, the potential for methylmercury poisoning following the consumption of contaminated fish remains high and is a human health concern.

1.3.4. Accumulation of methylmercury up the food chain.

Since methylmercury bioaccumulates and concentrates up the aquatic food chain, the highest concentrations are seen in the highest trophic level predators (e.g. tuna, swordfish, shark)(Oken & Bellinger, 2008; Shaw, 2012). The trophic level refers to the position the organism holds in the food chain; however, because the feeding habits of organisms are not confined to one trophic level, the mean value is often reported (Lim, & Persyn, 2013). The food chain is determined by the marine species within the ecosystem and consists of four trophic levels – (1) primary producers, (2) primary consumers, (3) secondary consumers, and (4) tertiary consumers (Figure 7)(Frank et al., 2005). The main distinction between the levels is that primary producers (e.g. plants, marine algae) absorb sunlight for energy to produce food through photosynthesis, whereas consumers (e.g. shrimp, mackerel, tuna, shark) must obtain their food from organisms lower in the food chain (Russell, Wolfe, Hertz, Starr, & McMillan, 2008). Tuna is a quaternary consumer that accumulates mercury and is frequently consumed by humans, and is therefore a human health risk.
Plankton (primary producer) is at the bottom of the food chain and absorbs methylmercury following the conversion from inorganic mercury by sediment bacteria (Shaw, 2012). Organisms higher up the food chain consume plankton leading to the bioaccumulation and biomagnification of methylmercury. Biomagnification is the process by which environmental contaminants increase in concentration at each trophic level (U.S. Geological Survey, 2000) (Table 3). In other words, the highest levels of methylmercury are found in tertiary consumers as these are large predatory game fish that bioaccumulate environmental contaminants over the lifespan (Krabbenhoft, & Rickert, 2013). Further, methylmercury is concentrated in adipose and muscle tissue indicating that fish higher in the food chain will contain the highest levels based on their body weight (Kannan et al., 1998). Researchers conclude that fish size is the best indicator of methylmercury content (Burger & Gochfeld, 2011). However, since methylmercury cannot be removed from the muscle tissue during the cooking process, consumption may
be detrimental to human health (U.S Department of the Interior). The amount of methylmercury ingested is dependent on diet.

<table>
<thead>
<tr>
<th>Marine Species</th>
<th>Trophic Level</th>
<th>Median Methylmercury Level (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plankton*</td>
<td>1</td>
<td>0.001</td>
</tr>
<tr>
<td>Shrimp</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>Tuna</td>
<td>4</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*Note. Data adapted from U.S. Food and Drug Administration (1990-2010).

1.3.5. Exposure to methylmercury in food – the New Zealand total diet food survey.

In light of evidence demonstrating the adverse human health effects of methylmercury, the World Health Organisation (WHO) have revised and amended their provisional tolerable weekly intakes (PTWIs) for dietary mercury exposure (New Zealand Total Diet Study (NZTDS), 2011). The current provisions are $1.6 \mu g/kg$ body weight methylmercury and $4 \mu g/kg$ body weight total mercury, excluding fish and shellfish (WHO, 2010; WHO, 2007). While these intakes reflect the differential toxicities of different mercury compounds (Shaw, 2012), they also highlight the potential risks associated with methylmercury consumption.

In New Zealand, the potential human health risks from methylmercury are high based on the estimated dietary exposure revealing that the ‘average’ individual consumes $0.33 \mu g/kg$ body weight per week (Table 4). This is much higher than the simulated methylmercury dietary exposures in China ($0.041 \mu g/kg$ body weight per week); however, this may be explained by the more frequent consumption of large predatory fish in New Zealand compared to other countries (i.e. UK, USA, Czech Republic)(NZTDS, 2011; Shaw, 2012). A recent non-total diet food survey found that 88% of the population incorporates fish in their diet once per month, whereas 45% are more regular consumers and eat fish at least once per week (Seafood Industry Council, 2007). Provided the simulated New Zealand diet excluded fish and shellfish, the estimated exposure to both inorganic mercury and methylmercury
would decrease significantly (Shaw, 2012). This demonstrates that fish is the main source by which humans are exposed to dietary mercury.

Table 4

<table>
<thead>
<tr>
<th>Simulated Diet</th>
<th>Estimated Exposure (µg/kg body weight/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inorganic mercury</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0.7</td>
</tr>
<tr>
<td>Excluding Fish</td>
<td>0.2</td>
</tr>
<tr>
<td>% PTWI (total diet)</td>
<td>5</td>
</tr>
<tr>
<td>PTWI (µg/kg bw/kg)</td>
<td>4</td>
</tr>
</tbody>
</table>

*Note.* Data adapted from Shaw (2012) and New Zealand Total Diet Study (2011).

The estimated dietary exposure to methylmercury for a simulated New Zealand diet does not exceed the provisional tolerable guidelines (21% of PTWI). However, depending on individual circumstances it may reach the point of being a toxicological concern, particularly for individuals who deviate from the ‘normal’ in terms of fish consumption (Ministry for the Environment, 2008). This is because the simulated diet is based on the estimated intake of fish consumed by the ‘average’ New Zealander. That is, individuals may be exposed to higher levels of methylmercury if they consume more fish than average (Shaw, 2012). As a result, the potential risk for adverse health effects will be enhanced. This raises the question of whether the benefits of consuming fish (i.e. dietary omega-3 fatty acids) outweigh the risks associated with exposure to environmental contaminants (i.e. mercury). However, despite the harmful neurological effects, recent evidence has lead researchers to conclude the health benefits of fish consumption surpass the potential risks among the general population (Hellberg et al., 2012; Mozaffarian, & Rimm, 2006; FAO/WHO, 2011). Populations that are more sensitive to environmental contaminants (i.e. pregnant women and young children) are however advised to monitor their consumption of fish and limit their consumption to fish species that are low in methylmercury but high in essential fatty acids as the benefit outweighs the risk for the fetus (Hellberg et al., 2012). The accumulation of mercury at the higher levels of the food chain starts with water contamination.
**1.3.6. Mercury levels in water.**

Although regulators conclude that the health benefits outweigh the potential risks among the adult population, dietary supplementation with fish oil may provide a safe alternative to obtaining high levels of omega-3 fatty acids in the absence of methylmercury. As discussed, the concentration of methylmercury present in fish is dependent on the trophic level, meaning that fish lower in the food chain generally contain less environmental contaminants (Fowler, Alexander, & Oskarsson, 2014). Recent evidence has further shown that methylmercury is dispersed in the ocean waters and bioaccumulates through the ocean layers (Lamborg et al., 2014). These distribution patterns appear to mimic the behaviour of methylmercury in the aquatic food chain. Due to the atmospheric deposition of mercury to the ocean (remember that mercury is released to the environment by natural and anthropogenic sources) the surface layers become supersaturated in elemental mercury compared to atmospheric levels. Through transformational processes, both inorganic and organic mercury (predominantly methylmercury) are transported from the surface ocean (>100 m)(high $\rightarrow$ low levels), concentrating through the thermocline (>1000 m) to the deep ocean waters (highest levels)(Lamborg et al., 2014; Mason et al., 2012; Strod et al., 2007). While this general trend is observed across the global ocean, the concentrations of mercury between the oceans are diverse. This may be due to the differential release of anthropogenic mercury in developed countries (Lamborg et al., 2014), though research has demonstrated that mercury is transported in the atmosphere over long distances as reflected by the presence of mercury in ecosystems remote from industrial activity (Strod et al., 2007).

Indeed, the levels of methylmercury in fish, sourced for the production of fish oil supplements, will depend on the location of their ecosystem in relation to the land. Information from fish oil companies revealed that most of the fish are sourced near the Peruvian Coast bordering the South Pacific Ocean (refer to section 3.2.2. Figure 9.) (M. Bosch, personal communication, April 23, 2015; Spokesperson from Nutra-Life, personal communication, May 20, 2015; Spokesperson from Healtheries, personal communication, May 25, 2015; Spokesperson from Red Seal, personal communication, May 20, 2015). The Peruvian Amazon is a popular region for artisanal gold mining and therefore, there is growing concern over the persistent environmental contamination (Yard et al., 2012). Research has shown that human exposure to mercury in artisanal mining regions is high –
particularly in Madre de Dios – since large amounts of elemental mercury are needed for the extraction of gold from ore deposits (Ashe, 2012; Yard et al., 2012). Following the extraction process, the mercury is evaporated and eventually deposited in the land and ocean leading to the bioaccumulation and biomagnification in the food chain (Yard et al., 2012).

In order to determine whether fish caught from the South Pacific Ocean are contaminated with methylmercury, personal communications were made with Dr. Katlin Bowman – a postdoctoral student at the University of California, Santa Cruz (UCSC) (K. Bowman, personal communications, May 8, 2015). Bowman has recently completed a study on the Peruvian Upwelling region of the South Pacific to measure the concentrations of total mercury and methylmercury at three ocean layers (Table 5). In general, the data provided are consistent with the average values of mercury in the ocean basin. In the thermocline waters, the levels of mercury were much lower than individual values in the North Atlantic, Southern Ocean, Artic, and Northeast Pacific. This indicates the environmental contamination in these regions is high (Lamborg et al., 2014) (Table 6). The data also appeared to demonstrate the general pattern of methylmercury bioaccumulation in the ocean (low levels in the surface layers $\rightarrow$ high levels in deeper waters). This indicates that fish sourced from the South Pacific Ocean may be less contaminated than fish caught from other regions of the world, thus, the mercury content in fish oil supplements might be lower than expected. In fact, one particular study examined the amounts of mercury in five over-the-counter fish oil supplements (Foran, Flood, & Lewandrowski, 2003). Cold vapor atomic absorption spectrometry was used for the determination of mercury. However, the analyses revealed that all supplements contained insignificant amounts, although the individual levels ranged from $<6 \mu g/L$ to $12\mu g/L$ mercury (Foran et al., 2003). These findings must be interpreted with caution because of the small sample of supplements analysed. Further research is necessary to ascertain the mercury content and the human health risks associated with supplements. The recent upsurge of interest in the use of fish oil supplements makes regulation of these dietary products ever important.
### Table 5

**Distribution and Average Concentrations of Total Mercury and Methylmercury in Peru Upwelling Regions**

<table>
<thead>
<tr>
<th>Region Description</th>
<th>Total Mercury (Picomole per kg)</th>
<th>Methylmercury (Picomole per kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelf Stations (&gt;200 m depth)</td>
<td>1.3 +/- 0.4</td>
<td>0.041 +/- 0.024</td>
</tr>
<tr>
<td>Upper 100 m (stations up to 1750 km west off coast)</td>
<td>0.74 +/- 0.70</td>
<td>0.076 +/- 0.070</td>
</tr>
<tr>
<td>Upper 1000 m (stations up to 1750 km west off coast)</td>
<td>0.88 +/- 0.54</td>
<td>0.086 +/- 0.063</td>
</tr>
</tbody>
</table>

*Note.* Data from Bowman, K. (2015).

### Table 6

**Concentrations of Total Mercury in Thermocline Waters in the Ocean Basin**

<table>
<thead>
<tr>
<th>Region</th>
<th>Total Mercury (Picomole per kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Atlantic^b^</td>
<td>0.41 +/- 0.14</td>
</tr>
<tr>
<td>Tropical Pacific^b^</td>
<td>0.82 +/- 0.35</td>
</tr>
<tr>
<td><strong>South Pacific (Peru)</strong>^a^</td>
<td><strong>0.88 +/- 0.54</strong></td>
</tr>
<tr>
<td>North Atlantic^b^</td>
<td>0.94 +/- 0.27</td>
</tr>
<tr>
<td>Southern Ocean^b^</td>
<td>0.95 +/- 0.057</td>
</tr>
<tr>
<td>Artic^b^</td>
<td>1.00 +/- 0.11</td>
</tr>
<tr>
<td>Northeast Pacific^b^</td>
<td>1.22 +/- 0.39</td>
</tr>
</tbody>
</table>

*Note.* Data from ^a^Bowman, K. (2015) and ^b^Lamborg et al. (2014).

#### 1.4. Dietary supplement regulations.

**1.4.1 Current dietary supplement regulations.**

Fish oil supplements in New Zealand are regulated as food under the Food Act 1981. They are also currently regulated under the Dietary Supplement Regulations 1985 enforced by the Ministry of Health. A dietary supplement is defined as a substance for oral use that contains dietary ingredients (e.g. vitamins, minerals, amino acids, concentrates) intended to supplement the diet (Ministry for Primary Industries, 2013). However, because fish oil supplements contain ingredients derived from an animal or animal product, they must also comply with regulations under the Animal Product Act 1999 or the Food Hygiene
Regulations 1974 and the Biosecurity Act 1993 (Ministry of Health, 2015). In order to ensure that fish oil supplements are safe for human consumption, they are also regulated in New Zealand under the Consumer Guarantees Act (1993) and the Fair Trading Act (1998). These laws deal exclusively with product safety and provide consumer protection that is paramount to the trade of goods and services (Ministry of Business, Administration and Employment, 2015).

Although regulated by legislation, there is no requirement for dietary supplements to obtain pre-market approval (New Zealand Food Safety Authority, 2004). Therefore, it can be argued that fish oil supplements may not be compliant with formal regulations enacted by current laws. For instance, the dietary supplement regulations describe many requirements relating to the composition and labelling of products so as to provide consumers with the necessary information to make an informed decision about its potential use. The product labelling requirements state that supplements must have the amounts of active and inactive ingredients contained within the product printed on the label to comply with legislation (Beattie, & Governor-General, 1985). However, research has indicated that many fish oil supplements sold in New Zealand do not contain the amounts of active ingredients that are advertised on the label (Albert et al., 2015). A recent study examining the quality and content of fish oil supplements sold over-the-counter in New Zealand revealed that only 3 of 32 brands contained amounts of omega-3 fatty acids consistent with product labels. Most of the supplements analysed contained <67% of the labeled content, which is considerably less than label claims (Albert et al., 2015). This clearly indicates a discrepancy between the label and actual content of fish oil supplements. The implication for consumers is that the recommended daily intakes may be too small to produce a significant benefit, leading to the cessation of supplementation. Further, the majority of fish oil supplements (83%) analysed by Albert et al., (2015) exceeded recommended oxidation levels, meaning they were highly oxidised, which only confirms the fact that over-the-counter dietary supplements are not actively regulated.

Because fish oil supplements are regulated as food, their manufactures are not permitted to make medicinal claims. Medicines in New Zealand are regulated under the Medicines Act 1981 and are defined as substances that have a ‘therapeutic purpose’ particularly with ‘treating or preventing disease’ (Beattie, D., & Governor-General, 1985; Shaw, 2003). Health benefits that are claimed or implied by a dietary supplement must be
supported by evidenced-based research (Ministry for Primary Industries, 2013). A number of fish oil supplement manufacturers claim that their preparations ‘may assist with… joint health by reducing inflammation and joint swelling’ and ‘… brain health by maintaining mental and cognitive function’. A dietary supplement claiming to reduce a non-communicable disease may be viewed as having a therapeutic purpose and may fall within the scope of the Medicines Act. This is particularly true when the claim involves ‘preventing or interfering with the normal operation of a physiological function’ to produce a health benefit, as would be the case with ‘reducing inflammation and joint swelling’.

Although current regulations are not specific to the psychological claims that can be implied by dietary supplements, the physiological processes necessary to produce a psychological benefit may be consistent with the Medicines Act definition. Fish oil products claiming to promote improvements to mental health may therefore be non compliant with current New Zealand regulations. Therefore, new regulations are being considered.

1.4.2. The natural health and supplementary products bill – 2016.

Because dietary supplements are not actively regulated in New Zealand, a discrete but determined movement for natural healthcare products has been gaining momentum in parliament over the past decade. The Natural Health and Supplementary Products Bill has progressed to its third reading in parliament (November, 2015) with the expectation that regulatory requirements will come into force mid 2016 (New Zealand Parliament, 2015). The Bill will be independent of legislation enforced by the Food Act and the Dietary Supplement Regulations (Ministry of Health, 2015), and is designed to actively regulate low-risk natural health and supplementary products to ensure they are safe for human consumption and are true to label content. It will also regulate the health benefits claimed by the company that manufactures them to ensure the claims are accurate and not a marketing ploy (Ministry of Health, 2015; Natural Health Products Bill, 2012). This is in contrast to current legislation, as the new bill states that legitimate health claims will be permitted on the proviso that they are supported by evidenced-based research – which is an exciting development for the natural health industry and for the New Zealand population. The exception is that the bill will limit the claims that can be made to specific conditions, which includes most DSM-5 disorders. However, the expectation is that customers will gain confidence in the effectiveness of natural products and will be able to make informed decisions about its
potential use based on their communicated benefits. The ability of consumers to make informed decisions on fish oil supplement products may be confounded if clinical trials were conducted with compounds dissimilar to those being sold over-the-counter.

1.5. Fish oil used in clinical trials.

In light of the evidence demonstrating the importance of omega-3 fatty acids for human health, fish oil supplements have emerged as one of the most popular dietary supplements on the global market (Albert et al., 2015). The effectiveness of fish oil supplementation has, therefore, been explored across many psychological domains, particularly the area of mood disorders, in the hope of finding a novel treatment approach for the prevention and/or management of psychiatric illness. The supplements used in clinical trials are high end products that are either provided by companies renowned for the global manufacture and/or distribution of fish oil supplements, or are formulated for research purposes. This is in contrast to over-the-counter formulations purchased from supermarkets, pharmacies or health food stores that are available to New Zealand consumers. To date, it has not been determined whether the fish oil used in research and over-the-counter supplements is comparable in terms of the dose and composition. Based on the literature, it can be assumed that the daily doses are different, with research doses being much higher than recommendations for over-the-counter supplements. In other words, the doses in research found to produce a significant reduction in symptoms of mood disorders may contain higher amounts of omega-3 fatty acids, meaning the doses in over-the-counter supplements may not be large enough to produce a psychological benefit. The potential difference between research and over-the-counter doses may be further enhanced by the fact that many fish oil supplements sold in New Zealand are not true to label, with most products containing significantly less omega-3 fatty acids than label content (Albert et al., 2015). On the condition that these preliminary findings are replicated, consumers of fish oil supplements may need to exceed recommended daily doses to obtain the desired mental and emotional health benefits – provided the dose-effect relationship is positive. A comparison of research and over-the-counter supplements (label versus actual content) will thus determine whether there are significant differences between the formulations used in research compared to over-the-counter supplements in terms of the
dose and composition of fish oil. So, how do omega-3 fatty acids act to regulate mood disorders?

1.6. Proposed mechanisms of action of fish oil for mood disorders.

Several mechanisms of action have been proposed by which fish oil supplements, containing omega-3 fatty acids, achieve pharmacological results in the context of mood disorders (Sinclair, Begg, Mathai, & Weisinger, 2007). Due to the complex nature of these neurobiological mechanisms, a snapshot of the extensive and growing literature will be provided to demonstrate the mood regulation effects of omega-3 fatty acids. The first mechanism is the role of omega-3 fatty acids in membrane function.

1.6.1. Membrane function.

One mechanism proposed relates to the incorporation of fatty acids in phospholipid membranes, as research has demonstrated that DHA plays an integral role in membrane fluidity, which can influence the function of proteins and receptors anchored within the membrane (Balanza-Martinez et al., 2011; Parker et al., 2006; Sinclair et al., 2007; Uauy & Dangour, 2006). The structures of proteins embedded in the lipid bilayer are sensitive to the effects of a changing microenvironment (Logan, 2003), thus changes in membrane composition may influence cell function through altered signaling pathways (Parker et al., 2006; Su, 2009). Membrane receptors present on the postsynaptic membrane bind to signaling molecules – neurotransmitters – and permit extracellular signals to affect internal cellular function (O’Conner & Adams, 2010). Changes in the structure of membrane receptors may interfere with normal neurotransmission processes and may trigger the complex cascade of events leading to a mood disorder. Incorporation of lipids in neuronal cell membranes is essential to maintaining normal brain function. Research has shown that DHA improves membrane fluidity and promotes neurotransmitter receptor binding (Balanza-Martinez et al., 2011).

1.6.2. Neurotransmitters.

It has been shown that a chronic dietary deficiency disrupts the chemical balance in the frontal cortex leading to an increase in serotonin and decrease in brain dopamine (Balanza-Martinez et al., 2011; Su, 2009). Increases in dopamine concentrations are
however observed in the nucleus accumbens (Balanza-Martinez et al., 2011; Sinclair et al., 2007) and may be due to loss of inhibitory control in the frontal cortex caused by reductions in dopamine (Logan, 2003). The frontal cortex dopamine reductions may be explained by the finding that vesicular monoamine transporter (VMAT2) levels in the frontal lobes are significantly diminished in omega-3 deficient rats (Logan, 2003). The VMAT2 is found on the presynaptic membrane and regulates the storage and release of monoamine neurotransmitters – dopamine, serotonin, norepinephrine, and epinephrine – to enable signal transduction and neurotransmission (Anlauf et al., 2004; Logan, 2003). This demonstrates the importance of omega-3 fatty acids for membrane function and ultimately mood disorders and suggests the differential depletion of omega-3 fatty acids in certain brain regions (Balanza-Martinez et al., 2011). Further, the adverse effects of dietary deprivation appear most prominent in the mesocortical dopaminergic pathway, as the frontal lobes were less active than the mesolimbic pathway in omega-3 deficient rats (Balanza-Martinez et al., 2011). Further, brain regions involved in cognition, motivation and emotion appear to be more vulnerable to the effects of chronic deprivation during gestation, based on the finding that the frontal cortex and temporal lobe of the developing rat were depleted of DHA relative to other regions (Balanza-Martinez et al., 2011; Soares & Mann, 1997). A long-term deficiency has repeatedly been found to cause a reduction in dopamine receptors (DA D$_2$) in the cerebral areas associated with depression, bipolar disorder, and schizophrenia (Soares & Mann, 1997; Su, 2009), thereby highlighting the importance of supplementation for maintaining neurotransmission in order to prevent the development of mood disorders. Interestingly, dietary supplementation of omega-3 fatty acids increased dopamine levels by 40% in the frontal cortex of rats and enhanced dopamine binding to the D$_2$ receptor (Sinclair et al., 2007). These findings confirm that DHA is imperative to the regulation of neurotransmitters linked to the pathogenesis of mood disorders – dopamine and serotonin (Balanza-Martinez et al., 2011; Sinclair et al., 2007; Su, 2009). Further, DHA is widely believed to regulate the protective neural proteins referred to as neurotrophins; in particular, brain-derived neurotrophic factor (BDNF).

1.6.3. Brain-derived neurotropic factor (BDNF).

A further mechanism proposed is that DHA regulates signal transduction processes that are crucial to neuronal differentiation and survival (Balanza-Martinez et al., 2011; Kim,
2007), indicating a neuroprotective role. In fact, recent evidence has demonstrated several neuroprotective effects that are postulated to explain the mood-regulating effects of DHA in depression and bipolar disorder (Balanza-Martinez, 2011; Logan, 2003). These mechanisms relate to the anti-inflammatory and antioxidant effects of DHA (see below), and the enhanced glucose transport and improved membrane function in the brain (Balanza-Martinez, 2011). Since these neuroprotective effects complement the neurodevelopmental effects of DHA, as mentioned earlier (see section 1.2.2), researchers have suggested an association between DHA and BDNF – a protein that belongs to the neurotrophin family – based on the premise that they are both involved in aspects of neural protection and development and are implicated in mood disorders (Balanza-Martinez, 2011). BDNF is a highly complex structure and plays an influential role in synaptic function, neuronal growth and connectivity, and neuroplasticity (Balanza-Martinez, 2011; Post, 2007; Sinclair et al., 2007). BDNF is widely distributed throughout the brain, though the highest concentrations are found in the cerebral cortex and hippocampus – brain regions associated with memory and modulation of emotional behaviour (Balanza-Martinez, 2011; Drevets, Price, & Furey, 2008; Sinclair et al., 2007). Studies have shown decreased serum BDNF levels in depressive disorders (Karege et al., 2005; Logan, 2003; Sanchez, 2010), which is consistent with the finding that chronic administration of antidepressants corrects abnormal serum levels leading to a reduction in symptoms of psychological illness (Sinclair et al., 2007). Animal studies have provided further evidence for the role of BDNF in the pathophysiology of depression by demonstrating enhanced BDNF levels in the hippocampal region following antidepressant therapy (Sanchez, 2010). Researchers have since suggested that omega-3 supplementation may reverse the psychological effects caused by a reduction in BDNF expression (Balanza-Martinez, 2011), based on the finding that a dietary deficiency decreased expression in the frontal cortex (Sanchez, 2010; Sinclair et al., 2007; Bhatia et al., 2011). Despite some controversial findings, observational studies have revealed low concentrations of omega-3 fatty acids in plasma and red blood cell membranes in patients with unipolar depression (Frasure-Smith, Lesperance, & Julien, 2004; Su et al., 2003). Provided low dietary intakes of omega-3 fatty acids influence development of mood disorders, supplementation may prove effective in regulating brain neurotrophins in regions of the brain implicated in the pathophysiology of depression and bipolar disorder (Balanza-Martinez et al., 2011). Thus, regulating brain neurotrophins may provide another
mechanism to explain their therapeutic effects in the treatment of mood disorders. Another way omega-3 fatty acids may operate to regulate mood disorders is through the moderation of brain inflammation.

1.6.4. Inflammation.

A large body of evidence supports that EPA modulates inflammatory and immune functions representing a novel mechanism to explain processes in which omega-3 fatty acids ameliorate symptoms of mood disorders (Miller et al., 2009; Su, 2009). Although the precise neurobiological mechanisms are unclear, research has consistently revealed that EPA competes with AA and prevents the incorporation into lipid membranes (Parker et al., 2006). Prevention of AA incorporation enhances membrane fluidity, and maintains signal transduction pathways and neurotransmitter systems – monoamine and glutamate systems. Studies have further shown that EPA inhibits the production of proinflammatory eicosanoids (i.e. prostaglandins (PGE$_2$), leukotriene (LTB$_4$), and thromboxane (A$_2$)) and prevents the release of proinflammatory cytokines relevant to depression (i.e. interleukin (IL) 1β, IL-2, IL-6, interferon gamma, and tumor necrosis factor (TNF-α))(Felger & Lotrich, 2013; Logan, 2003; Parker et al., 2006; Sinclair et al., 2007). This is consistent with the findings that patients with depression have elevated levels of inflammatory cytokines, levels that can be inhibited by some antidepressant medications (Sinclair et al., 2007). While the anti-inflammatory effects of EPA are well documented, the role of eicosanoids and cytokines in the pathophysiology of mood disorders has only recently been accepted (Miller et al., 2009). However, the evidence to support an association between neuroinflammation and depression is mounting (Felger & Lotrich, 2013; Miller et al., 2009). In contrast to the adaptive behavioural response following acute elevations in inflammatory cytokines, prolonged exposure has been found to produce changes in neurotransmitter systems due to the activation of inflammatory signaling pathways, which also causes a reduction in several growth factors (i.e. BDNF)(Felger & Lotrich et al., 2013). These persistent alterations are postulated to contribute to the pathophysiology of mood disorders (see below), which demonstrates the importance of effective therapeutic strategies that target the inflammatory response (Felger & Lotrich, 2013; Miller et al., 2009). Supplementation with omega-3 fatty acids may represent a novel treatment approach to the amelioration of
psychological symptoms by reducing neural inflammation in patients with elevated inflammatory biomarkers.

It is clear that there are several potential mechanisms of action that may explain the mood regulating effects of fish oil supplements containing omega-3 fatty acids. Future exploration of these proposed mechanisms is imperative to understanding the rationale for supplementation. The mechanisms discussed simply provide a basis for examining the therapeutic effects of fish oil supplements for mood disorders, of which there are many. This research examines three mood disorders, beginning with major depressive disorder.

1.7. A brief review of mood disorders.

1.7.1. Major depressive disorder.

There is worldwide concern for the growing prevalence of mental illness, with mood disorders proving to be one of the leading contributors to the global burden of disease (Leung, & Kaplan, 2009). Depression, in particular, presents a substantial health and financial burden and has emerged as a leading cause of disability worldwide (Leung & Kaplan, 2009; O’Neal et al., 2013; Sarris, Schoendorfer, & Kavanagh, 2009). According to the World Health Organisation, an estimated 350 million people are affected by depression around the world, which has increased from 121 million people in 2010 (Reddy, 2010; WHO, 2015). Further, the New Zealand Health Survey (2011/2012) revealed that 14.3% of adults in New Zealand have a history of depression (Mental Health Foundation, 2014), thus illustrating how prevalent mental disorders are amongst the global population. The impact of depression on disability-adjusted life years is expected to be much greater in the coming decades (Sarris et al., 2015; Sarris, Schoendorfer, & Kavanagh, 2009), which demonstrates the importance of finding effective treatments with minimal adverse effects. A major depressive disorder is characterised by episodes of depressed mood, loss of interest or pleasure and low energy that must persist for at least two weeks for a clinical diagnosis to be reached and be independent of adverse mood effects associated with substance abuse, bereavement or a medical illness (Sarris et al., 2009; WHO, 2015). A depressive episode can also be accompanied by feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration and must represent a change in mood from normal mood states. The chronic or recurrent nature of depression has the potential to interfere with normal everyday function – social, occupational and educational – and in extreme cases can lead to
self-harm and suicide (WHO, 2015a). Depression is especially troubling as it pertains to pregnancy.

1.7.2. Perinatal depression.

While the sociological effects of depression can be devastating, research has shown the consequences of maternal depression are much more serious and persist over the longer term for children in the family (Leung & Kaplan, 2009). The term maternal depression refers to the depressive episodes that peak during the first trimester of pregnancy (perinatal or antenatal) or within the 4-weeks post delivery (postpartum or postnatal) (Leung & Kaplan, 2009; Monzon, di Scalea, Pearistein, 2014; Sharma & Mazmanian, 2014). Research has shown the episodes of postpartum depression are comparable to a major depressive disorder and has been estimated to affect 12-16% of new mothers. There is a clear difference between postpartum depression and the baby blues in terms of the severity and duration of depressive episodes, with the baby blues affecting 70-80% of mothers in the first couple of weeks after childbirth (National Institute of Mental Health). Furthermore, perinatal depression has been associated with poor maternal health outcomes, which may be explained by evidence demonstrating that these vulnerable women are less likely to seek help from medical professionals yet are more likely to deviate from adaptive pregnancy behaviours towards drug and alcohol abuse (Leung & Kaplan, 2009). A growing body of evidence has demonstrated that postpartum depression has adverse effects on the infants functioning with poor outcomes observed across the cognitive, social, and developmental domains (Leung & Kaplan, 2009; Levant, 2011). Postpartum depression can threaten the mother-infant bonding process and can increase the risk of poor mental health outcomes in the offspring (Hirst & Mou-tier, 2010). In severe circumstances it may lead to maternal suicide or infanticide (Levant, 2011), thus illustrating the deleterious effects of depression, when left untreated. Another common mood disorder is bipolar disorder.

1.7.3. Bipolar disorder.

Depressive episodes are a distinctive feature of bipolar disorder – a chronic, recurrent mood disorder associated with significant disability, morbidity and in severe instances, premature mortality (Balanza-Martinez et al., 2011). A recent survey has revealed that 60 million people are affected by bipolar disorder worldwide – a condition that is characterised
by episodes of mania and depression, interspersed with periods of normal mood (Balanza-Martinez et al., 2011; WHO, 2015b). A manic episode is defined as a distinctive period of elevated or irritable mood that is abnormal and persistent over at least one week. The change in mood must be accompanied by three or more symptoms of mania – anxiety, disturbances in cognition and circadian rhythm, impulsivity, psychosis and psychomotor agitation (Balanza-Martinez et al., 2011; Krans, 2014; WHO, 2015b). The broad range of symptoms demonstrates that multiple neurobehavioral domains are affected, and thus extends beyond the core feature of mood dysregulation (Balanza-Martinez et al., 2011). While there are important distinctions between the types of bipolar disorder with respect to the intensity and duration of symptoms, research has shown that rapid-cycling bipolar disorder is a more severe form of the disorder (Table 7). However there is growing evidence to suggest that bipolar is a spectrum disorder, with subthreshold cases being undetected and untreated. This is despite there being moderate to severe episodes of mania and functional impairments of clinical significance (Merikangas et al., 2007). In contrast however, rapid-cycling disorder is a potentially devastating condition that is most prevalent in women and in those who endured their first episode during late adolescence (Krans, 2014; National Institute of Mental Health, 2012). It has been suggested that the poor functional outcomes associated with the disorder may be attributable to the persistent decline in cognitive and physical health over the long term (Balanza-Martinez et al., 2011). Effective treatments that focus on the prevention of cognitive deterioration will improve quality of life and may reduce the incidence of premature mortality. In addition, bipolar disorder is associated with several medical conditions, such as cardiovascular disease, neurological disorders, and metabolic dysfunction (i.e. diabetes mellitus). Such medical conditions tend to complicate the presentation and management of bipolar symptoms and worsen the treatment response, thus leading to poor life outcomes (Balanza-Martinez et al., 2011; Merikangas et al., 2007). It is clear that there are many medical avenues to be investigated in order to discover the most effective treatments for complex and multifaceted mood disorders such as depression and bipolar disorder (see the treatment section 1.10). There are a variety of potential variables associated with the etiology of mood disorders.
### Table 7

**Diagnostic Distinctions Between the Types of Bipolar Disorder (DSM-5)**

<table>
<thead>
<tr>
<th>Type</th>
<th>Episode Criteria</th>
<th>Episode Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bipolar I Disorder&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≥ 1 manic episode or mixed episodes – manic and depressive symptoms</td>
<td>≥ 7 days</td>
</tr>
<tr>
<td>Bipolar II Disorder&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≥ 1 hypomanic and ≥ 1 severe depressive episode</td>
<td>-</td>
</tr>
<tr>
<td>Otherwise Specified Bipolar and Related Disorder&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Meet criteria for hypomania and have a history of major depressive disorder or do not meet diagnostic criteria for full bipolar I disorder</td>
<td>≥ 4 days</td>
</tr>
<tr>
<td>Cyclothymic Disorder&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≥ 1 hypomanic episode and mild depressive episodes</td>
<td>≥ 2 years</td>
</tr>
<tr>
<td>Rapid-cycling Bipolar Disorder&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≥ 4 manic, hypomanic, major depressive episodes or mixed states</td>
<td>≤ 1 year</td>
</tr>
</tbody>
</table>

*Note.* Data adapted from <sup>a</sup>National Institute of Mental Health (2012) and <sup>b</sup>Tracy (2012).

#### 1.8. An overview of the etiology of mood disorders.

The etiology of mood disorders – depression, perinatal depression, and bipolar disorder – remains unclear. However, due to the complex nature of the conditions, researchers have proposed multiple causes that involve both genetic and environmental factors (Leung & Kaplan, 2009; Levant, 2011; Nestler et al., 2002). It is believed that genetic predispositions interact with an array of environmental factors to trigger the development of mood disorders (Leung & Kaplan, 2009; Levant, 2011).

#### 1.8.1. Genetic factors.

Based on the premise that genes influence mood, researchers have studied the genetic basis of depression and bipolar disorder in much depth (Harvard Medical School, 2009; Leung & Kaplan, 2009). A review of linkage studies reported evidence implicating specific genes with mood disorders and schizophrenia; however, replication of these findings is essential (Craddock & Forty, 2006). While the evidence of specific depression and bipolar disorder vulnerability genes is not conclusive, research has demonstrated that the risk of depression is higher in those who have inherited at least one short version of the serotonin-transporter gene (5-HTT). Thus, short versions make people more vulnerable to
depression during stressful life events (Harvard Medical School, 2009). In support of this finding, researchers have found that depressive episodes in response to stress were less severe in people with the protective variant of the CRHR1 gene, which codes for the corticotrophin-releasing stress hormone (Harvard Medical School, 2009). These findings illustrate the importance of genetic factors in mood disorders and the role of stress hormones in depression. Epidemiological studies have revealed that 40-50% of the risk for depression is attributable to genetic factors, indicating that depressive disorders are highly heritable (Nestler et al., 2002). However, the strongest evidence for the power of genetics is derived from family, twin, and adoption studies of bipolar disorder (Harvard Medical School, 2009; Smoller & Finn, 2003). Family studies have documented that the risk of bipolar disorder is highest for first-degree relatives of affected individuals, whereas, twin studies have provided substantial evidence to suggest that genes account for a large proportion of this familial aggregation (Smoller & Finn, 2003). In fact, heritability estimates for bipolar disorder are at 85% and 89% using narrow and broad concordance, respectively (McGuffin et al., 2003). However, the pattern of inheritance for genes linked to mood disorders remains unclear, although the uncertainties may be due to the complex nature of depression and bipolar disorder and the influence of multiple genes and their interactions with environmental factors (Leung & Kaplan, 2009).

1.8.2. Environmental factors.

Genetic factors enhance the predisposition for depression and bipolar disorder, whereas environmental factors trigger the cascade of neurological events in those with a genetic vulnerability for the disease (Leung & Kaplan, 2009). These environmental influences include stress (i.e. physical, mental, and emotional trauma), viral infections, hormonal disorders, medications (i.e. sedatives), and medical problems (Harvard Medical School, 2009; Leung & Kaplan, 2009; Nestler et al., 2002). In relation to the diverse range of environmental factors, researchers have proposed several mechanisms of action for the pathophysiology of mood disorders, thus the most prominent mechanisms will be of focus.

It has been well documented that acute and chronic stress – physical (e.g. giving birth, abuse), mental, and emotional stressors – causes activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to the release of glucocorticoids (cortisol) from the adrenal cortex (Nestler et al., 2002). Cortisol is the main hormone released in response to stress –
whether perceived or actual stress – and is regulated through the HPA axis (Randall, 2012). Acute stress enhances memory and cognition during potentially threatening situations, whereas chronic stress produces structural and functional brain changes that lead to pathological behaviours (McEwen, 2000; Nestler et al., 2002). Researchers have revealed that depression is associated with persistent elevations in serum cortisol in parallel to elevations in the corticotrophin-releasing factor (CRF) in cerebral spinal fluid. These findings indicate the mechanisms of the stress-response are dysregulated in depressive disorders (Levant, 2011; Nestler, 2002; Jolley, Elmore, Barnard, & Carr, 2007). While cortisol has direct involvement in multiple brain regions leading to behavioural changes, researchers have focused on the role of cortisol in the hippocampus, amygdala, and the nucleus accumbens – regions that regulate mood and motivation (Christoffel, Golden, & Russo, 2011; Nestler et al., 2002). Evidence has shown that sustained elevations in cortisol induce structural brain changes in the hippocampus, including damage to hippocampal neurons and reduced birth of neurons in the dentate gyrus (Nestler et al., 2002). While the precise mechanism for this damage remains to be fully elucidated, researchers postulate that it may involve the loss of dendritic arborization and dendritic spines, thus disrupting the connection between brain regions (Christoffel et al., 2011; McEwen, 2000). In contrast, chronic stress appears to enhance spine density and thickness of dendritic arborization in the amygdala and nucleus accumbens (Christoffel et al., 2011), indicating that sustained elevations in cortisol produce differential structural and functional brain changes. It has been proposed that the stress-induced changes in the hippocampus may explain the reductions in hippocampal volume associated with depression, whereas abnormal cognitive function may be attributed to the impairments in hippocampal function (Nestler et al., 2002). However, it remains unclear whether the dysregulation of the HPA axis is the predominant cause of depression or is secondary to alternative influences (Nestler et al., 2002), such as neuroinflammation.

In recent years, the role of inflammation in mood disorders has been recognised as an important contributor to the pathophysiology of disease (Levant, 2011). Researchers have demonstrated that chronic stress releases inflammatory mediators – proinflammatory cytokines (e.g. IL-1β, IL-6, TNF-α, and interferon-γ) – via upregulation of the immune system (Seematter, Binnert, Martin, & Tappy, 2004; Slavich & Irwin, 2014). Acute exposure to elevated inflammatory cytokines produces adaptive behavioural responses to enhance survival during threatening situations (Felger & Lotrich, 2013). In contrast, chronic exposure
to proinflammatory cytokines can induce profound mood and behavioural changes owing to the wide array of neuropsychiatric symptoms produced as a consequence of elevated levels (Felger & Lotrich, 2013; Levant, 2011; Slavich & Irwin, 2014; Smith, 1997). For instance, stress-induced activation of the immune system is associated with depressive symptoms, including low mood, anhedonia, fatigue, weight loss, cognitive dysfunction, psychomotor retardation and social-behavioural withdrawal (Miller et al., 2009; Setiawan et al., 2015; Slavich & Irwin, 2014). The mechanisms behind the cytokine mood and behavioural effects may be explained by the neural activation of inflammatory signaling pathways, which in turn, leads to changes in monoamine, glutamate and neuropeptide systems and reductions in BDNF (Felger & Lotrich, 2013). Observational studies have provided abundant evidence demonstrating elevated cytokines in patients with a major depressive disorder (Horrobin & Bennett, 1999; Howren et al., 2009). In fact, the inflammatory response to psychological stressors appears to be enhanced in patients with depression leading to augmented levels of IL-6 and activation of nuclear factor-kappaB (NFκB) – a proinflammatory-signaling pathway (Levant, 2011). In addition, experimental research provides substantial evidence to support the role of inflammation in the pathophysiology of mood disorders (Horrobin & Bennett, 1999). Studies have demonstrated that administration of cytokines can induce depressive and manic symptoms – fatigue, loss of interest, disturbed sleep and appetite, cognitive dysfunction and periods of euphoria – leading to a diagnosis of major depression or even bipolar disorder (Smith, 1997). However, researchers have yet to determine the causal relationship between inflammation and mood disorders, though it is believed the association will be complex (Kaplan, Rucklidge, Romjin, & McLeod, 2015). In summary, these findings provide compelling evidence for the role of neuroinflammation in mood disorders (Setiawan et al., 2015); however, future research is necessary to understand the detailed mechanisms involved. In addition to genetic and environmental influences, researchers have identified a number of social, psychological, and biological factors that enhance the risk for mood disorders (Leung & Kaplan, 2009).

1.8.3. Social, psychological and biological factors.

The social and psychological risk factors are diverse and have been studied in much depth. Examples of social influences include marital difficulties, family violence, substance abuse and stressful life events, whereas, psychological influences include a history of mood
disorders or psychiatric illness and mood symptoms during pregnancy (risk for postpartum depression) (Leung & Kaplan, 2009). Conversely, biological factors associated with the etiology of depression, perinatal depression and bipolar disorder include neurotransmitter function, hormonal disturbances, and the most recent influence, nutrient deficiencies (Leung & Kaplan, 2009). The influence of neurotransmitters in mood disorders follows.

1.8.3.1. Neurotransmitters.

Based on the proposed catecholamine or biogenic amine hypothesis for mood disorders (Barchas & Altemus, 1999; Kaplan et al., 2015), much research has been conducted to ascertain the role of neurotransmitters in the pathophysiology of depression and bipolar disorder. The basic premise is that abnormal mood states are caused by a chemical imbalance in catecholamine neurotransmitters (e.g. serotonin, dopamine, norepinephrine), though accumulating evidence has lead researchers to believe that the theory is too simple and vague as it fails to capture the complex nature of mood disorders (Kaplan et al., 2015; Leung & Kaplan, 2009). However, the general consensus is that neurotransmitters play a role when embedded in the current biological mechanisms of disease (Kaplan et al., 2015). The main neurotransmitters proposed to be involved in the pathophysiology of depression and bipolar disorder are serotonin and dopamine, with evidence demonstrating altered levels in brain regions that regulate mood and behaviour (Leung & Kaplan, 2009; Levant, 2011; Manji et al., 2003) (refer to section 1.6.2. for a detailed version). For instance, researchers have reported decreased levels of serotonin in the brain stem of postmortem depressives and suicide victims in addition to increased serotonin receptors in the frontal cortex (Levant, 2011). However, it is unknown whether these alterations in serotonin levels were due to the disorder or the treatment received prior to death. Nonetheless, the evidence to support a role for serotonin in bipolar disorder has been inconsistent and highly variable, with reports of increased and decreased levels. Further, most studies have reported no differences in serotonin levels between manic and depressed patients, which challenges the catecholamine theory of mood disorders (Manji et al., 2003). In order to ascertain the precise involvement of serotonin in mood disorders, researchers have examined the mood effects of tryptophan depletion – serotonin precursor – in healthy individuals and patients with depression (Manji et al., 2003). In theory, depletion should induce or enhance depressive symptoms (Rintamaki, & Partonen, 2011);
however, this depletion is only observed in patients with a predisposition for depression but not in healthy controls (Barchas & Altemus, 1999). Overall, the results of these studies have been inconsistent (Manji et al., 2003). It has therefore been proposed that there may be a subgroup of mood disorders that have abnormal neurotransmission systems, thus altered neurotransmitter levels will be apparent in this subgroup of patients (Barchas & Altemus, 1999). Furthermore, antidepressant medications that are intended to increase brain serotonin levels (i.e. selective serotonin reuptake inhibitors (SSRIs)) have had limited efficacy in alleviating symptoms of depression (Leung & Kaplan, 2009), thus demonstrating there are other complex neurobiological mechanisms involved in the pathogenesis of mood disorders (Manji et al., 2003). In light of this, researchers have focused on the dopaminergic system, as there is evidence to demonstrate a role for dopamine in depression and bipolar disorder (Harvard Medical School, 2009; Leung & Kaplan, 2009). Animal studies have reported that reduced dopaminergic function in the mesolimbic system leads to anhedonic behaviours, a defining characteristic of depression (Levant, 2011; Manji et al., 2003). However, the strongest evidence implicating dopamine in depression is the notable reductions in homovanillic acid – a dopamine metabolite – in the cerebrospinal fluid of depressed patients and suicide victims (Levant, 2011; Manji et al., 2003). Depression is also prevalent in Parkinson’s disease; a neurodegenerative condition that involves decreased dopaminergic neurons in the nigrostriatal region (Levant, 2011). It has been reported that manic episodes can alleviate symptoms of Parkinson’s disease (Manji et al., 2003), which may be explained by the finding that medications that increase brain dopamine (i.e. levodopa) can produce manic symptoms in some patients, though is typically observed in those with a history of bipolar disorder (Barchas & Altemus, 1999). In summary, neurotransmitters are an important part of the mechanisms involved in the etiology of mood disorders, but do not offer a full explanation of etiology as was once thought. The role of hormones must also be factored in.

1.8.3.2. Hormones.

It has been proposed that hormones are a key component involved in the pathophysiology of maternal depression (Leung & Kaplan, 2009). The sudden and dramatic changes in hormone levels – in particular estrogen and progesterone – following delivery has been found to enhance the vulnerability to depressive mood states (Hendrick, Altshuler,
& Suri, 1998). The postpartum period is therefore a crucial time for women who have a genetic or environmental predisposition for depression. Research has shown that estrogen and progesterone are produced by the placenta and rise steadily during pregnancy (100-fold and 20-fold increase, respectively) reaching high levels at full term. However, because the placenta is removed at delivery there is a sharp reduction in hormone levels (Hendrick et al., 1998; Leung & Kaplan, 2009). It has been documented that estradiol – a form of estrogen – augments neurotransmitter function through increased brain serotonin, meaning the rapid decrease after delivery may influence the development of postpartum depression. However, despite there being minimal evidence to support an association between changes in estrogen levels and the incidence of maternal depression (Leung & Kaplan, 2009), two studies revealed that estrogen supplementation reduced depressive symptoms during the postpartum period (Hendrick et al., 1998). However, the findings must be interpreted with caution based on the very small sample and the adjunctive treatment approach employed. Conversely, a prospective study was conducted to determine the psychological, environmental, and hormonal variables involved in postpartum mood disorders. Estradiol was the only variable associated with a diagnosis of postpartum depression (O’Hara, Schlechte, Lewis, and Verner, 1990), which suggests that changes in hormones may increase the risk for those who are already vulnerable to the disorder. While the abrupt reductions in progesterone following delivery are postulated to contribute to postpartum mood changes, the evidence has demonstrated a weak association at best (Zonana & Gorman, 2005). Several other hormones have been implicated in postpartum depression – prolactin, cortisol, oxytocin, thyroid, and vasopressin; – however, a detailed review is beyond the scope of the current study and can be found elsewhere (Zonana & Gorman, 2005). Based on the available evidence, the role of individual hormones remains uncertain, although in combination, they may induce depressive mood changes in vulnerable women. Nutrition, another important variable in the etiology of mood disorders must not be overlooked.

1.8.3.3. Nutrition.

In recent decades, evidence has accumulated that demonstrates a relationship between nutrition and mental illness (Sarris et al., 2015). A proposed mechanism that might explain the role of nutrition in mood disorders relates to the nutritional requirements of the brain (Sarris et al., 2015). Biochemical pathways are dependent on nutrients – amino acids,
fats, vitamins and minerals or trace elements – for brain structure and function. Thus, nutrients provide the foundation for a vast range of neurobiological processes that are involved in the pathophysiology of mood disorders (i.e. neurotransmission, immune and endocrine systems)(Leung & Kaplan, 2009; Sarris et al., 2015). There is credible evidence to support an association between specific nutrients (i.e. folate, vitamin B12, calcium, iron, selenium, zinc, and polyunsaturated fatty acids) and mood disorders (Leung & Kaplan, 2009). A review of correlational and intervention studies reported therapeutic effects of many vitamins (particularly B vitamins and vitamins C, D, and E) and minerals (calcium, chromium, iron, magnesium, zinc, and selenium) on mood states (Kaplan et al., 2007).

1.8.3.3.1. Example of individual nutrients involved in mood disorders.

The neurobiological mechanisms by which individual nutrients may influence abnormal mood are unclear (Kaplan et al., 2007). Though based on the evidence, researchers propose that S-adenosyl methionine (SAMe) – an endogenous sulfur-containing compound – is an important cosubstrate involved in the methylation of neurotransmitters (i.e. serotonin and dopamine) that influence mood regulation (Sarris et al., 2015; Sarris et al., 2009). Intervention studies have revealed antidepressant effects of SAMe through exogenous administration (Sarris et al., 2009). Zinc is an essential trace element involved in immune function, cytokine modulation and hippocampal neurogenesis through induced expression of BDNF (Nowak, Szewczyk, & Pilc, 2005; Rink & Kirchner, 2000; Sarris et al., 2009). A zinc deficiency has been associated with depressive disorders, though clinical trials have demonstrated an improvement in depressed mood following zinc supplementation (Levenson, 2006; Nowak et al., 2005). Research has documented that vitamin B9 (folate) is important for the biosynthesis of serotonin, dopamine, and norepinephrine and is involved as a cofactor in the methionine-homocysteine cycle (Miller, 2003). Thus, a folate deficiency would inhibit the production of these neurotransmitters leading to impaired neural function and an increased risk of mood disorders (Leung & Kaplan, 2009). Low folate levels have been reported in depressed patients (Sarris et al., 2015); however, supplementation with folate appears to reduce depressive symptoms and improve cognitive function in those with a deficiency and in poor responders to antidepressant medications (Fava & Mischoulon, 2009). Furthermore, vitamin D enhances the production of neurotransmitters associated with the pathophysiology of depression, and influences the expression of various
neurotrophins that are implicated in the survival and differentiation of neurons (McCann & Ames, 2007). There is strong evidence to support an association between low vitamin D levels and an increased risk for depression, with vitamin D therapy reducing symptoms of depression in populations (Anglin, Samaan, Walter, & McDonald, 2013; Howland, 2011). An extensive review of the roles of individual nutrients in mood can be found elsewhere (Kaplan et al., 2007; Sarris et al., 2009). Nutritional benefits of omega-3 fatty acids are examined separately in the following section.

### 1.9. Involvement of omega-3 fatty acids in the etiology of mood disorders.

There is abundant evidence to support the role of omega-3 fatty acids in the pathophysiology of mood disorders (see the proposed mechanisms for the therapeutic effects of omega-3 fatty acids discussed in great depth in previous sections (1.6.)). However, the association between low dietary intakes of omega-3 fatty acids and depression is controversial (Astorg, 2007; Hakkarainen et al., 2004; Grosso et al., 2014; Su et al., 2003). For instance, a study reported significantly lower concentrations of both EPA and DHA in the cell tissue of patients with depression (Su et al., 2003), which is supported by the finding that low dietary intakes of omega-3 fatty acid were associated with a depression diagnosis (Leung & Kaplan, 2009). In a study on postpartum depression, higher seafood intakes and higher DHA content in breast milk were predictive of lower depression prevalence, though the content of EPA in the mother’s milk was unrelated to mood (Hibbeln, 2002). This is an unexpected finding considering the evidence supporting the influential a role of EPA on mood and behaviour (Kidd, 2007). In contrast, a population-based study of males aged 50-69 years in Finland reported there was no association between dietary omega-3 fatty acid intakes and major depression (Hakkarainen et al., 2004). This finding appears consistent with two minor population-based surveys carried out in Australia and Greece (Grosso et al., 2014), which is further supported by a cross-sectional study revealing high intakes of both omega-3 and omega-6 fatty acids in those who reported depressed mood and anxiety (Hakkarainen et al., 2004). While the evidence to support a relationship between omega-3 fatty acids and mood disorders is controversial, an association remains biologically plausible based on the several neurobiological mechanisms proposed (Leung & Kaplan, 2009). As a consequence, researchers have postulated that the preventative role of dietary omega-3
fatty acids may be influenced by lifestyle factors, such as diet quality (Grosso et al., 2014; O’Neal et al., 2013).

**1.9.1. Diet quality.**

Many epidemiological studies have reported that healthy dietary patterns can be protective against the incidence of depression and suicide (O’Neal et al., 2013; Sarris et al., 2015). Indeed, results from the PREDIMED study revealed a non-significant reduction in the risk for depression in individuals assigned to the intervention group - a Mediterranean diet supplemented with nuts (Sanchez-Villegas et al., 2013). While a ‘healthy’ dietary pattern – vegetables, fruit, meat, fish, and whole grains – is associated with a reduced likelihood of a clinical depressive disorder, an ‘unhealthy’ dietary pattern comprising processed and nutrient depleted foods – fried foods, refined grains, sugary products, and beer – appears to increase the likelihood of depression and psychiatric illness (Jacka et al., 2010). An unhealthy dietary pattern is a defining characteristic of the Western diet, which has lead to a dramatic decline in the dietary intakes of omega-3 fatty acids and a sharp increase in omega-6 fatty acids (Logan, 2004). Evidence has demonstrated that a ‘pro-omega-6’ diet may enhance the release of proinflammatory eicosanoids, reduce levels of BDNF, and decrease neural membrane fluidity and neurotransmission (Sarris et al., 2009). Thus, a Western diet may contribute to the pathophysiology of mood disorders. The research to support an association between diet quality and mood disorders is extensive, thus detailed reviews can be found elsewhere (O’Neal et al., 2013; Sarris et al., 2015). However, a recent systematic review of diet quality, dietary patterns and depression found limited evidence to support a link between healthy ‘traditional’ diets and mood disorders (Quirk et al., 2013), indicating that a combination of lifestyle factors are involved in the pathophysiology of depression and bipolar disorder. As demonstrated, mood disorders are complex and multifactorial in nature, thus multiple factors (i.e. genetics, environmental, social, psychological, and biological) are likely to interact, triggering the pathophysiological changes necessary for the development of a mental illness. Treatment for mental illness is varied.
1.10. Current treatments for mood disorders.

1.10.1. Antidepressant pharmacological treatments – depression.

Mood disorders are rising in prevalence and pose a substantial health and financial burden worldwide (Leung & Kaplan, 2009). However, the current ‘gold standard’ medical treatments – antidepressants and antipsychotics – for these complex disorders are limited in their clinical effectiveness and have many side effects (Baghai et al., 2011; Kirsch et al., 2008; Su et al., 2009). Antidepressants are the most frequently prescribed medications worldwide and are believed to increase and prevent the reuptake of brain monoamines at the receptor level to exert their therapeutic effects (Ioannidis, 2008, Moncrieff & Cohen, 2006; Yildiz, Gonul, Tamam, 2002). Research has demonstrated that monoamine oxidase inhibitors (MAOIs) enhance the levels of serotonin, norepinephrine, and dopamine at the synapse by inhibiting the enzyme – monoamine oxidase – responsible for the metabolism of monoamine neurotransmitters (Yildiz et al., 2002). Further, tricyclic antidepressants (TCAs) act on the monoamines and catecholamines and have the potential to cause cardiac arrhythmias and seizures at high doses; though most TCAs have strong sedative effects and cause cognitive and behavioural impairments (Moncrieff & Cohen, 2006; Yildiz et al., 2002).

In contrast, the selective serotonin reuptake inhibitors (SSRIs) focus on inhibiting the reuptake of serotonin at the synapse leading to a sudden increase in brain serotonin (Yildiz et al., 2002). The reported side effects of SSRIs include, but are not limited to: anxiety, sleep disturbances, sexual dysfunction, and gastrointestinal discomfort (Yildiz et al., 2002), all of which are believed to contribute to the poor compliance of antidepressant medications (Logan, 2003). Though compared with TCAs, the adverse effect profiles of SSRIs are more favorable, and according to Hirst and Moutier (2010), have become the “mainstay of treatment for moderate to severe” major depression. More than 40 antidepressants are available on the global market (Su, 2009), with 39 involving serotonin transport (Yildiz et al., 2002). These proposed mechanisms are consistent with the chemical imbalance theory of depression; however, there is little evidence to support an association between abnormal monoamine levels and depressive disorders (Moncrieff & Cohen, 2006)(refer to section 1.7.1.2). Research conducted independent of pharmaceutical companies has provided contradictory evidence for reduced serotonin receptor binding in depressed patients (Moncrieff & Cohen, 2006) with some studies reporting no difference between patients who are medication-free and healthy subjects within the control group (Meyer et al., 2004;
Parsey et al., 2006). Parsey et al (2006) also revealed that antidepressant naïve patients had higher serotonin binding potential than control subjects indicating that the pathophysiology of depression extends far beyond the brain monoamine theory, which is deeply ingrained in our psyche.

According to Ioannidis (2008), a myriad of randomised controlled trials have been carried out to ascertain the therapeutic effects of antidepressant medications for major depressive disorders. Several hundred clinical trials have reported statistically significant benefits of antidepressant medications. However, meta-analyses have revealed only marginal therapeutic effects over placebo treatment (Kirsch et al., 2008; Pigott, Leventhal, Alter, & Boren, 2010). The general consensus among researchers is that the perceived efficacy of antidepressants has been inflated due to the selective reporting of clinical trials (Pigott et al., 2010; Turner, Matthews, Lindardatos, Tell, & Rosenthal, 2008). Consequent to the long-held concerns of public-minded researchers (Chan et al., 2004; Dickersin, 1997; Dickersin, 1990; Simes, 1986), the U.S Food and Drug Administration (FDA) has developed regimented protocols requiring registration and data archiving of all drug application trials intended to be used in support of attaining market approval (Pigott et al., 2010; Turner et al., 2008). The exact methods and means of analysis must be reported prior to the study to ensure the validity of the evidence from the clinical trials. This is done to prevent HARKing – “hypothesizing after the results are known” – (Pigott et al., 2010) and the selective reporting of results that are viewed as desirable to trial outcomes (Turner et al., 2008). Since the trial data are open to public review, several meta-analyses have been conducted to examine the efficacy of antidepressant medications using data from clinical trials submitted to the FDA (Pigott et al., 2010). A basic synopsis will be provided to demonstrate the selective publication of antidepressant trials and the implications for apparent efficacy (Ioannidis, 2008; Turner et al., 2008). Turner and colleagues (2008) performed a meta-analysis of 74 FDA-registered drug trials involving 12 antidepressant medications. The review found that 31% of these trials were not published in the literature. According to the FDA, a total of 38 studies had found a significant benefit for antidepressants and were viewed as positive trials, although one study remained unpublished. This meant that 36 studies did not find a significant benefit over placebo and were regarded as negative trials. Only three of these studies were published as having negative results. The remaining studies were either published in a way that implied a positive result (11 studies), or were never published in
research journals (12 studies). Prior to this investigation, it was believed that the vast majority of published antidepressant trials were positive; however, based on the FDA data only 51% of drug trials had found evidence of antidepressant efficacy (Turner et al., 2008). Further, the therapeutic benefits conveyed in the literature were inflated by 32% on average, indicating the perceived benefits are much higher than what the data suggest (Ioannidis, 2008). Provided the unpublished data is considered, the benefits of antidepressant medications fall short of the criteria for clinical significance (Kirsch et al., 2008). These findings are consistent throughout the literature and provide strong evidence for the selective publication of antidepressants (Pigott et al., 2010; Kirsch & Antonuccio, 2002; Ioannidis, 2008; Kirsch et al., 2008).

It has been proposed that antidepressant efficacy is influenced by the baseline severity of depression (Kirsch et al., 2008). Using relevant FDA-submitted data from clinical trials involving four new generation antidepressants, Kirsch and colleagues (2008) found the efficacy of these antidepressant drugs was marginal even in the most extreme cases of clinical depression. Though researchers concluded that the antidepressants were no more effective in severe major depression, the placebo simply became less effective (Ioannidis, 2008).

The placebo effect can be very powerful (Kirsch, 2010). Research has demonstrated that much of the improvement of antidepressants is due to the placebo effect, which has been shown to account for 50% of the therapeutic response. The remaining drug effect appears to be attributed to both spontaneous improvement and the pharmacological properties of the active medication (Kirsch, 2010). Individual expectations of improvement are central to the placebo effect. Evidence has shown that enhanced expectations of treatment outcomes can improve the effectiveness of the active drug. These expectations are related to the side effects of active medications. Thus patients who break blind during a clinical trial may expect less improvement than those who believe they have been assigned to the active drug. Kirsch (2010) has proposed that the drug-placebo difference may not be an antidepressant drug effect but rather an enhanced placebo effect produced in response to patients who have broken blind to their treatment condition (Kirsch, 2010). Future research is recommended to ascertain whether the modest improvement in severe depression is actually due to the placebo effect and highlights the urgency for effective
treatments with minimal side effects. Treatment for bipolar disorder, though similar in some aspects to treatment for depression, does have some differences.

1.10.2. Antidepressant pharmacological treatments – bipolar disorder.

The evidence submitted to the FDA repository demonstrates very clearly that antidepressant medications are ineffective for the treatment of depression (Ioannidis, 2008; Kirsch et al., 2008). However, much recent attention has focused on the therapeutic effects of antidepressants for bipolar disorder (Van Lieshout & MacQueen, 2010). These assertions are based on the premise that depressive episodes are a main feature of the disorder in that depression is a state that lasts for prolonged periods over the life course of the individual (Van Lieshout & MacQueen, 2010). A recent systematic review and meta-analysis have provided evidence to demonstrate the short-term efficacy of antidepressants in the treatment of bipolar depression (Gijsman, Geddes, Rendell, Nolen, & Goodwin, 2004). Conversely, a more recent meta-analysis showed no tangible benefits of reducing fluctuations between the manic and depressive states of bipolar disorder in either the short-term or long-term (Zhang et al., 2013). Due to the mixed results from numerous clinical trials, more research should be conducted to make generalizable conclusions regarding the use of antidepressant medications to treat bipolar disorder. Mood stabilisers are another treatment for bipolar disorder.

1.10.3. Mood stabilisers for bipolar disorder.

Much evidence has accumulated to support the use of mood stabilisers and atypical antipsychotics in the maintenance of bipolar disorder (Smith, Cornelius, Warnock, Bell, & Young, 2007). Based on current research, mood stabilisers such as lithium have emerged as the first line of treatment for this spectrum disorder either as monotherapy or adjunctive therapy with atypical antipsychotics (National Institute of Mental Health, 2012; Young, 2008). Lithium treatment has been shown to be more effective in reducing the relapse of manic episodes but less effective in diminishing the relapses in depression (Beynon, Soares-Weiser, Woolacott, Duffy, & Geddes, 2009; Geddes, Burgess, Hawton, Jamison, & Goodwin, 2004; Smith et al., 2007). However, a recent meta-analysis found that valproate – an anticonvulsant medication – was most effective in reducing incidents of depressive relapses (Beynon et al., 2009), leading researchers to conclude that traditional mood stabilisers may
be effective pharmacological treatments in the maintenance of bipolar disorder (Beynon et al., 2009; Young, 2008). While these medications have demonstrated treatment efficacy, research has documented that valproate has the potential to cause suicidal thoughts and behaviours. The FDA has since issued a warning regarding its clinical use and has recommended that patients be monitored carefully for abnormal mood and behavioural changes over the treatment duration (National Institute of Mental Health, 2012). Evidence has shown that lithium treatment can yield many adverse effects, which include, but are not limited to, restlessness, dry mouth, joint or muscle pain, acne, and unusual discomfort to cold temperatures (National Institute of Mental Health, 2012). These adverse effect characteristics challenge long-term compliance (Ketter, 2007) and thus illustrate the importance of finding effective treatments with both minimal and benign side effects for the maintenance of bipolar disorder. There is also evidence to support the use of atypical antipsychotics as both an alternative and adjunctive treatment to traditional mood stabilisers (Berk & Dodd, 2005). Based on current research, atypical antipsychotics are most effective in the prevention of manic episodes by reducing acute agitation and aggressive behaviours. However, there is some evidence for treatment efficacy in bipolar depression (Berk & Dodd, 2005). It has been reported that adjunctive therapy with atypical antipsychotics and valproate reduces the severity of manic episodes (Vacheron-Trystram, Braitman, Cheref, & Auffray, 2004). Yet, a recent systematic review and meta-analysis concluded that the effectiveness of adjunctive therapy is no more beneficial than monotherapy (Beynon et al., 2009). Further, olanzapine and quetiapine have both demonstrated significant effects in the maintenance of bipolar disorder (Berk & Dodd, 2005) with reduced manic relapses observed for olanzapine and lithium (Beynon et al., 2009; Smith et al., 2007).

Based on the evidence in the literature, mood stabilisers have distinctive mechanisms by which they produce different therapeutic profiles, meaning that certain medications are more efficacious than others (Smith et al., 2007). Due to the uncertainties surrounding the mechanisms of atypical antipsychotics, future research is necessary. While the side effect characteristics of these new generation antipsychotics are viewed as favorable compared to the conventional antipsychotic medications (i.e. typical)(Vacheron-Trystram et al., 2004), there is serious potential for major weight gain and changes in metabolism, leading to diabetes and high cholesterol (National Institute of Mental Health, 2012). Other side effects
include drowsiness, blurred vision, rapid heartbeat, and menstrual disturbances and tardive dyskinesia following long-term use (National Institute of Mental Health, 2012).

In conclusion, the available evidence demonstrates very clearly that pharmacological interventions are most effective in the prevention of manic relapses but not relapses into depressive states and have adverse effect profiles. Viewed together, these findings raise questions as to whether non-pharmacological interventions are more effective in the treatment of major depression and bipolar disorder than medications.

1.10.4. Non-pharmacological treatments for mood disorders.

There are several non-pharmacological treatments available for mood disorders; the most predominant are psychological interventions such as cognitive behavioural therapy, and interpersonal therapy (Sarris et al., 2009). Current evidence supports individual or group psychotherapy as a treatment for mild to moderate depression (Hirst & Moutier, 2010) and may be effective in the treatment of bipolar disorder (Driessen & Hollon, 2010). Cognitive behavioural therapy appears effective in the short-term treatment of depression and may complement treatment outcomes of pharmacotherapy in bipolar disorder (Driessen & Hollon, 2010). Nevertheless, a recent systematic review concluded that cognitive behavioural therapy produced only modest improvements in bipolar depression (Gregory, 2010). A research study found that interpersonal therapy administered in combination with antidepressant medication enhanced therapeutic outcomes in severe depressive disorders, but not in milder depressions (Thase et al., 1997). A randomised controlled trial provided evidence to support this finding and concluded the clinical advantages of combined therapy (psychodynamic supportive psychotherapy with antidepressants) in mild to moderate depressive disorders are unclear (de Jonghe et al., 2004). This assertion was made even though the combined benefits were evident to the patients (de Jonghe et al., 2004), which demonstrates that a therapeutic effect may be of clinical relevance but fall short of accepted criteria for statistical significance. However, the evidence on the efficacy of psychotherapy as either monotherapy or adjunctive therapy appears contradictory and is beyond the scope of the current review. These aforementioned findings simply provide the rationale for assessing the efficacy of dietary fish oil supplements as a novel treatment for mood disorders. A review of clinical trials on omega-3 fatty acids as a treatment option for major depression, perinatal depression and bipolar
disorder can be found below in section 1.12. Quantifying the number of people within the general population that use fish oil supplements is important to researchers and product manufacturers. Toward those objectives, surveys in western countries have been conducted.

1.11. Prevalence of fish oil supplements used in the general population.

Dietary supplement use has increased dramatically over the last decade (Bailey, Gahche, Miller, Thomas, & Dwyer, 2013; Bailey et al., 2010). The National Health and Nutrition Examination Survey (NHANES) reported that 49% of adults in America use dietary supplements on a daily basis, 33% of which reported that they use micronutrient supplements containing multi-vitamins and multi-minerals. The data from this survey revealed that the prevalence of dietary supplementation was higher among females (53%) than males (44%) (Bailey et al., 2010).

While micronutrient products are the most common supplements used to improve health and well being, there is evidence to demonstrate that fish oil supplements are the third most frequently reported dietary supplement used, following micronutrient and calcium formulations (Bailey et al., 2013). A recent National Health Interview Survey revealed that 7.8% of adults in America use fish oil supplements, which increased from 4.8% in 2007 (Clarke et al., 2015). This increase may be reflected by the accumulation of evidence pertaining to the human health benefits of fish oil.

In order to ascertain the motivations behind the personal use of dietary supplements, Bailey and colleagues examined the data provided by the 2007-2010 NHANES study, a representative population-based survey (Bailey et al., 2013). The analysis revealed that the main reasons for consuming dietary supplements were to ‘improve’ (45%) or ‘maintain’ (33%) general health and well being (Bailey et al., 2013), though the reported motivations were influenced by the health concerns of the individual. Older adults (≥60 years) were more inclined to describe heart, joint, and bone health as their reason for using over-the-counter supplements than younger individuals, and only 23% of adults reported using supplements based on their health care providers recommendations (Bailey et al., 2013).

The general consensus among researchers in this area of study is that consumers of dietary supplements tend to adopt a healthier lifestyle through engagement in regular
physical activity, eating a nutrient rich diet, and attending physician visits on a routine basis (Dickinson et al., 2012; Radimer et al., 2004). However, despite receiving many essential nutrients from food, studies have shown that nutrient intakes of supplement users and non-users often fall short of recommended guidelines for certain vitamins and minerals (Dickinson et al., 2012; Murphy, White, Park, & Sharma, 2007; Sebastian, Cleveland, Goldman, & Mosfegh, 2007). Suboptimal micronutrient levels may slow normal metabolic processes in the body, based on the premise that vitamins and minerals are involved in many coenzyme reactions and play a crucial role in neurotransmitter synthesis (Kaplan & Shannon, 2007). In cases where the nutrient content of the diet is great, but the intake is below recommended guidelines, there may be an inborn error of metabolism whereby the body cannot digest and absorb components of food (Thomas, 2004). The health implications of these inherited disorders are vast and in extreme cases may lead to brain and organ damage, episodes of a metabolic crisis, and even death (Thomas, 2004). However, supplementation with higher levels of specific micronutrients has been reported to correct the metabolic imbalance and restore normal body function (Kaplan & Shannon, 2007; Thomas, 2004).

Research into the health consequences associated with suboptimal nutrition and metabolic syndromes demonstrates the importance of dietary supplements, in particular, fish oil supplements for individuals who do not consume fish or seafood on a regular basis. This is because fish oil contains an abundance of essential fatty acids that play important roles in brain development and influence brain structure and function (refer to section 1.2)(Coletta et al., 2010; Innis, 2008; Surette, 2008; Valentine & Valentine, 2004). Although the body is able to synthesis omega-3 fatty acids from plant sources, the conversion of ALA to EPA and then DHA is very limited (Plourde & Cunnane, 2007; Swanson et al., 2012). Fish oil supplements are the most obvious alternative to obtaining high levels of omega-3 fatty acids for normal brain function. The importance of fish oil supplements is further enhanced by the fact that less than half (45%) of adults in New Zealand are regular consumers of fish (≥1 per week)(Seafood Industry Council, 2007).

The New Zealand Adult Nutrition Survey was therefore conducted to ascertain the prevalence of fish oil supplementation in the population (University of Otago and Ministry of Health, 2011). The survey defined a regular user as an individual who ‘consumed at least one supplement: daily, more than once per week or once per week’, whereas, occasional
use was defined as ‘any consumption less than once per week’. The reported findings indicated that 47.6% of the adult population had used a dietary supplement in the last year, 30.7% of which were considered regular users and 16.9% were occasional users of dietary supplements. Females were more inclined to report using supplements than males. In contrast to the low prevalence of fish oil supplementation in America, the New Zealand survey found that supplements containing ‘oils’ (fish oils, omega-3 products, flax/linseed oil and evening primrose oil) were the most reported dietary supplement (16.4%), closely followed by multi-vitamin and multi-mineral formulations (14.8%) (University of Otago and Ministry of Health, 2011). The high prevalence of fish oil supplementation is an unexpected finding based on the higher consumption of fresh water fish in New Zealand than other countries (Shaw, 2012)(see section 1.3.5). However, the general trend was comparable, such that the use of fish oil supplements increased with age and was higher among Pacific than Maori populations (University of Otago and Ministry of Health, 2011). Although the survey was not specific to fish oil, the data provides an indication of the prevalence of fish oil supplements in New Zealand and highlights the need for independent legislation. One component of the proposed Natural Health and Supplementary Products Bill in New Zealand would require manufacturers to conduct independent clinical trials of their products if they wish to claim therapeutic benefits associated with their use. Some independent clinical trials have been conducted to examine the efficacy of fish oil supplements for the treatment of mood disorders.


Decades of research on the neurobiological mechanisms of depression and bipolar disorder has enhanced our understanding of these complex and multifaceted disorders, yet these discoveries have not translated to improvements in treatment outcomes (Holtzheimer & Nemeroff, 2006; Weir, 2012). Current pharmacological treatments have demonstrated modest improvements in mood symptoms but cause a wide array of serious side effects that impede recovery and challenge compliance. It is of primary importance to both researchers and practitioners to have safe and well-tolerated treatments that have been proven effective in mood disorders.
A review of research on the efficacy of fish oil supplementation in adult populations indicates that augmentation leads to improvements in symptoms of major depression (Gertsik, Poland, Bresee, & Rapaport, 2012; Su et al., 2003) including postpartum depression (Su et al., 2008), and bipolar disorder (Frangou, Lewis, & McCrone, 2006; Stoll, Locke, Marangell, & Severus, 1999). Therapeutic effects resulting from fish oil supplementation have also been found in a multitude of psychiatric disorders such as schizophrenia, borderline personality disorder and attention-deficit/hyperactivity disorder (Peet & Stokes, 2005); however, these disorders are beyond the scope of the current review.

A systematic search of the scientific literature was performed to identify the research conducted into the effects of fish oil supplementation in patients with a mood disorder as mentioned above. The search revealed a large number of randomised controlled trials, systematic reviews, and supporting meta-analyses all of which are discussed in the following sections to provide the best demonstration of treatment effectiveness. At first glance the findings were positive and indicated a benefit of fish oil supplements in mood disorders but many studies found a non-significant effect of supplementation over placebo. The large heterogeneity in methodology was apparent throughout the literature, thus proving it difficult to reach a definitive conclusion regarding the efficacy of fish oil supplements in the treatment and/or management of mood disorders. The results of both positive and negative trials are outlined in the sections below. The review begins with major depressive disorder.

1.12.1. Major depressive disorder.
A major depressive disorder is a chronic or recurrent psychiatric mood disorder characterised by periods of depressed mood, loss of interest or pleasure and low energy, sometimes accompanied by feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration (WHO, 2015a). The social, physical and emotional impacts of depression can be devastating and have the potential to lead to suicidal thoughts and behaviours, especially when untreated. However, as discussed, current pharmacological interventions have not proven effective in the treatment of depression and are accompanied by adverse effects. Thus, supplementation with omega-3 fatty acids may represent a novel therapeutic approach based on the clinical trials that found a significant reduction in symptoms of major depression. While there is evidence to support the efficacy of omega-3 fatty acids in depression the findings are inconsistent. A search of the relevant
scientific literature revealed that 14 randomised controlled trials have been conducted to examine the independent and/or combined effects of omega-3 fatty acids either as monotherapy or adjunctive therapy with conventional medications. Of these clinical trials, five found a significant reduction in symptoms, four found no difference between active and placebo treatment, one found a dose-effect relationship (counted as three separate studies in statistical analysis) and the remaining trial found an improvement from one fatty acid but not the other (counted as two separate studies in statistical analysis).

Studies that reported a positive effect used the Hamilton Depression Rating Scale (HAM-D) as the primary outcome measure, although one of these studies measured the improvements in depressive symptoms with the Geriatric Depression Scale (GDS) due to their population of interest. The HAM-D is a clinician administered depression assessment scale that is designed to ascertain the severity of depression in patients diagnosed with a depressive disorder (Hamilton, 1960). The multiple item questionnaire probes responses to depressed mood, feelings of guilt, suicide, insomnia, agitation, psychomotor retardation, anxiety, weight loss and somatic symptoms (Hamilton, 1960). High scores are indicative of severe depression, thus treatment efficacy was based on the change in these scores from baseline to study endpoint, meaning omega-3 fatty acid supplements have been found to reduce the above-mentioned symptoms, which is seen as a positive outcome.

1.12.1.1. Positive clinical trials in major depression.

Since antidepressants are viewed as the ‘gold standard’ treatment for major depression, four out of the five studies that found a benefit of omega-3 supplements had examined the individual or combined effects in conjunction with maintenance medications, which means that a single trial was conducted with these essential nutrients as monotherapy. Rondanelli et al., (2010) investigated the therapeutic effects of omega-3 fatty acids in an elderly population that met diagnostic criteria for major depression or dysthymia to ascertain whether supplementation reduced depressive symptoms and improved quality of life. Italian women aged between 66 and 95 years received 1.67 g of EPA and 0.83 g of DHA (2.5 g/day) daily for a duration of eight-weeks, a formulation that was manufactured by a renowned company in Italy. In contrast, the control group received an identical dose of placebo containing paraffin oil. To determine treatment effectiveness, the researchers administered the GDS at baseline and following the intervention period and compared the
adjusted post-treatment means in the supplementation and placebo group. The effects of the intervention on health related quality of life was a secondary outcome measure and was assessed using the Short-Form 36-Item Health Survey (SF-36). The statistical analyses revealed a significant reduction in scores on the GDS in the intervention group (p < 0.017), which translated to a substantial difference in scores between the intervention and placebo groups (p < 0.017). Similar findings were reported for the SF-36 physical function (p < 0.001) and mental function scores (p < 0.001). An unexpected finding was that no improvements were observed in the placebo group, which may raise doubts about the study being a double blind clinical trial. Furthermore, compliance after treatment cessation was confirmed by the enhanced incorporation of both EPA and DHA in phospholipid membranes of red blood cells, indicating an important role in mood disorders. Overall, fatty acid augmentation was well tolerated and was associated with few side effects, such as gastrointestinal disturbances. These findings demonstrate that supplementation with small doses of omega-3 fatty acids leads to significant reductions in symptoms of depression and dysthymia in elderly populations and enhanced quality of life.

Of the four studies that found a positive effect of adjunctive therapy, only two studies explored the combined benefit of EPA and DHA, despite the neurobiological mechanisms demonstrating involvement of both omega-3 fatty acids in major depression. There are distinct methodological parallels between the two clinical trials (Su et al., 2003; Gertsik et al., 2012), which include the clinical disorder diagnosed (major depressive disorder), the intervention duration, primary outcome measure (HAM-D), placebo received (olive oil), and the reported treatment outcome (efficacious). However, the most distinctive feature was the dosage administered over the intervention period.

Su et al. (2003) compared the effectiveness of 4.4 g of EPA and 2.2 g of DHA (6.6 g/day) with matching placebo in conjunction with the maintenance medications prescribed prior to the study (fluoxetine - antidepressant) for eight-weeks. The omega-3 fatty acid capsules were produced in China Chemical and Pharmaceutical Company in Taiwan. Statistical analyses revealed significant reductions in HAM-D scores in the intervention group (-13.6 points) compared to the placebo group (-6.4 points), though improvements were observed in both groups. These differences were significant from the fourth week of the clinical trial (p < 0.001). In contrast to the study conducted by Rondanelli et al. (2010) that found enhanced incorporation of both EPA and DHA in erythrocyte membranes, only
the composition of DHA in red blood cell membranes increased from pre-treatment to post-treatment \( (p = 0.03) \). This finding is consistent with evidence that demonstrates an important role for DHA in membrane structure and function. These preliminary findings provide support for omega-3 fatty acid supplementation in combination with antidepressant medications in the short-term treatment of major depressive disorder.

In contrast to the very high dose that was administered in the aforementioned study, Gertsik et al. (2012) found a significant improvement in HAM-D scores resulting from 1.8 g of EPA and 0.4 g of DHA (2.2 g/day) in conjunction with citalopram antidepressant medication. The statistical analyses revealed that adjunctive therapy was more effective in reducing depressive symptoms than monotherapy, which involved citalopram plus placebo treatment containing 2 g olive oil per day – both the active and placebo capsules were obtained from Nordic Naturals. These differences were evident at week four \( (p = 0.018) \), week six \( (p = 0.007) \) and week eight of treatment \( (p = 0.006) \). While omega-3 supplementation with citalopram demonstrated treatment efficacy for major depression, there was no evidence to suggest that combined therapy accelerated the antidepressant response; however, the researchers postulated that a small difference between groups might not have been detected early in the treatment due to the power of the study.

These results provide evidence to support the remaining studies conducted by Nemets, Stahl and Belmaker (2002), Jazayeri et al., (2008), Peet and Horrobin (2002), and Mozaffari-Khosravi, Yassini-Ardakani, Karamati, and Shariati-Bafghi (2012) that showed a therapeutic effect of omega-3 supplementation in combination with maintenance medications for major depressive disorders. The main focus of the first three aforementioned studies was to investigate the efficacy of EPA but not DHA in conjunction with current antidepressant treatments. Nemets et al. (2002) reported significant reductions in HAM-D scores in the intervention group \(-12.4\) points) resulting from augmentation with 2 g EPA daily for the duration of four-weeks. A slight reduction was found in the placebo group \(-2.3\) points), but was significantly different from the intervention group at week two and remained this way for the treatment duration. These findings demonstrate that a small dose of EPA can produce a benefit for patients receiving concurrent antidepressants – paroxetine, fluoxetine, fluvoxamine, mirtazapine, and citalopram.
Furthermore, to determine whether the combined effect of EPA and fluoxetine leads to enhanced therapeutic outcomes, Jazayeri et al. (2008) allocated patients with a clinical major depression diagnosis to receive one of three treatments daily for eight-weeks, which included either 1 g EPA, or 20 mg fluoxetine, or their combination. Minami Nutrition, Belgium supplied the capsules containing EPA and treatment efficacy was determined by the change in HAM-D scores. A promising finding, based on the well-documented adverse effects of antidepressants, was that both EPA and fluoxetine were comparable in terms of reducing depressive symptoms. Research has consistently demonstrated that supplementation with EPA and/or DHA is well tolerated and may be associated with minor adverse events that are predominantly gastrointestinal in nature (i.e. nausea, diarrhoea, indigestion, constipation, and drowsiness), a notable improvement from current antidepressant medications. Further, the combined effect of EPA plus fluoxetine was superior to their separate therapeutic effects and was significant from the fourth week of treatment. These findings provide support for the efficacy of EPA in major depressive disorders and indicate that adjunctive therapy can enhance the treatment response and improve depressive symptoms. The implication is that antidepressant doses may be reduced with this novel treatment approach and may result in fewer adverse side effects.

There is substantial evidence to demonstrate the therapeutic effects of EPA and/or DHA in major depressive disorders, with doses found to be effective in the range of 1 g to 6.6 g. In order to determine the dose required to produce a pharmacological effect, Peet and Horrobin (2002) compared the treatment outcomes of depressed patients who received EPA at doses of either 1 (N = 23), 2 (N = 24), or 4 g/day (N = 23) for 12 weeks with those in the placebo group. Supplementation was in combination with maintenance antidepressant medications, which remained unchanged over the course of treatment. The primary outcome measures administered at baseline and at regular intervals were the HAM-D, the Montgomery-Asberg Depression Rating Scale (MADRS) and the Beck Depression Inventory (BDI). The statistical analyses revealed enhanced therapeutic outcomes in the group that received 1 g/day compared to the placebo group, these improvements were reported on all three psychometric assessments. In contrast, there was no significant benefit of receiving 2 g or 4 g of EPA daily, though a trend towards a therapeutic effect was observed in patients who were administered the higher dose. The researchers concluded that combination therapy with 1 g/day EPA was effective in ameliorating symptoms of major depression. This
finding provides evidence to support the treatment efficacy of low doses and is likely to be associated with fewer adverse events. However, while there is accumulating evidence to support the effectiveness of EPA in the treatment of major depression, there remains controversy as to whether the benefits of supplementation are attributed to the combination of both EPA and DHA or their separate pharmacological effects. In order to determine which fatty acid is responsible, Mozaffari-Khosravi et al., (2012) randomly assigned patients with mild to moderate depression to receive either 1 g/day of EPA or 1g/day of DHA, or placebo (coconut oil) in combination with maintenance medications for 12 weeks. The evidence demonstrated significant reductions in HAM-D scores from baseline to study endpoint in patients who received EPA. Based on the finding that response to treatment was higher in the EPA group (6 out of 21 patients) than in the DHA and placebo groups, the researchers concluded that EPA was more effective in the treatment of mild to moderate depression. However, further research is needed to support these preliminary findings and to ascertain whether they can be extended to severe depressive disorders.

Overall, there is evidence to support the role of both EPA and DHA in the treatment of major depressive disorders. Adjunctive therapy appears to enhance therapeutic outcomes and leads to a greater reduction in depressive symptoms than monotherapy. Based on the evidence, EPA appears more effective in the treatment of depression and can achieve beneficial effects at low doses. However, fatty acid supplementation may have limited efficacy in the real world due to the comprehensive exclusion criteria that was adopted by most studies. For instance, patients with a current comorbid psychiatric disorder were excluded from the study even though major depression is a complex and multifaceted condition associated with concurrent anxiety disorders. Future research is therefore needed to evaluate the therapeutic effects in patients with comorbid psychiatric disorders. As with most treatments, supplements and/or drugs, there are no panaceas.

1.12.1.2. Negative clinical trials in major depression.

Many intervention studies have provided evidence to demonstrate a therapeutic effect of omega-3 fatty acids in the treatment of major depression. However, an equal number of randomised controlled trials have reported an overall negligible benefit of supplementation for depressive disorders. A systematic search of the scientific literature
revealed that four clinical trials found a non-significant effect, of these; two studies examined the efficacy of EPA and/or DHA as monotherapy. Mischoulon et al. (2009) randomly assigned patients diagnosed with major depression to receive either 1 g/day of EPA or placebo for the duration of eight weeks ($N = 57$). The primary outcome measure was the change in HAM-D scores from baseline to study endpoint. Response to treatment was defined as a 50% or higher decrease in HAM-D scores. Statistical analyses revealed reductions in HAM-D scores in both the EPA (-10.3) and placebo group (-4.2); however, these differences failed to reach clinical significance, yet a trend towards improvement was evident. Plasma levels of EPA increased over the treatment duration and lead to a decrease in the omega-6:omega-3 fatty acid ratio. The researchers postulated that the small sample ($N = 57$) and low completion rates contributed to the non-significant finding, despite evidence of a clinical benefit.

Research has consistently demonstrated enhanced therapeutic an effect of EPA in the treatment of major depression; however, only one clinical trial was identified has having investigated the effectiveness of DHA as monotherapy. In contrast to the primary outcome measures of the aforementioned studies, Marangell et al. (2003) determined response to treatment based on the change in scores on the MADRS from baseline to study completion. Patients diagnosed with a major depressive disorder received either 2 g/day of DHA or placebo treatment for six weeks ($N = 36$). The analyses revealed that supplementation lead to greater reductions in MDRS scores than placebo, although the difference between groups was not statistically significant ($p = 0.23$). This finding provides evidence to support the clinical trial conducted by Mozaffari-Khosravi et al. (2012), which found a positive effect for EPA but not DHA in major depression.

The remaining two clinical trials identified during a search of the relevant literature evaluated the combined effects of both EPA and DHA as adjunctive therapy with maintenance medications (Grenyer et al., 2007; Lesperance et al., 2011). While there are distinctive methodological differences between these studies (i.e. dosage, placebo, trial duration, and psychometrics), the treatment outcomes were identical in that there was a trend towards statistical significance in the supplementation group. Grenyer et al. (2007) investigated the combined effect of 2.2 g of DHA and 0.6 g of EPA per day in patients diagnosed with major depressive disorder ($N = 83$), although it was mentioned that the population of interest was not initially deficient in omega-3 fatty acids. The received dose of
DHA was much higher than that of EPA, which may explain the negative outcome based on the abundance of evidence demonstrating superiority of EPA in the treatment of depression. The reported effect size was 2.73, which indicates that fish oil received in combination with maintenance antidepressant medications and a psychological intervention may have created a ceiling effect, whereby there was no additional variance for the fish oil group to demonstrate superiority over placebo. In contrast, Lesperance et al. (2011) evaluated the efficacy of 1.05 g/day EPA and 0.15 g/day DHA in a large population of patients with an episode of major depression ($N = 432$). Response to treatment in patients with a comorbid anxiety disorder (52.8%) was poor with no evidence of efficacy. Conversely, an improvement in depressive symptoms was observed in patients without comorbid anxiety disorders and was attributed to the pharmacological effects of EPA. These findings indicate that comorbid anxiety may hinder response to treatment and provides further evidence to support the role of EPA in the treatment of major depression.

Overall, the clinical trials that found no significant benefit of omega-3 fatty acid supplementation either administered higher doses of DHA than EPA and/or evaluated their pharmacological effects in depressed populations that had not received a major depressive disorder diagnosis. All studies demonstrated at trend towards improvement and reductions were evident on the primary and secondary outcome measures, indicating that depressive symptoms were reduced as a consequence to the clinical trial. Further, compliance was confirmed by the increased incorporation of fatty acids in red blood cell membranes or in the plasma of patients randomised to the intervention group. Supplementation was well tolerated and only minor adverse events were reported that pertained to gastrointestinal disturbances. Despite finding a non-significant benefit of omega-3 fatty acids in the treatment of depression, the results are valuable and provide an insight into the conditions that are necessary for a therapeutic effect. Positive outcomes are especially important for women bearing children.

1.12.2. Perinatal depression.

Perinatal depression, defined as “occurring during pregnancy or postpartum”, is a devastating illness that can have serious consequences for the affected mother and her child, especially when antidepressant treatment is discontinued (Freeman et al., 2008). However, there is growing concern surrounding the safety of antidepressants during
pregnancy, as recent evidence has suggested that chronic exposure during the third trimester may be associated with an enhanced risk of perinatal complications (Su et al., 2008). Due to these potential adverse events, expectant mothers tend not to continue with standard medications during pregnancy and lactation, which could lead to long term devastating effects for the mother and the development of her child. The discovery of safe and proven effective treatments is critical for women diagnosed with perinatal depression (Freeman et al., 2008; Su et al., 2008). Omega-3 fatty acid supplementation may prove a novel management and/or treatment approach to this population of interest.

A systematic search of the scientific literature revealed that three randomised controlled trials have been conducted to evaluate the efficacy of omega-3 fatty acid supplementation in the treatment of perinatal depression. Despite the large number of clinical trials that found a benefit in patients with a diagnosed major depressive disorder, only one of these three studies reported a significant improvement in symptoms resulting from omega-3 fatty acid supplementation.

1.12.2.1. Positive clinical trial for perinatal depression.

Su et al. (2008) found that a combined dose of 3.4 g/day (with 2.2 g EPA and 1.2 g DHA) lead to significant reductions in HAM-D scores from baseline to study completion in pregnant women with major depressive disorder (\( p = .019 \)). As compared to the placebo group (olive oil), patients in the intervention group had fewer depressive symptoms as reflected by a decrease in scores on the Edinburgh Postnatal Depression Scale (EPDS) and BDI. Based on these findings, omega-3 fatty acid supplementation may have therapeutic benefits in perinatal depression and has the potential to confer additional neurodevelopmental benefits to the growing fetus or infant. Overwhelmingly, the clinical trials reviewed herein indicated that 80% of the trials conducted had insignificant results.

1.12.2.2. Negative clinical trials for perinatal depression.

In contrast to these promising findings, Freeman et al. (2008) reported no significant benefit of omega-3 supplementation over placebo in perinatal women who met clinical criteria for major depressive disorder. Patients received either 1.1 g/day EPA and 0.8 g/day DHA or placebo (corn oil) for eight weeks \( (N = 59) \). A supportive psychotherapy intervention was offered to all patients in the clinical trial. The researchers found significant reductions in
HAM-D and EPDS scores in both the intervention and placebo group, which may be attributed to the psychotherapy that was received, meaning that a benefit from supplementation may not have been detected. While these findings provide little evidence of efficacy for omega-3 fatty acids, they demonstrate the potential for psychotherapy interventions as an alternative or adjunctive treatment for perinatal depression.

Rees, Austin, and Parker (2008) provide evidence to support this non-significant benefit of omega-3 supplementation in perinatal depression. Patients were either assigned to receive a daily dose of 2.05 g (0.414 g EPA and 1.638 g DHA), or a matching placebo containing sunola oil for six weeks ($N = 26$). Significant reductions from baseline on EPDS, HAM-D and MADRS scores were reported for both the intervention and placebo groups ($p < 0.001$); however, the between group differences were not statistical significant (EPDS -8.8 vs. -7.5; HDRS -11.8 vs. -9.3; MADRS -16.8 vs. -14.1, respectively). A current comorbid anxiety disorder was not found to influence treatment response thus the researchers attributed the negative findings to the mild to moderate forms of depression in perinatal women, which appears consistent with studies demonstrating that omega-3 supplemention is more effective in major depression.

Overall, the evidence to demonstrate a benefit of omega-3 supplementation in perinatal depression is limited. However, it appears that the response to treatment is enhanced in populations with severe depressive disorders, although further research is clearly needed in this area to reach a definitive conclusion. Clinical trials designed to assess the efficacy of omega-3 fatty acids in the treatment of bipolar disorder have been conducted.

1.12.3. **Bipolar disorder.**

Bipolar disorder is a chronic and recurrent mood disorder that is characterised by episodes of mania and sometimes depression, interspersed with periods of normal mood. Current medications appear to be effective in the treatment of bipolar disorder but are complicated by serious side effects that have the potential to cause detrimental health outcomes. Based on the evidence demonstrating treatment efficacy of omega-3 fatty acids in major depressive disorders, researchers have investigated the potential therapeutic effects in the maintenance of bipolar disorder. Four double blind randomised controlled
trials were identified during a systematic search of the relevant literature, of these studies; two reported a significant benefit of omega-3 fatty acids over placebo.

1.12.3.1 Positive clinical trials.

Stoll et al. (1999) conducted a preliminary study to ascertain whether the omega-3 fatty acids have mood stabilising effects in patients with a diagnosed bipolar disorder – types I or II. Patients were randomised to receive either 6.2 g EPA and 3.4 g DHA or placebo containing olive oil for 16 weeks, in combination with maintenance medications. The primary outcome measure was the duration of time the patients remained in the study, which was used to determine the treatment response. Numerous psychometric assessments were also administered at regular intervals over the treatment period (i.e. Young Mania Rating Scale (YMRS), HAM-D, Clinical Global Impression Scale (CGI), and the Global Assessment Scale). Statistical analyses revealed that the period of remission was significantly longer in the treatment group than in the placebo group (p = 0.002) and reduced bipolar symptoms as reflected by a significant decrease in scores on almost every assessment. Overall, omega-3 supplementation was well tolerated despite a few minor gastrointestinal disturbances in some patients. These preliminary findings indicate a role for omega-3 fatty acids in the prevention of both manic and depressive relapses, though more research is needed. Chiu et al. (2005) reexamined the data reported by Stoll et al., (1999) and discovered that some patients in the supplementation group relapsed into a manic state, which indicates that omega-3 fatty acids may be more beneficial in the prevention of a depressive relapse but not for a manic relapse (Chiu, Huang, Chen, and Su, 2005).

While episodes of mania are the defining feature of bipolar disorder and are often dramatic in nature, the depressive phases are endured for prolonged periods over the life course and contribute to poorer outcomes (Frangou et al., 2006). In order to examine the efficacy of EPA in bipolar depression, Frangou et al. (2006) compared the therapeutic outcomes in patients who were randomised to receive either 1 g/day or 2 g/day EPA or placebo (liquid paraffin) as adjunctive treatments with maintenance medications. As compared to the placebo group, significant reductions in HAM-D and CGI scores from baseline to study completion were reported for both intervention groups. However, there was no difference in clinical outcomes between the groups that received 1 g/day and 2 g/day (counted as two separate studies in statistical analysis), which appears consistent with
the results of Peet and Horrobin (2002). This indicates that improvements in depressive disorders can be achieved with low doses of EPA either as monotherapy or adjunctive therapy with standard medications. Half of the clinical trials reviewed in this study showed positive results and the other half were insignificant.

1.12.3.2. Negative clinical trials.

The remaining two clinical trials that were identified during the literature search found no evidence of a therapeutic effect in bipolar disorder. In the study conducted by Chiu et al. (2005) a combined dose of 0.44 g/day EPA and 0.24 g/day DHA in conjunction with valproate resulted in decreased scores on the YMRS (N = 14); however, the improvement was not statistically different from the placebo group (olive oil) on all outcome measures. A few minor adverse effects were reported in some patients, though these may have been attributed to valproate treatment. However, the researchers concluded that augmentation with omega-3 fatty acids was not effective in the acute treatment of bipolar mania. However, due to the very small sample, these preliminary results must be interpreted with caution.

Despite the evidence to support a beneficial effect of EPA in major depressive disorder, Keck et al. (2006) reported that adjunctive therapy with 6 g/day EPA (N = 116) was not effective in the treatment of bipolar depression and rapid cycling bipolar disorder. A large number of patients (54%) exited the trial before the final assessment, the main reason being poor treatment response. Overall, these findings provide little evidence to support the positive results reported by Stoll et al., (1999), although the beneficial effect that was published in the literature appears questionable, as a revision of the data indicated that EPA was less effective in reducing symptoms of mania.

In conclusion, there is some evidence to support a role for omega-3 fatty acids in the maintenance of bipolar disorder. However, the benefits appear specific to bipolar depression but not the manic episodes. Due to the lack of randomised controlled trials, further research is needed to provide the best indication of treatment efficacy.

In general, the doses used in clinical trials that were found to be effective were small and ranged from 1 g to 9.6 g per day with the most common dose being 1 g of EPA (Table 9). The majority of clinical trials deemed positive administered EPA in combination with standard medications, indicating that adjunctive therapy with mainly EPA was beneficial in
reducing depressive symptoms. However, the evidence remains controversial, as the doses used in clinical trials that were not effective were also small raising questions as to whether the doses are statistically different between positive and negative trials. Meta-analyses conducted by independent researchers on existing clinical trials related to omega-3 fatty acids and mood disorders provides additional layers of impartiality and objectivity to extant relevant literature, thereby strengthening or weakening research findings.


The clinical trials outlined in the sections above have provided conflicting evidence to support the efficacy of omega-3 fatty acids in the treatment of major depression, perinatal depression and bipolar disorder. Several meta-analyses of randomised controlled trials have therefore been conducted to evaluate the therapeutic benefits of omega-3 fatty acids in these mood disorders, in order to reach a more definitive conclusion regarding treatment efficacy. Results of the comprehensive meta-analyses revealed that omega-3 fatty acids significantly improved clinical outcomes in patients with diagnosed major depression or bipolar disorder compared to placebo, but was not effective in those without a depressive diagnosis or perinatal depression. In other words, the severity of depression at baseline was a predictor of treatment effectiveness (Appleton, Rogers, & Ness, 2010; Appleton et al., 2006; Grosso et al., 2014; Lin and Su, 2007; Ross, Seguin, & Sieswerda, 2007; Sarris et al., 2012). Grosso et al. (2014) reported that the combined effect size estimate in diagnosed major depression was 0.56, compared to 0.74 in bipolar depression and 0.24 in pregnant women with major depressive disorder. These standardised mean differences provide an indication of the magnitude of the effect, though these are expected to differ across meta-analyses depending on inclusion and exclusion criteria. However, the general consensus among researchers is that a final conclusion cannot be drawn due to considerable heterogeneity across trials and publication bias, as demonstrated in Table 9, though several factors have been identified to influence treatment response. For instance, studies that administered fish oil preparations containing mainly EPA, rather than DHA, were more likely to report significant improvements in clinical outcomes (Grosso et al., 2014). Based on individual trials, a meta-analysis had reported that high doses ($\geq 4$ g/day EPA) were more efficacious in reducing depressive symptoms than moderate or low doses; however, this pooled difference was not significant (Lin & Su, 2007), meaning that therapeutic effects can
be achieved at low doses. In contrast, studies that administered DHA alone found no benefit in treatment outcomes, indicating that EPA alone or in combination with DHA is more effective in reducing symptoms of depressive disorders. As compared to the efficacy of monotherapy, analyses revealed that adjunctive therapy with omega-3 fatty acids and maintenance medications significantly enhanced clinical outcomes. Viewed together, predictors of treatment response include more severe depressions, and preparations containing mainly EPA and adjunctive therapy with standard medications (Grosso et al., 2014; Lin & Su, 2007).

While there is substantial heterogeneity across clinical trials, the available evidence has demonstrated a modest beneficial effect of omega-3 fatty acids in the management and/or treatment of major depression and bipolar disorder. Further research is clearly needed before they can be recommended as an effective treatment for these complex and multifaceted disorders. This research study strives to fill part of this research gap through testing several hypotheses.


The independent variables being investigated in this research are the amounts of both omega-3 fatty acids and mercury in over-the-counter fish oil supplements sold over-the-counter in New Zealand. The dependent variables are the effective treatments of major depressive disorder and bipolar disorder.

Five hypotheses are proposed and addressed in this study:

HYPOTHESIS 1: The doses of omega-3 fatty acids in over-the-counter supplements are smaller than those used in clinical research trials.

HYPOTHESIS 2: The actual amounts of omega-3 fatty acids in over-the-counter fish supplements are less than the label claim.

HYPOTHESIS 3: There are detectable levels of mercury in over-the-counter fish supplements.

HYPOTHESIS 4: The benefits of omega-3 fatty acids are exceed the potential risk of mercury.

HYPOTHESIS 5: The analysed fish oil supplements are effective in the treatment of mood disorders for a small population.

The objectives of this research are captured in the research hypotheses –
Research Aims and Objectives

The aim of the current research is to investigate whether the ingredients and dosages in dietary fish oil supplements sold over-the-counter are consistent with the doses used in clinical trials that were effective in the treatment of major depressive disorder and bipolar disorder. The ingredients and dosages used in over-the-counter fish oil products will be determined from the most popular fish oil supplements available over-the-counter in New Zealand. The over-the-counter fish oil supplement sample will be drawn from New Zealand supermarkets, health food stores and pharmacies and will be based on sales data provided by the parent organisations of these stores. The ingredients and dosages used in clinical trials that focus on adult populations with a clinical diagnosis of depression and bipolar disorder will be found in literature search of papers published in peer-reviewed journals. A further aim of this research is to measure the fatty acid composition of over-the-counter fish oil supplements as compared to the amounts claimed on product labels. The composition of fatty acids in the fish oil supplement selected will be determined from independent chemical analyses. The amounts reported from the analysis will be compared to the amounts of omega-3 fatty acids printed on product labels. A further aim of this research is the determination of mercury levels in fish oil supplements as this will permit a risk and benefit analysis between the risk associated with mercury and the mood regulation benefits of omega-3 fatty acids. The amounts of mercury in over-the-counter fish oil products selected will be determined from an independent chemical analysis carried out by an accredited laboratory in New Zealand. The final research aim is to determine whether the over-the-counter fish oil supplements may be an effective treatment option for major depression and bipolar disorder using the extant literature as a frame of reference.
2. Method
Using the extant literature as a frame of reference from which to test the hypotheses, the most popular fish oil supplements sold in New Zealand at the time of the search will undergo relevant chemical analyses. The purpose of the chemical analyses is to determine the fatty acid composition as compared to the amounts of essential fatty acids stated on product labels in a comparative analysis. A second purpose of the chemical analyses is to determine the mercury content in the fish oil supplements selected.

2.1. Selection of Clinical Trials to be Included in the Study

A systematic search of the scientific literature was performed to identify clinical trials published in PubMed/MEDLINE, EMBASE, PsychINFO, and the Cochrane Database of Systematic Reviews up to May 2015 using the following search terms: (omega-3, or polyunsaturated fatty acids or PUFA or EPA or DHA, or fish oil) in combination with the following key words: (depression, or depressive disorder, or mood disorder, or depressed mood, or bipolar disorder, or perinatal depression, or postpartum depression). The search was limited to literature in English and relevant randomised controlled trials and meta-analyses. Retrieved articles were identified and screened by reading the abstract to determine the relevance to the current study. Reference lists from the relevant articles and review papers were inspected for any additional studies not found in the previous literature search. The process of identification and inclusion of clinical trials is shown in Figure 8. The inclusion criteria for studies in this review include the following: (1) randomised design, (2) double-blind, placebo controlled trial, (3) administration of omega-3 fatty acid supplement, (4) patients who met DSM criteria for a mood disorder OR (5) a baseline score ≥ 15 on the HAM-D, (5) exploring changes in depressive symptoms as either primary or secondary outcome, (6) adult populations, and (7) treatment period lasting at least four weeks. The exclusion criteria for this review include the following: (1) not a double-blind, placebo controlled trial, (2) patients without a DSM mood disorder diagnosis, AND/OR (3) a baseline score ≤ 14 on the HAM-D, (4) child and adolescent populations (aged 6 – 19 years), (5) mood disorder with Alzheimer’s disease AND/OR Schizophrenia AND/OR Parkinson’s disease AND/OR cardiovascular disease, AND/OR diabetes, (5) sample size ≤ 10.

The clinical outcome of interest in this study was a change from baseline to endpoint scores on a psychometric assessment in patients receiving omega-3 fatty acid supplements
compared to patients taking placebo. Treatment response was when there was a group difference on the primary outcome. In other words, clinical trials in this study were considered effective if the difference in scores on the primary outcome measures between the fish oil and placebo groups were statistically significant (p < 0.05).

The preferred rating scales used to measure a change in mood symptoms in the clinical trials reviewed were the HAMD, MADRS, and BDI for major depression, HAM-D, and EPDS for perinatal depression, and the HAMD, YMRS, and CGI for bipolar disorder. A total of 22 studies were included in the review as having evaluated the efficacy of fish oil supplements in the prevention, management and/or treatment of mood disorders. Of these studies, 13 of them examined patients with major depressive disorder; three were conducted with women with perinatal depression, two with healthy pregnant women to assess the effectiveness in the prevention of postpartum depression and five studies included patients with bipolar disorder. The next task for this research was to select the 10 most popular fish oil supplements sold over-the-counter in New Zealand.
2.2. Top 10 Fish Oil Supplement Investigation

2.2.1. Fish oil dietary supplement products.

A fish oil dietary supplement for the purpose of this study was defined as any product consumed orally that labeled itself as a ‘fish oil, odourless fish oil, or omega-3 fish oil supplement’ and contained EPA and DHA from the bodies of deep water fish (e.g. sardines, pilchards, anchovies, mackerel, tuna, and salmon). Marine oils from alternative sources (e.g. krill, calamari, and algae) were omitted due to their compositional differences. Similarly, plant sources of omega-3 (e.g. flax and chia seeds, walnuts, and rapeseed oil) fall outside the scope of the current study as research consistently demonstrates an inability to efficiently convert ALA to EPA and then DHA in the body.

Although all over-the-counter fish oil supplements that were available for purchase over-the-counter in supermarkets, pharmacies and health stores in New Zealand were eligible for inclusion in the study, only the top 10 best selling fish oil supplements sold at the time of the search (April 2015) were selected. In order to select the most popular fish oil supplements in New Zealand, formal letters were written to the chief executive officers or managing directors of four large health-food companies in New Zealand (Foodstuffs New Zealand Ltd, Progressive Enterprises Ltd, Green Cross Health Ltd, and Health 2000 Retail Ltd). The letters requested data on the annual sales of fish oil supplements sold across all stores owned and operated by the company (Table 8).

Of the four companies that were contacted, only two responded to the letters via email – Foodstuffs New Zealand Ltd and Green Cross Health Ltd. Excel spreadsheets depicting the top ranking fish oil supplements for each company, between March 2014 and March 2015, revealed the total product units sold across all stores in New Zealand. The data were collated and arranged in ascending order based on the total units sold in order to determine the current top 10 fish oil supplements. However, an agreement with Foodstuffs Ltd and Green Cross Health Ltd means the raw data provided remain commercial-in-confidence. Correspondence with the manufacturers of the fish oil supplement products was also conducted and is discussed in the next section.
### Table 8

<table>
<thead>
<tr>
<th>Foodstuffs New Zealand Ltd¹</th>
<th>Progressive Enterprises Ltd¹</th>
<th>Green Cross Health Ltd²</th>
<th>Health 2000 Retail Ltd³</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAK ‘n SAVE</td>
<td>Countdown</td>
<td>Amcal</td>
<td>Health 2000</td>
</tr>
<tr>
<td>New World</td>
<td>Fresh Choice</td>
<td>Life Pharmacy</td>
<td></td>
</tr>
<tr>
<td>Four Square</td>
<td>Supervalue</td>
<td>Radius</td>
<td>Unichem</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Care Chemist</td>
</tr>
</tbody>
</table>

**Note.**

¹Supermarket  
²Pharmacy  
³Health food store

### 2.2.2 Communications with fish oil supplement companies.

Dietary supplements sold in New Zealand must comply with the New Zealand Dietary Supplement Regulations 1985, which extends to supplement labelling (Beattie & Governor-General, 1985). While the labels of fish oil products appear to be in compliance, ‘mercury tested’ encompasses the extent to which consumers are informed of mercury levels in over-the-counter fish oil supplements, including those selected for analysis. To determine whether the potential for mercury toxicity is smaller following the consumption of fish oil supplements than deep-water fish, two emails were sent to the companies that manufacture the fish oil products selected for analysis in this study. The first email probed for information regarding the mercury levels in the fish oil supplements, whereas the second email enquired about the companies’ testing processes involved in the removal of mercury and other heavy metals from the fish oil used in the supplements. The responses to these emails are provided in the results section.

Furthermore, based on the fact that mercury exists in the environment and bioaccumulates in marine species leading to considerable differences in amounts between predatory and non-predatory fish, it is important to establish the species used for the production of fish oil and the location they were caught from. When information was not available on the website of the companies that manufacture the fish oil supplements analysed in this study, a private message was sent through the website or social media page of each company. The responses to these emails can be found in the results section. Though
information regarding the fish species was provided, independent chemical analyses were needed to identify the amounts of omega-3 fatty acids and mercury levels in the fish oil capsules analysed.

2.3. Measurement of Omega-3 Fatty Acids in the Fish Oil Supplements

2.3.1. Analytical methodology.

Analysis of the fish oil supplements was performed using Gas Chromatography (GC) to measure amounts of EPA and DHA per capsule. AOAC Official Methods 991.39 (AOAC International, 2005) was used by AsureQuality Ltd, Auckland, a New Zealand GLP (Good Laboratory Practice) accredited laboratory approved by International Accreditation New Zealand (IANZ) where an approximate 0.025 g of fish oil from each fish oil capsule was accurately weighed into a glass tube containing an internal standard (i.e. 25 mg of C23:0 methyl or ethyl ester) diluted with isoctane (2,2,4-trimethylpentane) to allow identification peaks based on their retention time. Fatty acid samples were derivatized to methyl esters and specifically analysed by GC equipped with a flame ionization detector. Colleagues at AsureQuality Ltd. calculated the amounts of omega-3 fatty acids in the samples using GC peak areas for the test and internal standards correcting for the ester derivitisation used to make the fatty acids volatile or GC analysis (methodology and calculations described in full in AOAC International, 2005).

2.4. Measurement of Mercury in the Fish Oil Supplements

2.4.1. Analytical methodology.

Mercury was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The analysis was performed at Hill Laboratories Ltd, a New Zealand GLP accredited laboratory approved by International Accreditation New Zealand (IANZ). The American Public Health Association (APHA) Standard Method 3125B (Standard Methods, 2009) was used for the determination of heavy metals.

In short, measurement of mercury involved acid digestion of the lipid contents from the proprietary capsules using nitric and hydrochloric acids at 85°C for 1 hr. Elements in the sample were ionised using an inductively coupled plasma, followed by mercury determination by mass spectrometry.
A potential problem with this methodology is the high temperature used for the acid digestion. As methylmercury is extremely volatile (boiling point = 92°C), any mercury present in the sample may evaporate during the process prior to ICP-MS analysis. However, Hill Laboratories Ltd confirmed unequivocally that no mercury was lost during the acid digestion process, based on results of independent studies conducted by laboratory analysts (D. Day, personal communications, June 4, 2015). ICP-MS is an excellent methodology and allows direct determination of heavy metals at extremely low levels (0.001 µg/kg) with high sensitivity and enhanced reliability.
3. Results
3.1. Clinical Research Trials Identified During a Literature Search

Based on the systematic search and review of peer reviewed literature in the psychological area of mood disorders and in accordance with criteria used to select clinical research trials for this study, 22 existing research studies were found. From the 22 clinical trials identified, the compositional ingredients and dosages in the fish oil formulations evaluated were compared to the results of the independent chemical analysis conducted on the fish oil supplements in this project. Data in Table 9 shows the ingredients and dosages of omega-3 fatty acids used in selected clinical research and shows heterogeneity across clinical trials, which extends to the placebo used in the control group. The choice of placebo for some of these clinical trials was olive oil – a monounsaturated fatty acid that offers protection against various chronic diseases such as cardiovascular disease (Kris-Etherton et al., 2002b). Based on the relationship between depression and cardiovascular disease (Joynt, Whellan, & O’Conner, 2003), the choice of olive oil as placebo may be problematic and could influence study outcome. However, it is unclear from the data whether olive oil supplementation was an influential factor in clinical trials with fish oil that were no more effective than placebo in the treatment of mood disorders, as both positive and negative trials used olive oil as placebo. Moreover, the data in Tables 10 and 11 capture the discrepancies in the efficacy of fish oil supplementation for patients with mood disorders. Clinical trials showed that larger doses of omega-3 fatty acids were not necessarily more effective than smaller doses (Tables 10 and 11). The top 10 fish oil supplements used in this study was selected based on sales data.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Disorder</th>
<th>No. of Patients</th>
<th>Mean Age (years)</th>
<th>Duration (weeks)</th>
<th>Intervention</th>
<th>EPA Daily Dose</th>
<th>DHA Daily Dose</th>
<th>Placebo</th>
<th>Mono vs. Adjunct Therapy</th>
<th>Outcome measure</th>
<th>Statistical Significance vs. Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rondanelli et al.</td>
<td>2010</td>
<td>MDD (only women)</td>
<td>46</td>
<td>83.95</td>
<td>8</td>
<td>Omega-3</td>
<td>1.67 g</td>
<td>0.83 g</td>
<td>Paraffin oil</td>
<td>Mono</td>
<td>GDS, SF-36</td>
<td>Yes</td>
</tr>
<tr>
<td>Mozaffari-Khosravi et al.</td>
<td>2013</td>
<td>Depression - moderate</td>
<td>27</td>
<td>37.5</td>
<td>12</td>
<td>EPA</td>
<td>1 g</td>
<td>-</td>
<td>Coconut oil</td>
<td>Mono</td>
<td>HAM-D2</td>
<td>Yes</td>
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<tr>
<td>Su et al.</td>
<td>2003</td>
<td>MDD</td>
<td>28</td>
<td>39</td>
<td>8</td>
<td>Omega-3</td>
<td>4.4 g</td>
<td>2.2 g</td>
<td>Olive oil</td>
<td>Adjunct</td>
<td>HAM-D2</td>
<td>Yes</td>
</tr>
<tr>
<td>Nemets et al.</td>
<td>2002</td>
<td>MDD</td>
<td>20</td>
<td>53</td>
<td>4</td>
<td>EPA</td>
<td>2 g</td>
<td>-</td>
<td>NR</td>
<td>Adjunct</td>
<td>HAM-D2</td>
<td>Yes</td>
</tr>
<tr>
<td>Peet and Horrobin</td>
<td>2002</td>
<td>MDD</td>
<td>23</td>
<td>43.5</td>
<td>12</td>
<td>EPA</td>
<td>1 g</td>
<td>-</td>
<td>Liquid paraffin</td>
<td>Adjunct</td>
<td>HAM-D2, BDI, MADRS4, HAM-D2, BDI3, MADRS4, CGI5</td>
<td>Yes</td>
</tr>
<tr>
<td>Gertsik et al.</td>
<td>2012</td>
<td>MDD</td>
<td>42</td>
<td>40.5</td>
<td>8</td>
<td>Omega-3</td>
<td>0.9 g</td>
<td>0.2</td>
<td>Olive oil</td>
<td>Adjunct</td>
<td>HAM-D2</td>
<td>Yes</td>
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<tr>
<td>Jazayeri et al.</td>
<td>2008</td>
<td>MDD</td>
<td>60</td>
<td>34.8</td>
<td>8</td>
<td>EPA, EPA + Fluoxetine</td>
<td>1 g</td>
<td>-</td>
<td>Rapeseed oil</td>
<td>Adjunct</td>
<td>HAM-D2</td>
<td>Yes</td>
</tr>
<tr>
<td>Mozaffari-Khosravi et al.</td>
<td>2013</td>
<td>Depression - moderate</td>
<td>27</td>
<td>34</td>
<td>12</td>
<td>DHA</td>
<td>-</td>
<td>1 g</td>
<td>Coconut oil</td>
<td>Mono</td>
<td>HAM-D2</td>
<td>No</td>
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<tr>
<td>Marangell et al.</td>
<td>2003</td>
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<td>36</td>
<td>47</td>
<td>6</td>
<td>DHA</td>
<td>-</td>
<td>2 g</td>
<td>NR*</td>
<td>Mono</td>
<td>HAM-D2, MADRS4</td>
<td>No</td>
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<tr>
<td>Mischoulon et al.</td>
<td>2009</td>
<td>MDD</td>
<td>57</td>
<td>45</td>
<td>8</td>
<td>EPA</td>
<td>1 g</td>
<td>-</td>
<td>Paraffin oil</td>
<td>Mono</td>
<td>HAM-D2</td>
<td>No</td>
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</table>

*NR: Not reported
<table>
<thead>
<tr>
<th>Study Authors</th>
<th>Year</th>
<th>Disorder Type</th>
<th>N 1</th>
<th>N 2</th>
<th>Treatment 1 Dosage</th>
<th>Treatment 2 Dosage</th>
<th>Adjunct Treatment</th>
<th>Outcome Measure 1</th>
<th>Outcome Measure 2</th>
<th>Outcome Measure 3</th>
<th>Outcome Measure 4</th>
<th>Outcome Measure 5</th>
<th>Outcome Measure 6</th>
<th>Outcome Measure 7</th>
<th>Outcome Measure 8</th>
<th>Outcome Measure 9</th>
<th>Outcome Measure 10</th>
<th>Outcome Measure 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peet and Horrobin</td>
<td>2002</td>
<td>MDD</td>
<td>24</td>
<td>44</td>
<td>12 EPA 2 g</td>
<td>-</td>
<td>Liquid paraffin</td>
<td>Adjunct</td>
<td>HAM-D^4, MADRS^4</td>
<td>BDI^3</td>
<td>MADRS^1</td>
<td>BDI^3</td>
<td>HAM-D^2</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Peet and Horrobin</td>
<td>2002</td>
<td>MDD</td>
<td>23</td>
<td>47.4</td>
<td>12 EPA 4 g</td>
<td>-</td>
<td>Liquid paraffin</td>
<td>Adjunct</td>
<td>HAM-D^4, MADRS^4</td>
<td>BDI^3</td>
<td>MADRS^1</td>
<td>BDI^3</td>
<td>HAM-D^2</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Grenyer et al.</td>
<td>2007</td>
<td>MDD</td>
<td>83</td>
<td>45</td>
<td>16 Omega-3 0.6 g</td>
<td>2.2 g</td>
<td>Olive oil</td>
<td>Adjunct</td>
<td>BDI^3</td>
<td>HAM-D^2</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
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<tr>
<td>Lesperance et al.</td>
<td>2011</td>
<td>MDD</td>
<td>432</td>
<td>-</td>
<td>8 Omega-3 1.05 g</td>
<td>0.15 g</td>
<td>Sunflower oil</td>
<td>Adjunct</td>
<td>BDI^3</td>
<td>HAM-D^2</td>
<td>IDS^6</td>
<td>MADRS^4</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Perinatal Depression</td>
<td>Su et al. 2008</td>
<td>Perinatal MDD</td>
<td>36</td>
<td>31</td>
<td>8 Omega-3 2.2 g</td>
<td>1.2 g</td>
<td>Olive oil</td>
<td>Mono</td>
<td>HAM-D^4, EPDS^5</td>
<td>BDI^3</td>
<td>MADRS^4</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<td>Perinatal Depression</td>
<td>Rees et al. 2008</td>
<td>Perinatal MDD</td>
<td>26</td>
<td>33</td>
<td>6 Omega-3 0.4 g</td>
<td>1.6 g</td>
<td>Sunflower oil</td>
<td>Mono</td>
<td>HAM-D^4, EPDS^5</td>
<td>MADRS^4</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td></td>
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<tr>
<td>Perinatal Depression</td>
<td>Freeman et al. 2008</td>
<td>Perinatal MDD</td>
<td>59</td>
<td>30</td>
<td>8 Omega-3 1.1 g</td>
<td>0.8 g</td>
<td>Corn oil + 1% fish oil</td>
<td>Mono</td>
<td>HAM-D^4, EPDS^5</td>
<td>MADRS^4</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
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<tr>
<td>Bipolar Disorder</td>
<td>Frangou et al. 2006</td>
<td>Bipolar Disorder I or II</td>
<td>24</td>
<td>47.8</td>
<td>12 EPA 1 g</td>
<td>-</td>
<td>Paraffin oil</td>
<td>Adjunct</td>
<td>HAM-D^2</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Bipolar Disorder</td>
<td>Frangou et al. 2006</td>
<td>Bipolar Disorder I or II</td>
<td>25</td>
<td>46</td>
<td>12 EPA 2 g</td>
<td>-</td>
<td>Paraffin oil</td>
<td>Adjunct</td>
<td>HAM-D^2, YMRS^5</td>
<td>CGI^5</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Bipolar Disorder</td>
<td>Stoll et al. 1999</td>
<td>Bipolar Disorder</td>
<td>30</td>
<td>43</td>
<td>16 Omega-3 6.2 g</td>
<td>3.4 g</td>
<td>Olive oil</td>
<td>Adjunct</td>
<td>HAM-D^2, YMRS^5</td>
<td>CGI^5</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Bipolar Disorder</td>
<td>Keck et al. 2006</td>
<td>Bipolar Disorder</td>
<td>116</td>
<td>45</td>
<td>16 EPA 6 g</td>
<td>-</td>
<td>Liquid paraffin</td>
<td>Adjunct</td>
<td>IDS-C^10</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
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<tr>
<td>Disorder I or II</td>
<td>Chiu et al. 2005</td>
<td>Bipolar Disorder</td>
<td>14</td>
<td>-</td>
<td>4</td>
<td>Omega-3</td>
<td>0.44 g</td>
<td>0.24 g</td>
<td>Olive oil</td>
<td>Adjunct</td>
<td>YMRS&lt;sup&gt;8&lt;/sup&gt;</td>
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</tr>
</tbody>
</table>

**Note.**
* NR = Not Reported
1. The Geriatric Depression Scale Short Form (GDS-SF)
2. Hamilton Depression Rating Scale (HAM-D)
3. Beck Depression Inventory (BDI)
4. Montgomery-Asperg Depression Rating Scale (MADRS)
5. Clinical Global Impressions Scale (CGI)
6. Inventory of Depressive Symptoms (IDS)
7. Edinburgh Postnatal Depression Scale (EPDS)
8. Young Mania Rating Scale (YMRS)
9. Global Assessment Scale (GAS)
10. Inventory of Depressive Symptoms-Clinician Rated (IDS-C)
11. Positive and Negative Syndrome Scale (PANSS)
12. Clinical Global Impressions-Bipolar Illness (CGI-BP)
Table 10

**Effective Doses of Omega-3 Fatty Acids in Clinical Trials for the Treatment of Mood Disorders**

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Intervention</th>
<th>Combined Dose</th>
<th>Mono vs. Adjunct</th>
<th>Statistical Significance vs. Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peet and Horrobin</td>
<td>2002</td>
<td>EPA</td>
<td>1 g</td>
<td>Adjunct</td>
<td>Yes</td>
</tr>
<tr>
<td>Mozaffari-Khosravi et al.</td>
<td>2013</td>
<td>EPA</td>
<td>1 g</td>
<td>Mono</td>
<td>Yes</td>
</tr>
<tr>
<td>Jazayeri et al.</td>
<td>2008</td>
<td>EPA</td>
<td>1 g</td>
<td>Adjunct</td>
<td>Yes</td>
</tr>
<tr>
<td>Frangou et al.</td>
<td>2006</td>
<td>EPA</td>
<td>1 g</td>
<td>Adjunct</td>
<td>Yes</td>
</tr>
<tr>
<td>Gertsik et al.</td>
<td>2012</td>
<td>Omega-3</td>
<td>1.1 g</td>
<td>Adjunct</td>
<td>Yes</td>
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<tr>
<td>Frangou et al.</td>
<td>2006</td>
<td>EPA</td>
<td>2 g</td>
<td>Adjunct</td>
<td>Yes</td>
</tr>
<tr>
<td>Nemets et al.</td>
<td>2002</td>
<td>EPA</td>
<td>2 g</td>
<td>Adjunct</td>
<td>Yes</td>
</tr>
<tr>
<td>Rondanelli et al.</td>
<td>2010</td>
<td>Omega-3</td>
<td>2.5 g</td>
<td>Mono</td>
<td>Yes</td>
</tr>
<tr>
<td>Su et al.</td>
<td>2008</td>
<td>Omega-3</td>
<td>3.4 g</td>
<td>Mono</td>
<td>Yes</td>
</tr>
<tr>
<td>Su et al.</td>
<td>2003</td>
<td>Omega-3</td>
<td>6.6 g</td>
<td>Adjunct</td>
<td>Yes</td>
</tr>
<tr>
<td>Stoll et al.</td>
<td>1999</td>
<td>Omega-3</td>
<td>9.6 g</td>
<td>Adjunct</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Note.* The doses of omega-3 fatty acids used in clinical trials are ordered from smallest to largest.
### Table 11

**Ineffective Doses of Omega-3 Fatty Acids in Clinical Trials for the Treatment of Mood Disorders**

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Intervention</th>
<th>Combined Dose</th>
<th>Mono vs. Adjunct</th>
<th>Statistical Significance vs. Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeman et al.</td>
<td>2008</td>
<td>Omega-3</td>
<td>1.9 g</td>
<td>Mono</td>
<td>No</td>
</tr>
<tr>
<td>Rees et al.</td>
<td>2008</td>
<td>Omega-3</td>
<td>2.05 g</td>
<td>Mono</td>
<td>No</td>
</tr>
<tr>
<td>Grenyer et al.</td>
<td>2007</td>
<td>Omega-3</td>
<td>2.8 g</td>
<td>Adjunct</td>
<td>No</td>
</tr>
<tr>
<td>Lesperance et al.</td>
<td>2011</td>
<td>Omega-3</td>
<td>1.2 g</td>
<td>Adjunct</td>
<td>No</td>
</tr>
<tr>
<td>Chiu et al.</td>
<td>2005</td>
<td>Omega-3</td>
<td>.68 g</td>
<td>Adjunct</td>
<td>No</td>
</tr>
<tr>
<td>Peet and Horrobin</td>
<td>2002</td>
<td>EPA</td>
<td>2 g</td>
<td>Adjunct</td>
<td>No</td>
</tr>
<tr>
<td>Peet and Horrobin</td>
<td>2002</td>
<td>EPA</td>
<td>4 g</td>
<td>Adjunct</td>
<td>No</td>
</tr>
<tr>
<td>Mischoulon et al.</td>
<td>2009</td>
<td>EPA</td>
<td>1 g</td>
<td>Mono</td>
<td>No</td>
</tr>
<tr>
<td>Marangell et al.</td>
<td>2003</td>
<td>DHA</td>
<td>2 g</td>
<td>Mono</td>
<td>No</td>
</tr>
<tr>
<td>Mozaffari-Khosravi et al.</td>
<td>2013</td>
<td>DHA</td>
<td>1 g</td>
<td>Mono</td>
<td>No</td>
</tr>
<tr>
<td>Keck.</td>
<td>2006</td>
<td>EPA</td>
<td>6 g</td>
<td>Adjunct</td>
<td>No</td>
</tr>
</tbody>
</table>

*Note.* The doses of omega-3 fatty acids used in clinical trials are ordered from smallest to largest.
3.2. Top 10 Over-the-counter Fish Oil Supplements Analysed

3.2.1. Determination and purchase of top 10 dietary fish oil supplements.

Annual sales data provided by Foodstuffs New Zealand Ltd and Green Cross Health Ltd determined the top 10 fish oil supplements that would later undergo analysis (Table 12). The supplements were purchased over-the-counter from stores owned and operated by Foodstuffs New Zealand Ltd and Green Cross Health Ltd such as Life Pharmacy (see products 1-6, Table 12), Amcal (see product 7, Table 12), and PAK’n SAVE supermarket (see products 8-10, Table 12). Products with a use by date between 15 and 35 months from the date of the search were purchased, as determined by product availability in store.

The ingredients and recommended daily dosage for each over-the-counter fish oil supplement were recorded, including the batch number and country of origin, an excerpt from the full table can be seen in Appendix A. Importantly, fish oil supplements sold over-the-counter contain a combination of natural fish oil, marine triglycerides as either free fatty acids, ethyl esters and/or re-esterified triglycerides, and antioxidants. The amount of EPA and DHA as marine triglycerides within each product capsule was recorded from the supplement label for comparison purposes (label claims versus actual contents).

Purchased fish oil supplements were stored in a cool, dark environment to minimise oxidative and light induced changes to the capsule component. Preparation of the supplements for analysis involved allocating three fish oil capsules from each supplement to one of 10 glass jars, all individually wrapped in aluminum foil and sealed. Each sample was assigned an alphabetical letter corresponding to the product label to ensure the brand of supplement remained unknown to the analyst. The batch was enclosed in bubble wrap and placed inside a cardboard box to minimise the risk of breakage.
Table 12

Top 10 over-the-counter fish oil supplements from data supplied by Foodstuffs New Zealand Ltd and Green Cross Health Ltd

<table>
<thead>
<tr>
<th>Rank</th>
<th>Supplement Name</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Go Healthy Fish Oil Reflux Free 1500 mg</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Nutra-Life Omega-3 Fish + Vitamin D 1500 mg</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Good Health Omega-3 Health Guard 1000 mg</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Natures Own Fish Oil Odourless 2000 mg</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Go Healthy Fish Oil Advanced Omega Pc 1550 mg</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Go Healthy Fish Oil Odourless 2000 mg</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Sanderson Fish Oil 2000 mg</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Healtheries Fish Oil 1000 mg</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Healtheries Fish Oil 1500 mg</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Red Seal Fish Oil 1000 mg</td>
<td></td>
</tr>
</tbody>
</table>

3.2.2. Communications with fish oil companies regarding product labelling.

To determine whether over-the-counter fish oil supplements are a safer alternative to the consumption of deep-water fish, two emails were sent to the companies that manufacture the fish oil supplements selected for analysis in this study, as shown in Table 12. To reiterate, the first email asked for information regarding the mercury levels in the fish oil supplements; however, no response was received (note that Red Seal was inadvertently excluded from this email group). A possible explanation for the lack of response may be an attempt from the companies to avoid communications with the public that may raise questions about the safety of their product. Nevertheless, the second email enquired about the companies testing processes to ensure the removal of mercury and other heavy metals from the fish oil used in the supplements. Of the 10 companies that were contacted, four replied – Nutra-Life, Nature’s Own, Healtheries, and Red Seal. The greater response rate may be explained by the fact that the email informed the companies of the independent analyses and subsequent results of the current study. The email also provided an opportunity to reveal the various processes that are performed prior to the product reaching the market. For instance, the company literature relating to the fish oil purification process employed by Nature’s Own revealed the extent of the procedures involved in the testing and processing of the fish oil (Appendix B). This process appears consistent with that
carried out by Red Seal. Following the oil refinement process, the Therapeutic Goods and Administration states that any fish oil product must contain less than 0.5 mg/kg of mercury (Therapeutic Goods and Administration, 2012); however, a more rigorous standard of less than 0.1 mg/kg can be used.

A search was conducted to determine the species of fish used for the production of fish oil and to establish where the fish were caught. When the information was not available on the company website, a message was sent through the website or social media page to request the location the fish were sourced from. The search revealed that all 10 fish oil supplement companies extract the crude oil from similar species of fish (e.g. sardines) that are caught in similar regions of the world (Table 13)(Figure 9). However, the names of the fish provided by the companies and exact species are not known, in some cases, the common name could be ambiguous (e.g. mackerel). Further, the amount of mercury in marine species is determined by the position the organism holds in the food chain, known as the trophic level. That is, the higher the position in the food chain, the greater the amount of mercury in fish adipose tissue. The species of fish selected for the production of the fish oil supplements in this study are trophic level 2 (e.g. sardines), trophic level 3 (e.g. sardines, pilchards, anchovies), and tropic level 4 (e.g. mackerel, tuna, salmon), which means a detectable amount of mercury in the fish oil is expected to be present prior to the initial oil refinement process. Independent chemical analysis of the fish oil supplements in this study is instrumental in assessing several concerns such as mercury levels and actual amounts of omega-3 fatty acids per capsule.

<table>
<thead>
<tr>
<th>Brand</th>
<th>Fish</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Go Healthy</td>
<td>Sardines, Anchovies, Salmon</td>
<td></td>
</tr>
<tr>
<td>Nutra-Life</td>
<td>Sardines, Pilchards, Anchovies, Mackerel</td>
<td>Chile/Peru/Morocco</td>
</tr>
<tr>
<td>Good Health Omega-3</td>
<td>Sardines, Anchovies, Mackerel</td>
<td>South Pacific Ocean</td>
</tr>
<tr>
<td>Natures Own</td>
<td>Sardines, Anchovies</td>
<td>South America/Pacific</td>
</tr>
<tr>
<td>Sanderson</td>
<td>Sardines, Pilchards</td>
<td>Chile</td>
</tr>
<tr>
<td>Healtheries</td>
<td>Sardines, Sardinella, Pilchards, Anchovies, Mackerel, Tuna, Salmon, Sardines</td>
<td>South America/Africa</td>
</tr>
<tr>
<td>Red Seal</td>
<td>Sardines</td>
<td>Chile/Peru/Morocco</td>
</tr>
</tbody>
</table>
3.2.3. Analysis of omega-3 fatty acids in the 10 fish oil supplements (analysis 1).

GC analysis was employed to determine the actual amount of omega-3 fatty acids per fish oil capsule. Results from the analyses revealed that all 10 top selling fish oil supplements contained higher amounts of EPA than DHA (Table 14). Further, there appears to be only a small difference between products in terms of the individual fatty acid contents, as demonstrated by a reported range of 9 mg and 6 mg for EPA and DHA respectively*, despite large differences between label claims. For instance, Healtheries Fish Oil 1000 mg (Brand B) contains 184 mg EPA and 119 mg DHA, whereas, Nature’s Own Odourless Fish Oil 2000 mg (Brand F) contains 176 mg EPA and 114 mg DHA (Table 14). Despite label claims made by Nature’s Own, Healtheries fish oil capsules contain more omega-3 fatty acids irrespective of capsule content. A direct comparison between label and actual amount of both EPA and DHA shows the extent of the differences (Table 14). The percentage differences were calculated and showed that five out of 10 supplements contain a moderate percentage of the claimed omega-3 fatty acid content. Considerable differences were observed for Nature’s Own Fish Oil, which contains 51.1% and 52.5% less EPA and
DHA, respectively, whereas the difference seems less apparent for Nutra-Life Fish Oil, which contains 31.5% and 36.7% less EPA and DHA, respectively. These findings support initial indications regarding the large discrepancy between label and actual content of fish oil supplements. Few products, however, contain amounts higher than or equal to label content, thereby creating divide between supplement brands.

Though the evidence indicates a clear distinction between amounts of omega-3 fatty acids, statistical analyses were conducted through IBM SPSS (Statistical Package for the Social Sciences) Statistics version 21 to evaluate whether the actual amounts of omega-3 fatty acids are statistically different from label claims. Given that the data did not follow a normal distribution, a two-tailed Mann-Whitney test was performed for each omega-3 fatty acid. Results of the analyses were largely in the expected direction, whereby the actual amount of EPA and DHA per capsule was considerably less than label content. The effect size was calculated for each independent analysis using N (total number of fish oil supplements) and the computed Z score. In general, 0.1 represents a small effect size, 0.3 a medium effect size and 0.5 a large effect size. Upon initial inspection of the data, two values were viewed as possible outliers; however, there was insufficient evidence to presume the values were derived from a different population to that of interest.

Despite large observable differences, the actual amounts (Mdn 181.5) of EPA per fish oil capsule did not differ significantly overall from the labeled content (Mdn 270), U(30.5), z -1.485, ns, r = -0.33 (2dp). However, the actual amount (Mdn 115) of DHA per capsule was significantly less than the amount stated on the label (Mdn 180), U(15.5), z -2.636, p = .008, r = -0.59 (2dp) (Table 15).

While the analyses provide valuable information regarding the significance level, the individual percentage differences for each fish oil product more accurately reflects the extent of the difference between label and actual amounts of omega-3 fatty acids. That is, the variation for each fish oil supplement appears less meaningful when viewed collectively and compared against an independent group.

Furthermore, the combined amount of EPA and DHA per fish oil capsule was calculated. As shown, half of the fish oil supplements contain between 48 – 69% of the claimed omega-3 fatty acid content (Figure 10). Conversely, four of the remaining five products clearly do meet label claims. Healtheries Fish Oil 1000 mg (Brand B) exceeds label content, whereas Sanderson Fish Oil (Brand J) contains the exact amount stated on the
label. This demonstrates the importance of individual differences, as opposed to group differences. Due to the fact that five of the fish oil supplements analysed were found to contain much less omega-3 fatty acids than their product labels, the products were reanalysed with different batch numbers.

(Note: *Exclude Brand H and Brand J)

Table 14

Comparison Between Label and Actual Amounts of Omega-3 Fatty Acids in Over-The-Counter Fish Oil Supplements

<table>
<thead>
<tr>
<th>Fish Oil Supplement</th>
<th>EPA Label (mg)</th>
<th>EPA Actual (mg)</th>
<th>% Diff.</th>
<th>DHA Label (mg)</th>
<th>DHA Actual (mg)</th>
<th>% Diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Red Seal 1000 mg</td>
<td>180</td>
<td>181</td>
<td>+0.5</td>
<td>120</td>
<td>114</td>
<td>-5</td>
</tr>
<tr>
<td>B. Healtheries 1000 mg</td>
<td>180</td>
<td>184</td>
<td>+2</td>
<td>120</td>
<td>119</td>
<td>-0.83</td>
</tr>
<tr>
<td>C. Healtheries 1500 mg</td>
<td>270</td>
<td>182</td>
<td>-32.6</td>
<td>180</td>
<td>114</td>
<td>-36.7</td>
</tr>
<tr>
<td>D. Go Healthy 1500 mg</td>
<td>270</td>
<td>177</td>
<td>-34.4</td>
<td>180</td>
<td>116</td>
<td>-35.6</td>
</tr>
<tr>
<td>E. Good Health 1000 mg</td>
<td>180</td>
<td>177</td>
<td>-1.6</td>
<td>120</td>
<td>118</td>
<td>-1.7</td>
</tr>
<tr>
<td>F. Natures Own 2000 mg</td>
<td>360</td>
<td>176</td>
<td>-51.1</td>
<td>240</td>
<td>114</td>
<td>-52.5</td>
</tr>
<tr>
<td>G. Go Healthy 1550 mg</td>
<td>275</td>
<td>181</td>
<td>-34.2</td>
<td>185</td>
<td>113</td>
<td>-39</td>
</tr>
<tr>
<td>H. Go Healthy 2000 mg</td>
<td>360</td>
<td>320</td>
<td>-11.1</td>
<td>240</td>
<td>210</td>
<td>-12.5</td>
</tr>
<tr>
<td>I. Nutra-Life 1500 mg</td>
<td>270</td>
<td>185</td>
<td>-31.5</td>
<td>180</td>
<td>114</td>
<td>-36.7</td>
</tr>
<tr>
<td>J. Sanderson 2000 mg</td>
<td>360</td>
<td>360</td>
<td>0</td>
<td>240</td>
<td>240</td>
<td>0</td>
</tr>
</tbody>
</table>

Note:
- Fish oil supplements highlighted in bold contain considerably less omega-3 fatty acids
- Alphabetical letters refer to the fish oil product code allocated prior to analysis

Table 15

Median and Dose Distribution Properties of Omega-3 Fatty Acids Across Label and Actual Composition

<table>
<thead>
<tr>
<th>Omega-3 Fatty Acid</th>
<th>Label Amount Median (SD)</th>
<th>Actual Amount Median (SD)</th>
<th>Label Amount Minimum-Maximum</th>
<th>Actual Amount Minimum-Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA</td>
<td>270 mg (73.5)</td>
<td>181.5 mg (68)</td>
<td>180 – 360 mg</td>
<td>176 – 360 mg</td>
</tr>
<tr>
<td>DHA</td>
<td>180 mg (49.0)</td>
<td>115 mg (46.85)</td>
<td>120 – 240 mg</td>
<td>113 – 240 mg</td>
</tr>
</tbody>
</table>
Figure 10. EPA and DHA in over-the-counter omega-3 fatty acid supplements expressed as actual content as a percentage of label content. Key: A = Red Seal 1000 mg, B = Healtheries 1000 mg, C = Healtheries 1500 mg, D = Go Healthy 1500 mg, E = Good Health 1000 mg, F = Nature's Own 2000 mg, G = Go Healthy 1550 mg, H = Go Healthy 2000 mg, I = Nutra-Life 1500 mg, J = Sanderson 2000 mg.

3.2.4. Re-analyses of five fish oil supplements (analysis 2).

Given the large discrepancy between label and actual content of the omega-3 fatty acids, re-analyses were conducted by AsureQuality. This was done to check whether the results obtained are representative of analysed fish oil supplements sold over-the-counter in New Zealand. Eligibility criteria required different batch numbers to be assigned to establish whether inconsistencies are evident across batches or confined to a single batch.

Five supplements were included in the analysis on the basis that they contained notably less EPA and DHA than the amounts claimed on product labels (Table 16). Re-analyses revealed minimal variation between products. As previously demonstrated in the first analysis, EPA and DHA amounts per capsule ranged between 6 mg and 2 mg; respectively, despite label disclosures suggesting large differences based on total capsule content of fish oil (Table 17).
Table 16

Five Fish Oil Supplements Selected for Re-Analysis

<table>
<thead>
<tr>
<th>Brand</th>
<th>Supplement Description</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Brand C. Healtheries Fish Oil 1500 mg</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Brand D. Go Healthy Odourless Fish Oil 1500 mg</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Brand F. Nature’s Own Odourless Fish Oil 2000</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Brand G. Go Healthy Go Fish Oil 1550 mg</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Brand I. Nutra-Life Fish Oil + Vitamin D 1500 mg</td>
<td></td>
</tr>
</tbody>
</table>

The percentage difference between label and actual content of omega-3 fatty acids per fish oil capsule was calculated to enable a direct comparison between the results of the five fish oil supplements analysed twice due to large discrepancies (first analysis vs. second analysis) (Table 17). While there is a small percentage difference of EPA and DHA between analyses, the results support previous findings and thus confirm that some fish oil supplements do not contain the amounts of omega-3 fatty acids that are advertised by the company. Healtheries Fish Oil (brand C) contained 35.2% and 35.6% less EPA and DHA per fish oil capsule than the amounts stated on product labels. In comparison, Nature’s Own Fish Oil contained 51.7% and 52.1% less EPA and DHA than label content as revealed in the second analysis. These discrepancies are evident across batches and do not appear to be confined to a single batch.

Though the differences are not batch related there is variation between the two different batches of fish oil (Figure 11). Greater amounts of EPA can be seen in the second analysis, whereas greater amounts of DHA are present in the first analysis. These findings indicate biological variability between batches.
Table 17

<table>
<thead>
<tr>
<th>Fish Oil Supplement</th>
<th>EPA Label (mg)</th>
<th>EPA Actual (mg)</th>
<th>% Diff.\textsubscript{1}</th>
<th>% Diff.\textsubscript{2}</th>
<th>DHA Label (mg)</th>
<th>DHA Actual (mg)</th>
<th>% Diff.\textsubscript{1}</th>
<th>% Diff.\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Healtheries 1500 mg</td>
<td>270</td>
<td>175</td>
<td>-32.6</td>
<td>-35.2</td>
<td>180</td>
<td>116</td>
<td>-36.7</td>
<td>-35.6</td>
</tr>
<tr>
<td>D. Go Healthy 1500 mg</td>
<td>270</td>
<td>181</td>
<td>-34.4</td>
<td>-33</td>
<td>180</td>
<td>115</td>
<td>-35.6</td>
<td>-36</td>
</tr>
<tr>
<td>F. Natures Own 2000 mg</td>
<td>360</td>
<td>174</td>
<td>-51.1</td>
<td>-51.7</td>
<td>240</td>
<td>115</td>
<td>-52.5</td>
<td>-52.1</td>
</tr>
<tr>
<td>G. Go Healthy 1550 mg</td>
<td>275</td>
<td>176</td>
<td>-34.2</td>
<td>-36</td>
<td>185</td>
<td>115</td>
<td>-39</td>
<td>-37.8</td>
</tr>
<tr>
<td>I. Nutra-Life 1500 mg</td>
<td>270</td>
<td>175</td>
<td>-31.5</td>
<td>-35.2</td>
<td>180</td>
<td>117</td>
<td>-36.7</td>
<td>-35</td>
</tr>
</tbody>
</table>

*Note.*
- % Diff.\textsubscript{1} = First Analysis, % Diff.\textsubscript{2} = Second Analysis (Re-Analysis)
- Alphabetical letters refer to the letters used in Table 14

*Figure 11.* Percentage variability of omega-3 fatty acids between two different batches of commercial fish oil supplements.

### 3.2.5. Summary of fish oil supplement analyses.

Half of the fish oil supplements analysed contains considerably less omega-3 fatty acids per fish oil capsule than the amounts stated on product labels. Results of the Mann-Whitney test largely supported the observed percentage differences for individual fish oil
products. However, few supplements contain appropriate amounts of EPA and DHA per capsule and are consistent with product labels. Further reconciliation attempts to match label claims with clinical research trial findings and the amounts of omega-3 fatty acids found in the independent chemical analyses required a comparative analysis of dosages.

### 3.2.6. Dose comparison between research and over-the-counter supplements.

Past studies investigating the effectiveness of supplementation on mental health demonstrate that the doses used in research are typically much higher than the doses found in over-the-counter supplements (Rucklidge, Shaw and Harris, 2014). The implication for consumers is that the dose in over-the-counter supplements may be insufficient to produce a pharmacological effect. A daily dose comparison between clinical trials and over-the-counter fish oil supplements was conducted to ascertain whether the amount of omega-3 fatty acids present in fish oil capsules is sufficient to produce an effect. The clinical trials were deemed effective in reducing symptoms of mood disorders (i.e. major depression, perinatal depression, and bipolar disorder)(Table 10). The daily dose in over-the-counter supplements was determined by the recommended daily dose of three capsules and the maximum recommended daily dose of seven capsules for brain health and was calculated based on label and actual content of omega-3 fatty acids per fish oil capsule. A dose of three and seven fish oil capsules was used across all the fish oil supplements analysed in this study. With regards to the five fish oil supplements that were re-analysed, the amounts of omega-3 fatty acids per fish oil capsule from the first and second analysis were averaged to give an accurate representation of capsule content. For the remaining five supplements that were not re-analysed, the actual amounts were used to calculate the daily dose for brain health.

As demonstrated, the daily dose in research appears higher than in over-the-counter supplements, irrespective of the two clinical trials that administered a very high dose of omega-3 fatty acids (Figure 12). Five clinical trials used a daily dose comparable to over-the-counter supplements, of these, four administered pure EPA rather than a combination of both EPA and DHA. Further, the daily dose difference between research and over-the-counter supplements is greater when the actual amount of omega-3 fatty acids is considered, as half of the supplements contained considerably less EPA and DHA per capsule than label claims.
A two-tailed Mann-Whitney test was used to establish whether the doses in research supplements are significantly different from the labels and actual amounts of omega-3 fatty acids in over-the-counter supplements. With regards to the recommended dose of three fish oil capsules per day, statistical analyses revealed non-significant differences between research doses (Mdn 2000 mg) and doses in over-the-counter supplements based on the amounts of omega-3 fatty acids printed on product labels (Mdn 1350 mg), U(35), z -1.419, ns, r = -0.31 (2dp). However, the doses of omega-3 fatty acids used in research (Mdn 2000 mg) were significantly higher than the actual amounts of omega-3 fatty acids present in over-the-counter fish oil supplements (Mdn 885 mg), U(10), z -3.181, p = .001, r = -0.69 (2dp). These results indicates that three fish oil capsules may not provide appropriate amounts of omega-3 fatty acids to effectively ameliorate symptoms of major depression, perinatal depression and bipolar disorder.

Conversely, the dose of omega-3 fatty acids used in research appears more similar to the dose in over-the-counter supplements provided that seven fish oil capsules are consumed per day (Figure 13). Upon first inspection, over-the-counter supplements seem to contain higher daily doses of omega-3 fatty acids, based on their labeled content, than in research. This difference, in favour of over-the-counter supplements, disappears when daily doses in research are compared to the amounts of omega-3 fatty acids present in the fish oil supplements.

Consistent with recent observations, statistical analyses reveal that doses of omega-3 fatty acids used in the clinical research studies (Mdn 2000 mg) do not differ significantly from the label content of the fish oil products analysed (Mdn 3150 mg), U(30), z -1.774, ns, r = -0.39 (2dp). Similarly, the doses of omega-3 fatty acids used in clinical trials do not differ significantly from the amounts (Mdn 2065) of omega-3 fatty acids in seven over-the-counter fish oil supplements, U(36), z -1.343, ns, r = -0.29 (2dp). Irrespective of the two clinical trials that used very high doses, the findings suggest that seven over-the-counter fish oil capsules may provide appropriate amounts of omega-3 fatty acids per day to produce pharmacological effects consistent with that observed in clinical trials. Because the clinical research trials reviewed were non-determinant with respect to effective doses of omega-3 fatty acids for mood disorders, further analysis was needed to determine a dosage amount that would produce a psychological benefit to patients with such disorders.
Figure 12. Dose comparison between clinical trials that found a significant pharmacological effect and the label vs. actual amount of omega-3 fatty acids present in over-the-counter supplements based on the recommended daily intake of three fish oil capsules for brain health. Blue = Label content, Red = Actual content. * Pure EPA was used in these clinical trials, rather than a combination of both EPA and DHA. M = Monotherapy, A = Adjunctive therapy.

Figure 13. Dose comparison between clinical trials that found a significant pharmacological effect and the label vs. actual amount of omega-3 fatty acids present in over-the-counter supplements based on the maximum recommended daily intake of seven fish oil capsules for brain health. Blue = Label content, Red = Actual content. * Pure EPA was used in these clinical trials, as opposed to a combination of both EPA and DHA. M = Monotherapy, A = Adjunctive therapy.
3.2.7. Effective vs. non-effective clinical trials.

A dose comparison between effective clinical trials and clinical trials that were not effective (Table 11) was conducted to determine the appropriate daily dose necessary to significantly reduce symptoms of mood disorders. Clinical trials that found a significant reduction in symptoms were expected to have administered a higher daily dose of omega-3 fatty acids than clinical trials that were not effective. However, the graph revealed that the daily doses are comparable across all trials, irrespective of whether supplementation produced a significant benefit over placebo (Figure 14). All three clinical trials that used pure DHA were found not effective. The graph also showed that even high doses of omega-3 fatty acids received in combination with conventional medications was not an accurate predictor of a significant reduction in symptoms.

Results from the two-tailed Mann-Whitney test provide evidence that the similarities in doses used in effective and non-effective clinical trials. The doses that produced a significant reduction in symptoms (Mdn 2000) were not significantly different from the doses that did not have a positive pharmacological effect (Mdn 2000)(Table 18), U(59), Z -.100, ns, r = -0.021 (2dp). In conclusion, daily dose cannot be used to differentiate between clinical outcomes; therefore, questions remain surrounding the efficacy of fish oil supplements for the treatment of mood disorders.
Figure 14. Dose comparison between clinical trials that found a significant pharmacological effect and a non-significant effect of omega-3 fatty acids for mood disorders. *Pure EPA was used in these clinical trials. **Pure DHA was used in these clinical trials.

<table>
<thead>
<tr>
<th>Clinical Trial</th>
<th>Dose Median (SD)</th>
<th>Minimum-Maximum Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective</td>
<td>2000 mg (2797)</td>
<td>1000 – 9600 mg</td>
</tr>
<tr>
<td>Non-Effective</td>
<td>2000 mg (1562)</td>
<td>680 – 6000 mg</td>
</tr>
</tbody>
</table>

3.2.8. Summary of research and over-the-counter supplements dose comparison.

In summary, a daily dose of seven fish oil capsules purchased over-the-counter may provide sufficient amounts of omega-3 fatty acids to produce a beneficial effect for individuals with a mood disorder. However, the daily doses used in clinical trials that did not find a significant symptom reduction were similar to the daily doses used in clinical trials that found a significant benefit. This creates uncertainty surrounding the effectiveness of omega-3 fatty acids for the treatment and management of mood disorders. Thus far, this paper has explored omega-3 fatty acids in over-the-counter fish oil supplements. The next phase of the research was to assess the mercury content in the product capsules under review.
3.3. Amounts of Mercury in Top 10 Over-the-counter Fish Oil Supplements

3.3.1. Theoretical intakes of mercury following consumption of fish oil supplements.

Mercury is a neurotoxic heavy metal that occurs naturally in the environment and bioaccumulates in marine species, and consequently leads to high concentrations being consumed by humans. Given the potentially detrimental effects of mercury on the central nervous system, theoretical daily intakes were calculated for each fish oil supplement to ascertain whether the amount of mercury consumed (seven capsules, five capsules, three capsules or one capsule daily) would exceed recommended dietary guidelines. Theoretical worst-case intakes of mercury are based on the limit set by the TGA of 0.5 mg/kg and the *daily-recommended dosage* printed on product labels. The following calculations were performed for all 10 fish oil supplements:

1. Theoretical worst-case Hg dose = 

   \[
   \text{Weight of fish oil in capsule (mg)} \times \text{(No. of capsules consumed daily)} \times 0.5 \text{ (TGA limit of Hg mg/kg)}
   \]

   \[
   1000
   \]

   Theoretical worst-case Hg dose

2. 70 kg (Internationally accepted average body weight)

3. Theoretical worst-case Hg dose (mg/kg body weight/day)

The theoretical intake of mercury was dependent on the total number of capsules consumed daily. Individuals who consume more fish oil capsules will be exposed to greater amounts of mercury. Based on the daily omega-3 fatty acid recommendations printed on
product labels, 0.08 mg/kg bw/day is the maximum amount of mercury consumed from the fish oil supplements, provided that the limit set by the TGA and daily-recommended dose have not been exceeded (Table 19). In comparison to the PTDI for mercury of 0.23 mg/kg bw/day, theoretical intakes are much lower, especially when fewer than seven capsules are consumed per day. Similarly, 0.56 mg/kg bw/week is the maximum amount of mercury consumed per week as a result of fish oil supplementation, which is less than the PTWI of mercury – 1.6 mg/kg bw/week. As a consequence of these theoretical intakes, it would be expected that fish oil supplements purchased over-the-counter will be safer for humans than deep-water fish, even though fish contain many nutrients important to human health. To further investigate this theory required a chemical analysis of the fish oil supplements by a qualified laboratory.

<table>
<thead>
<tr>
<th>Table 19</th>
<th>Theoretical Intakes of Mercury from Fish Oil Supplements Based on the Limit Set by the TGA of 0.5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish Oil Supplement</td>
<td>Seven Capsules</td>
</tr>
<tr>
<td>A. Red Seal</td>
<td>0.05 (0.35)</td>
</tr>
<tr>
<td>B. Healtheries</td>
<td>0.05 (0.35)</td>
</tr>
<tr>
<td>C. Healtheries</td>
<td>0.08 (0.56)</td>
</tr>
<tr>
<td>D. Go Healthy</td>
<td>0.08 (0.56)</td>
</tr>
<tr>
<td>E. Good Health</td>
<td>0.05 (0.35)</td>
</tr>
<tr>
<td>F. Natures Own</td>
<td>0.1 (0.7)</td>
</tr>
<tr>
<td>G. Go Healthy</td>
<td>0.08 (0.56)</td>
</tr>
<tr>
<td>H. Go Healthy</td>
<td>0.1 (0.7)</td>
</tr>
<tr>
<td>I. Nutra-Life</td>
<td>0.08 (0.56)</td>
</tr>
<tr>
<td>J. Sanderson</td>
<td>0.1 (0.7)</td>
</tr>
</tbody>
</table>

Note.
- PTDI (Methylmercury) = 0.23 mg/kg bw/day, PTWI (Methylmercury) = 1.6 mg/kg bw/week
- Alphabetical letters refer to the letters used in Table 14

3.3.2. Amounts of mercury in fish oil supplements.

The measurement of mercury was performed by Hill Laboratories using ICP-MS to determine the amounts of mercury present in the fish oil supplements in order to ascertain
whether high doses of fish oil supplements is a safe alternative to conventional therapies. Despite theoretical calculations indicating small, yet notable, daily intakes of mercury, the analyses revealed amounts of mercury below the 0.010 mg/kg Limit of Detection (LoD) (Table 20). The risk of adverse health effects resulting from mercury intake is extremely low based on the finding that the top fish oil supplements contain mercury below the limit set by the TGA. The results, summarised below, also provide support for the companies testing processes involved in the removal of mercury and other heavy metals.

<table>
<thead>
<tr>
<th>Fish Oil Supplement</th>
<th>Mercury (mg/kg as rcvd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Red Seal Fish Oil 1000 mg</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>B. Healtheries Fish Oil 1000 mg</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>C. Healtheries Fish Oil 1500 mg</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>D. Go Healthy Odourless Fish Oil 1500 mg</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>E. Good Health Omega-3 Fish Oil 1000 mg</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>F. Nature’s Own Odourless Fish Oil 2000 mg</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>G. Go Healthy Go Fish Oil 1550 mg</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>H. Go Healthy Go Fish Oil 2000 mg</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>I. Nutra-Life Fish Oil + Vitamin D 1500 mg</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>J. Sanderson Fish Oil 2000 mg</td>
<td>&lt; 0.010</td>
</tr>
</tbody>
</table>

*Note. LoD – 0.010 mg/kg as rcvd*

3.3.3 Summary of the measurement of mercury in fish oil supplements.

The calculated theoretical worst-case intakes of mercury were indicative of a small, yet measurable, intake of mercury on a daily basis. Notwithstanding this finding, the amounts measured fall well below provisional tolerable intake guidelines. However, ICP-MS showed that fish oil supplements contain below the LoD of mercury and may therefore provide a safe alternative to fish and seafood consumption.
4. Discussion
This section is an overall discussion on the results from the literature review and independent chemical analyses related to the omega-3 fatty acids and mercury in the fish oil supplements. Other components of this discussion include a risk and benefit analysis between omega-3 fatty acids and mercury, strengths and limitations of this study, implications of these findings, and directions that future research might consider. This discussion is limited in scope to the mood disorders of depression and bipolar disorder.

4.1. Clinical Research Trials Included in This Study

All research that is reliant on subjective assessment may be biased to some extent. Assumptions made in research are not always correct and the clinical research trials included in this review likely contain biases with respect to either positive or negative results, which may have influenced publication decisions. Some of the biases may include: sample bias, response bias, publication bias, and perhaps funding bias; however, it is unknown which biases were present and to what extent for each study. This section addresses these biases.

Sample bias refers to a sample population in research that does not include all members of the target population. In the clinical trials reviewed in this study, sample bias may be the result of several factors. For example, research participants were frequently referred to the clinical trials by their respective medical practitioners. Basic assumptions are that the practitioners and researchers were competent in their diagnoses. A further assumption is that many participants had medical insurance and as a result were able to seek medical attention. The assumption regarding medical insurance is that individuals lacking such insurance were likely not included as participants through physician referral. In some clinical trials, recruitment was self-initiated versus a doctor referral. Individuals who volunteered themselves for these clinical research trials may have had different motivations, which influenced their participation, such as recognition for the need for treatment or an alternative treatment, an expectation or desire for mental health improvement, and a willingness to commit time and effort. Commitments of time and effort may have affected the number of participants.

Sample size bias results when an insufficient amount of research participants are involved in a study. The effect of sample size bias is that findings are not typically generalisable. Most of the clinical trials reviewed in this study had between 20 and 99
participants, which may be considered underpowered. The implication of a study being underpowered is that findings may not be generalisable to relevant population. Moreover, underpowered trials can result in negative outcomes because the sample size is simply too small to detect a significant difference between intervention and placebo groups. Important to consider is the possibility that an underpowered study can result in bidirectional skews; that is, treatment outcomes can be positive or negative.

Another form of sample bias is response bias. This type of bias occurs when the self-reporting participant conveys responses to the researcher that are inflated and influenced by what they believe the researcher wants to hear and/or what the participant would like to believe. Response bias often occurs when participants are less than truthful with researchers. The implication of response bias is that results may be skewed.

Each of the bias types presented are methodological shortcomings, which can culminate in publication bias either individually or as an aggregated whole. Publication bias occurs when researchers focus on publishing statistically significant outcomes rather than null findings. Possible reasons for publication bias are many, including the desire of the researchers to become published, to satisfy expected results of research funding entities, to minimise attention directed at errors in research methodology, or even to reduce exposure of negative outcomes related to the subject matter of the clinical trials. For example, pharmaceutical research on antidepressant drugs selectively reported findings favorable to the company, while not reporting findings that were adverse to company interests. Publication bias is well documented in research, especially as it pertains to antidepressant medication as discussed in Pigott et al. (2010), Kirsch and Antonuccio, (2002), Ioannidis (2008), and Kirsch et al. (2008). The meta-analyses conducted on the clinical trials included in this research study indicated there was publication bias, thus making it difficult to draw meaningful conclusions regarding the efficacy of fish oil supplementation for the treatment of mood disorders (Grosso et al., 2014 & Lin & Su, 2007). To summarise, research bias is inherent in all research endeavors. Yet, it is publication bias, which represents perhaps one of the most problematic bias types because agenda often trumps public knowledge through the use of selective reporting, obfuscation, and the undue influence of research funding entities. Notwithstanding the bias associated with research, excluding publication bias, the clinical trial researchers implemented a variety of methods to control for bias, such as double blind experiments, participant randomisation, placebo controlled design, and the
use of eligibility and exclusion criteria. The next section is a discussion on the results from independent laboratory tests performed on 10 of the most popular fish oil supplements sold over-the-counter in New Zealand.

4.2. Omega-3 Fatty Acids

This study explored the potential use of over-the-counter fish oil supplements in the management and/or treatment of depression, perinatal depression and bipolar disorder. To address this, three aims were investigated across this section. The first aim was to determine the most popular fish oil supplements sold in New Zealand stores. The second aim was to measure the fatty acid composition and compare the amounts in the fish oil capsules to the amounts stated on product labels. The third aim was to compare the doses in over-the-counter fish oil supplements to the doses used in research that were effective in the amelioration of depressed mood. This final comparison was based on the actual and label content of omega-3 fatty acids in over-the-counter fish oil supplements to provide an indication as to whether the amounts in the capsules are sufficient to exert a therapeutic benefit.

In this section, the fatty acid composition in over-the-counter fish oil supplements will be described and discussed in relation to labeled content. A further discussion will focus on the doses used in both research and commercial settings. This discussion begins with the products labels and actual amounts of omega-3 fatty acids in over-the-counter supplements.

4.2.1. Label vs. actual content of omega-3 fatty acids in fish oil supplements.

The actual amount of EPA and DHA per capsule in the majority of the over-the-counter fish oil supplements analysed in this research was less than the amount stated on the product labels. Considerable differences were observed for half of the supplements, which contained between 48 – 69% of the claimed fatty acid content. Conversely, the remaining fish oil products analysed contained amounts that were similar to label content, thus demonstrating a notable divide between the different brands of supplements. All the fish oil supplements included in the study contained higher amounts of EPA than DHA. This is a promising finding considering that fish oil preparations with mainly EPA are recognised
as a predictor of treatment efficacy in clinical research trials. Yet, the effectiveness will be
dependent on the actual amount in the products rather than the claimed amount.

While the evidence indicated a clear distinction between the labels and the actual
content of EPA and DHA within the capsules analysed, a non-parametric, two-tailed Mann-
Whitney test revealed that the amounts of DHA per capsule were significantly less (p =
0.008) than the amounts stated on the labels but the amounts of EPA were not statistically
different to label content. However, in this case the individual percentage differences for
each fish oil product provided a more accurate indication of the differences between the
labels and the actual amounts of EPA and DHA per capsule. These observable discrepancies
were replicated in a second analysis of five of the products and were confirmed to be
representative of the top 10 fish oil supplements sold in New Zealand stores. The
significance of this finding is that the difference was not confined to a single batch,
supporting the study conducted by Albert et al. (2015), which found that most of the fish oil
supplements analysed contained less than 67% of the amounts stated on the labels.

While the results from this research are consistent with the results of Albert et al.
(2015), their study sample size was much larger with 32 products analysed, and the
individual percentage differences were more pronounced across a greater number of
supplements. Since the brands of fish oil products analysed in Albert et al. (2015) were not
disclosed to the public for reasons unknown, a direct comparison between the products
analysed therein and those in this research could not knowingly be conducted. However, it
is possible that the same products were analysed in both studies. Overall, most fish oil
supplements sold over-the-counter in New Zealand do not contain the amounts of omega-3
fatty acids that are stated on the labels. Therefore, an argument can be made that these fish
oil supplements are not compliant with the product labelling laws under dietary supplement
regulations, requiring the amounts of active and inactive ingredients contained within
products to be printed on the labels.

However, considering that fish oil is vulnerable to oxidation due to the large number
of double bonds in the fatty acid chain (Albert et al., 2015)(which can undergo an oxidative
ultra violet catalyst free radical chain reaction), the amounts of EPA and DHA in the
supplements may have degraded during the manufacturing and encapsulation process as a
consequence of exposure to oxygen. The presence of light and heat accelerates the
oxidation process and causes fatty acids to be replaced with a combination of different
oxidative markers leading to reduced levels in over-the-counter fish oil supplements (Anonymous, 2007). The unstable nature of fish oil may explain the large discrepancy between labels and actual content of fatty acids in the supplements purchased and analysed. Albert et al. (2015) provided evidence to support this explanation and reported that most of the supplements analysed exceeded recommended oxidation levels, in particular the peroxide value and anisidine value, which are combined to give an estimate of the total oxidation value. These findings appear consistent with a survey of retail fish oil supplements in New Zealand that found 4 of 29 products exceeded recommended total oxidation levels, though the information regarding the specific oxidation markers and methodology was limited (Anonymous, 2007). Antioxidants are known to reduce oxidative damage and are therefore added to the fish oil capsules to inhibit further oxidation and prolong the shelf life of the supplement. A study measuring the peroxide values revealed that fish oil was more stable when tocopherol (vitamin E – 0.05%) was added and improved the oxidative stability by 22% in fish oil intended for domestic use (Pak, 2005). This finding suggests that antioxidants used in the preparation of fish oils are limited in their ability to prevent oxidation, but depending on the concentration, may extend the storage time leading to reduced adverse health effects that may be caused by oxidative damage.

There have been few human intervention studies to date that have examined biological mechanisms of oxidised fish oil. However, there is evidence to demonstrate involvement of lipid peroxidation in the pathogenesis of human disease (Albert et al., 2013). Based on the available evidence, researchers have postulated that ingested omega-3 fatty acid peroxides may result in a complex cascade of events leading to lipid membrane peroxidation and oxidative stress. Reduced fatty acid composition in cell membranes alters the fluidity of the membrane, protein transport, and cellular signaling, which have all been implicated in the pathophysiology of major depressive disorder, perinatal depression, and bipolar disorder. Research has shown that oxidative stress enhances the activation of neural pathways involved in the production of proinflammatory cytokines (Albert et al., 2013). Prolonged exposure to proinflammatory cytokines has been found to produce changes in neurotransmitter systems and to adversely interact with neuroendocrine functions and neural plasticity; processes that are involved in mood disorders (Felger & Lotrich, 2013; Miller et al., 2009). These harmful effects of lipid peroxides were produced as a consequence of administering high doses of oxidised fish oil when tested on animals than
humans would normally consume (Albert et al., 2013). The one human trial that investigated the health effects of oxidised and nonoxidised fish oil reported there was no difference in in vivo markers of oxidative stress, lipid peroxidation or inflammation (Ottestad et al., 2012). Limitations to the study included the short intervention (7 weeks) and the failure to measure specific inflammatory markers such as prostaglandins and cytokines that are important to health outcomes. While the evidence indicates that short term exposure to oxidised fish oil may not cause adverse health effects, individuals who purchase over-the-counter fish oil supplements tend to be regular consumers and continue to consume them over the long term in order to achieve a pharmacological benefit. Further research is clearly needed to evaluate the effects of chronic exposure to oxidised fish oils.

Based on the evidence that fish oil supplements sold over-the-counter in New Zealand are highly oxidised leading to the degradation of fatty acids (Albert et al., 2015), it remains possible that product labels were an accurate reflection of the amounts contained in the products at the time of encapsulation. In other words, the large discrepancies may be due to oxidation rather than an intentional decision made by the manufacturing company to reduce costs. Importantly, oxidised fish oil might not cause harm to the consumer, but may be less effective in the treatment of disease due to the degradation of omega-3 fatty acids.

Furthermore, the fatty acids in fish oil supplements are presented as either free fatty acids, ethyl esters, or re-esterified triglycerides. However, based on the vague ingredient information printed on certain product labels there is clearly concern that the supplements analysed contained fatty acids in the form of phospholipids. Thus it was unclear whether the analytical method (AOAC 991.39) carried out in this research measured all the fatty acids present in the capsules analysed. There is a possibility that the large differences in fatty acids found between the labels and the actual composition in fish oil supplements may have been attributed to the fatty acids not known to be present. AsureQuality, the GLP accredited laboratory used in this study, confirmed that the method used measured both EPA and DHA in the form of triglycerides and phospholipids, thus confirming that the results obtained were correct. Overall, most of the analysed fish oil supplements sold over-the-counter in New Zealand do not contain the amounts of omega-3 fatty acids that are advertised on the labels. Further analyses were conducted to compare the doses used in research and the doses provided in over-the-counter fish oil supplements.
4.2.2. Dose comparison between research and over-the-counter supplements.

The median daily doses in research supplements were not found to be greater than in over-the-counter supplements based on the labels and actual content of fatty acids measured. These findings were confirmed through a non-parametric, two-tailed Mann-Whitney test that revealed, overall, non-significant differences between the research and over-the-counter doses. This conclusion was based on the recommended minimum dose of three fish oil capsules with a maximum dose of seven fish oil capsules for brain health.

4.2.2.1. Differences between label and actual chemical composition of three capsules.

Based on label analysis, the doses of omega-3 fatty acids in clinical trials were statistically higher ($p = 0.001$) in concentration than the capsules analysed by AsureQuality. In accordance with the minimum recommended dosage beneficial for brain health printed on the product labels, a multiplier of three was used for each of the 10 product capsules analysed. It was found that three capsules did not contain high enough dose of omega-3 fatty acids to be effective. However, it is possible that the four fish oil supplements analysed in this research whose concentration levels were true to label may provide doses that are comparable to those used in the clinical research cited in the literature review.

4.2.2.2. Differences between label and actual chemical composition of seven capsules.

Though there are differences between the label and the actual content in the products, consumption of seven fish oil capsules provides sufficient amounts of essential nutrients to produce a therapeutic benefit as indicated in clinical research. The aggregate amounts of omega-3 fatty acids in seven capsules are high enough to offset the label discrepancies. Thus, a non-parametric, two-tailed Mann-Whitney test revealed no significant difference between the actual content in the fish oil supplements using seven capsules analysed by AsureQuality from those in the clinical research trials.

4.2.2.3. Inferences from the comparative analysis.

The most important implication of these revelations may be the ability to generalise the findings from the clinical research trials regarding the concentration levels of omega-3
fatty acids to fish oil supplements sold over-the-counter in New Zealand as it pertains to benefits derived from taking the supplements according to product recommendations. That is, if the patient takes seven capsules they should have a benefit. Whereas, if the minimum dosage of three capsules is taken then the amounts of omega-3 fatty acids may not be sufficient to produce a pharmacological effect. For depressed adult populations, this means that fish oil supplements available in supermarkets, health food stores and pharmacies may produce a therapeutic benefit in patients with major depression and/or bipolar disorder. While three fish oil capsules may not provide sufficient amounts of omega-3 fatty acids to ameliorate symptoms of depression, the supplements analysed in this research that were true to label may achieve a therapeutic benefit at the minimum recommended dosage. Conversely, regardless of whether the labels were reflective of the actual amounts of omega-3 fatty acids contained in each capsule, the maximum recommended dosage of seven fish oil capsules has the potential to improve depressive symptoms, but may cause minor adverse events that are predominantly gastrointestinal in nature (i.e. nausea, diarrhoea, indigestion, constipation, and drowsiness). Apart from these side effects, high doses of fish oil was generally well tolerated in clinical research studies, indicating that over-the-counter supplements may represent a novel approach in the management and/or treatment of these complex and multifaceted disorders.

Several mechanisms have been proposed to explain the therapeutic effects of fish oil containing optimal fatty acid concentrations relevant to mood disorders (see section 1.6). While these proposed mechanisms demonstrate important roles for both EPA and DHA in major depression and bipolar disorder, evidence from clinical intervention trials revealed that pure DHA was not effective in reducing depressive symptoms, yet fish oil formulations with mainly EPA improved clinical outcomes and enhanced treatment response. As compared to the amounts of DHA in the fish oil supplements analysed, the amounts of EPA were greater regardless of whether the content was consistent with label claims. Thus, providing that a minimum of three fish oil capsules is consumed daily, over-the-counter supplements may ameliorate symptoms of the investigated mood disorders either as monotherapy or adjunctive therapy with standard medications. However, the clinical research studies reviewed revealed that supplementation with omega-3 fatty acids augments the efficacy of antidepressants medication. Depressed populations who ingest over-the-counter fish oil supplements may experience a greater reduction in symptoms
when taken in combination with prescribed antidepressants. Further research is needed to understand the precise mechanisms of action by which omega-3 fatty acids enhance the efficacy of antidepressant medications. Remaining still is the question of what is an effective dose of omega-3 fatty acids beneficial for the treatment of mood disorders.

**4.2.3. A comparison between effective and non-effective doses in research.**

The effective doses of omega-3 fatty acids used in clinical research for the treatment of depression and bipolar disorder were found to be ineffective in the amelioration of depressive symptoms and mania in other research trials. Results from a non-parametric, two-tailed Mann-Whitney test confirmed observable similarities related to the amounts of omega-3 fatty acids used in the clinical trials as reported in the literature reviewed. The results indicate non-significant differences between the doses of EPA and/or DHA that reduced mood symptoms and doses that did not produce significant benefits over placebo. Despite strong evidence supporting the mood regulation effects of omega-3 fatty acids, the most important implication of this non-significant difference in dosages is that the findings challenge the efficacy of fish oil supplementation in depressed populations and raises questions as to whether they should be recommended as an effective treatment for all patients with depression and/or bipolar disorder.

Based on the literature, there are several plausible explanations for the differential therapeutic outcomes in patients with a clinical mood disorder. The first relates to the etiology of depression and bipolar disorder, which is believed to involve a complex interaction between genetic predispositions and environmental factors but remains to be fully elucidated due to the multifaceted nature of the conditions (Leung & Kaplan, 2009; Levant, 2011; Nestler et al., 2002). A number of social, psychological, and biological factors have also been identified to increase the risk for mood disorders (Leung & Kaplan, 2009). To be sure, the exact causes of mood disorders are complicated.

Though omega-3 fatty acids may be beneficial for a subgroup of depressed populations, future clinical research should more diligently investigate the causation of the mood disorders prior to commencing treatment. The reason this is important is to ensure the appropriate treatment for the individual. Treatment must be tailored for each patient to achieve optimal therapeutic outcomes.
Once the cause of the mood disorder has been identified, a treatment regime including omega-3 fatty acids might be implemented. There are some cases for which omega-3 dietary deficiencies have been suggested as a cause of mood disorders and were treated effectively with supplements containing omega-3 fatty acids. The literature reviewed revealed comparable amounts of omega-3 fatty acids administered to patients and produced different results. The differences in clinical outcomes may be correlated to compositional baseline differences of EPA and/or DHA in each patient prior to the ingestion of supplements. This interpretation is supported by evidence where chronic dietary deficiency in omega-3 fatty acids has been found to trigger neural changes leading to the mood disorders. Hence, depression caused by the adverse effects of a dietary deficiency may be effectively treated with omega-3 fatty acid supplements; whereas, patients not deficient in omega-3 fatty acids may show less improvement. However, deficiency in omega-3 fatty acids may have another underlying variable—absorption.

It has been shown that different forms of omega-3 fatty acids are absorbed at different rates. Ethyl esters, the least well absorbed of the fatty acids, were used in some of the clinical research trials reviewed; this may explain the different therapeutic outcomes produced by similar doses of different omega-3 fatty acid forms. Important to consider is the fact that some over-the-counter supplements contain omega-3 fatty acids in the form of ethyl esters, thereby indicating that these supplements may be less beneficial in the treatment of mood disorders. According to Lawson and Hughes (1988) the absorption of ethyl esters is improved when consumed with a high fat meal due to the stimulation of pancreatic enzymes that aid digestion and encourage intestinal absorption. Future clinical research trials should examine the efficacy of omega-3 fatty acids as ethyl esters and triglycerides paired with a high fat meal in the treatment of major depression and bipolar disorder. Genetic factors may also play a role in absorption.

Inborn errors of metabolism might adversely affect absorption of vital nutrients. These inherited conditions can increase the risk of a mood disorder; however, research has shown that these genetic predispositions may be treated with supplementation with high doses of specific micronutrients and essential fatty acids. Due to the poor bioavailability of ethyl esters, omega-3 fatty acids in the form of triglycerides may be more beneficial for patients with this genetic disorder. These metabolic imbalances may explain why some patients in clinical trials responded to treatment but not others, which provides support for the fact
that supplementation with omega-3 fatty acids may not be an effective treatment for all patients with depression and bipolar disorder. A lack of standardisation in the manufacture of fish oil supplements results in differing amounts of ingredients contained in the capsules than sometimes stated on product labels.

The over-the-counter fish oil supplements analysed in this study and in the study conducted by Albert et al. (2015) contained amounts of omega-3 fatty acids that were less than the amounts stated on product labels. Fish oil is prone to oxidation even with the addition of antioxidants, thus it is possible that the fish oil capsules administered to patients in clinical trials deemed to be not effective in the treatment of the investigated mood disorders may have contained less omega-3 fatty acids than the anticipated dose printed in peer reviewed journal articles. Because no independent laboratory analysis was conducted on the fish oil supplements in the clinical trials and instead the researchers relied on manufacturer disclosure, the exact amounts remain unknown. Therefore, it is difficult to conclusively state whether the dosages found to be effective in clinical trials are accurate. Due to the variation in amounts of omega-3 fatty acids between over-the-counter fish oil products, generalisations cannot be made regarding the exact dosage that produces a benefit. The patient’s mindset also influences response to treatment.

Research has shown that patients who believe the treatment will provide therapeutic benefits have a higher probability of experiencing an improvement (Kirsch, 2010). This placebo effect, as it is known, is widely recognised in the research community as a viable factor in treatment found to be beneficial. People with mood disorders who use over-the-counter fish oil supplements are more likely to realise benefits when their expectations for improvement are high. Side effects associated with fish oil supplements might indicate to the patient that the supplements are working which in turn may improve the effectiveness of the product leading to reductions in mood symptoms, vis-à-vis, the placebo effect.

There is some conjecture surrounding the use of olive oil as placebo because it is rich in polyunsaturated fatty acids (e.g. oleic acid). Therefore, a high intake of olive oil may lead to an increased production of omega-3 fatty acids from which oleamide, a psychoactive lipid, is synthesised in mammals (Puri, 2000). It is therefore possible that the placebo might have a positive psychological mood effect in patients with a diagnosed mood disorder. However, of the 22 clinical research trials reviewed, six studies used olive oil as placebo; four of these studies found a significant benefit of omega-3 fatty acids over placebo. In this
study, the choice of placebo does not appear to influence study outcome, however, this deserves further investigation and could form the basis of a future study.

In conclusion, the clinical research trials reviewed provide evidence in support of fish oil supplements may not be an effective treatment for all patients with major depression and bipolar disorder. These are complex and multifaceted disorders that are not fully understood in terms of their etiology, thus a single nutrient cannot be a miracle cure for all populations with a mood disorder. The effectiveness of over-the-counter fish oil supplements may vary across depressed adult populations depending on individual circumstances. However, factors that may influence the efficacy of over-the-counter fish oil supplements relate to the chemical form that omega-3 fatty acids are presented in, the daily dosage that is consumed, the metabolic function of the consumer and their expectations for improvement. More research is clearly needed to ascertain the predictors of treatment efficacy. A major concern prior to this research was the potential for exposure to methylmercury resulting from ingestion of fish oil supplements.

4.3. Discussion on Mercury

Fish contain many nutrients that are essential to human health including omega-3 fatty acids. However, due to the release of mercury from natural and anthropogenic sources to the environment, fish is a major source of human exposure to dietary mercury. Since mercury is a neurotoxic heavy metal that causes detrimental effects to the developing brain and central nervous system. This study explored the potential risk of mercury associated with fish oil supplements sold over-the-counter in New Zealand. To address this concern, the main research aim was to determine the amounts of mercury present in the fish oil capsules analysed in this study in order to explore whether over-the-counter fish oil supplements are a safe alternative for the treatment of major depressive disorders, perinatal depression and bipolar disorder.

In this discussion, the measurement and determination of mercury in the over-the-counter fish oil supplements analysed will be described and discussed in relation to the analytical methodology performed. A discussion of the implications of these results for the general population and individuals with a clinical mood disorder will follow. The discussion begins with a review of findings, based on correspondence with the product manufacturers in this study, regarding the detectable amounts of mercury allowed per fish oil capsule.
4.3.1. Theoretical vs. actual amounts of mercury in over-the-counter fish oil supplements.

Theoretically, fish oil supplements sold over-the-counter in New Zealand may contain small, yet detectable, amounts of methylmercury. Theoretical calculations were based on the limit of mercury (0.5 mg/kg) set by the TGA and the daily-recommended dose of omega-3 fatty acids claimed to be beneficial on product labels. The maximum combined amount of methylmercury in seven fish oil capsules is much lower than the PTDI and PTWI, therefore, dietary exposure to methylmercury is even lower when fewer than seven fish oil capsules are consumed per day. Alternatively, those who exceed the recommended daily dose of fish oil will be exposed to higher methylmercury doses. However, the limit of mercury implemented by the regulatory agency, Therapeutic Goods and Administration, is the maximum amount of mercury that is considered acceptable for human consumption per fish oil capsule. Hence, it is possible that the amounts of mercury in over-the-counter fish oil supplements may be minimal. Importantly, mercury is released into the environment from natural and anthropogenic sources and exists in various forms – organic, inorganic, and elemental. This suggests that humans are exposed repeatedly to mercury in different forms throughout the life course. The theoretical worst-case intakes simply reflect one source by which humans are exposed to mercury and while they appear small, the cumulative effect may exceed the threshold and produce a neurotoxic effect that is detrimental to central nervous system functioning. However, based on these theoretical worst-case intakes, the expectation is that the over-the-counter fish oil supplements analysed in this study will contain amounts of methylmercury that are below the provisional tolerable thresholds that are regarded as safe for human health.

Despite theoretical calculations indicating a small risk of exposure to methylmercury, the results from the ICP-MS analysis performed at Hill Laboratories revealed that the amounts of mercury in the fish oil supplements analysed were below the 0.010 mg/kg LoD. In other words, the methylmercury in the fish oil products analysed was not detected. Thus, there is minimal risk of mercury toxicity as a result of over-the-counter fish oil supplements. This is particularly important when high doses of fish oil are consumed to achieve a possible pharmacological benefit for mood disorders.
Despite fish containing an abundant of nutrients important for human health, this finding indicates that over-the-counter fish oil supplements may provide a safer alternative to fish and seafood consumption, particularly during critical periods of development (i.e. pregnancy and early childhood). The absence of mercury in the fish oil supplements analysed provides support for their use in the management and treatment of major depression, perinatal depression, and bipolar disorder. However, mercury is not the only environmental contaminant present in fish oil.

Research has demonstrated that heavy metals (e.g. mercury, lead, and cadmium), dioxins, and polychlorinated biphenyls bioaccumulate in the aquatic food chain attaining high concentrations in large predatory fish. Thus, it is possible that the fish oil supplements analysed in this study may have contained measurable amounts of these environmental contaminants; however, it was beyond the scope of the current study to analyse the fish oil capsules for each impurity that may be present. However, based on the discrepancy between the theoretical amounts of methylmercury and the actual amounts provided by Hill Laboratories, it is conceivable that the concentrations of other contaminants would also be negligible. This assertion is based on the premise that companies that manufacture fish oil supplements perform comprehensive testing and processing of crude oil to ensure the final fish oil product is high quality and safe from environmental pollutants.

These environmental contaminants are removed from the crude fish oil during the molecular distillation process, thus, because the fish oil supplements analysed in this study contained no traceable amounts of methylmercury, it is expected that other contaminants would also be successfully removed. Therefore, the results from this study provide support for the companies’ purification processes involved in the removal of pollutants from fish oil used in over-the-counter products. This has important implications for human health based on the evidence that chronic exposure to a cocktail of contaminants may cause detrimental health outcomes, especially during prenatal and early postnatal development.

It is important to remember that although the fish oil supplements analysed do not contain measurable amounts of methylmercury, there is a high chance that individuals might also consume large predatory fish in order to achieve optimal therapeutic outcomes, which may increase the risk for mercury toxicity. Dietary exposure to methylmercury reflects only one source by which humans are exposed, meaning that repeated exposure from multiple sources over the life course may lead to the accumulation in adipose tissue.
and result in adverse neurotoxic effects. Mercury exposure should therefore be monitored carefully.

In conclusion, the most popular fish oil supplements sold over-the-counter in New Zealand pose no risk of methylmercury toxicity, which may be explained by the extensive testing and purification processes that are performed prior to encapsulation. The removal of environmental contaminants means that fish oil supplements may provide a safe alternative to standard medications in the treatment of mood disorders, provided that they contain sufficient amounts of omega-3 fatty acids to produce a positive pharmacological effect. Further research is needed to replicate these preliminary findings in order to ascertain whether the results of this study can be generalised to the remaining fish oil supplements sold over-the-counter in supermarkets, health food stores and pharmacies in New Zealand. Intuitively there is no risk of mercury toxicity associated with fish oil supplements. However, a risk and benefit analysis is still warranted.

4.4. Risk and Benefit Analysis

Fish is the major source of human exposure to methylmercury – a neurotoxin that can interfere with central nervous system function in adult populations and cause adverse neurological effects in the developing fetus and young infant. Research has shown that chronic low level exposure to methylmercury during prenatal development contributes to decreased birth weight, poor cognitive function and developmental delays, whereas in adulthood, chronic exposure has the potential to disrupt neurocognitive function such as attention, fine motor function, and verbal learning and memory (Yokoo et al., 2003). These negative effects are due to the fact that methylmercury mimics methionine and therefore crosses the blood brain barrier attaining a toxicological significant concentration in the brain. Provided that the fish oil products analysed in this study contained detectable amounts of methylmercury per capsule, fish oil supplementation would reflect one source by which humans would be exposed to low levels of methylmercury over prolonged periods and in theory, may have the potential to cause the aforementioned adverse effects. However, because the analysed fish oil products do not contain traceable amounts of methylmercury, there is minimal risk to human health as a consequence of fish oil supplementation. Thus, the neurological benefits of omega-3 fatty acids for mood disorders clearly outweighs the low risk associated with methylmercury, regardless of whether fish oil
capsules contain amounts of essential fatty acids that are consistent with product labels. However, based on the literature reviewed, a minimum of three and maximum of seven over-the-counter fish oil capsules may provide a dose that is sufficient to achieve positive pharmacological results. Therefore, over-the-counter fish oil supplements have the potential to ameliorate symptoms of depression and bipolar disorder. However, the efficacy of treatment will be dependent on the individual circumstances of the depressed individual based on the premise that a single cause for these mood disorders does not exist, and thus, there is no panacea for treatment.

In conclusion, over-the-counter fish oil supplements may provide therapeutic benefits in patients with major depression and bipolar disorder and pose minimal risk to human health, thus the benefits outweigh the potential risk in relation to methylmercury. These preliminary findings are promising; however, further research is needed to determine which populations will respond to treatment and the predictors that enhance clinical outcomes. The ultimate goal, is personalised treatment. As with all scientific research there are both strengths and limitations to be found. A discussion of those attributes follows.

4.5. Strengths and Limitations

4.5.1. Omega-3 fatty acid study strengths.

There are several strengths of this study on omega-3 fatty acids and treatment for mood disorders. The first of these strengths relates to the large number of randomised double blind, placebo controlled trials that have been reviewed. This study design is considered the gold standard trial for evaluating the effectiveness of medical interventions. Due to the many randomised controlled trials that are published in the scientific literature, only these study types were included for analysis in this study. The reason for inclusion was because the randomised placebo controlled trials provided a more accurate indication of the efficacy of omega-3 fatty acid supplements and the dose that is required to produce a therapeutic benefit in patients with a mood disorder. Thus, causation between the intervention and the reported clinical outcomes can be inferred with a degree of confidence. The results from clinical research can therefore be generalised to over-the-counter fish oil supplements available in New Zealand, thereby representing a novel approach to the treatment of mental illness.
A further strength of this study was the inclusion of the 10 most popular over-the-counter fish oil supplements sold in New Zealand at the time of the search. These fish oil products were selected based on the annual sales data provided by Foodstuffs New Zealand Ltd and Green Cross Health Ltd. In other words, the fish oil supplements analysed were not selected at random, but through a systematic search of the most common products purchased in supermarkets and pharmacies across New Zealand. Thus, the results from the independent analyses will be of relevance to a large proportion of individuals who consume fish oil supplements on a regular basis, but not those who do not purchase the brands analysed in this study.

An important strength of this study is that the fish oil supplements selected were analysed by AsureQuality, a New Zealand GLP accredited laboratory approved by IANZ. The reason this accreditation is important is because all chemical laboratories registered with the IANZ are compliant with chemical testing and calibration criteria, which means that the results obtained from the omega-3 fatty acid analyses are correct and therefore reliable. The implication is that the results provided by AsureQuality can be confidently disclosed to the public without concern for their repercussions from companies that manufacture the fish oil products analysed in this study.

A separate, but related, strength of this study is the replication of the initial results that revealed large inconsistencies between the label and actual content of omega-3 fatty acids in half of the supplements analysed. The fish oil products that contained much less omega-3 fatty acids than their label claims were repurchased with a different batch number to ascertain whether the discrepancies were batch related or consistent across batches. Since the results were replicated in the second analysis, this meant the differences were correct and may be generalised to the remaining fish oil products that were not reanalysed. Overall, these findings can be viewed as being accurate and reliable, that said, limitations existed.

4.5.2. Omega-3 fatty acid study limitations.

While there are many strengths to this study on omega-3 fatty acids and mood disorders, there are a number of limitations that have been identified. The first of these limitations is the considerable heterogeneity across the clinical research trials reviewed. Each of the clinical trials included in the analysis differed in terms of their methodology (e.g.
daily dose, composition of fatty acids, placebo administered, trial duration, primary outcome measures, and the mood disorder) and reported clinical outcomes. Thus, the general consensus is that a definitive conclusion cannot be drawn in relation to the efficacy of omega-3 fatty acid treatment and the dose required to produce a therapeutic benefit in patients with depression and bipolar disorder. However, the results from these research trials do provide an indication of the predictors that influence treatment response.

A further limitation of this study relates to the selection of the fish oil supplements included for analysis. Prior to the selection, formal letters were sent to the chief executive officer or managing director of Foodstuffs New Zealand Ltd, Green Cross Health Ltd, Progressive Enterprises Ltd and Health 2000 Retail Ltd, to which the former two companies responded and provided annual sales data for fish oil dietary supplements sold across their stores in New Zealand. However, there was no response to the letters from the latter two companies, which meant the fish oil products selected were only the top 10 fish oil supplements sold in stores owned by Foodstuffs New Zealand Ltd and Green Cross Health Ltd, rather than being the top 10 fish oil supplements sold in New Zealand. Though it is possible that the results from this analysis are applicable to a broad range of fish oil products, as reflected by the study conducted by Albert et al. (2015) that analysed 32 supplements and found large differences between their labels and actual content of fatty acids, the results are specific to the fish oil supplements analysed in this study. This may limit the ability to confidently generalise the results to other fish oil supplements available over-the-counter in New Zealand.

A wide range of over-the-counter fish oil supplements were analysed in this study, however, the sample was relatively small due to the monetary constraints of a Master’s thesis. The study sample comprised only 10 fish oil products, which is a limitation to the study, as the analysis did not include all fish oil supplements available on the New Zealand market. Though it is possible that the products analysed in this study are representative of most fish oil supplements sold over-the-counter, based on the findings reported by Albert et al. (2015), but clearly further research is needed.

Again, due to monetary constraints, AsureQuality measured the capsules from each fish oil product in duplicate, rather than the preferred analytical method of being measured in triplicate. This preferred method is to better determine the biological variation between fish oil capsules derived from a single fish oil product in order to accurately report the
omega-3 fatty acid content per fish oil supplement. Though the capsules were measured in duplicate, the analysis results will still be correct.

4.5.3. Mercury study strengths.

A strength of this mercury analysis was that the fish oil supplements were analysed for mercury at Hill Laboratories, an IANZ accredited laboratory, which gives regulatory credibility to the findings. Since all accredited laboratories are compliant with chemical testing and calibration protocols, the results can be viewed as an accurate reflection of the mercury content in the analysed fish oil products. Thus, the results are from a reliable source and can be used to calculate exposure to mercury from over-the-counter fish oil supplements.

4.5.4. Mercury study limitations.

There are two limitations involved in this fish oil supplement analysis for methylmercury. The first limitation is that the fish oil products included in this study were only analysed for mercury, but not other heavy metals and environmental pollutants that may have been present in the capsules. Though it was beyond the scope of this study to analyse the fish oil supplements for each environmental contaminant, a definitive conclusion regarding their long-term safety cannot be drawn from this study alone. In other words, the results from this study are specific to the risk of exposure to methylmercury resulting from over-the-counter fish oil supplements purchased over-the-counter in New Zealand.

The second limitation to this study is that the fish oil supplements were analysed only once and were not reanalysed with a different batch. This is an important limitation because it cannot be determined whether the results of the mercury analysis are confined to a single batch or consistent across batches. Due to it being unknown, it is possible that other batches of fish oil may contain detectable amounts of mercury per fish oil capsule and thus, pose a risk to human health. However, since all fish oil products analysed did not contain traceable amounts of mercury, it would be unexpected to find amounts of mercury higher than the 0.010 mg/kg LoD in different batches of fish oil, especially since the companies that manufacture fish oil supplements purify the crude oil to remove all environmental contaminants. Though to be sure, replication of these findings is necessary to verify the
findings from this research. Moreover, future research questions may be generated from replicating the analyses conducted.

4.6. Implications and Future Directions

4.6.1. Implications.

The results of this study have important implications for both the general population and populations with a clinical mood disorder diagnosis. The first implication is that over-the-counter fish oil supplements have the potential to be a novel treatment for individuals with major depression and bipolar disorder. Fish oil supplements can provide a natural treatment option for adults with mood disorders that in some cases can augment the pharmacologic effect of antidepressant medications, or can act alone to ameliorate psychological symptoms. It is important that both populations are informed of the potential therapeutic effects of over-the-counter fish oil supplements. The reason that this is important is because fish oil products may provide an equally effective, yet safer alternative to standard medications based on extant literature reviewed. For instance, the fish oil administered in clinical research trials was generally well tolerated and was associated with few minor adverse events when consumed at high doses. It is expected that fish oil supplements purchased over-the-counter will also have similar adverse effect profiles as the fish oil used in research. Thus, the reported side effects of fish oil supplements appear less severe than those caused by antidepressants, mood stabilisers and second generation antipsychotics. Provided the dose of fish oil consumed is sufficient to achieve pharmacological results, significant improvements in mood symptoms are possible for a subgroup of patients with depression and bipolar disorder.

However, it is important that the public understands that fish oil supplements are not a panacea for treatment and their effectiveness will be dependent on each patient’s individual circumstances. The complex interaction between genetic predispositions and environmental influences, in addition to social, psychological and biological factors, means that the cause(s) for the mood disorder will be different for each patient. Depending on the reason for the ailment, then fish oil supplements might be an appropriate treatment option. However, treatment efficacy will also be influenced by the dose of fish oil consumed daily.

The general public may be unaware that most fish oil supplements sold over-the-counter in New Zealand do not contain the amounts of fatty acids that are stated on
product labels. This result has important implications for the consumer, especially for patients with mood disorders who supplement their diet with fish oil to maintain their mental and cognitive function. Thus, if the fish oil capsules ingested do not contain the appropriate amounts of omega-3 fatty acids, then the minimum recommended dose of three fish oil capsules for brain health may not be sufficient to exert a therapeutic effect. For the general population, the discrepancy between the labels and actual content of omega-3 fatty acids per fish oil capsule means that the doses recommended to assist with joint, heart, and brain health may be insignificant to prevent the onset of disease. This is a concern. However, the most obvious remedy is that a greater number of fish oil capsules are consumed per day in order to be confident that a therapeutic effect can be achieved.

The fish oil supplements analysed in this study contained no detectable amounts of methylmercury per capsule. This result has important implications for adult populations who consume over-the-counter fish oil products, in particular, pregnant women with a clinical mood disorder. Research has shown that fish oil contaminated with mercury can be detrimental to central nervous system function due to the accumulation of methylmercury in the adult and neonatal brain. However, since the fish oil supplements analysed do not contain mercury, they pose no risk to human health and represent a safer alternative to pharmacological drugs during pregnancy and lactation. They may also confer additional neurological benefits to the developing fetus and young infant. Thus, it is imperative that women of childbearing age are informed of these safer and potentially effective treatment options. Though omega-3 fatty acids are widely documented as beneficial to many aspects of health, future research should be conducted to explore operational aspects of omega-3 fatty acids and the composition and amounts of fatty acids beneficial for the psychological treatment of mood disorders. Additional ideas for future research are considered in the next section.

4.6.2. Future directions.

4.6.2.1. A holistic approach to mood disorders.

Future research should focus on the larger picture related to the cause(s) of depression and bipolar disorder, a holistic approach that includes causative factors such as genetics, environment, social, psychological and biological influences because of the importance of the interaction between these variables for mood disorders. A holistic view of
the causes of mental illness may facilitate a more directed approach toward a treatment more beneficial for the patient. Proven effective treatments for each patient is a priority for both researchers and general practitioners.

**4.6.2.2. Biological mechanisms of fish oil for mood disorders.**

A further direction for future research involves determining how fish oil supplements can regulate mood and ameliorate symptoms of mental illness. Several mechanisms by which fish oil supplements achieve pharmacological results for depression and bipolar disorder have been proposed. However, the precise mechanisms of action remain unclear. The research to date has provided conflicting evidence regarding the efficacy of fish oil supplements in the treatment of mood disorders, and as a consequence, there are uncertainties surrounding the accuracy of the mechanisms proposed. A possible explanation for these controversial findings may be the varied fish oil formulations used in research. These supplements varied in both ingredients and dosages of essential fatty acids, which makes it difficult to ascertain the composition of fish oil that is most effective for mood disorders. An advantage of these different formulations is that they provide an indication of the ingredients and dosages that are essential to the treatment of depression and bipolar disorder. Future research should aim to identify the most effective fish oil formulation through extensive testing of different fatty acid combinations (e.g. EPA, DHA, EPA + DHA) at different dosages to ascertain the formula that will produce optimal therapeutic outcomes.

**4.6.2.3. Efficacy of different forms of omega-3 fatty acids for mood disorders.**

The effectiveness of fish oil supplements in the treatment of mood disorders is expected to be different across products with respect to the absorption of different fatty acid forms. Because over-the-counter fish oil supplements contain essential fatty acids as either free fatty acids, ethyl esters, or re-esterified triglycerides, future research could also focus on the difference between clinical outcomes in relation to these forms of omega-3 fatty acids. In other words, research should determine the form that is most effective in reducing psychological symptoms in order to provide the best possible treatment for the patient. A separate but related direction for future research may be to evaluate the efficacy of fish oil supplements when combined with a high fat meal, compared to placebo. This recommendation is based on the premise that a meal containing 44 grams of total fat
enhances the absorption of fatty acids in ethyl ester and triglyceride forms (Davidson et al., 2012; Lawson & Hughes, 1988). In theory, improved absorption should augment the pharmacological effect of fish oil supplements and reduce symptoms of depression and bipolar disorder. Thus, extensive and systematic testing is required to determine the optimal fat content of the meal that will enhance clinical outcomes in patients with a mood disorder.

4.6.2.4. Depressed populations most likely to benefit from omega-3 fatty acids.

A chronic dietary deficiency of omega-3 fatty acids has been implicated in the pathophysiology of depression and bipolar disorder. Provided that dietary deprivation is the primary cause of the ailment, then fish oil supplementation is the most obvious treatment option. Future research should therefore determine whether fish oil supplements are more effective in alleviating psychological symptoms in populations that are deficient in omega-3 fatty acids than in patients who are not deficient prior to the commencement of treatment. It has been demonstrated that a diet rich in omega-6 fatty acids may also contribute to the pathophysiology of mood disorders due to the complex cascade of events caused by chronic exposure to these proinflammatory nutrients. Thus, a further direction for future research may involve measuring the incorporation of both omega-3 and omega-6 fatty acids in red blood cell membranes to determine whether patients with a clinical mood disorder have an imbalance of these nutrients at baseline. The premise is that supplementation will correct the balance to maintain normal brain function. In order to evaluate the efficacy of fish oil supplements in patients with a fatty acid imbalance, the measures performed at baseline must be repeated following the cessation of treatment. These latter research directions may provide an indication of the predictors of treatment efficacy, which may be valuable toward developing treatments that are tailored to each patient.

4.6.2.5. Replication of positive clinical trials.

Replications of effective clinical research trials are also required for the recommendation of fish oil supplements as a psychological treatment for mood disorders. These replications need to evaluate the long-term therapeutic effects of fish oil supplement administration, as a benefit may take longer to achieve in some patients but not in others. Explorations into the maintenance treatment effects of supplementation will provide an
indication as to whether the doses of fish oil can be gradually reduced over the long term but not cause depressive or manic relapses in patients. If the treatment effects are maintained over extended periods without continuous administration, then the reported side effects should also be reduced as well as the accumulated cost of purchasing over-the-counter supplements.

4.6.2.6. Independent chemical analysis of larger sample of fish oil supplements.

Limitations in the sample size of fish oil supplements in this study provides an opportunity for future research to analyse a broad range of over-the-counter fish oil supplements in New Zealand to determine their concentrations of both omega-3 fatty acids and mercury. Results from a larger sample of over-the-counter fish oil supplements would provide a more accurate representation of the amounts of ingredients contained in fish oil products and the amounts of environmental contaminants present. The implication of this recommendation is that it would provide a better picture as to the number of capsules required to produce a therapeutic benefit. In order to confirm that fish oil supplements are safe for human consumption then further research is needed to analyse the fish oil capsules for individual environmental pollutant that might be present. The results of these future studies would enable a comprehensive risk and benefit analysis to be carried out and will provide valuable information to the consumer regarding the safety of fish oil supplements for both the general population and populations with a clinical mood disorder.

5. Conclusions

This study identified the most popular fish oil supplements sold over-the-counter in New Zealand, and has investigated the amounts of omega-3 fatty acids and mercury through independent laboratory analyses. A review of the extant literature has shown that fish oil supplements have the potential to ameliorate mood symptoms in certain adult populations with a clinical depression or bipolar disorder diagnosis. This research has demonstrated that most over-the-counter fish oil supplements contain amounts of omega-3 fatty acids that are considerably less than the labels claim but nevertheless, revealed that a dose of three and seven fish oil capsules is consistent with the doses found to be effective in clinical research studies. The implication for this finding is that over-the-counter fish oil supplements may provide a natural treatment option for the effective amelioration of
psychological symptoms. Furthermore, it was found that fish oil supplements do not contain detectable amounts of methylmercury and thus, pose minimal risk to human health. However, more research should be performed.

Future research should focus on replicating clinical research trials that were found to be effective in the treatment of mood disorders. For example, research could be conducted to identify the most effective fish oil composition for the treatment of depression and bipolar disorder. Additionally, it is important to validate the findings from previous clinical research trials. Validation of the existing findings is important as a first step in devising personalised treatment plans since “one size does not fit all.”


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# Appendices

## Appendix A

### Purchased Fish Oil Supplement Information

<table>
<thead>
<tr>
<th>Brand</th>
<th>Country of Origin</th>
<th>Batch No.</th>
<th>Date of purchase</th>
<th>Use by</th>
<th>Batch No.</th>
<th>Date of purchase</th>
<th>Use by</th>
<th>Total EPA + DHA</th>
<th>RRP* ($NZ)</th>
<th>Label - mercury tested</th>
<th>Reply</th>
<th>Recommended Daily Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanderson Fish Oil 2000 mg</td>
<td>NZL</td>
<td>7167</td>
<td>May-15</td>
<td>Apr-18</td>
<td></td>
<td></td>
<td></td>
<td>600 mg</td>
<td>$0.05</td>
<td>Yes</td>
<td>NA</td>
<td>1-2 capsules (general health) 3 capsules (heart, circulation and brain health) 5 capsules (joint health)</td>
</tr>
<tr>
<td>Go Healthy Fish Oil 1550 mg</td>
<td>NZL</td>
<td>V4328</td>
<td>May-15</td>
<td>Sep-17</td>
<td>J5021</td>
<td>Aug-15</td>
<td>Jan-18</td>
<td>460 mg</td>
<td>$0.06</td>
<td>Yes</td>
<td>No</td>
<td>1-3 capsules</td>
</tr>
<tr>
<td>Good Health Omega-3 Fish Oil 1000 mg</td>
<td>NZL</td>
<td>S35B</td>
<td>May-15</td>
<td>Nov-17</td>
<td></td>
<td></td>
<td></td>
<td>300 mg</td>
<td>$0.05</td>
<td>Yes</td>
<td>Yes</td>
<td>1-2 capsules (children) 1-3 capsules (adults)</td>
</tr>
<tr>
<td>Natures Own Odourless Fish Oil 2000 mg</td>
<td>AUS</td>
<td>3204</td>
<td>May-15</td>
<td>Nov-17</td>
<td>14455</td>
<td>Aug-15</td>
<td>Feb-18</td>
<td>600 mg</td>
<td>$0.05</td>
<td>Yes</td>
<td>No</td>
<td>1-2 capsules (general wellbeing) 3 capsules (heart, brain and eye health) 5 capsules (joint health)</td>
</tr>
<tr>
<td>Red Seal Fish Oil 1000 mg</td>
<td>NZL</td>
<td>Y4183</td>
<td>May-15</td>
<td>Oct-17</td>
<td></td>
<td></td>
<td></td>
<td>300 mg</td>
<td>$0.05</td>
<td>Yes</td>
<td>NA</td>
<td>1-3 capsules</td>
</tr>
<tr>
<td>Brand</td>
<td>Country</td>
<td>Code</td>
<td>Printed</td>
<td>Expired</td>
<td>Strength</td>
<td>Price</td>
<td>Stock</td>
<td>Will Deliver</td>
<td>Recommended Uses</td>
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<tr>
<td>Nutra Life Fish Oil</td>
<td>AUS/NZ</td>
<td>98938</td>
<td>May-15</td>
<td>Aug-16</td>
<td>300 mg</td>
<td>$0.05</td>
<td>Yes</td>
<td>No</td>
<td>2-4 capsules (heart and cholesterol maintenance) 2-7 capsules (healthy brain and eye function, and general wellbeing) 6 capsules (joint health) 7 capsules (symptomatic relief of dry skin and eczema) 1 capsule (general wellbeing)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish Oil Plus Vitamin D 1500 mg</td>
<td></td>
<td>99617</td>
<td>Aug-15</td>
<td>Mar-18</td>
<td>450 mg</td>
<td>$0.05</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healtheries Fish Oil</td>
<td>NZL</td>
<td>89632</td>
<td>May-15</td>
<td>Sep-17</td>
<td>450 mg</td>
<td>$0.07</td>
<td>Yes</td>
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<td>1 capsule (general wellbeing) 2-4 capsules (heart and brain health) 4 capsules (joint and skin health)</td>
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<td>1500 mg</td>
<td></td>
<td>98357</td>
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<td>Sep-17</td>
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<td>Go Healthy Fish Oil</td>
<td>NZL</td>
<td>G4057</td>
<td>May-15</td>
<td>Apr-17</td>
<td>600 mg</td>
<td>NA</td>
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<tr>
<td>Go Healthy Go Fish Oil Odourless 1500 mg</td>
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<td>J5072</td>
<td>May-15</td>
<td>Jan-18</td>
<td>450 mg</td>
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<tr>
<td>1500 mg</td>
<td></td>
<td>K5039</td>
<td>Aug-15</td>
<td>Feb-18</td>
<td>450 mg</td>
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<td>Yes</td>
<td>NA</td>
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<td>Healtheries Fish Oil</td>
<td>NZL</td>
<td>84185</td>
<td>May-15</td>
<td>May-17</td>
<td>300 mg</td>
<td>$0.05</td>
<td>Yes</td>
<td>NA</td>
<td>1-2 capsules (general wellbeing) 3-6 capsules (heart and brain health) 6 capsules (joint and skin health)</td>
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</tr>
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<td>1000 mg</td>
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Appendix B. Testing and Processing of Fish Oil Prior to Encapsulation

Unmatched Quality

Sent to Consumer Healthcare to launch Consumer Healthcare business with a large portfolio of vitamins, minerals and supplements over the past seven years. We believe that the company’s dedication to the development of high-quality and innovative products is what sets us apart from other companies in the industry. Our commitment to quality is reflected in all of our products, which are developed to the highest standards to meet the needs of our customers and the health industry.

Testing and Processing

At Sanso Consumer Healthcare, we take great care to ensure that our fish oil products meet the highest quality standards. Our testing and processing procedures are designed to produce high-quality oil that is free from contaminants and impurities. The entire process is carefully monitored to ensure that the final product meets our high standards of quality.

What do we test for?

When a fish oil product is received at our facility, it undergoes a thorough inspection to ensure that it meets our quality standards. We test for a variety of parameters, including levels of contaminants such as heavy metals, pesticides, and pollutants, as well as other quality attributes such as purity, potency, and stability.

Environmental pollutants

Environmental pollutants are not only harmful to the environment, but also to the health of consumers. We test for these pollutants to ensure that our products are safe and do not pose any health risks.

Sustainably sourced

Our fish oil products are sourced sustainably from responsibly managed fisheries. We are committed to using only sustainable and responsible fishing practices to ensure the long-term health of our oceans and the environment.

Unmatched quality from source to shelf

Our commitment to quality is reflected in every stage of the production process, from sourcing and processing to packaging and distribution. We take pride in our ability to provide consumers with the highest-quality fish oil products available on the market.