



# Risk Profile: Risk Assessment of Fish Oil and Oxidised Fish Oil

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## **Scientific Interpretive Summary**

### **Risk Assessment of Fish Oil and Oxidised Fish Oil**

This risk assessment was commissioned by the Ministry for Primary Industries (MPI) to further investigate the safety of fish oils and oxidised fish oils in response to a New Zealand study published in January 2015 on fish oil supplements. This study suggested that most supplements contained oils that had significant levels of oxidation. The study also claimed that over 50% of these fish oils exceeded industry guidelines for maximum levels of oxidation. However it is important to note that these industry guidelines are based on palatability of the products and not consumer safety. They do not give any indication of what level of oxidation of fish oils pose a potential health risk or not.

To look into this further, MPI commissioned two Dunedin-based toxicologists to produce a scientific report covering:

- 1) a review of the literature for more recent safety data on fish oils and oxidised fish oils;
- 2) a risk assessment based on available data; and
- 3) an evaluation of whether robust safety standards for oxidation of fish oils can now be derived.

The report found there is no evidence of any food safety concerns for consumers of fish oils or fish oil supplements if consumers follow manufacturers' recommended dose instructions found on product labels. It also found that fish oils contain fatty acids with double bonds, and will oxidise naturally over time. The chemical products of this oxidation process are only toxic if dietary intakes are sufficiently high. The level of oxidation products in the fish oils on the New Zealand market is not high enough to pose a risk to consumers.

# **RISK ASSESSMENT OF FISH OIL AND OXIDISED FISH OIL**

## **A Report to the Ministry for Primary Industries (MPI)**

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**April, 2015**

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<sup>1</sup> Please note: While Dr Temple was formerly the Director of, and Dr Beasley is employed by, the National Poisons Centre, this study was not carried out under the auspices of the National Poisons Centre and the views portrayed in the report do not reflect those of the National Poisons Centre or the University of Otago.

## **Executive Summary**

Recently, fish oil supplements on the NZ market were evaluated for quality and content, and only 3% had the level of fish oil claimed to be present. One reason why the levels were lower than claimed was because a significant percentage was oxidised. This is of concern, because when fatty acids are oxidised, toxic substances (such as peroxides, aldehydes, and ketones) are formed.

The research question was whether such oxidised fish oils pose any risk of toxicity when consumed, and in particular whether there is any risk to the fetus when consumed during pregnancy.

One randomised placebo-controlled trial examined the effects of oxidised versus nonoxidised oil in humans, over seven weeks. No difference was found in markers of *in vivo* lipid peroxidation or antioxidant activity, or liver function tests. This suggested that oxidised fish oils may not be associated with acute oxidative toxicity. However this was a short study and did not assess pathological markers associated with atherosclerosis, such as oxidised LDL or carotid artery intimal thickness. Nor did it investigate potential reproductive effects, the main subject of the current report.

There are however a number of animal experimental studies examining such effects; but only in a minority is it possible to glean the doses of omega-3 fatty acids given, on a mg/kg basis. One such study was that by Damgaard et al, 2003. Our risk assessment based on its (plus other relevant ) data suggested that in adult female mink the NOAEL was ~ 318 mg/kg for the two omega-3 polyunsaturated fatty acids (w-3 PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) combined.

On the other hand the body weight of the kits was affected at day 28 after birth and at weaning. This has also been noted by other researchers, though such weight deficits may be because feed with an increased percentage of w-3 PUFAs is less palatable and digestible, (rather than due to any direct toxicity) at such doses. However given the above finding, it may be prudent to consider the estimated level of 318 mg/kg as a lowest observed adverse effect level (LOAEL) rather than a NOAEL as regards effects on offspring.

Such a NOAEL or LOAEL (318 mg/kg) is ~ 42 times higher than the current acceptable or recommended daily intake of EPA and DHA combined, which for pregnant women is 450 mg, or about 7.5 mg/kg body weight. This provides considerable grounds for reassurance, even though the difference is less than one hundred fold.

The U.S. FDA concluded that use of menhaden (fish-based) oil as a food ingredient is generally regarded as safe (GRAS), provided that the combined daily intake of DHA and EPA does not exceed 3 grams/day. This generally corresponds to < 60 mg/kg for a human adult, which is about one fifth of the above proposed NOAEL.

However human and indeed animal data appear not extensive enough to serve as the basis for derivation of an acceptable daily intake (ADI) of fish oil, including at any specific level of oxidation (PV, AV, or Totox values). In 2010 it was observed that knowledge does not allow setting and recommending of maximum acceptable peroxide and anisidine values for the large variety of refined fish oils. This situation does not appear to have changed.

Given the paucity of specific evidence, it is still not possible to know whether marine oils are safe after high levels of oxidation. Long-term safety studies are required, looking at appropriate disease outcomes and surrogates thereof, and relating these to the oxidative state.

## Introduction

Intake of fish and fish oils has been linked to a reduced risk of coronary heart disease (CHD) and CHD deaths. Supplements are recommended for those who do not include fish in their diet, and are used in clinical practice to prevent CHD and in the treatment of mild-to-moderate hypertriglyceridemia.<sup>2</sup> The major benefit demonstrated has been a reduction of risk of CHD, but fish oils also have anti-inflammatory properties, and have been investigated for possible beneficial effects on brain function.<sup>3</sup> Consumers take fish oil for a variety of reasons, but particularly because it has shown promise for lowering inflammation, improving cognition, and lowering cardiovascular disease risk.

Fish oils contain significant quantities of omega-3 long-chain polyunsaturated fatty acids (omega-3 PUFA or n-3 PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), that are considered the metabolically active compounds.

However n-3 PUFA are highly prone to oxidation, due to the large number of double bonds within the fatty acid chain. This may cause deterioration in fat quality during storage of fatty fish products. Further oxidation of PUFA can also occur *in vivo*. As fish oils oxidise, such as during storage, unoxidised fatty acids diminish and are replaced by a complex “soup” of lipid peroxides (and hydroperoxides<sup>1</sup>) and secondary oxidation products (aldehydes and ketones).<sup>4</sup> Addition of antioxidants reduces but does not prevent oxidation.<sup>5</sup>

While specific oxidation species are difficult to measure, the degree of oxidation can be described by measuring the peroxide value (PV or POV) and the anisidine value (AV). The PV (POV) reflects the primary oxidation products (lipid peroxides), while the anisidine value (AV) reflects secondary oxidation products (aldehydes and ketones). Together these parameters are used to estimate the total oxidation value, Totox,<sup>3</sup> which has been defined as  $(2 \times \text{PV}) + \text{AV}$ .<sup>6</sup>

A number of organisations have recommended maximum levels for these indices. However these industry standards are based on palatability, as it is considered that there are insufficient data to set standards based on health effects.<sup>7</sup>

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<sup>2</sup> Ottestad I, et al. Oxidised fish oil does not influence established markers of oxidative stress in healthy human subjects: a randomised controlled trial. *British Journal of Nutrition* 2012; 108: 315-26.,

<sup>3</sup> Turner R, et al. Are the health benefits of fish oils limited by the products of oxidation? *Nutrition Research Reviews* 2006; 19: 53-62.

<sup>4</sup> Albert BB, et al. Fish oil supplements in New Zealand are highly oxidised and do not meet label content of n-3 PUFA. *Scientific Reports* 2015; 5: 7928.

<sup>5</sup> Zuta P, et al. The effect of alpha-tocopherol on the oxidation of mackerel oil. *Food Chemistry* 2007; 100: 800-7.

<sup>6</sup> Albert BB, et al. Oxidation of marine omega-3 supplements and human health. *Biomed Research International* 2013; ID 464921.

<sup>7</sup> EFSA Panel on Biological Hazards (BIOHAZ). Scientific opinion on fish oil for human consumption. Food hygiene, including rancidity. *EFSA J* 2010; 8: 1874

It has been stated that specifications for food-grade oils usually indicate a maximum peroxide value of 2 mEq O<sub>2</sub>/kg and a p-anisidine value of 10 mEq O<sub>2</sub>/kg, but most commercially available fish oils do not meet these specifications.<sup>2</sup> More recently it was suggested that the PV and AV should not exceed 10 meq/kg and 20 meq/kg respectively.<sup>8</sup>

### **Research Question**

Recently, fish oil supplements on the NZ market were evaluated for quality and content, and only 3% had the level of fish oil claimed to be present. One reason why the levels of fish oils were lower than claimed was because they were significantly oxidised, with 83% exceeding “acceptable” peroxide values and 50% exceeding “acceptable” Totox values. This is of concern, as it is known that when fatty acids are oxidised, toxic substances (such as peroxides, aldehydes, and ketones) are formed.

The question requiring consideration is whether such oxidised fish oils pose any risk of toxicity when consumed, and in particular whether there is any risk to the fetus when consumed during pregnancy. Thus the objective was to carry out a risk assessment of fish oils and oxidised fish oils.

### **Research Findings**

There are insufficient interventional human studies that examine potential biological functions of oxidised marine n-3 oils; however there is evidence that lipid peroxidation is involved in human disease. In addition, animal studies show that oxidised lipids may cause organ damage, inflammation, carcinogenesis, and advanced atherosclerosis. These deleterious effects should not be ignored, particularly when marine n-3 oils are taken during pregnancy, early childhood, and old age, and for long periods of time.

Animal studies have provided clear evidence that oxidised lipids are harmful, though typically this has involved using higher doses of oils than humans consume, or administering oxidation products in non-physiological ways.<sup>9</sup>

Chronic feeding of oxidised PUFAs to rats led to growth retardation, intestinal irritation, liver and kidney enlargement, haemolytic anaemia, decreased vitamin E, increased lipid peroxides, and inflammatory changes in the liver, cardiomyopathy, and potentially malignant colon cell proliferation.<sup>8</sup>

A major secondary oxidation product of omega-3 oils is the aldehyde 4-hydroxyhexenal (HHE), which when injected intravenously has caused liver damage in test animals. It is

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<sup>8</sup> Council of Europe. European Pharmacopoeia, 6<sup>th</sup> ed. Suppl 6.3. Strasbourg: 2008.

<sup>9</sup> Esterbauer H. Cytotoxicity and genotoxicity of lipid-oxidation products. American Journal of Clinical Nutrition 1993; 57(5): 779S-785S.

chemically similar to the omega-6 oxidation product, 4-hydroxynonenal (HNE), which is known to be highly toxic and causes DNA damage.

There is increasing evidence that *in vivo* oxidation of LDL has a role in atherosclerosis. Given that ingested peroxides are transported in LDL, it is possible that they could have a role in enhancing LDL oxidation and atherosclerosis. This is supported by a study in rabbits where addition of fish oil to a high cholesterol diet led to rapid atherosclerosis.<sup>10</sup> It was speculated that if this is due to oxidation of LDL, ingested marine n-3 could be atherogenic in humans.<sup>5</sup>

One randomised placebo-controlled trial examined the effects of oxidised versus nonoxidised oil in humans, over seven weeks. No difference was found in markers of *in vivo* lipid peroxidation (including HHE and HNE), markers of antioxidant activity, C-reactive protein, or liver function tests.<sup>1</sup> This result suggested that oxidised marine n-3 may not be associated with acute oxidative toxicity. However this was a short study and did not assess important pathological markers associated with atherosclerosis, such as oxidised LDL or carotid artery intimal thickness.<sup>5</sup>

It has recently been suggested that given the paucity of specific evidence (as at 2013), it is impossible to know whether marine oils are safe after oxidation.<sup>5</sup> These authors concluded that long-term safety studies were required, looking at appropriate disease outcomes and surrogates thereof, and relating these to the oxidative state.

The omega-3 supplementation literature is highly conflicting, especially in the area of effects on cardiovascular parameters. Varying degrees of oxidation may be a major cause of these conflicting results.<sup>5</sup>

## Human studies

Human data is not extensive enough to allow the derivation of an acceptable daily intake of oxidised fish oil, including at any specific PV, AV, or Totox values. In 2010 the European Food Safety Authority (EFSA) noted that knowledge at that time did not allow setting and recommending of maximum acceptable peroxide and anisidine values for the large variety of refined fish oils.<sup>6</sup>

One study found that a daily intake of 8 g of oxidised n-3 fish oil (with a PV of 18 mEq O<sub>2</sub>/kg oil), did not influence a variety of *in vivo* markers of oxidative stress, lipid peroxidation, and inflammation in healthy subjects after use for three or seven weeks.<sup>1</sup> Such reliable and established markers included C-reactive protein (CRP; an inflammatory marker) and urinary levels of 8-iso-PGF<sub>2a</sub>, a secondary oxidation product. (The comparison groups took 8g of non-oxidised fish oil or 8 g of high oleic sunflower oil).

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<sup>10</sup> Thierry J, Seidel D. Fish oil feeding results in an enhancement of cholesterol-induced atherosclerosis in rabbits. *Atherosclerosis* 1987; 63(1): 53-6.



This finding is in accord with some other studies showing no change in 8-iso-PGF2a measured in plasma and spot urine samples in healthy subjects, but on the other hand differs from other studies showing decreased 8-iso-PGF2a after intake of n-3 fatty acids, when measured in a 24 hour urine collection. However the authors of the above study<sup>1</sup> also noted no change in other antioxidant and oxidative stress markers (including alpha-tocopherol, total glutathione, catalase and glutathione peroxidase activity) after n-3 supplementation. They commented “in the present study a daily intake of n-3 FA (approximately 0.7 g EPA and 0.9 g DHA), regardless of oil quality, does not seem to affect lipid peroxidation or oxidative stress in healthy subjects”.

(Changes in plasma 4-HHE and 4-HNE were not significantly different between the randomised groups after 3 or 7 weeks of intervention. Also no significant differences in EPA, DHA, and docosapentaenoic acid between the fish oil and oxidised fish oil groups was observed).

Studies have shown that hydroperoxides are converted into secondary oxidation products during the digestion process, and that the latter are absorbed, at least partially. However in this study, no increase in secondary oxidation products (such as 8-iso-PGF2a, 4-HHE, or 4-HNE) was found.

The oxidised fish oil (oxidation was by oxygen sparking) had moderately elevated oxidation values relative to the non-oxidised fish oil. The oxidised oil had a Totox value of 45 (PV 18, AV 9) while the non-oxidised oil had a Totox value of 11 (PV 4, AV 3).

The authors however acknowledged the short intervention period and modest sample size, and observed that the spectrum of oxidation products generated (by oxygen sparking) may be different from those formed by the spontaneous degradation of n-3 supplements. They also noted that further, larger studies would be necessary to assess the degree to which their results are also valid in subjects with elevated background levels of inflammation and/or oxidative stress.

## **Animal studies**

Damgaard et al<sup>11</sup> noted that in one study in minks, feed intake and growth had been impaired by feeding poor quality fish oil.<sup>12</sup> However high dietary levels of fresh fish oil had no adverse effect on weight gain and were tolerated quite well by males during the

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<sup>11</sup> Damgaard BM, et al. Effects of high dietary levels of fresh or oxidised fish oil on performance and blood parameters in female mink (*Mustela vison*) during the winter, reproduction, lactation and early growth periods. Acta Agric Scand Sect A, Animal Sci 2003; 53: 136-46.

<sup>12</sup> Borsting CF, et al. Inclusion of oxidised fish oil in mink diets. 1. The influence on nutrient digestibility and fatty-acid accumulation in tissues. Journal of Animal Physiology and Animal Nutrition. 1994; 72: 132-45.

growing-furring period.<sup>13, 14</sup> It was also found that feeding diets of which up to 50% of the dietary fat was derived from fish did not reduce body weight and resulted in good pelt length and fur quality.<sup>15</sup>

Rouvinen et al<sup>16</sup> found no differences in reproduction results, kit growth, and haematological indices between females fed 30% whole herring or 30% lean haddock scrap during the reproduction and nursing periods. Tauson<sup>17</sup> investigated a diet with 95% fat derived from fish during the reproduction and lactation periods, and found that the diet was associated with a normal reproductive performance and normal kit survival during the lactation period; however kit growth was inferior during the lactation period. Borsting et al 1998)<sup>11</sup> found that fish oil as the sole dietary fat source to female mink in the breeding season resulted in a smaller litter size and severe negative effects on kit growth performance and on the health of the kits. Clausen et al found that high dietary levels of fatty herring scrap used for females during the winter, reproduction, and lactation periods had no negative effects on reproduction results, though the growth rate of the kits was reduced as the proportion of dietary fat derived from fish was increased.<sup>18</sup>

In adult mink, highly oxidised fish oil has caused iron-deficiency anaemia,<sup>19</sup> while mink fed high levels of marine lipids suffer from iron-deficiency anemia induced by suboptimal levels of vitamin E, which latter was probably caused by high levels of dietary PUFA.<sup>20</sup> Anemia in mink fed rancid fat in combination with vitamin E deficiency is usually explained by disorders in the cell membrane structures, including the membranes of erythrocytes.<sup>21</sup> The number of platelets was also shown to decrease with increasing proportions of fat derived

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<sup>13</sup> Borsting CF, et al. Effects of high amounts of dietary fish oil of different oxidative quality on performance and health of growing-furring male mink (*Mustela vison*) and of female mink during rearing, reproduction and nursing periods. Journal of Animal Physiology and Animal Nutrition 1998; 79: 210-23.

<sup>14</sup> Rouvinen KI, et al. Effects of high dietary levels of silver hake and Atlantic herring on growing-furring performance and blood clinical-chemistry of mink (*Mustela vison*). Canadian Journal of Animal Science 1997; 77: 509-17.

<sup>15</sup> Clausen TN, et al. Fatty acids and defatted fish products for mink in the growing-furring period. I. Effects on growth and pelt characteristics. Acta Agric Scand Sect A, Animal Sci 1999; 49: 225-34.

<sup>16</sup> Rouvinen KI, et al. Female fertility, embryonic and pre-weaning survival and early growth of offspring of mink fed herring and vitamin E. Research Report 1993. Nova Scotia Agricultural College; Canada.

<sup>17</sup> Tauson AH. High dietary level of polyunsaturated fatty acids and varied vitamin E supplementation in the reproductive period of mink. Journal of Animal Physiology and Animal Nutrition 1994; 72: 1-13.

<sup>18</sup> Clausen TN, et al. Fatty and defatted herring scrap fed to mink (*Mustela vison*) in the reproduction and nursing periods. Canadian Journal of Animal Science 1999; 77: 457-65.

<sup>19</sup> Engberg RM, Borsting CF. Inclusion of fish oil in mink diets. 2. The influence on performance and health considering histopathological, clinical-chemical, and haematological indices. Journal of Animal Physiology and Animal Nutrition 1994; 72: 146-57.

<sup>20</sup> Tauson A-H, Neil M. Fish oil and rapeseed oil as main fat source in mink diets in the growing-furring period. Journal of Animal Physiology and Animal Nutrition. 1991; 65:84-95..

<sup>21</sup> Chow CK. Oxidative damage in the red cells of vitamin E-deficient rats. Free Radic Res Comm 1992; 16: 247-58.

from fish in the diet of male mink in the growing period and female mink in the lactation period.<sup>16,22</sup>

In humans, a dietary supply of PUFA has resulted in decreased platelet counts, decreased platelet aggregation and increased bleeding time.<sup>23</sup>

Damgaard et al<sup>10</sup> investigated the effects of high dietary levels of fresh or moderately oxidised fish oil on performance and blood parameters in female minks during the winter, reproduction, and lactation periods, and evaluated the effects of the diets on kits' performance during the lactation and early growth periods. Minks were divided into five groups depending on their assigned dietary regimen; whether receiving fresh fish oil stored frozen, fresh fish oil ensiled, oxidised fish oil stored frozen, oxidised fish oil ensiled, or soya oil (the control group). In the diets with fish oil, fish lipids accounted for 72% of the dietary fat compared with 14% of dietary lipids in the control diet.

A limitation of the study (for regulatory purposes) was that the daily doses of fish oil (fresh or oxidised) were not determined or at least described. The oxidised oil had a peroxide value (PV) of ~ 60 meq O<sub>2</sub>/kg oil. (The oxidation of the oil was carried out in open vessels at room temperature. No Totox value was given). The fresh oil had a PV of 10 meq O<sub>2</sub> per kg oil. Both batches of fish oil were mixed with defatted herring scrap. Half of each mixture was stored frozen and the other half ensiled. In the control diet the oil source was soya oil. The diets were supplemented with 84 mg alpha-tocopherol (vitamin E) per kg diet.

They found that the number of live (and stillborn) kits was not differentially affected by the different diets during the winter and pregnancy periods. The number of kits at weaning and number of kits lost during the lactation period were similarly not affected by the experimental diet fed either during the winter and pregnancy period or the lactation and early growth periods. The body weights of the females during the winter and lactation periods were not affected by the experimental diets.

However the body weights of the kits was affected by the experimental diets at day 28 after birth and at weaning. At day 28, the weight of the kits in the control group and the "fresh-silated" group were higher than that in the other groups; however at weaning, the weight of the kits in the control group remained higher, but the weight of the kits in the "fresh-silated" group was lower than that in the other groups.

The haemoglobin concentration was highest in the "oxidised-frozen" group, and lowest in the "fresh-silated" and control groups. The erythrocyte numbers and haematocrit values were not significantly different across the four experimental diets; neither were the

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<sup>22</sup> Damgaard BM, et al. Fatty fish and defatted fish products for male mink (*Mustela vison*) in the growing-furring period. II. Effects on haematological and clinical-chemical parameters, vitamin E status and fatty acid composition. Acta Agric Scand; Sect A, Animal Sci 2000; 50: 19-29.

<sup>23</sup> Lorenz R, et al. Platelet function, thromboxane formation and blood pressure control during supplementation of the western diet with cod liver oil. Circulation 1983; 67: 504-11.

leucocyte count or monocyte and eosinophil percentages. The percentage of neutrophils was highest for the “fresh-silated” and “oxidised-silated” groups, lower for the “fresh-frozen” and “oxidised-frozen” groups, and lowest for the control group. The platelet count was lower for all four experimental groups, but the “oxidised” groups did not have a lower count than the “fresh” groups. However in the kits, the platelet count was lower in the oxidised fish oil than in the fresh fish oil groups. In the adults (females) the concentration of alpha-tocopherol (vitamin E) was not influenced by the experimental diets, but in the (male) kits the levels of alpha (and gamma) tocopherol were lower in the experimental groups than the control group.

In agreement with Tauson<sup>16</sup> and Clausen et al,<sup>17</sup> there were no differences in the number of live and stillborn kits per litter, indicating that prolonged feeding with high amounts of fish lipids fed either fresh or moderately oxidised had no adverse effects on the breeding result. This is in contrast to Borsting (1998)<sup>12</sup> who found that fish oil fed as the only dietary fat source to mink in the breeding season resulted in smaller litter sizes at delivery. Damgaard et al also found that the number of kits at weaning and kit mortality during the lactation period were not influenced by the diet fed during the winter and pregnancy periods or that fed during the lactation and early growth periods. Tauson<sup>16</sup> also found that a high percentage of fat from fish (95%) also resulted in normal kit losses during the lactation period.

The Damgaard study results indicate that irrespective of oxidation quality and storage conditions, high dietary levels of fish oils had no negative influence on the female adult animals. On the other hand, the body weight of the kits was negatively influenced by high dietary levels of fish oil. These findings are consistent with those of Borsting et al<sup>12</sup>, Clausen et al<sup>17</sup> and Tauson.<sup>16</sup> The latter concluded that reduced weights at weaning of kits fed herring oil may have been because diets with high amounts of fish oil are less palatable than more conventional diets. A further explanation for the reduced growth of kits fed herring oil may be that it has a higher percentage of saturated fatty acids than has soya oil, and due to poor fat digestion, saturated fatty acids may have lesser bioavailability than unsaturated fatty acids.

Of note, the retarded kit growth in the groups fed herring oil mixtures was most pronounced in the group fed fresh ensiled herring, not in the oxidised oil groups.<sup>10</sup>

In humans, platelet counts have been shown to decrease with increasing levels of n-3 PUFA in the diet.<sup>22</sup> This is consistent with the findings of Damgaard et al 2003,<sup>10</sup> where in the adults the platelet count was lower for all four experimental groups. However only in the kits was it found that the platelet count was lower in the oxidised fish oil than in the fresh fish oil groups. Also in humans, platelet aggregation was reduced and bleeding time

prolonged after a high intake of fish fat;<sup>24</sup> however no comparison was made in this study between fresh and oxidised fish oils.

Damgaard et al<sup>10</sup> found that the plasma level of alpha tocopherol (vitamin E) in the females was high and unaffected by the experimental treatments, indicating that the high levels of moderately oxidised fish oil caused no depletion of vitamin E body stores. They added 84 mg vitamin E per kg diet, which corresponded to the level found sufficient to protect growing mink kits fed highly oxidised marine fat against symptoms of vitamin E deficiency.<sup>25</sup> Borsting et al 1998<sup>12</sup> found that moderately oxidised fish oil as the sole fat source to lactating females caused depletion of vitamin E stores. However Clausen et al<sup>17</sup> found that 78% of dietary fat from fish fed to lactating females had no negative effects on vitamin E plasma levels. Damgaard 2003<sup>10</sup> found that in the control group, kits had substantially lower levels of alpha-tocopherol than adult females, and that it was particularly low for those kits receiving high levels of fish oil in the diets.

In the Damgaard study there were only limited effects on haematological indices. This contrasts with some previous results where moderately oxidised fish oil (as the sole fat source) caused anaemia in female mink at the end of the nursing period. However Tauson<sup>16</sup> and Clausen et al<sup>17</sup> did not find any negative effects on haematological indices in lactating females fed high amounts of fish fats. (Anaemia caused by oxidised fat is usually explained by disorders in the cell membrane structures, leading to increased fragility of the erythrocytes, and consequently haemolytic anaemia).

Borsting et al<sup>12</sup> noted that previous studies had shown that high dietary amounts of fresh fish oil without antioxidative stabilisation negatively affect the performance and health of adult mink. Reduced liver weights and a progressive anemia were also observed in some cases. They observed that anaemia caused by oxidised fat is usually explained by disorders in cell-membrane structures, including that of erythrocytes. This may lead to increased red cell fragility, and thus haemolytic anemia.

The pathological findings were more severe and appeared earlier when the mink were fed highly oxidised fish oil. However growing mink kits have been quite resistant to dietary oxidative stress if vitamin E supplementation is adequate (at 60 to 80 mg dl-alpha-tocopherol acetate per kg of feed).<sup>24</sup>

In the Borsting et al (1998)<sup>12</sup> study, four groups of mink were fed either fresh fish oil (with a POV value 10 meq O<sub>2</sub>/kg oil) or the same oil oxidised to POV values of 30, 70, or 100 meq O<sub>2</sub>/kg oil respectively (with ethoxyquin added for stabilisation). Fish oil represented 12% of the diet during the growing-furring period, and 3% during the reproduction period. However

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<sup>24</sup> Thorngren M, Gustafson A. Effects of 11-week increase in dietary eicosapentaenoic acid on bleeding time, lipid and platelet aggregation. Lancet 1981; 4: 1190-3.

<sup>25</sup> Engberg RM, et al. On the utilization, retention and status of vitamin E in mink (*Mustela vison*) under dietary oxidative stress. Journal of Animal Physiology and Animal Nutrition 1993; 69: 66-78.

the actual dietary intakes were not given in gravimetric amounts (rather in terms of energy intakes), and hence the doses of fish oil involved could not be calculated.

All females were mated with males fed a standard diet. The average POV of the oils were 11, 38, 70 and 103 meq O<sub>2</sub>/kg respectively for the four groups. The amount of PUFA in the oil decreased from 23.6% in the fresh oil to 23.1%, 22.7%, and 22.4% in the oils fed to groups 2,3, and 4. The content of free fatty acids in the fresh oil was 10 g/kg and this level was almost unaffected by the oxidation.

Unsurprisingly, the plasma concentration of alpha-tocopherol was inversely related to the POV value of the oil used in the four groups. There were no significant differences between the groups with respect to plasma levels of the enzymes alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK), and glutathione peroxidase (GSH-P), or the concentrations of total plasma lipids and triglycerides. Neither were there significant differences in a number of haematological indices, including packed cell volume, haemoglobin, or red cell number. The leucocyte count was slightly higher in the group receiving the fresh oil.

The PUFA-content in the oil decreased slightly in response to oxidation, from 31.7% in the fresh oil group to 30.1%, 29.8%, and 29.6% in the oils fed to groups 2,3, and 4 respectively. The average POV of the oils in this period were 3, 30, 76 and 107 meq/O<sub>2</sub>/kg respectively.

When the kits started to eat the experimental diets at 4 to 5 weeks, their growth was retarded and the experiment had to be cancelled before the end of the lactation period.

The lack of disappearance of PUFA during storage of the oxidised fish oils was in contrast to the extensive losses of these fatty acids in fish oils oxidised to 200 or 400 meq O<sub>2</sub>/kg oil stored for about 10 weeks in an earlier experiment.<sup>11</sup> This finding indicates that the addition of adequate amounts of ethoxyquin after oxidation is able to prevent or at least postpone further oxidation of fatty acids, even in fish fat oxidised to quite high levels of peroxides.

The authors concluded that high intakes of ethoxyquin stabilised fish oil with varying oxidative quality caused some adverse effects on the growth and health of growing male mink kits. The effects were not as pronounced as in a previous experiment with adult mink fed fish oil with a higher POV.<sup>11</sup>

This study was however limited by the lack of quantitative estimates of fish oil or PUFA intake, on a gram per kilogram body weight basis.

Kobayashi et al found that feeding a diet containing fish oil to rats at doses of 15% body weight induced a significant proliferation of peroxisomes compared to that with a safflower oil diet. Feeding n-3 enriched fish oil at 10% body weight did not accelerate the onset of bleeding in stroke-prone spontaneously hypertensive rats. These findings were considered

re-assuring, given the only minimal findings in these animals despite their being fed levels several fold higher than the anticipated maximal intake in humans.<sup>26</sup>

Rabbani et al undertook a 13 week study in rats, studying the effects of high dose fish oil concentrates.<sup>27</sup> The oils comprised corn oil, pure menhaden oil (MO), different mixtures of corn oil with menhaden oil, and pure MaxEPA<sup>®</sup> fish oil concentrate (FOC). Menhaden oil is crude fish oil, containing a total of 29.9% n-3 fatty acids, including 9.2% DHA and 12.9% EPA. The MaxEPA<sup>®</sup> FOC provided a total of 35% omega-3 fatty acids, including 12% DHA and 17.8% EPA.

Twenty rats of each sex were allotted to one of six groups (240 animals). The rats were gavaged daily with the oils and mixtures thereof in a volume equal to “0.5% of an animal’s body weight”, or 5mL/kg/day for 13 weeks. The oils had a specific gravity of 0.93 g/mL, suggesting that the dose would have been 4.65 g/kg/day. This suggests the Menhaden Oil provided daily doses of 0.43 g/kg DHA (4.65 x 9.2%) and 0.6 g/kg EPA (4.65 x 12.9%), while the MaxEPA<sup>®</sup> provided daily doses of 0.56 g/kg DHA (4.65 x 12%) and 0.83 g/kg EPA (4.65 x 17.8%). In terms of total n-3 fatty acids, Menhaden oil provided 1.39 g/kg (4.65 x 29.9%) per day and MaxEPA<sup>®</sup> provided 1.63 g/kg (4.65 x 35%) per day. However there is some uncertainty around this as the authors do not estimate the doses given in g/kg body weight.

However the “human equivalent” of the highest dose was said to be about 19 g of n-3 fatty acids per day for MO and 23 g of n-3 fatty acids per day for MaxEPA<sup>®</sup> for a 65 kg human.<sup>26</sup> In the Menhaden oil, DHA plus EPA comprised 74% of the n-3 fatty acids  $[(9.2 + 12.9)/29.9]$ , while in the MaxEPA<sup>®</sup>, DHA and EPA comprised 85% of the n-3 fatty acids  $[(12 + 17.8)/35]$ . Therefore the human equivalent doses for DHA plus EPA in this study was 14 g (19 x 74%) for the menhaden oil and 19.6 g (23 x 85%) for MaxEPA<sup>®</sup>.

While such doses of fish oil demonstrated some benefit on reduction of total serum cholesterol, the authors concluded that rats, especially females, given subchronic and high doses of fish oil concentrate developed potentially harmful changes. These included increased red cell deformity, increased relative liver and spleen weights, and reductions in mean platelet volume, absolute amount of serum HDL, serum iron and vitamin E concentrations.

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<sup>26</sup> Kobayashi T, et al. Assessment of the possible adverse effects of oils enriched with n-3 fatty acids in rats; peroxisomal proliferation, mitochondrial dysfunctions and apoplexy. *Nutritional Biochemistry* 1996; 7: 542-8.

<sup>27</sup> Rabbani PI, et al. Subchronic toxicity of fish oil concentrates in male and female rats. *Journal of Nutrition Science and Vitaminology* 2001; 47: 201-12.

However the doses of DHA and EPA involved (adjusted on a weight to weight basis to humans) appeared to exceed current maximum recommended doses by a fairly wide margin.

DHA specifically is also present at high concentrations in some algal derived oils, and Schmitt et al conducted a prenatal developmental toxicity study in rats using such a DHA-rich algal oil (containing ~ 42% DHA). The authors commented that “dosage levels were 400, 1000, and 2000 mg/kg/day administered at a dosage volume of 5 mL/kg” (for three different groups of female Sprague Dawley rats). In the abstract they stated that the dosage level of 2,000 mg/kg was considered the NOAEL for maternal toxicity and embryo/fetal development.<sup>28</sup> This may correspond to a DHA level of 840 mg/kg (2,000 x 42%).

In a dietary combined one generation/90 day reproductive toxicity study, the NOAEL for systemic toxicity was considered to be 50, 000 ppm (in feed) for F0 male and female and F1 male systemic toxicity, and 25,000 ppm for F1 female systemic toxicity. F0 reproductive performance values, estrous cycle length, gestation length, parturition, and the numbers of former implantation sites and unaccounted-for sites were unaffected by the algal oil exposure.<sup>27</sup>

## **Risk assessment**

### **Hazard identification**

Fish oil has been found to have beneficial effects but also deleterious effects in human and experimental animal studies. In the latter, considerable emphasis has been placed on reproductive effects. Damgaard et al<sup>10</sup> found that high dietary levels of fresh or moderately oxidised fish oil could be used for mink females during the winter and reproduction periods without any negative effects on performance, health, and reproduction results. However high levels of fish oil resulted in lower kit weights at weaning.

### **Exposure Assessment**

Exposure assessment is difficult in this specific situation because dietary amounts of PUFAs including EPA and DHA used in relevant studies have not generally been detailed or expressed in terms of mg per kg body weight, and this critical statistic needs to be estimated in other ways.

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<sup>28</sup> Schmitt D. et al. Toxicologic evaluations of DHA-rich algal oil in rats: developmental toxicity study and 3-month dietary toxicity study with an *in utero* exposure phase. Food and Chemical Toxicology 2012; 50: 4149-57.



In one (adult) mink study, daily feed consumption averaged 40 g dry matter per kg body weight for males and 53 g dry matter per kg body weight for females.<sup>29</sup> Adult female mink weigh between 0.7 to 1.1 kg while males weigh 0.9 to 1.6 kg.<sup>30</sup> For adult females, using the lowest weight (0.7 kg) yields an estimate of daily feed consumption of 37.1 g (53 x 0.7).

In the Damgaard et al (2003) study, the diet with fish oil contained 140 g defatted herring scrap per kg of feed; that is, it contained 14% as herring scrap, itself containing 30% herring oil. Thus the herring oil constituted 4.2% of the diet by weight. Applying this proportion to the above information regarding food consumption by adult female mink gives an estimate for herring oil consumption of 1.56 grams (or 2.23 g/kg for a 0.7 kg animal).

One analysis revealed that herring oil has a total n-3 PUFA content of 21.4% (w/w). The 20:5 n3 (ie EPA) content was 7.5%, while the 22:6 n3 (ie DHA) content was 6.8%.<sup>31</sup>

Therefore in the Damgaard (2003) study, the estimated EPA consumption is 1.56 g x 0.075 = 117 mg and that for DHA is 1.56 g x 0.068 = 106 mg. Or, in a 0.7 kg adult female mink, 167 mg/kg EPA and 151 mg/kg DHA, with a combined total of 318 mg/kg.

This calculation is summarised below:

Total diet (female mink) = 53 g/kg = 53,000 mg/kg

“Fish oil”: 14% of total diet = (53 x 0.14) g/kg = 7.42 g/kg = 7,420 mg/kg

Herring oil: (30% of 14%) = 4.2% of total diet = 2,226 mg/kg

EPA: (7.5% x 4.2%) = 0.315% of total diet = 167 mg/kg

DHA (6.8% x 4.2%) = 0.286% of total diet = 151 mg/kg

EPA + DHA = 0.6% of total diet = (53,000 mg/kg) x 0.006 = 318 mg/kg

(Correction to dry dietary matter alone by excluding the water component (5.9%) in the above diet gives a value of 14.9% for the defatted herring scrap, or 4.46% herring oil. Applying this to the above estimate for food consumption by adult female mink yields an estimate of 1.65 grams or 2,360 mg/kg herring oil; or 177 mg/kg EPA and 160 mg/kg DHA, with a combined total of 337 mg/kg. This results in only a ~ 6% difference in the dose estimates of these PUFAs).

## Dose-Response Relationships

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<sup>29</sup> Bleavins MR, Aulerich RJ. Feed consumption and food passage time in mink (*Mustela vison*) and European ferrets (*Mustela putorius furo*). *Laboratory and Animal Science* 1981; 31(3): 268-9.

<sup>30</sup> [www.biokids.umich.edu/critters/Neovison\\_vison/](http://www.biokids.umich.edu/critters/Neovison_vison/)

<sup>31</sup> Opstvedt J. Fish lipids in animal nutrition. Norwegian Herring Oil & Meal Industry Research Institute, 5033 Fyllingsdale, Norway.me

There are insufficient quantitative data for a detailed dose-response assessment. However the Damgaard (2003) study appears to yield a no observed adverse effect level (NOAEL) for a number of effects; ie 318 mg/kg. The number of live as well as stillborn kits per litter was not affected by the experimental diets fed to the mothers during the winter and pregnancy periods. The number of kits at weaning and the number of kits lost during the lactation period were also not affected by the diet fed during the winter and pregnancy period or during the lactation and early growth periods.

On the other hand the body weight of the kits was affected at day 28 after birth and at weaning. This has also been noted by Borsting (1998), Clausen (1999) and Tauson (1994). This suggests that in some respects the above estimated dose should be considered as a lowest observed adverse effect level (LOAEL) rather than a universal NOAEL.

However Tauson concluded that it was likely diets with high amounts of fish oil were less palatable than more conventional diets, such that dietary intakes (and hence body weights) may be lower for the high fish oil groups. In some studies the experimental groups also had a higher content of saturated as opposed to unsaturated fatty acids in their diets relative to the control group. It was suggested that in growing kits the saturated fatty acids may have been less digestible, which might help explain the reduced growth of kits fed herring oil.<sup>10</sup> The apparent hypothesis was that the reduced kit weights may not reflect a direct toxic effect of the fish oil.

The Damgaard et al study also did not reveal any obvious distinction in reproductive toxicity between fresh and oxidised oils in terms of risk of adverse reproductive outcomes. This represents some grounds for reassurance (at least with fish oils with PV or Totox values no more than 60 meq O<sub>2</sub>/kg as used in that study). However this is a single study and more work is required before the relative toxicities of fresh fish oil and fish oils of various Totox values can be quantified.

As noted earlier, Borsting et al<sup>12</sup> found that some pathological findings were more severe and appeared earlier when the mink were fed highly oxidised fish oil. Four groups of mink were fed either fresh fish oil (with a POV value 10 meq O<sub>2</sub>/kg oil) or the same oil oxidised to POV values of about 30, 70, or 100 meq O<sub>2</sub>/kg oil respectively. There were no significant differences between the groups with respect to plasma levels of the enzymes ALT, AST, CK, and GSH-P<sub>x</sub>, or the concentrations of total plasma lipids and triglycerides. Neither were there significant differences in a number of haematological indices, including packed cell volume, haemoglobin, or red cell number. The leucocyte count was however slightly higher in the group receiving the fresh oil.

Also the growth of the kits was retarded when they started to eat the experimental diets at 4 to 5 weeks. Unfortunately however this experiment had to be cancelled before the end of the lactation period, which precluded any study of the effects of “high” levels of oxidation.

## **Risk characterisation**

The current acceptable or recommended daily intake of EPA and DHA combined of 450 mg in pregnant women represents about 7.5 mg/kg body weight. This is substantially lower (ie about ~ 42 –fold less) than the above estimated NOAEL (or LOAEL for offspring) of 318 mg/kg for EPA and DHA combined. This would seem to provide considerable grounds for reassurance, even though the difference is less than one hundred fold.

The U.S. FDA concluded that use of menhaden (fish-based) oil as a food ingredient is generally regarded as safe (GRAS), provided that the combined daily intake of DHA and EPA does not exceed 3 grams/day.<sup>27</sup> This generally corresponds to < 60 mg/kg for a human adult, which is about one fifth of the above proposed NOAEL.

However human and indeed animal data appear not extensive enough to serve as the basis for derivation of an acceptable daily intake (ADI) of fish oil, including at any specific level of oxidation (PV, AV, or Totox values). In 2010 it was observed that knowledge does not allow setting and recommending of maximum acceptable peroxide and anisidine values for the large variety of refined fish oils.<sup>6</sup> This situation does not appear to have changed.