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**SUMMARY OF PUBLISHED RESEARCH ON THE
BENEFICIAL EFFECTS OF FISH CONSUMPTION
AND OMEGA-3 FATTY ACIDS FOR CERTAIN
NEURODEVELOPMENTAL AND
CARDIOVASCULAR ENDPOINTS**

SECTION A: CARDIOVASCULAR

SECTION B: NEURODEVELOPMENTAL

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Introduction

This report serves as a companion document to the Food and Drug Administration (FDA) draft report entitled “Report of Quantitative Risk and Benefit Assessment of Commercial Fish, Focusing on Fetal Neurodevelopmental Effects (Measured by Verbal Development in Children) and on Coronary Heart Disease and Stroke.” The information contained in this document represents an in-depth overview of the scientific literature regarding the health effects of fish and of the long chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid on cardiovascular disease and neurodevelopment.

Fish provide a source of easily digestible protein of high biological value, micronutrients including vitamins A and D, the minerals iodine and selenium, and high levels of the amino acids taurine, arginine and glutamine (EFSA 2005; He and Daviglus 2005). Additionally, many fish provide a uniquely rich food source of long chain omega-3 fatty acids (also called n-3) long-chain polyunsaturated fatty acids (n-3 LC PUFA), most notably docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). A number of research studies have reported associations between consumption of fish, fish oil, or n-3 LC PUFA and reduced risk of cardiovascular events such as heart attack and stroke (Kris-Etherton et al., 2002). Moreover, the n-3 LC PUFA, docosahexaenoic acid, has been shown to be essential for development of the central nervous system (EFSA 2005, page 30). Consequently, there is considerable interest in whether there is an association between fetal, infant or child neurodevelopment and maternal intake of fish or n-3 LC PUFA during pregnancy and lactation (SACN 2004).

There are a number of original (or primary) scientific studies on the health effects associated with consumption of fish or n-3 LC PUFA. The primary studies most relevant to the evaluation of the risks and benefits of fish consumption have been summarized and evaluated previously in a number of recent scientific reports and review articles (or secondary sources). Thus, the approach used to develop this document was primarily to inventory these secondary sources and to highlight any findings related to the health effects of fish consumption and of the n-3 LC PUFA found in fish on cardiovascular disease and neurodevelopment. Research has addressed the possible association of fish or of n-3 LC PUFA consumption with numerous other health outcomes, including neuropsychiatric disorders (including depression and psychotic disorders), cognitive decline and Alzheimer’s Disease, neurodegenerative disorders, cancer risk reduction, and reduced risk of chronic degenerative diseases related to immune and auto-immune or musculo-skeletal function, acute macular degeneration and other visual impairments, although consideration of these outcomes is beyond the scope of this document. When available, the document also identifies reports of quantitative dose-response relationships which may be used in risk and benefit assessment modeling.

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Summary of Scientific Information in Section A: Effect of Fish Consumption and Omega-3-Fatty Acids on Cardiovascular Disease

Published reports have made conclusions about the relationship between fish or n-3 LC PUFA consumption and coronary heart disease (CHD) risk:

- Primary and secondary prevention, randomized clinical trial of fish oil consumption. The recently published large-scale clinical trial called the Japan EPA Lipid Intervention Study (JELIS) from Japan included over 18,000 men and women (Yokoyama et al., 2007). Almost 15,000 participants had no record of coronary artery disease (primary prevention). Results showed a 19 percent decrease in major coronary events (fatal plus nonfatal) for all subjects, a 19 percent decrease for secondary prevention subjects and an 18 percent decrease for primary prevention subjects. The decrease in risk was similar in magnitude for primary and secondary prevention, but was not statistically significant for primary prevention alone ($p = 0.13$). For the full study and for both subgroups, there was no significant decrease in sudden cardiac death or coronary death alone, probably reflecting that the high baseline fish intake in Japan is above a possible threshold for effect on risk of sudden death or CHD death.
- Secondary prevention, randomized clinical trials of fish or fish oil consumption. The large, well-conducted secondary prevention trial, GISSI, included over 10,000 men and found a 15 percent decrease in all deaths plus nonfatal heart attacks and strokes, a 26 percent decrease in cardiovascular deaths plus nonfatal heart attacks and strokes and a 45 percent decrease in sudden death, all significant (1999; Marchioli et al., 2002) Results of the DART1 study were consistent with GISSI, but results differed for the poor quality DART2 study (Burr et al., 2003; Burr et al., 1989).
- Meta-analyses of randomized controlled trials of fish or fish oil consumption. Mozaffarian and Rimm (Mozaffarian and Rimm 2006) conducted a meta-analysis including five randomized controlled trials and 15 prospective cohort studies of fish or fish oil intake and CHD death among >300,000 subjects. There was a significant, 17 percent decrease in total CHD mortality. A total 36 percent reduction in risk was estimated for intakes of 250 mg/day EPA/DHA.
- Observational studies of blood levels of n-3 LC PUFA and CHD risk. As summarized by (SACN 2004) and others, additional evidence for the cardiovascular benefits of fish and fish oil consumption is provided by several cohort or case control studies that found decreased CHD risk associated with higher blood levels of DHA and EPA. SACN stated that, "Taken together, these data support the hypothesis that n-3 LC PUFA are responsible for the observed inverse association between fish consumption and sudden cardiac death."

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- Meta-analyses of observational studies of fish consumption and risk of cardiovascular disease. There are several meta-analyses of observational studies of fish consumption and risk of CHD or stroke with fairly consistent results among the meta-analyses. The prospective studies of CHD death included more than 200,000 men and women and the prospective studies of stroke also included more than 200,000 men and women. For example, the meta-analyses of He et al (He et al., 2004a; He et al., 2004b) found a 15 percent decreased risk of CHD death and a 13 percent decreased risk of stroke associated with fish intake once per week compared with less than once per month.
- A meta-analysis (Studer et al., 2005) of 97 studies, with 137,140 individuals in intervention and 138,976 individuals in control indicated that the benefits of n-3 LC PUFAs were comparable to (or greater) than the benefits of statins for overall mortality.

In 2002, FDA reviewed the credible scientific evidence available at that time to support a qualified health claim for the labeling of foods. The Agency concluded that supportive but not conclusive research showed that consumption of EPA and DHA omega-3 fatty acids may reduce the risk of coronary heart disease.

Summary of Scientific Information in Section B: Effect of Fish Consumption and Omega-3 Fatty Acids on Neurodevelopmental Outcomes

This section summarizes recent reports and review articles and presents an overview of current scientific information on the association of maternal fish consumption with infants' and children's visual and cognitive neurodevelopment.

Randomized trials of maternal supplementation in pregnancy and lactation: Among the few available studies of maternal supplementation, women in the Helland et al. (Helland et al., 2003) study were supplemented with fish or fish oil providing 1.2 grams of DHA per day during pregnancy and lactation and increased infant DHA blood levels were demonstrated biochemically (Eilander et al., 2007). Limitations included uncertain effect of the corn oil control supplement, the small subset of the population that received follow up IQ testing at age four years, and uncertain differences in background n-3 LC PUFA intake between Norwegian and U.S. women. However, the 4.1 point higher average K-ABC Mental Processing IQ scores of the children of fish oil supplemented mothers supports the plausibility of measurable neurodevelopmental benefits of maternal seafood consumption and gives one example of magnitude of dose-response. Additional maternal supplementation trials would be helpful to replicate this result, and to add features such as detailed background n-3 LC PUFA status, supplementation in pregnancy alone or including lactation, various levels of fish oil supplement dose, and several years of complete, planned follow up testing.

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Randomized trials of infant formula supplementation: The complexity and inconsistency of the literature on supplementation of infant formula with DHA is a barrier to demonstrating the plausibility of measurable neurodevelopmental benefits for infants and children. The potential for estimating a quantitative dose-response from these data is limited. Among the factors that differed across the randomized trials were: infant population (preterm or term birth), timing of supplementation (beginning at birth or after period of breastfeeding; duration of a few months to one year), test formula composition (presence of arachidonic acid (AA); levels of DHA, AA and ALA), additional breastfed comparison group, neurodevelopment outcome (vision, cognitive, general development, other), visual acuity testing (behavioral or electrophysiologic), neurodevelopment testing (global or targeted assessment), age at testing (early infancy to three years or older). Systematic reviews and meta-analyses evaluated the randomized trials in subgroups according to various study conditions, and generally found the evidence for neurodevelopmental benefit of DHA supplemented formula to be inconsistent and inconclusive (Lewin et al., 2005; Simmer 2001; Simmer and Patole 2004; Simmer et al., 2008a; Simmer et al., 2008b; Smithers et al., 2008). Studies were grouped differently in different systematic reviews, and newer studies were available for more recent reviews, making comparisons difficult across reviews.

The analysis of Lauritzen et al. (Lauritzen et al., 2001) concentrated on a single age at testing (four months) and identified formula composition and visual acuity method as likely sources of heterogeneity among trials. These authors recommended that future trials use conditions from previous positive trials, including DHA as 0.36 percent of lipids in test formula and electrophysiologic method for visual acuity testing. The meta-regression of Uauy et al (Uauy et al 2003) quantified the dose-response for DHA equivalents in 12 comparisons from seven controlled trials of term infant visual acuity at four months of age (Table 3). Morale et al. (2005) analyzed visual acuity at age 12 months in studies from a single laboratory and found a linear dose-response for duration of supply of LC PUFA from formula supplemented with DHA as 0.36 percent of lipids, breastfeeding, or both (Table 12). Birch et al. (2005) designed a trial to carry out the Lauritzen et al. recommendations regarding DHA level in test formula and electrophysiological visual acuity as well as adequate sample size (greater than 20 per group). Supplemented infants had significantly better visual acuity at six, 17, 39 and 52 weeks of age and better stereoacuity at 17 weeks.

Most studies showed little evidence of a positive effect of supplemented formula on infant neurodevelopment using global tests, such as the Bayley scales. A few studies reported positive effects using more specific, focused developmental assessments, but these assessment methods were not adopted by other research groups (Willatts et al., 1998). The study of Birch et al. (Birch et al., 1998) did find a positive effect of supplemented formula for four months using Bayley's MDI at 18 months of age. In a follow up at four years of age, infants supplemented with DHA plus AA had mean Wechsler Performance, Verbal and Full Scale IQ scores that were 4.4, 5.7 and 6.5 points higher, respectively, than scores of control infants (Birch et al., 2007). However, the

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statistical significance of this comparison was not tested directly but in a research design including a breastfed group and a DHA (with no AA) supplemented group. A secondary analysis of the IQ comparison for DHA plus AA supplemented and control infants from Birch et al. (Birch et al., 2007) would show whether the result is statistically significant and if not significant, what sample size would be needed to replicate the results with adequate power.

Cohen et al (Cohen et al., 2005a; Cohen et al., 2005c) pooled the results of nine unique trials of supplemented formula and neurodevelopmental outcomes. Based on the average DHA level in supplemented formulas, the authors estimated an effect size of 4.6 IQ points for each one percent DHA (as percent of lipids) in infant formula (Table 9). In the supplemented formula of Birch et al. (2007), the DHA level was 0.36 percent, giving a Full Scale IQ effect size of 18 points ($6.5/0.36$) per one percent DHA in formula, considerably larger than the 4.6 point effect size of Cohen et al. (2005a, 2005c) Cohen and coauthors reported only a point estimate and did not state whether their result was significantly different from no effect.

Observational studies: Observational studies, such as prospective cohort studies, consider outcomes related to participants' actual diets. In a project on the Risks and Benefits of Seafood, maternal fish consumption, rather than infant or maternal fish oil supplementation, is the actual exposure most relevant to assessing potential health benefits for infant and child neurodevelopment. Although an observational study cannot conclusively demonstrate a cause and effect relationship, possible confounding variables that are known and measured can be adjusted for in the statistical analysis.

In three cohort studies, children's visual function (Williams et al., 2001) and neurodevelopment (Daniels et al., 2004; Oken et al., 2005) were positively associated with mother's fish intake during pregnancy, with adjustment for covariates. The association with visual function is consistent with certain analyses of supplemented infant formula (Birch et al., 2005; Lauritzen et al., 2001; Morale et al., 2005; Uauy et al., 2003). Recent results estimated a positive, quantitative association between maternal fish consumption and children's developmental scores in Project Viva in the United States at three years of age (Oken et al 2008a) and in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort in the United Kingdom at eight years of age (Hibbeln et al., 2007a, 2007b) (Table 14). The magnitude of the quantitative estimates from observational studies is considerably larger than estimates based on infant supplementation studies and a series of assumptions (Cohen et al., 2005a, 2005c).

In both observational cohorts, the positive association of neurodevelopment with mothers' fish intake was stronger with additional adjustment for maternal mercury exposure. Independent, negative associations of neurodevelopment with maternal mercury exposure were also observed, but these were smaller than the independent, positive associations with maternal fish intake (Hibbeln et al., 2007a; Hibbeln et al., 2007b; Oken et al., 2008a). This was in contrast to the Cohen et al. (Cohen et al., 2005a)

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risk benefit assessment, which used different types of data and evidence for DHA benefits of fish intake (Cohen et al., 2005a) and for risks of mercury (Cohen et al 2005c). As discussed in the FDA draft risk and benefit assessment report, Cohen et al. (2005c) may have overestimated the dose-response for the risks of maternal methylmercury exposure. However, a possible underestimate of the dose-response for the benefits of maternal DHA intake in Cohen et al. (2005b) should not be overlooked. The quantitative estimates of neurodevelopmental DHA benefits of Cohen et al. (2005a, 2005b) have been used in other analyses (Guevel et al., 2008), but should be viewed with caution because of their inconsistency with the cohort studies.

The identification of independent neurodevelopmental benefits of maternal seafood intake and risks of maternal mercury exposure in Project Viva and the ALSPAC cohort are consistent with recent data from established cohort studies of maternal mercury exposure and children's neurodevelopment. Structural equation modeling of neurodevelopmental test data at seven and 14 years of age from the Faroe Islands showed that, after mutual adjustment for both variables, there was an independent, positive association with maternal fish intake as well as a negative association with maternal mercury exposure (Budtz-Jorgensen et al., 2007). Preliminary results from the Seychelles nutrition cohort suggested that children's developmental tests were positively associated with maternal n-3 LC PUFA blood levels and negatively associated with maternal hair mercury (Myers et al., 2007). These associations were stronger when mutually adjusted for the other variable.

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SECTION A

OVERVIEW OF SCIENTIFIC INFORMATION ON THE EFFECT OF FISH CONSUMPTION AND OMEGA-3 FATTY ACIDS ON CARDIOVASCULAR DISEASE

(a) Introduction

A number of studies have found associations between the consumption of fish, fish oil, or n-3 LC PUFA and reduced risk of cardiovascular events, such as heart attack and stroke (Kris-Etherton et al., 2002). Among the health effects associated with fish or n-3 LC PUFA consumption, the cardiovascular health effects have been reported by a large, recognized body of science. Numerous observational studies in healthy populations have investigated the possible association of fish consumption with decreased cardiovascular risks. The table below lists a number of recent reports and recommendations summarizing the body of science on the cardiovascular benefits of fish or n-3 LC PUFA consumption. Also listed are several recent systematic reviews, meta-analyses and risk assessments.

The reports and reviews have in common that they are directed towards the cardiovascular benefits of fish or n-3 LC PUFA consumption, but they vary regarding the specific purpose of the review and the particular scientific questions addressed. The reports also vary by the methods and detail of the scientific review, the criteria for inclusion or exclusion of studies, whether the analysis is qualitative or quantitative, and the criteria for evaluating studies and drawing conclusions. Thus, from a large, recognized body of scientific data, the conclusions and recommendations may differ based on the purpose of each report and the methods of the review.

Table A-1. Recent reports and reviews on the cardiovascular health benefits of fish or n-3 LC PUFA consumption (see reference list for full citations)

Reports and Recommendations, 2000 through 2005

- **United States, non-government organizations:**
 - AHA (American Heart Association), 2000, 2002
 - NAS IOM (National Academy of Sciences, Institute of Medicine) Dietary Reference Intakes, 2002
- **United States, government organizations:**
 - AHRQ (Agency for Health Care Research and Quality), DHHS, 2004

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- 2005 Dietary Guidelines Advisory Committee, 2004
- 2005 Dietary Guidelines for Americans, 2005
- FDA (Food and Drug Administration), Qualified Health Claims, 2004
- **International, government and non-government organizations:**
 - SACN (Scientific Advisory Committee on Nutrition), United Kingdom, 2004
 - EFSA (European Food Safety Authority) 2005
 - WHO (World Health Organization), 2003
 - ISSFAL (International Society for the Study of Fatty Acids and Lipids), 2004
 - European Society of Cardiology, 2003

Report and Recommendation, 2006

- **United States, non-government organization:**
 - NAS IOM, Seafood Choices, 2006

Other Recent Systematic Reviews, Meta-Analyses and Risk Assessments

- He et al., meta-analysis of fish consumption and coronary heart disease death, 2004
- He et al., meta-analysis of fish consumption and stroke, 2004
- Whelton et al., meta-analysis of fish consumption and coronary heart disease, 2004
- Hooper et al, Cochrane Collaboration, review and meta-analysis of omega-3 fatty acids and prevention and treatment of cardiovascular disease, 2004, 2006
- Harvard Risk/Benefit Papers, American Journal of Preventive Medicine, 2005
 - König et al.(2005) meta-analysis of fish consumption and coronary heart disease death
 - Bouzan et al. (2005) meta-analysis of fish consumption and stroke
 - Cohen et al. (2005a) assessment of risk and benefit of fish consumption
- Mozaffarian & Rimm, clinical review of scientific evidence for adverse and beneficial health effects of fish consumption, 2006

(In contrast to the large, recognized body of science reporting the cardiovascular health benefits of seafood consumption, a few studies have suggested that methylmercury in fish may increase cardiovascular risk. As described in the reports and reviews listed in Table A-1, numerous observational studies in healthy populations have investigated the possible association of fish consumption with decreased heart disease risk. Many of these studies focused on fish consumption itself, rather than on n-3 fatty acids or other nutrients or components in fish. Thus, the cardiovascular benefits associated with fish consumption in these studies are the net result of beneficial components in fish together with possible risk from harmful components such as methylmercury. To provide context on the relationship between the cardiovascular benefits of fish consumption and possible cardiovascular risk of methylmercury in fish, research on the possible cardiovascular risk of methylmercury in fish is discussed briefly later in this section.)

(b) Conclusions and Recommendations from Some Recent Reports

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American Heart Association

The American Heart Association (AHA) Dietary Guidelines in 2000 noted the scientific evidence for a cardioprotective effect of food sources of n-3 LC PUFA, EPA and DHA, beyond the effect of changes in serum lipid profiles. The AHA recommended consumption of at least two servings of fish per week “to confer cardioprotective effects” (Krauss et al., 2000). In 2002, the AHA updated its 1996 scientific review of fish consumption and cardiovascular disease (Kris-Etherton et al., 2002). The 2002 report summarized new findings from epidemiologic studies and clinical trials. It also described possible physiologic mechanisms by which n-3 LC PUFA may reduce risk for cardiovascular disease, including prevention of cardiac arrhythmia (irregular heartbeat), prevention of blood clot formation, lowering of blood triglycerides, and slowing the development of atherosclerotic plaques. The report concluded that n-3 fatty acids have been shown in epidemiological and clinical trials to reduce the incidence of cardiovascular disease and that large-scale epidemiological studies show benefit from consumption of plant and marine food sources of n-3 fatty acids.

The 2002 AHA report concluded that the collective data supported the 2000 AHA recommendation to include at least two servings of fish per week (particularly fatty fish) in a healthy diet for the general population. Consumption of a variety of fish was recommended to achieve cardiovascular health outcomes while minimizing potentially adverse effects of environmental pollutants such as methylmercury. The AHA further recommended that heart disease patients consume one gram of n-3 LC PUFA (EPA plus DHA) per day. Although obtaining n-3 fatty acids from food sources was preferable, the AHA recognized that n-3 fatty acid dietary supplements might be needed to obtain the 1 gram per day recommended for heart patients.

In 2006, AHA published its Diet and Lifestyle Recommendations, Revision 2006 (AHA 2006). One of the nine diet and lifestyle recommendations for cardiovascular disease risk reduction was, “Consume fish, especially oily fish, at least twice a week.” The AHA stated that “The consumption of 2 servings (about 8 ounces) per week of fish high in EPA and DHA is associated with a reduced risk of both sudden death and death from coronary artery disease in adults.” The references cited were the AHA 2002 report (Kris-Etherton et al., 2002) and the updated report from the Agency for Healthcare Research and Quality (Wang et al., 2006), discussed below. Furthermore, AHA recognized that in addition to providing EPA and DHA, regular fish consumption may facilitate the displacement of other foods higher in saturated and trans fatty acids, such as fatty meats and full-fat dairy products.

Institute of Medicine of the National Academies: Dietary Reference Intakes

The Institute of Medicine (IOM) considered recommendations for intake of n-3 fatty acids in its 2002 report, “Dietary Reference Intakes: Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids” (IOM, 2002). One of a series of

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IOM reports on dietary reference intakes (DRIs) for various nutrients, this report is also called the “IOM Macronutrient Report” because it covered the energy-yielding macronutrients. The Macronutrient Report separated its evaluation and recommendations into two aspects: first, the metabolic requirements for specific nutrients and second, quantitative guidance on proportions of energy sources (such as protein, carbohydrate and fat) to decrease chronic disease risk, while ensuring sufficient intakes of essential nutrients.

Metabolic requirements for n-3 fatty acids

The IOM reviewed scientific evidence showing that the n-3 PUFA, alpha-linolenic acid (ALA), which is an 18-carbon fatty acid, is an essential nutrient. Specifically, ALA cannot be synthesized by humans, and must be supplied by dietary means. Deficiency of ALA results in adverse clinical symptoms, including poor growth and neurological abnormalities. Although there were some available data on treating certain hospitalized patients with ALA to correct ALA nutrient deficiencies, there were no similar data for correcting ALA deficiency in otherwise healthy individuals. Therefore, the IOM Macronutrient Committee could not determine an Estimated Average Requirement (EAR) for ALA, and also could not set a Recommended Dietary Allowance (RDA) for ALA, which by definition depends on the EAR. Instead, the IOM set a level for Adequate Intake (AI) of ALA, based on median intake of the population. For adults age 19 and older, the AI for ALA was set at 1.6 grams/day for men and 1.1 grams/day for women. The Committee stated that, in the United States, an ALA deficiency is basically unknown in free-living populations. (The AI for pregnancy and lactation and for infants and children is discussed later, in the section on neurodevelopment.)

Although humans cannot synthesize n-3 fatty acids from other types of fatty acids, humans can use the 18-carbon n-3 fatty acid, ALA, as a starting material to synthesize the n-3 LC PUFAs, EPA and DHA, which are 20-carbon and 22-carbon fatty acids, respectively. The IOM stated that small amounts of EPA and DHA can contribute towards reversing a deficiency of n-3 fatty acids. EPA and DHA can contribute up to 10 percent of the total n-3 fatty acid requirement, and therefore up to this percent can contribute towards the AI for ALA.

The IOM reviewed possible adverse effects of macronutrients, including n-3 fatty acids. The committee stated that the long chain n-3 fatty acids, DHA and EPA, are more biologically potent than their precursor, ALA. Therefore, interest in possible adverse effects has focused on DHA and EPA. The report concluded that there is evidence to suggest that high intake of EPA and DHA may impair immune response and result in excessively prolonged bleeding times. However, the data were not sufficient for IOM to set an Upper Limit (UL) for EPA or DHA. The committee recommended that dietary supplements of EPA and DHA be used with caution because information on possible adverse effects is incomplete.

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Quantitative guidance on proportions of energy sources to decrease chronic disease risk

The 2002 Macronutrient Report reviewed the scientific evidence on macronutrients and chronic disease for setting Acceptable Macronutrient Distribution Ranges (AMDRs). There may be increased risk of chronic disease and insufficient intakes of essential nutrients when intake is above or below the AMDR.

In reviewing the literature on low n-3 PUFA diets, the IOM noted that, “Growing evidence suggests that dietary n-3 polyunsaturated fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) reduce the risk of coronary heart disease and stroke.” Possible biological mechanisms included preventing arrhythmias, reducing atherosclerosis, decreasing platelet aggregation, decreasing plasma triglycerides, increasing high-density lipoprotein (HDL) cholesterol, affecting endothelial function, decreasing inflammatory molecules called eicosanoids and moderately decreasing blood pressure. The IOM reviewed the epidemiological evidence (prospective cohort studies and case control studies) of fish, fish oil or n-3 fatty acid intake and clinical outcomes of coronary heart disease or stroke. The committee also reviewed studies of the effect of fish oil supplementation on intermediate markers such as triglyceride and other lipid levels and platelet aggregation. The committee reviewed three randomized controlled trials (RCTs) of fish or fish oil intervention and coronary heart disease (Burr et al., 1989; Singh, 1997 and GISSI, 1999) and one RCT of dietary advice plus a special margarine containing ALA (de Lorgeril, 1994, 1999).

The report concluded that ALA, EPA and DHA may provide beneficial health effects when consumed at moderate levels. However, the AMDRs were not set based on the literature review of low n-3 PUFA diets, including the literature on cardiovascular disease. The IOM estimated the AMDR for ALA by using the AI for ALA as the lower end of the intake range. For adults, the ALA AI of 1.1 g/d for women and 1.6 g/d for men would correspond to about 0.6 percent of energy (calories). The highest intake of ALA from food in North America corresponds to around 1.2 percent of energy. This was set as the upper end of the AMDR intake range. Thus the AMDR for ALA was set at 0.6 to 1.2 percent of energy. Because EPA and DHA can contribute up to 10 percent of the AI for ALA, as noted above, the AMDR for EPA plus DHA was set at 0.06 to 0.12 percent of energy.

Agency for Healthcare Research and Quality

In 2004, the Agency for Healthcare Research and Quality (AHRQ) of the U.S. Department of Health and Human Services (DHHS), through its Tufts New England Medical Center Evidence-Based Practice Center, conducted systematic reviews of the scientific literature and published three related evidence-based reports on the health benefits of n-3 fatty acids on cardiovascular diseases. The AHRQ cardiovascular reviews were supported by the National Institute of Health Office of Dietary Supplements and were part of a series of reviews summarizing the current evidence on the health effects of

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omega-3 fatty acids. The purpose of the AHRQ series of reviews was to inform the research community and the public on the effects of omega-3 fatty acids on various health conditions, and to help define the agenda for future research.

The first AHRQ cardiovascular report reviewed cell culture, tissue and whole animal studies to evaluate the physiologic effects of n-3 fatty acids on cardiac arrhythmia or on biologic processes related to arrhythmia, such as cardiac ion channels, pumps or exchange mechanisms in cell organelles (Jordan et al., 2004). Based on a systematic literature review and combined analysis of published studies (meta-analysis), Jordan et al. found that fish oil supplementation in rat studies has anti-arrhythmic effects compared with n-6 fatty acid supplementation (typically from vegetable oils). In experiments which attempted to deliberately induce arrhythmia in rats, those supplemented with fish oil were protected because they had less ventricular tachycardia and fibrillation. Considering studies in rats as well as in other species (monkeys, dogs, rabbits and pigs), the authors concluded that fish oil supplementation might have anti-arrhythmic effects compared with n-6 fatty acid or monounsaturated fatty acid supplementation. The authors could draw no conclusions from the literature on organ and tissue studies of the biological mechanism by which n-3 fatty acids might protect against arrhythmia, because of small numbers of specific studies and conflicting results among studies.

The second AHRQ report reviewed the evidence on the role of n-3 fatty acids on intermediate markers and risk factors for cardiovascular disease in humans (Balk et al., 2004). In a systematic review of the literature, Balk and coauthors concluded there is strong evidence that fish oils have a strong beneficial effect on blood triglycerides that is dose dependent and similar in various populations. The report also found a very small beneficial effect of fish oils on blood pressure and possible beneficial effects on coronary arteries after angioplasty, on exercise capacity in atherosclerosis patients and on heart rate variability in patients with recent heart attack. No beneficial effects were found on serum lipids, C-reactive protein (a marker for inflammation), markers for thrombosis (or clotting), or glucose tolerance. There was also no consistent evidence of adverse effects of fish oils on glucose tolerance.

This report also considered how dietary intake of n-3 fatty acids affects blood and tissue n-3 fatty acid levels, intermediate markers of n-3 fatty acid intake. In meta-analyses and meta-regressions of published studies, Balk and coauthors found a dose-response relationship in humans between n-3 LC PUFA consumption and change in blood levels of EPA and DHA in the blood components studied: plasma or serum phospholipids, platelet phospholipids, or red blood cell membranes. Supplementation of one gram of EPA and/or DHA corresponded to approximately a one percent increase in EPA plus DHA in serum or plasma phospholipids, platelet phospholipids or red blood cell membrane phospholipids. The meta-regression equations were:

For 15 randomized trials with 28 n-3 LC PUFA arms:

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Percent change in plasma/serum phospholipid EPA plus DHA level = 0.93 x grams (EPA plus DHA) per day + 1.4. R-squared = 0.45, $p < 0.001$.

For a subset of 12 studies, omitting four studies that reported only EPA and not DHA levels:

Percent change in plasma/serum phospholipid EPA plus DHA level = 1.24 x grams (EPA plus DHA) per day + 0.89. R-squared = 0.63, $p < 0.001$.

For 12 randomized trials with 21 n-3 LC PUFA arms:

Percent change in platelet phospholipid EPA plus DHA level = 0.74 x grams (EPA plus DHA) per day + 1.16. R-squared = 0.52, $p < 0.001$.

For a subset of 10 studies, omitting 2 studies that reported only EPA and not DHA levels:

Percent change in plasma/serum phospholipid EPA plus DHA level = 0.80 x grams (EPA plus DHA) per day + 1.25. R-squared = 0.72, $p < 0.001$.

For nine randomized trials with 12 n-3 LC PUFA arms:

Percent change in erythrocyte membrane phospholipid EPA plus DHA level = 1.05 x grams (EPA plus DHA) per day + 2.69. R-squared = 0.39, $p < 0.02$.

The third AHRQ report considered the effect of dietary or supplemental n-3 fatty acids on clinical outcomes of cardiovascular disease, such as heart attack and stroke (Wang et al., 2004). This report also determined the average intake of n-3 fatty acids in the U.S. population and in various subpopulations. In this systematic literature review, Wang and coauthors screened 7,464 study abstracts, retrieved and screened 768 full scientific articles, further examined 118 of these articles and identified 39 unique studies for inclusion in the final review. Included studies reported mortality or cardiovascular disease clinical outcomes with at least one year of follow-up duration. The studies looked at consumption of fish or n-3 fatty acids (including supplements) and their effects on cardiovascular disease outcomes either in the general population (primary prevention) or in people who already had cardiovascular disease (secondary prevention). In addition to studies of fish and of the n-3 LC PUFA found in fish and fish oil, this review also included studies of the 18-carbon n-3 PUFA found in plant foods and vegetable oil, called alpha-linolenic acid (ALA).

The primary prevention studies of the general population included 22 prospective cohort studies, 4 case control studies, one cross sectional study and one randomized controlled trial (RCT). For secondary prevention, there were 11 RCTs and one prospective cohort study in people with cardiovascular disease. The authors concluded that

“Overall, the evidence from the primary and secondary prevention studies supports the hypothesis that consumption of omega-3 fatty acids (EPA, DHA, ALA), fish, and fish oil reduces all-cause mortality and various CVD outcomes such as sudden death, cardiac death (coronary or MI death), and MI, although the

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evidence is strongest for fish or fish oil.” [CVD is cardiovascular disease and MI is myocardial infarction, or heart attack]

The report also found that overall methodological quality of the studies on fish oil (EPA plus DHA) was good, but the RCT data for ALA was poor. Possible adverse events associated with fish oil or ALA supplementation were minor. Results were inconsistent for the 16 studies of primary or secondary prevention of stroke.

Wang and coauthors found that there was an imbalance in the type of studies with evidence of primary compared with secondary prevention. For primary prevention, the evidence of cardiovascular benefits was from prospective cohort studies of both men and women. For secondary prevention, most of the evidence was from one large clinical trial (RCT), which did not include women. The authors recommended further research on RCTs in the general population and in countries with different background diets and risk factors. They also recommended careful assessment in future studies of background diet and risk factors, as well as of types of fish and fish preparation and the dietary ratio of n-3 to n-6 fatty acids. Additionally, studies should attempt to determine what foods or food groups are reduced (replaced) in diets with high fish intake; for example, sources of saturated fat such as meat and cheese may be reduced.

The three AHRQ reports have been published in the form of review articles in scientific journals (Matthan et al., 2005; Balk et al., 2006; Wang et al., 2006). The article on cardiovascular disease outcomes updated the literature review to July 2005 (Wang et al., 2006). This review concluded:

“Evidence suggests that increased consumption of n-3 FAs from fish or fish-oil supplements, but not of ALA, reduces the rates of all-cause mortality, cardiac and sudden death, and possibly stroke. Evidence of the benefits of fish oil is stronger in secondary- than in primary-prevention settings. However, no benefits of FA supplementation were seen in patients with an ICD [implantable cardioverter defibrillator], and adverse effects appear to be minor.”

2005 Dietary Guidelines Advisory Committee

In August, 2004, the 2005 Dietary Guidelines Advisory Committee presented to the Secretaries of the Department of Health and Human Services (HHS) and the Department of Agriculture (USDA) its scientific report with recommendations for the 2005 revision of “Dietary Guidelines for Americans” (2005 Dietary Guidelines Advisory Committee, 2004). The Advisory Committee used an evidence-based approach to develop its recommendations for updating Dietary Guidelines for Americans, a document published periodically to provide science-based advice to promote health and reduce chronic disease risk through diet and physical activity. One of the nine key messages developed by the Advisory Committee was “Choose fats wisely for good health”. The recommendations under this message included keeping intake of saturated fat, trans fat

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and cholesterol very low, and keeping total fat intake within a recommended range (20 to 35 percent of calories for adults). Another recommendation under this message addressed intake of fish, DHA and EPA for prevention of cardiovascular disease and stated:

“A reduced risk of both sudden death and CHD death in adults is associated with the consumption of two servings (approximately eight ounces) per week of fish high in the n-3 fatty acids called eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). To benefit from the potential cardioprotective effects of EPA and DHA, the weekly consumption of two servings of fish, particularly fish rich in EPA and DHA, is suggested.”

The Advisory Committee also recommended that pregnant and lactating women and children consult current consumer advisories to avoid eating fish with a high mercury content and to limit their consumption of fish with a moderate mercury content.

In general, the Advisory Committee used systematic reviews of the scientific literature to address research questions formulated and refined by the Committee and its working subcommittees. In the section on Fats, the Committee asked seven major questions related to different types of fat and how they are related to health. Question Six asked by the Committee was, “What are the relationships between n-3 fatty acids and health?” One conclusion of the Committee was about ALA, and stated that “intake between 0.6 to 1.2 percent of calories will meet requirements for this fatty acid and may afford some protection against CVD outcomes.” The Committee based this conclusion on the IOM’s AMDR for ALA. The Committee’s second conclusion was the same as the recommendation stated above regarding fish, EPA and DHA and heart disease. The conclusion statement added that “other sources of EPA and DHA may provide similar benefits; however, further research is warranted.”

The Advisory Committee review regarding EPA, DHA and fish was based in part on the 2002 IOM Macronutrient Report summarized above (IOM, 2002). The Advisory Committee noted that, although humans can convert ALA to EPA and DHA, this occurs slowly and the conversion rates are not well understood. Therefore, fish high in EPA and DHA are an important source of these fatty acids. The Advisory Committee noted the IOM statements about EPA and DHA and heart disease risk and the AMDR for EPA and DHA, as summarized above.

The Advisory Committee stated that its conclusion regarding fish and heart disease risk was based on epidemiologic studies of fish consumption in healthy populations, particularly as reviewed in the AHRQ report on cardiovascular disease summarized above (Wang et al., 2004). The Advisory Committee noted that the 22 prospective cohort studies reviewed by Wang and coauthors were conducted in many parts of the world, and that there were various methodological differences among the studies. Nevertheless, the

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Advisory Committee found that, “Despite some limitations, if viewed together, these studies provide evidence that is highly applicable to the U.S. population.”

The Advisory Committee reviewed the cohort studies to evaluate how much fish consumption and corresponding DHA and EPA intake was needed to provide a cardioprotective effect. The Committee found that on average, in five U.S. studies, the lowest risk of clinical coronary events was associated with EPA plus DHA intake of 496 mg/day (or about 3.5 g/week), with a range of 246 to 919 mg/day. This corresponds to consumption of about eight ounces per week (or two four-ounce servings per week) of fish that the Committee called high n-3 fish, containing on average about 1.6 grams DHA plus EPA per serving (discussed further below). The Committee noted there is some evidence that consuming more than two servings of fish per week may provide additional cardioprotective benefit, and cited as examples a cohort study by Mozaffarian and coauthors (2003) and meta-analyses of fish consumption and CHD mortality and stroke (He et al., 2004a; He et al., 2004b). However, the Committee noted that the AHRQ report (Wang et al., 2004) also found insufficient evidence to define an optimal intake of n-3 fatty acids.

The Advisory Committee reviewed the secondary prevention RCTs (Burr et al., 1989; GISSI, 1999; Singh et al., 1997), and noted that the cardio preventive effect of fish oil supplements in these trials provides evidence that the active cardioprotective dietary factor in fish is EPA and DHA. This would explain the cardioprotective effects of fish consumption in the epidemiologic (cohort and case control) studies.

The Committee stated:

“Fish is a good source of nutrients including protein, the B-vitamins and minerals such as potassium, phosphorous, and selenium and also is low in calories. Since fish is low in saturated fat, it provides a means to reduce saturated fat intake when substituted for foods such as red meats and full-fat dairy products.”

As requested by the Committee, USDA used modeling to examine predicted changes in U.S. nutrient intake due to approximately doubling current fish consumption to a total intake of eight ounces of fish per week (Advisory Committee Report, Appendix G-3). The model classified fish into two groups, having more or less than 500 mg EPA plus DHA per three ounces. In the model based on current dietary patterns, one ounce of low n-3 fish such as cod, haddock or snapper, would provide 105 mg EPA plus DHA and one ounce of high n-3 fish such as mackerel, salmon or trout, would provide 407 mg EPA plus DHA. Current consumption is about 80 percent low n-3 fish. Following these proportions and increasing total fish intake to eight ounces per week would provide about 208 mg EPA plus DHA per day in a 2000 calorie dietary pattern. Changing fish consumption to eight ounces of high n-3 fish per day per day would provide 512 mg EPA plus DHA in a 2000 calorie dietary pattern. This can be compared with the Committee’s estimate that in five U.S. studies, the lowest risk of clinical coronary events was

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associated with EPA plus DHA intake of approximately 496 mg/day (or about 3.5 g/week), with a range of 246 to 919 mg/day. Therefore, the Committee suggested that fish high in n-3 fatty acids should be emphasized to achieve the recommendation for fish consumption.

With respect to recommendations for increased fish intake, a hypothetical question is often asked regarding the effect on the overall profile of nutrients other than EPA and DHA, when fish is substituted for other foods. In the USDA models, the impact on other nutrients of substituting more fish or high n-3 fish for the same amount of meat and poultry was quite small. According to the analysis, there was no decrease in intake of vitamins and minerals when eight ounces of fish per week was substituted for meat, poultry or beans. The analysis also showed little or no change in total fat, saturated fat and cholesterol, when eight ounces of total fish or high n-3 fish was incorporated into the baseline dietary patterns. Substitution of fish for meat or poultry might be expected to result in lower intake of saturated fat and possibly total fat. However, the baseline dietary patterns in the models were specialized patterns used in modeling nutrient adequacy by USDA. For modeling purposes, these patterns use the lowest fat forms of foods in the food group, and food composites are therefore nutrient-dense versions of the food group (Advisory Committee Report, Part D, Section 1: Aiming to meet recommended intakes of nutrients). For the special analysis on fish intake, the fish items were re-grouped using n-3 fatty acid content (EPA plus DHA). However, the baseline composites of the meat, poultry and beans food subgroups were retained. Therefore, the analysis compared total fish items based on current consumption or high n-3 fish items against meat, poultry and beans composites chosen to be low in fat. In this situation, fat and saturated fat did not change when fish was substituted for meat or poultry in the models. (Additionally, Mozaffarian and Rimm (2006) noted that displacement of other foods, such as meat and dairy products, by fish probably does not account for the observed health benefits of fish. The foods replaced would vary widely among individuals and across cultures, and corresponding changes in fat and saturated fat would not account for the magnitude of decreased CHD risk associated with fish consumption.) In the special analysis for the Advisory Committee, the comparison also showed that it was unlikely that dietary substitution of eight ounces of fish per week would have an adverse effect of decreased intake of vitamins and minerals, even when the fish was substituted for nutrient dense versions of meat, poultry or beans

In reviewing the scientific evidence, the Advisory Committee also considered positions taken by other expert groups, including AHA and the Scientific Advisory Committee on Nutrition (United Kingdom), the World Health Organization and the European Society for Cardiology (discussed below), and the National Cholesterol Education Program and the American Diabetes Association (as follows):

- National Cholesterol Education Program—recommends fish as a food item for people to choose more often (NCEP, 2002; Table V.2–6).

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- American Diabetes Association—two to three servings of fish per week provide dietary n-3 polyunsaturated fats and can be recommended (Franz et al., 2004).

In summary, the Committee stated:

“Collectively, the evidence presented above provided the basis for recommending two servings of fish per week to decrease risk of heart disease. A conservative estimate is that two servings of fish high in n-3 fatty acids per week may reduce the risk of coronary death, primarily sudden death, by as much as 30 percent (Hu et al., 2002) among adults. Fish is recommended rather than supplements because epidemiologic and some RCT data demonstrate benefits of fish; it is a good source of n-3 fatty acids and many other nutrients; and it is low in calories and saturated fatty acids.”

Dietary Guidelines for Americans 2005

In January, 2005, HHS and USDA published Dietary Guidelines for Americans 2005 (U.S. DHHS and USDA 2005), based on the scientific report of the 2005 Dietary Guidelines Advisory Committee. The 2005 Dietary Guidelines identified 23 key recommendations for the general public and 18 for special populations, all grouped into nine general topics. Under the topic, Fats, the four recommendations for the general public include: 1) limit intake of saturated fat to 10 percent of calories, cholesterol to 300 mg/d and keep trans fat intake as low as possible; 2) keep total fat intake between 20 to 35 percent of calories, with most fats coming from sources of polyunsaturated and monounsaturated fatty acids, such as fish, nuts and vegetable oils; 3) select lean, low fat or fat-free choices in meat, poultry, dry beans and milk or milk products and 4) limit intake of fats and oils high in saturated and trans fat and choose products low in these fats and oils. The key recommendations mention fish consumption as a source of polyunsaturated fatty acids, but did not mention fish or n-3 fatty acids, DHA and EPA, in connection with heart disease prevention.

In the Dietary Guidelines chapter on Fats, the text of the Discussion states:

“Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega-3 fatty acids that are contained in fish and shellfish. Fish that naturally contain more oil (e.g., salmon, trout, and herring) are higher in EPA and DHA than are lean fish (e.g., cod, haddock, and catfish). Limited evidence suggests an association between consumption of fatty acids in fish and reduced risks of mortality from cardiovascular disease for the general population.”

The text under Considerations for Specific Population Groups further states:

“Evidence suggests that consuming approximately two servings of fish per week (approximately eight ounces total) may reduce the risk of mortality from coronary

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heart disease and that consuming EPA and DHA may reduce the risk of mortality from cardiovascular disease in people who have already experienced a cardiac event.”

The report also recommends that women of childbearing age, pregnant and lactating women and young children consult advisories from regulatory agencies regarding methylmercury and other environmental contaminants in fish.

U.S. Food and Drug Administration

In 2000, the FDA responded to a court decision in *Pearson v. Shalala*¹ (“Pearson”) directing the agency to consider the health claim “Consumption of omega-3 fatty acids may reduce the risk of coronary heart disease” in dietary supplement labeling. The court held that First Amendment does not permit FDA to reject health claims that the agency determines to be potentially misleading unless the agency can also reasonably determine that a disclaimer (qualified health claim) would not eliminate the potential deception.

Based on review of the available intervention trials for omega-3 fatty acids and reduced CHD risk (GISSI, 1999; von Schacky et al., 1999; Singh et al., 1997; Burr et al., 1994), FDA concluded that there was a relationship between the intake of EPA/DHA and reduced risk of CHD in a diseased population (i.e., subjects diagnosed with CHD or had a recent myocardial infarction). There were no intervention studies to demonstrate a causal relationship between EPA/DHA and reduced risk of CHD in the general healthy population. From the agency’s review of the observational data, FDA concluded that the data were mixed for a relationship between fish intake and CHD risk. Furthermore, FDA stated that observational studies reflect the total diet of individuals, and as such, show the association of many factors in addition to n-3 LC PUFAs. FDA noted that the physiological effects (e.g., bleeding time and platelet aggregation) of n3 LC PUFA were similar in healthy individuals and individuals who had a prior coronary event, and therefore the evidence suggested that the benefit of n-3 LC PUFAs on CHD risk could be extrapolated from subjects with existing heart disease to the general population. Based on the evidence reviewed, FDA concluded that there was insufficient evidence to meet the significant scientific agreement standard for an authorized health claim (FDA, 1999) about a relationship between n-3 LC PUFA intake and reduced risk for the general population. FDA concluded, however, that the weight of the scientific evidence for the relationship outweighed the scientific evidence against it. Therefore, FDA issued a qualified health claim that reflected the level of scientific evidence to support the claim. In 2002, the following qualified health claim was issued through a letter of enforcement discretion for the labeling of dietary supplements (FDA 2002):

¹ *Pearson v. Shalala*, 164 F.3d 650, 659 (D.C. Cir. 1999)

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“Consumption of omega-3 fatty acids may reduce the risk of coronary heart disease. FDA evaluated the data and determined that, although there is scientific evidence supporting the claim, the evidence is not conclusive.”

On December 20, 2002, the agency announced its intention to extend the *Pearson* decision to conventional human food and provide for qualified health claims for such foods. In 2004, FDA responded to two petitions requesting a health claim on n-3 LC PUFA and CHD for the labeling of conventional foods (FDA 2004 [Wellness] and FDA 2004a [Martek]).

FDA evaluated the safety of long-chain n-3 fatty acids in conventional foods because FDA requires that substances must be safe and lawful in order to be the subject of a health claim (except for claims about decreased intake of a substance). FDA concluded that the consumption of long chain n-3 fatty acids in conventional foods and dietary supplements (including the use of EPA and DHA as food ingredients) was safe, provided that daily intakes of EPA and DHA from conventional food and dietary supplement sources do not exceed 3.0 grams per day.

Regarding the association between n-3 LC PUFA and CHD, FDA found that the scientific evidence from randomized clinical trials (or intervention studies) available since the 2000 review did not show a relationship between n3 LC PUFAs and reduced risk of CHD in the general population. FDA noted the overall lack of primary prevention intervention studies for EPA and DHA intake and CHD risk, and observed that CHD risk reduction in a general healthy population cannot be predicted from secondary prevention studies in diseased populations. Primary prevention evidence was available from 10 observational studies published since FDA’s 2000 review. Of these, FDA did not consider two studies that only provided total fish consumption without estimates of fish type or portion size in order to estimate corresponding intake of n-3 LC PUFAs. FDA found the remaining eight new studies were of moderate to high quality. FDA stated:

“Observational studies provide only supportive rather than direct evidence for a relationship. For these reasons, FDA considers observational studies as less persuasive than intervention studies conducted in a general healthy population for establishing a substance-disease relationship. Nevertheless, primary prevention of CHD in healthy populations by EPA and DHA omega-3 fatty acids was observed in the majority of observational studies reviewed, which included two large prospective cohorts conducted in the US, the Nurses' Health Study (n=84,688; 16 year follow-up; Hu et al., 2002) and the U.S. Physicians Health Study (n=20,551; 11 to 17 year follow-up; Albert et al., 1998, 2002). In sum, the majority of observational studies consistently observed an associated CHD risk reduction from intake of EPA and DHA estimated from the diet in men and women in populations relevant (three studies) or less relevant (two studies) to the general U.S. population.”

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FDA's evaluation of the strength of the evidence stated:

“Thus, FDA is not changing its position from that outlined in the October 31, 2000 letter on the EPA and DHA omega-3 fatty acid and CHD qualified claim that there is sufficient suggestive evidence that the benefit on CHD reported in CHD patients (i.e., secondary prevention) (reviewed in the October 31, 2000 letter) applies to the general population because of: (1) The primary CHD prevention in the general population associated with EPA and DHA consumption from fish in observational studies; and, (2) intervention studies demonstrating similar physiological effects of EPA and DHA in both the diseased and general populations. FDA still concludes that the weight of the scientific evidence for a health claim for EPA and DHA omega-3 fatty acids outweighs the scientific evidence against such a claim. The most significant change in the available body of evidence since 2000 is the additional observational studies, the majority of which consistently reported an associated benefit in CHD risk from EPA and DHA consumption from fish.”

Overall, FDA concluded for the labeling of conventional foods and dietary supplements:

“Based on FDA's consideration of the scientific evidence and other information submitted with your petition, and other pertinent scientific evidence and information, FDA concludes that there is sufficient evidence for a qualified health claim, provided that the qualified claim is appropriately worded so as to not mislead consumers. Thus, FDA will consider exercising enforcement discretion for the following qualified health claim:

“Supportive but not conclusive research shows that consumption of EPA and DHA omega-3 fatty acids may reduce the risk of coronary heart disease. One serving of [Name of the food] provides [] gram of EPA and DHA omega-3 fatty acids. [See nutrition information for total fat, saturated fat, and cholesterol content.]

Dietary supplements may declare the amount of EPA and DHA per serving in "Supplement Facts," instead of making the declaration in the claim.”

Scientific Advisory Committee on Nutrition, United Kingdom

In the United Kingdom, the Food Standards Agency (FSA) requested advice on the benefits and risks of fish consumption, particularly oily fish, from the Scientific Advisory Committee on Nutrition (SACN) and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT). A report, Advice on Fish Consumption: Benefits and Risks, was published in 2004 by the joint SACN/COT Subgroup (SACN 2004). The report had the purpose of combining the nutritional considerations of fish consumption from SACN and the toxicological considerations of

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contaminants in fish from COT; weighing the nutritional benefits against possible risks; and developing dietary advice for the public.

The starting point for the SACN review was the 1994 advice on fish consumption and n-3 LC PUFA intake from the U.K. Cardiovascular Review Group of the Committee on Medical Aspects of Food Policy (COMA) (1994). The 1994 advice was for individuals to consume at least two portions of fish weekly, of which one should be oily, and to increase the population average consumption of n-3 LC PUFA from about 0.1 g/day to about 0.2 g/day. The SACN report reviewed two aspects of health benefits of fish consumption: the effects of n-3 LC PUFA on early human growth and cognitive function and the relationship of fish consumption and cardiovascular disease.

The SACN review of cardiovascular benefits covered human population studies with disease end points—prospective cohort studies and RCTs, as well as a small number of case control studies. The report stated that evidence about fish consumption and thrombotic stroke (stroke cause by blood clots) was equivocal, and that the review would focus on heart disease outcomes rather than stroke. However, SACN noted that studies had observed no significant association between fish or fish oil consumption and hemorrhagic stroke (stroke caused by bleeding).

SACN tabulated and evaluated 23 prospective cohort and case control studies of fish or n-3 LC PUFA consumption and coronary heart disease (CHD) and four RCTs of fish or n-3 LC PUFA intervention and CHD outcome. Among the 23 observational studies, there were 16 reports from prospective cohort studies that measured fish or n-3 LC PUFA intake using dietary questionnaires, five reports from studies that measured blood levels of DHA and EPA as intermediary markers of fish intake, and two reports from a case control study that measured DHA levels in adipose tissue. SACN stated that, overall, the prospective cohort studies suggest that those who consume fish have a lower risk of CHD than those who do not, and that the risk reduction appears to be dose-dependent in high risk populations. Men with higher DHA levels in adipose tissue in the Euramic case-control study or with higher DHA blood levels in a Finnish cohort study had lower risk of myocardial infarction (heart attack) (Guallar et al 2002, Rissanen et al 2000). (In these two studies, the reduced risk associated with n-3 LC PUFA was attenuated by high mercury content in fish. However, SACN emphasized that the two studies suggesting that mercury attenuated the beneficial association of LC n-3 PUFA still reported a positive association between fish consumption and CHD.)

Additional evidence for the cardiovascular benefits of fish consumption came from cohort or case control studies measuring blood levels of DHA and EPA. In a case-control study nested in the Physicians' Health Study cohort, higher blood levels of DHA and EPA were associated with decreased risk of sudden cardiac death (Albert et al 2002). A case control study nested in the Multiple Risk Factor Intervention Trial cohort found higher serum DHA levels associated with decreased CHD risk (Simon et al., 1995). These results were consistent with a case control study in Washington State that found

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that higher red blood cell membrane DHA and EPA was associated with lower risk of primary cardiac arrest (Siscovick et al., 1995). SACN stated that, “Taken together, these data support the hypothesis that LC n-3 PUFA are responsible for the observed inverse association between fish consumption and sudden cardiac death.”

The SACN report noted that there were no primary prevention RCTs on fish or fish oil intervention and CHD prevention. Three secondary prevention trials showed that fish consumption or fish oil supplementation reduce coronary mortality in patients who have previously had a heart attack (Burr et al., 1989, 1994; Singh et al., 1997; GISSI 1999). A recent RCT found an adverse effect of fish consumption or fish oil supplements on cardiac mortality in men with CHD who had angina (Burr et al., 2003). However, the SCAN report found problems with the design of this study. Thus, SACN concluded that the secondary prevention trials provide evidence that increased fish or fish oil consumption would decrease mortality in patients who have had a heart attack. As recognized by the 1994 COMA report, SACN stated that it is difficult to extrapolate the secondary prevention results to primary prevention in a “healthy” population. However, the SACN noted that the U.K. population is at high risk for CHD, and that almost 30 percent of the English population has some form of cardiovascular disease.

In summary, the SACN report concluded that the majority of the evidence base, including three out of four of the RCTs, suggests that fish consumption and n-3 LC PUFA intake reduce risk from cardiovascular disease.

Conclusions were based on the review of cardiovascular benefits together with the review of n-3 LC PUFA and human growth and development, discussed below. The Committee endorsed the previous recommendation for consumption of at least two portions of fish a week, of which one should be oily. This was considered a minimal achievable objective, considering the low background fish consumption in the U.K. Although the Committee concluded that it may be beneficial for individuals to consume more fish than this guideline, they were unable to identify a level for increased consumption. They stated it would be inappropriate to discourage fish consumption at higher levels than the recommendation, unless there was an upper limit beyond which people should not consume. The Committee concluded that

“An increase in population oily fish consumption to one portion a week, from the current levels of about a third of a portion a week, would confer significant public health benefits in terms of reduced risk of CVD.”

The Committee revised the 1994 COMA recommended intake of n-3 LC PUFA, 0.2 g/day, increasing the recommendation to 0.45 g/day, consistent with the intake corresponding to the fish consumption guidelines. The Committee recommended additional research on the response of different body pools, including fat stores, cells and plasma constituents, to doses of n-3 LC PUFA intake.

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European Food Safety Authority

The European Food Safety Authority (EFSA) formed an Interpanel working group (the Panel) to respond to a request from the European Parliament for a scientific assessment of the human health risks related to consumption of wild and farmed fish. The EFSA published its report in June, 2005, titled, “Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the European Parliament Related to the Safety Assessment of Wild and Farmed Fish.” The executive summary of the report stated:

“There is evidence that fish consumption, especially of fatty fish (one to two servings a week) benefits the cardiovascular system and is suitable for secondary prevention in manifest coronary heart disease.”

The European Parliament request to EFSA concerned the health risks related to human consumption of fish marketed in the European Union, including wild and farmed fish, such as salmon and other carnivorous species farmed in substantial amounts. Information was specifically requested on adverse health effects associated with persistent organic pollutants in fish, and the assessment was specifically requested to cover the consumption of Baltic herring. The EFSA interpretation of its charge noted that risk management is a function of the European Commission. Therefore, the EFSA report gives a scientific opinion on the health risks related to human consumption of wild and farmed fish. The specific topics addressed by the report are:

- The influence of season and life history stage of fish on the nutrient levels and the contaminant levels in fish (selecting the right fish comparator)
- The quality of feed and feeding practices and its impact on the pattern of contaminants in fish
- A comparison of the nutritional composition of wild and farmed fish
- The beneficial effects associated with fish consumption
- An evaluation of relevant contaminants in fish and comparison with health based guidance values for risk characterization
- An overall impact and risk assessment of the consumption of Baltic herring.

In an appendix, the report reviewed the metabolism and function and the physiological requirement for n-3 LC PUFA in humans (Annex 2). This review noted that there is a dietary requirement for n-3 ALA and stated that there is no consensus on the need for an intake of preformed DHA in adults, although data indicated that DHA is conditionally indispensable for preterm infants (discussed in the section on neurodevelopment in this report). The report noted that the Scientific Committee on Food (SCF) of the European Commission in 1993 set population reference intakes for total n-3 fatty acids at 0.5 percent of energy intake (calories) for adults and recommended that total n-3 fatty acid

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intake not exceed five percent of energy intake. The Panel also summarized various national and international recommended intakes of fish or n-3 LC PUFA.

The EFSA Panel summarized the evidence for the cardiovascular health benefits of LC n-3 PUFA from three major, recent reviews—the AHRQ report (Wang et al., 2004) and the U.K. SACN report (2004), both discussed above, and a Cochrane Collaborative review (Hooper et al., 2004), discussed below.

The Panel noted that the U.K. SACN report (2004) considered that the results of prospective cohort studies regarding fish consumption and the risk of thrombotic stroke were equivocal. The Panel cited results of a meta-analysis of 8 observational studies that found reduced relative risk (RR) for any type of stroke for fish consumption once per week compared with once per month or less: RR 0.87 (95 percent confidence interval, 0.77 to 0.98; p for trend = 0.06) (He et al., 2004). Nevertheless, the Panel concluded that, regarding fish consumption and stroke, “the overall evidence is not conclusive and does not permit definition of a dose-response relationship.”

Regarding risk of coronary heart disease, the Panel noted that SACN concluded that the evidence from prospective cohort studies indicates that fish consumption is associated with decreased risk. However, not all studies were supportive of this relationship. The Panel report reviewed and summarized several possible reasons for different findings among observational studies, including level of study population risk for CHD, length of follow-up period, lack of adjustment for contaminant exposures, such as high mercury in fish, and type of fish meal. The panel cited a meta-analysis of fish consumption and CHD death, including 222,364 individuals in 13 cohorts with average of 11.8 years of follow-up (He et al., 2004b, discussed below). In the meta-analysis:

“...individuals who consumed fish had a lower mortality from coronary heart disease than individuals not consuming fish or less than once per month. The pooled multivariate relative risks for coronary heart disease mortality were 0.89 (95 percent CI, 0.79 - 1.01) for fish intake one - three times per months, 0.85 (95 percent CI, 0.76 - 0.96) for fish once per week, 0.77 (95 percent CI, 0.66 - 0.89) for fish two to four times per week, and 0.62 (95percent CI, 0.46 - 0.82) for fish five or more times per week. Each 20 g/day increase in fish intake was related to a seven percent lower risk of mortality from coronary heart disease (P for trend = 0.03) (He et al., 2004b).”

The EFSA Panel noted that there are no randomized controlled trials of fish or fish oil and primary prevention of coronary heart disease. The report reviewed the secondary prevention trials, and noted three trials that found decreased risk of cardiovascular events in heart disease patients: one study using fish or fish oil (Burr et al., 1989) and two studies using fish oil (Singh et al, 1997; GISSI, 1997). Another secondary prevention trial, using fish or fish oil, had poor methodological quality and found higher risk of cardiac death in those treated with fish or fish oil (Burr et al., 2003). The Panel noted

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that the overall analysis of the secondary prevention randomized controlled trials shows a beneficial effect of dietary and supplemental n-3 LC PUFA on coronary heart disease.

The Panel noted the different conclusions of the systematic reviews of n-3 fatty acids and cardiovascular disease in the AHRQ report (Wang et al., 2004) and the Cochrane Collaboration review (Hooper et al., 2004). As discussed above, Wang and coworkers concluded that consumption of n-3 fatty acids from fish or fish oil reduced all cause mortality and various cardiovascular disease outcomes. Similarly, the SACN report (discussed above) concluded that both randomized controlled trials in secondary prevention as well as prospective epidemiologic evidence support beneficial effects of fish or fish oil on mortality in heart disease patients and beneficial effects of fish consumption on risk of cardiovascular disease in the general population. In contrast, Hooper and coworkers concluded that it is not clear that dietary or supplementary n-3 fatty acids alter total mortality or combined cardiovascular events in people with or at high risk of cardiovascular disease or in the general population (discussed below). The Panel noted that one difference between the analyses of Wang and coworkers and Hooper and coworkers was the inclusion of one particular study of poor methodological quality (Burr et al., 2003). (Another difference, not explicitly noted by the Panel, was that Hooper and coworkers excluded the prospective observational studies from the analysis, and based their evaluation solely on the randomized controlled trials, as discussed below.) Overall, the Panel found:

“The Panel observed that the result of the meta-analysis by Hooper et al. (2004) maybe partly explained by the inclusion of one big randomized controlled trial on the use of fish oil (Burr et al., 2003), which shows some methodological deficits, and concludes that there is sufficient evidence of beneficial effects associated with fish consumption for populations with or at risk of cardiovascular disease.”

The Summary and Conclusions of the EFSA report chapter on Beneficial Effects Associated with Fish Consumption stated:

“Fish is an important source of proteins of high biological value, LC n-3 PUFA, essential minerals, especially iodine, selenium and calcium, and vitamins, especially vitamins A and D and B12. LC n-3 PUFA are not essential in human nutrition beyond the foetal and neonatal period, but may be conditionally essential in immature and young infants.”

Regarding cardiovascular disease, the chapter Summary and Conclusions stated:

“There is evidence that fish consumption, preferably of fatty fish - one to two servings of 130 g per week, which may correspond to an LC n-3 PUFA intake ranging from 1.86 g for one meal (0.26 g/day) up to 9.7 g LC n-3 PUFA for two meals/week (1.38 g/day), depending on the type of fish - and, alternatively, fish oil or isolated LC n-3 PUFA (about one gram of LC n-3 PUFA/day) benefit the

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cardiovascular system and are suitable for secondary prevention in manifest coronary artery disease. Nevertheless, results from both epidemiologic and interventional studies suggest that health benefits are associated with the consumption of certain levels of EPA/DHA from fish and fish oils also in the healthy population. The expected benefits include a decrease in the risk of cardiovascular disease and stroke and improved neurodevelopmental and perinatal growth in infants.”

World Health Organization

In 2003, the World Health Organization (WHO) published “Diet, Nutrition and the Prevention of Chronic Diseases”, a report of a Joint WHO/FAO Expert Consultation (WHO, 2003). (FAO is the Food and Agriculture Organization of the United Nations.) The WHO report presented population nutrient intake goals for preventing diet-related chronic diseases. Recommended intake of total fat was 15 percent to 30 percent of energy (calories), with less than 10 percent of energy from saturated fat, less than one percent of energy from trans fatty acids, six percent to 10 percent of energy from polyunsaturated fat, and the remainder from monounsaturated fat. The recommended polyunsaturated fat intake was provided by five percent to eight percent of energy from n-6 polyunsaturated fatty acids and one to two percent of energy from n-3 polyunsaturated fatty acids.

The WHO Expert Consultation used a modification of the criteria of the World Cancer Research Fund (WCRF) to describe the strength of the evidence for relationships of diet and nutrition with increased or decreased risk of chronic disease (WHO, 2003 page 54; WCRF, 1997). Briefly, the categories for strength of the evidence were:

- convincing evidence, based on epidemiological studies showing consistent associations with little or no evidence to the contrary; a substantial number of studies including prospective observational studies and, where relevant, randomized controlled trials of consistent size, duration and quality with consistent results; biological plausibility;
- probable evidence, based on epidemiological studies showing fairly consistent associations, but perceived shortcomings in the evidence or some evidence to the contrary, which precludes a more definite judgment;
- possible evidence, based mainly on findings from case-control and cross-sectional studies; insufficient randomized controlled trials, observational studies or non-randomized controlled trials; and
- insufficient evidence, based on findings of a few studies which are suggestive, but insufficient to establish an association between exposure and disease.

Regarding cardiovascular diseases (CVD), the WHO report stated that these are the major contributor to the global burden of noncommunicable disease. WHO attributes one third of all global deaths (15.3 million per year) to CVD. The WHO report categorized the

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strength of the evidence for dietary and nutritional factors associated with increased or decreased risk of CVD as follows:

“Convincing associations for reduced risk of CVD include consumption of fruits (including berries) and vegetables, **fish and fish oils (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA))** (emphasis added), foods high in linoleic acid and potassium, as well as physical activity and low to moderate alcohol intake. While vitamin E intake appears to have no relationship to risk of CVD, there is convincing evidence that myristic and palmitic acids, trans fatty acids, high sodium intake, overweight and high alcohol intake contribute to an increase in risk.

A “probable” level of evidence demonstrates a decreased risk for a-linolenic acid, oleic acid, NSP², wholegrain cereals, nuts (unsalted), folate, plant sterols and stanols, and no relationship for stearic acid. There is a probable increase in risk from dietary cholesterol and unfiltered boiled coffee.

Possible associations for reduced risk include intake of flavonoids and consumption of soy products, while possible associations for increased risk include fats rich in lauric acid, b-carotene supplements and impaired fetal nutrition.

The WHO report noted that most but not all observational studies showed that fish consumption is associated with a reduced risk of coronary heart disease, and that

“Regular fish consumption (1--2 servings per week) is protective against coronary heart disease and ischaemic stroke and is recommended. The serving should provide an equivalent of 200--500 mg of eicosapentaenoic and docosahexaenoic acid. People who are vegetarians are recommended to ensure adequate intake of plant sources of a-linolenic acid.”

International Society for the Study of Fatty Acids and Lipids

In 2004, the International Society for the Study of Fatty Acids and Lipids (ISSFAL) published a policy statement on Recommendations for Intake of Polyunsaturated Fatty Acids in Healthy Adults (ISSFAL 2004). This report found an absence of well-controlled studies definitively establishing the requirement in adults of the essential n-6 polyunsaturated fatty acid, linoleic acid. However, based on studies in infants and on changes in plasma levels of fatty acids in adults, the report concluded that two percent of energy (calories) from linoleic acid is adequate for healthy adults.

Regarding a healthy intake of the essential n-3 polyunsaturated fatty acid, ALA, ISSFAL considered ten studies of ALA intake and cardiovascular endpoints, and concluded that the data suggested that a healthy ALA intake is approximately 0.7 percent of energy

² Non-starch polysaccharides

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(calories). ISSFAL noted that this recommendation is consistent with the 2002 IOM Macronutrient Report, although somewhat lower than the one percent of energy recommendation of some international groups.

The ISSFAL report also addressed the minimum recommended intake of EPA plus DHA for cardiovascular health. For this purpose, ISSFAL considered epidemiologic studies conducted in the U.S. and meeting certain criteria: population was free of CHD at baseline; disease endpoint was CHD death, primary cardiac arrest, and/or sudden cardiac death; risk was assessed across a range of intakes of EPA+DHA; confounding was controlled by multivariate analysis to estimate relative risk or odds ratios. The ISSFAL review identified 8 studies in 6 study populations meeting these criteria. A beneficial association between EPA+DHA intake and heart disease risk was found in five of the six study populations. Results from the 6 study populations were combined in a meta-analysis: Hu et al. (2002); Nurses' Health Study; Albert et al. (1998); Physicians' Health Study; Siscovik et al. (1995); Seattle Primary Cardiac Arrest Study; Dolecek et al. (1992); Multiple Risk Factor Intervention Trial (MRFIT); Mozaffarian et al. (2003); Cardiovascular Health Study; Albert et al. (2002); Health Professionals Follow-up Study. ISSFAL tabulated the relative risks or odds ratios from these studies, but did not describe the detailed methods used in the meta-analysis. The meta-analysis found a 37 percent decreased risk of CHD death for the highest compared with the lowest quintile of EPA+DHA intake, statistically significant, $p = 0.03$. Intake in the highest quintile was 566 mg EPA+DHA per day. Thus, regarding EPA and DHA, ISSFAL stated:

“Based on large prospective population studies and well-controlled case-control studies, an intake of about 500 mg of EPA+DHA per day would be expected to significantly reduce risk for death from CHD in healthy adults. This intake is both safe and achievable by diet alone, even for pregnant and lactating women for whom mercury intake can be an issue.”

In June, 2006, ISSFAL requested an opinion from a panel of experts regarding the meta-analysis by Hooper et al. (2006), described below. The panel reported a number of concerns with the Cochrane analysis of Hooper and coauthors, and concluded that:

“In our view, the weight of the evidence available in May of 2006 is sufficient to conclude, even in light of the Cochrane analysis, that EPA and DHA reduce risk for cardiovascular diseases. Taken together, we see no reason why ISSFAL should revise its previous (2004) statement on omega-3 fatty acids and cardiovascular disease in light of the Cochrane analysis.”

European Society of Cardiology

In 2003, European Guidelines on Cardiovascular Disease Prevention in Clinical Practice were published by the Third Joint Task Force of European and other Societies, consisting

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of representatives from the European Society of Cardiology, seven other clinical societies, and invited experts. The Guidelines were intended for clinical practice and were targeted at those at highest risk of cardiovascular disease (CVD), which includes not only coronary heart disease (CHD), but also stroke and peripheral artery disease. The Guidelines gave an overview of the current patterns of CVD and CVD risk factors in Europe, and noted that WHO has identified three components of prevention strategies for comprehensive CHD prevention (WHO 1982). The first component is a population strategy, to change, in the entire population, the lifestyle and environmental factors that underlie the mass incidence of CHD. The second component is a high risk strategy, to identify high risk individuals and reduce their risk factor levels. The third component is a secondary prevention strategy, to prevent recurrent CHD events and disease progression in those with clinically established CHD. The Guidelines are targeted for clinical practice for the second and third components of CHD prevention. However, the Guideline report acknowledged the importance of the first component, population prevention, because even small changes at the population level will affect the health of many people. Most cases of CHD and stroke occur in the large number of people with only modestly elevated risk factor levels.

For population prevention, the Task Force endorsed international and European strategies for tobacco control, and the WHO recommendations for obesity prevention. The Task Force also endorsed the Population Dietary and Physical Activity Goals of the European Heart Network, an alliance of 30 national heart foundations and organizations committed to the prevention of cardiovascular disease in Europe (European Heart Network 2002). The Population Dietary Goals include four goals for which scientific evidence is strong and public health gain large. One of these is to keep saturated fat intake less than 10 percent of energy (calories) and trans fat less than two percent of energy. (The other three goals involve fruit and vegetable intake, salt intake and weight management). The Population Dietary Goals also include two goals for which scientific evidence is moderate and public health gain moderate. One of these is to consume four to eight percent of energy from n-6 polyunsaturated fat, two grams per day of n-3 ALA and 200 mg per day of n-3 LC PUFA. (The other goal is to keep total fat less than 30 percent of energy).

For clinical prevention of those at highest CVD risk, the Task Force critically reviewed the literature on CVD risk factors and interventions to modify risk factors. Regarding ALA, the review noted that several prospective epidemiologic studies showed higher ALA intake was associated with lower risk of fatal cardiovascular events. Also, a clinical trial of secondary prevention showed a reduction in coronary and all cause mortality after 46 months on a Mediterranean diet enriched with ALA (de Lorgeril 1999). Regarding EPA and DHA, the review noted that several prospective epidemiologic studies found lower risk of fatal coronary occurrences and sudden death among those regularly consuming fish compared with non-consumers. Two secondary prevention clinical trials (Burr 1989; Marchioli 2002) also showed benefits of fish advice or fish oil supplements on risk of mortality, coronary mortality or fatal coronary events. A systematic review

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found a reduction in overall mortality, fatal heart attack and sudden death with dietary or supplemental n-3 fatty acids in people with CHD (Bucher 2002). The Task Force stated:

“All these results suggest a significant protective effect of EPA and DHA on fatal cardiovascular events. These effects are partly due to regulation of the heart rate.”

The Task Force noted the importance of dietary changes in the overall management of CVD risk in clinical practice. The Task Force stated that cost effectiveness of dietary intervention is favorable compared with other common interventions, and recommended that:

“This diet should be encouraged in all those at increased risk of cardiovascular disease, not just in those referred with established coronary heart disease, stroke or other clinical manifestations of atherosclerosis.”

The dietary advice was grouped into five recommendations covering 1) balance of varied diet and regular exercise, including eating from each major food group; 2) encouraging fruits and vegetables, cereals and grain products, skimmed dairy products, fish and lean meat; 3) eating n-3 fatty acids from fish and some vegetable oils; 4) replacing some saturated and trans fat with monounsaturated and polyunsaturated fatty acids of vegetable and fish origin; 5) adjusting energy intake to maintain ideal weight. The detailed recommendations regarding fatty acid intake were:

“Eating omega-3 FA’s—from seafood and some vegetable oils—seems to be particularly appropriate as it provides great protection against fatal cardiovascular accidents. It is the most important dietary advice for those with cardiovascular disease as fish or fish oil supplements is dramatically protective, and rapidly so.”

“Other important elements of the diet include replacement of some saturated and trans-FA’s with MUFA’s and PUFA’s of vegetable and seafood origins. The intake of lipids will have to represent approximately 30 percent of energetic intake. The intake of saturated fats must not exceed 30 percent of total lipids. The intake of cholesterol must be less than 300 mg/day.”

The role of n-3 fatty acids was highlighted in the Executive Summary of the Guidelines:

“Oily fish and omega-3-fatty acids have particular protective properties.”

The European Society for Cardiology also addressed dietary risk factors in its Task Force Guidelines on the Management of Acute Myocardial Infarction (Van de Werf 2003) and on Sudden Cardiac Death (Priori 2001). The sudden cardiac death Task Force noted that no distinction was observed between risk of sudden cardiac death and overall risk of CHD in epidemiologic studies that found decreased CHD risk with lower intake of

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saturated fat and greater intake of polyunsaturated fat. However, a specific decreased risk of sudden cardiac death was found in the U.S. Physicians' Health Study, a prospective epidemiologic study of over 20,000 men aged 40 to 84 years, with no previous history of heart attack. For those who ate fish at least one per week, the relative risk of sudden cardiac death was 0.48 (95 percent CI, 0.24 to 0.96, $p = 0.04$) compared with those who ate fish less than once per month. Estimated n-3 fatty acid intake from fish was also associated with decreased risk of sudden cardiac death. In this study, fish consumption was not associated with total myocardial infarction or non-sudden cardiac death.

The myocardial infarction Task Force reviewed the secondary prevention clinical trials of Mediterranean diet/ALA and fish advice or fish oil supplements (de Lorgeril 1999; Burr 1989; GISSI 1999). The Task Force recommendations for secondary prevention of myocardial infarction included Mediterranean-type diet and supplementation with one gram per day of n-3 polyunsaturated fatty acids from fish oil. The usefulness or efficacy of these dietary recommendations was considered to be Class I, evidence and/or general agreement that a given treatment is beneficial, useful and effective. The strength of the evidence was rated as level B, data derived from a single randomized clinical trial and/or a meta-analysis or from non-randomized studies.

Institute of Medicine of the National Academies: Seafood Choices, Balancing Benefits and Risks

In 2006, the IOM released a report, "Seafood Choices, Balancing Benefits and Risks," which examined relationships between benefits and risks associated with seafood to help consumers make informed choices. The report focused on marine species, i.e., "seafood." It used a qualitative approach to balancing benefits and risks of seafood intake by population groups.

As part of its task to analyze and balance the benefits and risks of seafood consumption, the IOM report reviewed and evaluated the scientific literature on the benefits associated with nutrients from seafood.

The report noted that the high nutritional quality of seafood makes it an important component of a healthy diet. In particular, seafood is high in protein and low in saturated fat and cholesterol. Seafood also contributes selenium to the American diet and is unique among animal protein foods as a source of the n-3 LC PUFA, DHA and EPA. The report noted that the roles of DHA and EPA in maintaining health and preventing chronic diseases have not been completely elucidated. Additionally, the report noted that optimal levels for EPA and DHA intake are not defined, and that required intake levels for omega-3 fatty acids were not established by the IOM Macronutrient report. However, the report concluded that:

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“...there is evidence to support an overall benefit to the general population for reduced risk of heart disease among those who eat seafood compared to those who do not, and there may be benefits from consuming EPA and DHA for adults at risk for coronary heart disease.”

In an appendix, the report tabulated the studies reviewed (including original studies as well as review articles), noting the author, study type, subjects, exposure, exposure amount and results. The table also classified the conclusion of each study by designating the conclusion as: B, evidence of benefit; N, evidence of no association or no clear association; A, evidence of an adverse effect; and N/A, conclusion not available, data presented for background only. Studies regarding adult cardiovascular diseases were tabulated in the areas of cardiovascular outcomes (45 studies), stroke (17 studies), lipid profile (14 studies), blood pressure (eight studies), arrhythmia (five studies) and other cardiac indicators (two studies). Studies were also tabulated for other chronic diseases, including diabetes, adult asthma and allergies, cancer and aging/other neurological outcomes.

The report noted the absence of randomized controlled trials of seafood or omega-3 fatty acid consumption in subjects representative of the general population, and indicated that such studies might be impractical because the small number of expected cardiovascular events would result in large samples sizes and lengthy follow-up periods. The committee discussed the secondary prevention studies of Burr et al. (1989), GISSI (1999) and Burr et al. (2003). The report noted that, although Burr et al. (1989) found a significant reduction in all-cause mortality at two years in the group with increased fish intake, an extended follow up did not suggest substantial long term survival benefit (Ness et al. 2002). Additionally, Burr et al. (2003) found higher cardiovascular mortality in the group with increased fish intake or n-3 LC PUFA supplements. In the GISSI study, those receiving EPA/DHA supplements had significantly decreased risk of death, non-fatal heart attack and non-fatal stroke combined; all-cause mortality; and cardiovascular death. However, those taking supplements were not significantly different from controls in risk of at least one cardiac event (cardiac death, resuscitation, recurrent heart attack, or unstable angina). Moreover, the report suggested that the study of Singh et al. (1997) should be removed from consideration of the evidence because of serious concerns about this trial and related publications from this research group. Overall, the review concluded that the randomized trials showed conflicting results for an effect of EPA/DHA on cardiovascular events and non long-term protective effect of seafood intake in subjects with a previous history of CHD. The committee noted that this conclusion was consistent with the findings of the systematic review by Hooper et al. (2004, 2006) (discussed below).

The IOM report noted the availability of a number of observational studies of the association of fish or EPA/DHA intake with cardiovascular deaths or events, both in the general population and in those with a history of CHD. The text summarized the results of meta-analyses by Whelton et al. (2004), He et al. (2004) and König et al. (2005).

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Overall, the quantitative results of the meta-analyses suggest that fish consumption is associated with decreased risk of CHD or death, especially in the general population without a history of CHD. However, the report expressed caution because observational studies may be subject to residual confounding. Therefore, it is possible that seafood intake is a marker for a healthy lifestyle, and does not prove a causal association between seafood consumption and cardiovascular protection.

Some observational studies are also available for the association between fish consumption and stroke. The IOM report summarized meta analyses by He et al. (2004) and Bouzan et al. (2004), a review by Skerrett and Hennekens (2003) and recent results from the Cardiovascular Health Study (Mozaffarian et al., 2005). Overall, the report stated that these observational studies provided inconclusive results for an association between fish intake and stroke. The results suggest that fish intake may influence stroke risk, but identification of mechanisms or alternate explanations for the results requires further study. The type of fish preparation is not recorded in many observational studies, but may be an important effect modifier, as found in the Cardiovascular Health Study.

Overall, the IOM report stated that results from individual studies are not consistent and results from critical reviews are not clearly supportive of a cardioprotective effect of EPA/DHA. The committee assessed the level of evidence for cardiovascular health benefits using the criteria of the Oxford Centre for Evidence Based Medicine. An association of increase in DHA/EPA intake with mortality and cardiovascular events in people with a history of heart attack was supported by randomized controlled trials, evidence level 1b. Association of higher fish intake with cardiovascular mortality and events was supported by meta-analyses of observational studies, evidence level 2a/3a. Association of DHA/EPA intake with cardiovascular mortality and events (apparently in the general population) and of higher fish intake with stroke was supported by contradictory evidence or insufficient evidence on which to base recommendations, evidence level 3b. The report stated that the Oxford criteria do not account for study quality and that a limitation of the IOM Seafood Choices report is that the committee's selection of studies reflects its subjective assessment of quality and importance. For an alternate approach to the assessment and synthesis of evidence, the report referred the reader to the AHRQ reports discussed above.

The executive summary of the report presented the following primary findings for benefits associated with nutrients from seafood for adult chronic diseases:

“Observational evidence suggests that increased seafood consumption is associated with a decreased risk of cardiovascular deaths and cardiovascular events in the general population. Evidence is insufficient to assess if this association is mediated through an increase in EPA and DHA consumption and/or a decrease in saturated fat consumption and/or other correlates of seafood consumption.

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“Evidence is inconsistent for protection against further cardiovascular events in individuals with a history of myocardial infarction from consumption of EPA/DHA-containing seafood or fish oil supplements. The protection evidenced by population (observational) studies has not been consistently observed in randomized clinical trials.”

“Evidence for a benefit associated with seafood consumption or fish oil supplements on blood pressure, [and] stroke...is inconclusive. Whereas observational studies have suggested a protective role of EPA/DHA for each of these diseases, supportive evidence from randomized clinical trials is either non-existent or inconclusive.”

An additional finding stated in the chapter on health benefits was:

“Based on three recent meta-analyses of observational studies (Table 3-2), there appears to be a linear association between seafood consumption and primary prevention of cardiovascular disease; the committee did not find strong scientific evidence to suggest a threshold of consumption, such as two servings per week, below which seafood consumption provides no benefit and above which increasing consumption provides no additional benefits.”

The Committee incorporated the above recommendations into guidance for different populations groups, including:

“Adolescent males, adult males, and females who will not become pregnant:

- a. May reduce their risk for cardiovascular disease by consuming seafood regularly, e.g., two 3-ounce servings per week;
- b. Who consume more than two servings a week should choose a variety of types of seafood to reduce the risk for exposure to contaminants from a single source;

“Adult males and females who are at risk of coronary heart disease:

- a. May reduce their risk of cardiovascular disease by consuming seafood regularly, e.g., two 3-ounce servings per week;
- b. Although supporting evidence is limited, there may be additional benefits from including high EPA/DHA seafood selections
- c. Who consume more than two servings a week should choose a variety of types of seafood to reduce the risk for exposure to contaminants from a single source”

The Committee decided there was insufficient evidence to set an upper limit on the amount of seafood consumed each week by the general public, except where research supports such recommendations.

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(c) Results and Conclusions of Some Recent Meta-Analyses and Published Quantitative Estimates of Cardiovascular Benefits of Fish

In addition to these reports and recommendations on the cardiovascular benefits of fish or n-3 LC PUFA consumption, several systematic reviews, meta-analyses and risk assessments have been published in the peer-reviewed scientific literature.

A systematic review is a review of a focused scientific or clinical question, using systematic and explicit methods to search for and identify articles, abstract information from the articles, and combine the information either qualitatively or quantitatively (West, 2002; Khan, 2001). Compared with a traditional, narrative review, the systematic procedures of a systematic review prevent bias and the explicit, transparent methodology of a systematic review permits the review to be replicated (West, 2002; Khan, 2001; Petitti, 2000). The process of using statistical techniques to quantitatively combine the information obtained in a systematic review is called a meta-analysis (West, 2002; Khan, 2001; Petitti, 2000).

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Key Distinctions Between Narrative and Systematic Reviews, by Core Features of Such Reviews, and Including Selected References on Reviews and Evaluation of Scientific Evidence (Excerpted from West et al., 2002)

This table (adapted from Cook et al., 1997) depicts the differences between systematic and narrative reviews. The major difference between these two approaches to synthesizing the clinical or scientific literature is that a systematic review attempts to minimize bias by the comprehensiveness and reproducibility of the search for and selection of articles for review.

What is a systematic review? According to Cook and colleagues, a systematic review is a type of scientific investigation of the literature on a given topic in which the "subjects" are the articles being evaluated. Thus, before a research team conducts a systematic review, it develops a well-designed protocol that lists: (1) a focused study question, (2) a specific search strategy, including the databases to be searched, and how studies will be identified and selected for the review according to inclusion and exclusion criteria, (3) the types of data to be abstracted from each article, and (4) how the data will be synthesized, either as a text summary or as some type of quantitative aggregation or meta-analysis. These steps are taken to protect the work against various forms of unintended bias in the identification, selection, and use of published work in these reviews.

In contrast, what is a narrative review? A narrative review is similar to a systematic review but without all the safeguards to control against bias.

Core Feature	Narrative Review	Systematic Review
Study question	Often broad in scope.	Often a focused clinical question.
Data sources and search strategy	Which databases were searched and search strategy are not typically provided.	Comprehensive search of many databases as well as the so-called gray literature. Explicit search strategy provided.
Selection of articles for study	Not usually specified, potentially biased.	Criterion-based selection, uniformly applied.
Article review or appraisal	Variable, depending on who is conducting the review.	Rigorous critical appraisal, typically using a data extraction form.
Study quality	If assessed, may not use formal quality assessment.	Some assessment of quality is almost always included as part of the data extraction process.
Synthesis	Often a qualitative summary.	Quantitative summary (meta-analysis) if the data can be appropriately pooled; qualitative otherwise.
Inferences	Sometimes evidence-based.	Usually evidence-based.

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Note that the AHRQ report discussed above (Wang et al., 2004) is a systematic review with detailed description of methodology. But the AHRQ report does not provide a quantitative meta-analysis because the authors considered that the studies were too heterogeneous to combine in a quantitative estimate.

Of the recently-published meta-analyses, one used results from randomized clinical trials, five meta-analyses used results from observational studies, and one meta-analysis combined results from observational studies and randomized clinical trials:

Meta-analysis of randomized clinical trials

- Hooper et al, 2004, 2006 (Cochrane Collaboration)

Meta-analyses of observational studies

- Coronary heart disease
 - He et al., 2004a
 - Whelton et al., 2004
 - König et al., 2005
- Stroke
 - He et al., 2004b
 - Bouzan et al., 2005

Meta-analysis of combined observational studies and randomized clinical trials

- Coronary heart disease
 - Mozaffarian & Rimm, 2006

Highlights of Selected Randomized Clinical Trials

As background for discussion of meta-analyses involving randomized trials, this subsection will briefly summarize the highlights of three randomized clinical trial studies that were pivotal to the outcome of the meta-analyses.

GISSI-Prevenzione Study

A large, multi-center trial of fish oil supplements for secondary prevention of heart disease was conducted in Italy by the GISSI Prevenzione investigators group (GISSI, 1999; Marchioli et al., 2002). Subjects were 11,323 men who previously had a heart attack in the three months preceding the trial and were randomly assigned to fish oil supplements or controls. The supplements provided approximately 850 mg DHA plus EPA per day. After three and a half years, those taking omega-3 supplements, compared with controls, had a statistically significant 15 percent decreased risk of all deaths plus nonfatal heart attacks and strokes (95 percent CI, two percent to 26 percent lower risk), a 20 percent decreased risk of cardiovascular deaths plus nonfatal heart attacks and strokes (95 percent CI, six percent to 32 percent lower risk) and a 45 percent decreased risk of

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sudden death (95 percent CI, 23 percent to 61 percent lower risk). The decreased risk of sudden death became statistically significant after only four months of taking omega-3 supplements.

The AHRQ review rated the quality of the GISSI study as good (B), indicating “susceptible to some bias, but not sufficient to invalidate the results. A study in this category has some deficiencies but none likely to cause major bias. The study may be missing information making assessment of the limitations and potential problems difficult.” The qualitative conclusion of the AHRQ report states that “the data for secondary prevention appear to be reliable but they are derived from one very large study”, referring to the GISSI study.

Diet and Reinfarction Trial (DART)

In the DART secondary prevention study in Wales, 2,033 men who had had a recent heart attack were randomized to advice to consume two servings (about 200 to 400 grams) of oily fish per week or no fish advice (Burr et al., 1989). Subjects who could not tolerate fish were given supplements called Maxepa, and asked to consume three per day, to provide 0.5 g EPA per day. During follow-up, subjects received reinforcement of dietary advice from study dietitians. Dietary questionnaires were completed by all subjects and more detailed dietary intake information and blood plasma levels of EPA were determined in a subset of subjects at six months and two years. On average, the questionnaires showed that weekly EPA intake of the subjects in the fish advice group was 2.3 g at six months and 2.4 g at two years, compared with 0.7 and 0.6 g, respectively, in the no fish advice group. In 107 men given fish advice and 96 men not given fish advice, geometric mean percentages of EPA in blood plasma at two years were significantly different, 0.59 percent and 0.46 percent, respectively ($p < 0.01$). In the fish advice group, 14 percent took the EPA capsules at six months and 22 percent took the capsules at two years.

After two years, the fish advice group had a statistically significant 29 percent decreased risk of total mortality compared with the no fish advice group (95 percent CI, 8 percent to 46 percent decreased risk). The fish advice group also had a 16 percent decreased risk of total ischemic heart disease (IHD) events (nonfatal heart attacks plus IHD deaths) that was not statistically significant (95 percent CI, seven percent increased risk to 33 percent decreased risk). Secondary analysis showed that the lower total mortality was attributable to a statistically significantly lower heart disease (IHD) mortality in the fish advice group. The AHRQ review rated the quality of the 1989 Burr study as poor (C), corresponding to “significant bias that may invalidate the results. A study with serious errors in design, analysis, or reporting. These studies may have large amounts of missing information or discrepancies in reporting.”

Diet and Angina Randomized Trial (DART 2)

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In another secondary prevention study in Wales, 3,114 men with angina (chest pain) were randomized to advice to consume two servings of oily fish per week or no fish advice (Burr et al., 2003). This study is also called the Diet and Angina Randomized Trial or DART 2. Subjects who could not tolerate fish were given supplements called Maxepa, to provide three grams of EPA per week. Subjects were classified with angina if they were taking nitrate medication and reported chest pain on exertion. About half the subjects had a history of heart attack and about 12 percent had diabetes.

Subjects were recruited in two phases, separated by a 12 month interruption of funding. In Phase I, 1,111 subjects were recruited in 1990 to 1992. In Phase II, 2,003 subjects were recruited in 1993 to 1996. In Phase II, half of those in the fish advice group were randomized to the supplements providing three grams of EPA per week. Dietary intake questionnaires were administered at six months in Phase I subjects and in a subset of Phase II subjects. Blood plasma levels of EPA at six months were determined in a subset of Phase I subjects. In those completing the dietary questionnaires, the average weekly EPA intake of the fish advice group at six months was 2.65 g higher than the intake of the no fish advice group. In 39 subjects given fish advice, the blood plasma EPA increased by 1.23 g/dl from baseline to six months, compared with a slight decline of 0.16 g/dl in 29 subjects in the no fish advice group

Mortality was determined in 1999, after three to nine years of follow-up. Survival analysis showed that the fish advice group had a 15 percent higher risk of total mortality compared with the no fish advice group, not statistically significant (95 percent CI, four percent decreased risk to 36 percent increased risk, $p = 0.13$). The fish advice group also had a 26 percent increased risk of cardiac death that was marginally statistically significant (95 percent CI, 0 percent to 58 percent increased risk, $p = 0.047$) and a statistically significant 54 percent increased risk of sudden death (95 percent CI six percent to 23 percent, $p = 0.025$). Secondary analysis showed that the increased risk of cardiac death and sudden death in the fish advice group was confined to the Phase II subjects and was greater in the subjects who were randomized to EPA supplements in Phase II. Compared with the no fish advice group, the randomized supplement subgroup had a statistically significant 45 percent increased risk of cardiac death and 84 percent increased risk of sudden death, whereas the dietary fish subgroup (Phase I and II combined) had non-statistically significant increased risks of 20 percent and 43 percent, respectively. Information was not given on the proportion of those in the dietary fish subgroup who were given EPA supplements because they did not tolerate fish. The AHRQ review rated the quality of the 2003 Burr study as poor (C), corresponding to “significant bias that may invalidate the results. A study with serious errors in design, analysis, or reporting. These studies may have large amounts of missing information or discrepancies in reporting.”

Meta-analysis of randomized clinical trials: Hooper et al., 2004, 2006 (Cochrane Collaboration)

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A systematic review and meta-analysis of the association of omega-3 fatty acids with mortality, cardiovascular disease, and cancer was published in 2004 by the Cochrane Collaboration (Hooper et al., 2004). In 2006, the major results of the extensive Cochrane report were published in the British Medical Journal (BMJ) (Hooper et al., 2006).

Methods: The primary question of the meta-analysis was whether dietary or supplemental omega-3 fatty acids alter total mortality, cardiovascular events, cancers or other adverse events. The systematic review covered randomized clinical trials of adults, with at least six months of follow-up, that included diet advice or dietary supplements to promote omega-3 fatty acid intake, including ALA as well as EPA and DHA, and that recorded outcomes of mortality or cardiovascular events. To identify possible adverse effects, cohort studies of dietary or supplemental omega-3 fatty acids, with at least six months of follow up, were also reviewed. Cohort studies were included if they reported intake of omega-3 fatty acids or “oily fish” but not included if they only reported intake of “fish” in general. The data extracted from the cohort studies were for the highest compared with the lowest level of omega-- fatty acid intake. Relevant databases were searched systematically for randomized controlled trials and cohort studies published through February, 2002.

Analysis of Randomized Controlled Trials: The review identified 15 trials enrolling a total of 33,193 subjects and having 1,995 deaths. There was not a statistically significant decrease in relative risk for total mortality in the omega-3 fatty acids groups, RR = 0.87 (95 percent CI, 0.73 to 1.03, p = 0.11). There was significant heterogeneity, p = 0.04. In the subgroup of 12 studies that looked at marine omega-3 fatty acids only (excluding ALA studies), there were 19,142 subjects and 1,855 deaths. In this subgroup, the relative risk of total mortality was also not significantly decreased, RR = 0.86 (95 percent CI, 0.70 to 1.04, p = 0.12) and there was heterogeneity of borderline significance, p = 0.05. When the study of dietary advice and angina (Burr et al., 2003; DART 2) was omitted, analysis of the remaining 14 studies showed that the omega-3 groups had a significant, 17 percent decrease in mortality risk (95 percent CI, 9 percent to 25 percent decreased risk) and the heterogeneity was removed, p = 0.52. Results were not reported for the marine omega-3 subgroup with the 2003 Burr study removed.

The review also identified 18 trials enrolling a total of 33,625 subjects and having 2,628 cardiovascular events. The relative risk for total cardiovascular events in the omega-3 fatty acids groups was not statistically significant, RR = 0.95 (95 percent CI, 0.82 to 1.12, p = 0.57). The results showed significant heterogeneity, p less than 0.0001. This result was little changed in the marine omega-3 fatty acid subgroup, or by omitting the 2003 Burr study.

In secondary analyses, there was a nonsignificant, 15 percent decreased risk of cardiovascular death and a nonsignificant, 14 percent decreased risk of fatal myocardial infarction, roughly similar to the results for total mortality. Secondary analysis also showed little effect on nonfatal heart attack, RR = 1.04 with wide confidence intervals. There was no significant effect on sudden death in six pooled studies, RR = 0.85 with

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wide confidence intervals and heterogeneity. However, the GISSI study suggested significant protection from sudden death by omega-3 fatty acid consumption and the 2003 Burr study showed significantly increased risk of sudden death. There was a non significant increased risk of stroke in nine pooled studies including 31,255 subjects and 243 strokes, RR = 1.17 with wide confidence intervals. For these secondary analyses, results were not reported separately for the marine omega- subgroup.

Analysis of Cohort Studies: The review identified only three included cohort studies that reported total mortality, enrolling 3,801 subjects and having 318 deaths. There was a significant, 35 percent decreased risk of death in the high omega-3 group (95 percent CI, 12 percent to 52 percent decreased risk) with no significant heterogeneity. None of the cohort study analyses were reported separately for the marine omega-3 subgroup. The review also identified seven included cohort studies of total cardiovascular events, with 69,702 subjects and 1,929 cardiovascular events. There was a nonsignificant decreased risk in the high omega-3 group, RR = 0.91 (95 percent CI, 0.73 to 1.13) and significant heterogeneity, p less than 0.0001.

In the secondary analysis of nine cohort studies of cardiovascular deaths, there were 109,303 subjects and 1,772 cardiovascular deaths. There was a significant, 21 percent decreased risk in the high omega-3 group (95 percent CI, one percent to 31 percent decreased risk, p = 0.04). There was significant heterogeneity. There was only one included cohort study for fatal myocardial infarction (Yuan et al., 2001) and for sudden death (Albert, 1998). Each showed a significantly decreased risk for the high omega-3 group. The three pooled cohort studies for nonfatal myocardial infarction showed little effect of high omega-3 consumption, RR = 0.93 with wide confidence intervals.

Hooper et al Discussion and Conclusions: Hooper et al. noted the inconsistency between the 2003 Burr et al. study of dietary advice and angina (DART 2) and the other clinical trials in the meta-analysis. In general, when the 2003 Burr study was omitted from subgroup and sensitivity analyses, the analysis showed that the omega-3 group had a significantly decreased risk of death or cardiovascular outcome and heterogeneity was removed. However, after an extensive discussion of the characteristics of the 2003 Burr study, Hooper et al. (2004) found no justification for disregarding that study in evaluating the results of the meta-analysis.

The meta-analysis of the cohort studies was included in order to identify possible adverse outcomes that might be missed in a clinical trial (none were found). Additionally, the results of the cohort study analysis were mentioned briefly in comparison with the analysis of the randomized trials. The 2006 BMJ paper (Hooper et al., 2006) included graphs of the cohort study analyses of the primary health outcomes, total mortality and total cardiovascular events. However, the significant 21 percent decreased risk of cardiovascular death mentioned above was a secondary outcome and was not stated in the BMJ paper (Hooper et al., 2006). The potential for confounding in the cohort studies was discussed extensively and the authors concluded that they were uncertain whether the

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cohort studies could adequately adjust for confounding by lifestyle and health factors (Hooper et al., 2004). Additionally, the results of the cohort study analyses were not consistent with the main results of the randomized trial analyses (note that these included the 2003 Burr study) (Hooper et al., 2004). Therefore, the results of the cohort study analyses did not play a great part in the conclusions of Hooper et al., 2004 or 2006.

The practical implications found by the authors, as stated in Hooper et al., 2004, are:

“It is not clear that dietary or supplemental omega-3 fats reduce or increase total mortality, combined cardiovascular events, or cancers in people with, or at risk of, cardiovascular disease or in the general population. Neither were robust significant effects seen for any secondary outcome events. As no significantly increased risks of any events (total mortality, cancers, strokes) were seen there is no need for people to stop eating oily fish or taking supplemental sources of omega-3 fats if they are currently doing so.”

The authors further concluded that existing dietary recommendations for the general public to eat more oily fish, and for people who have had heart attacks to consume higher amounts, should continue for the present. However, this advice should be reviewed regularly. Additionally, a high intake of omega-3 fatty acids should not be recommended for people with angina but who have not had a heart attack. The authors also concluded that additional, high quality randomized controlled trials on omega-3 fatty acids and health outcomes are needed, particularly in healthy populations (primary prevention). However, large and expensive trials would be needed for primary prevention.

Responses to Hooper et al. Meta-analysis (Cochrane Collaboration): Numerous comments and criticisms of the methodology and conclusions of the meta-analysis by Hooper et al. (2006) were published in letters to the British Medical Journal (Geleijnse et al. 2006; He and Song 2006) and posted as electronic comments (Twisselmann 2006; Electronic responses 2006). Comments criticized the analysis of randomized trials because it inappropriately combined different types of studies to obtain a quantitative estimate, including: primary and secondary prevention studies, trials of dietary supplements and dietary advice, fish or plant sources of omega-3 fatty acids, fatal and nonfatal cardiovascular event outcomes. Comments also criticized the methodologically poor DART 2 study and its inclusion in the analysis, noting that removal of this study changed the conclusion to clear benefit from no benefit. Comments pointed out that the systematic literature review had a cutoff date of 2002 and was not updated between the Hooper et al. 2004 report and the 2006 publication, yet included the poor quality DART 2 study (Burr et al., 2003) which was still unpublished in 2002. The analysis was criticized for basing conclusions on numerical estimates that were significantly heterogeneous, an indication that unlike studies have been inappropriately combined.

Comments also criticized the inclusion criteria for observational studies, stating that included studies reported estimates of dietary intake of omega-3 fatty acids, but other

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studies that reported fish intake or biomarkers of omega-3 fatty acid intake were excluded. Analysis of cohort studies by comparing the lowest and highest quintile of intake, regardless of range of intake in the study population, was criticized as a source of heterogeneity. Comments cited a published commentary that found problems with previously published Cochrane reviews of diet and chronic disease (Truswell 2005). The commentary by Truswell noted that most of the evidence base in nutrition is observational, especially cohort studies. Truswell criticized the methodology of two earlier Cochrane reviews by the Hooper et al. group, which focused on randomized clinical trials of dietary fat intake and cardiovascular disease and of health effects of salt intake.

In their reply, Hooper et al. (Electronic responses 2006) emphasized that the main conclusion of their meta-analysis was not that omega-3 fatty acids offer no protection from heart disease, but rather that the relative risk for mortality suggested benefit (RR = 0.87), but the result was not statistically significant, indicating uncertainty of benefit. The authors also emphasized that their review does not question the U.K. health eating advice for consumption of two portions of fish per week by the general public, of which one should be oily. The authors stated they are less sure about higher, therapeutic doses of omega-3 fatty acids for people with heart disease. Hooper et al. pointed out that several of the subgroup analyses and sensitivity analyses suggested by the comments had been presented in the longer Cochrane report (Hooper et al., 2004). Regarding cohort studies, the authors stated again their concern about the potential for confounding. They clarified that, contrary to many of the comments, they did include cohort studies that measured exposure using biomarkers of omega-3 fatty acids or intake of oily fish, as well as studies that estimated intake of omega-3 fatty acids. They did, however, exclude cohort studies that reported intake of fish in general, because their goal was to study the effect of omega-3 fatty acids, not the effect of fish in general.

Comparison of Scientific Panels and Hooper et al. Meta-analysis: As noted above, in general the conclusions of the scientific panels gave little weight to the poor quality secondary prevention study, the DART 2 trial (Burr et al., 2003). Results of the DART 2 trial were noted in the reviews by AHRQ (Wang et al., 2004), SACN (2004), the Dietary Guidelines Advisory Committee (2004) and EFSA (2005). The EFSA report specifically stated that the weight accorded to the DART 2 study accounts for the difference between the conclusions of Hooper et al. (2004) and those of other reports such as Wang et al. (2004) and SACN (2004). A panel of experts for ISSFAL (2006) reported a number of concerns with the DART 2 trial and the Hooper et al. (2006) meta-analysis, similar to the comments summarized above. The ISSFAL panel concluded that the weight of the evidence is sufficient to conclude, even in light of the Cochrane analysis, that EPA and DHA reduce risk for cardiovascular diseases. The literature review by AHRQ was updated to June, 2005, and generally confirmed the earlier conclusions (Wang et al., 2006). Wang et al. (2006) noted the agreement between their conclusions and those of the more narrowly defined meta-analyses of He et al. (2004a and b) but did not compare their results with the Cochrane review (Hooper et al. 2004 and 2006).

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Highlights of Recently Published Randomized Clinical Trials

This subsection will summarize the results of several recently published randomized clinical trials that contribute to the body of evidence from intervention studies.

Intervention Studies of Patients with Cardiac Defibrillators. Three randomized trials evaluated the effect of fish-oil consumption in patients with implantable cardioverter defibrillators (Raitt et al., 2005; Leaf et al., 2005; Brouwer et al., 2005). The results for all-cause mortality and cardiac death were summarized by Wang et al. (2006), in an update to the 2004 AHRQ report, and later corrected and re-summarized by Lau et al., (2006). Raitt et al. (2005) followed 100 patients taking 1.3 g/d DHA plus EPA from fish oil for two years compared with 100 controls. Patients taking fish oil had non-significant decreased risk of all-cause mortality, RR 0.4 (95 percent CI 0.13 to 1.23) and cardiac death, RR 0.4 (95 percent CI 0.08 to 2.01). Results were similar in the study by Brouwer et al. (2006) of 273 patients taking 0.8 g/d EPA plus DHA from fish oil compared with 273 controls. Patients taking fish oil for one year had non-significant decreased risk of all-cause mortality, RR 0.57 (95 percent CI 0.24 to 1.34) and cardiac death, RR 0.46 (95 percent CI 0.18 to 1.20). Leaf et al. (2005) studied 200 patients taking 2.6 g/d EPA plus DHA compared with 202 controls. In contrast with the other two studies, they found little effect of fish oil for one year on all-cause mortality, RR 1.09 (95 percent CI 0.51 to 2.34) or cardiac death, RR 1.01 (95 percent CI 0.18 to 1.20). However, Leaf et al. found that patients taking fish oil had a near-significant decreased risk of the primary study endpoint, time to first defibrillator shock for arrhythmia or death from any cause, RR 0.72 (95 percent CI 0.51 to 1.01, $p = 0.057$).

Japan EPA Lipid Intervention Study (JELIS). The JELIS study followed 18,645 patients from throughout Japan who had high total cholesterol, 6.5 mmol/L (251 mg/dl) or greater (Yokoyama 2007). All patients received statin therapy to lower blood lipids, and half were randomized to receive 1.8 g/d EPA. The cohort included 3,664 patients with documented coronary artery disease; of these, 1,050 had a history of myocardial infarction. The primary endpoint was any major coronary event, including sudden cardiac death, fatal and non-fatal myocardial infarction, and other non-fatal events including unstable angina, angioplasty, stenting or coronary bypass surgery. After 4.6 years of followup, the patients taking EPA had a 19 percent decreased risk of major coronary events (95 percent CI 5 to 31 percent, $p = 0.011$). For those with documented coronary disease (secondary prevention), there was also a decreased risk of major coronary events with EPA consumption, Hazard Ratio 0.81 (95 percent CI 0.66 to 1.00, $p = 0.048$). For those without previous documented coronary disease (primary prevention), there was a decreased risk of major coronary events with EPA consumption, but the result was not statistically significant, Hazard Ratio 0.82 (95 percent CI 0.63 to 1.06, $p = 0.132$). The authors concluded that EPA is a promising treatment for prevention of major coronary events, especially non-fatal coronary events, in Japanese patients with high blood cholesterol.

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GISSI-HF (Heart Failure) Trial. The GISSI-HF trial followed 6,975 patients in Italy who met inclusion criteria for clinical evidence of heart failure (GISSI-HF Investigators 2008). There were 3,494 patients randomized to one g/d EPA plus DHA and 3,481 randomized to placebo. The co-primary endpoints were: 1) time to death and 2) time to death or admission to hospital for cardiovascular reasons. Results were adjusted for admission to hospital for heart failure in the previous year, previous pacemaker and aortic stenosis. After 3.9 years of followup, the patients taking EPA plus DHA had an adjusted nine percent decreased risk of death (95 percent CI, 0.2 to 16.7 percent, $p = 0.041$) and an adjusted eight percent decreased risk of death or admission to hospital for cardiovascular reasons (95 percent CI, 0.1 to 15.1 percent, $p = 0.009$). The authors concluded that a simple and safe treatment with n-3 PUFA can provide a small beneficial advantage in patients with heart failure in the context of usual care.

Meta-analyses of Observational Studies

As mentioned in the Introduction, one purpose of this summary document is to identify reports of quantitative dose-response relationships for the association between fish intake and cardiovascular health benefits with potential for use in risk and benefit assessment modeling. Five recent meta-analyses have provided quantitative estimates of the association between fish consumption and coronary heart disease or stroke from systematic reviews of observational studies—prospective cohort studies (and sometimes including some case control studies) (Table A-2).

- Coronary heart disease
 - He et al., 2004a
 - Whelton et al., 2004
 - König et al., 2005
- Stroke
 - He et al., 2004b
 - Bouzan et al., 2005

In comparison with randomized clinical trials, the subjects in observational studies consume their usual diets; dietary intake of fish or fish oil supplements is not randomly assigned by the investigator. Therefore, an observational study cannot conclusively demonstrate a cause and effect relationship. Observed relationships between fish intake and cardiovascular disease could be due to another, confounding variable of participant characteristics or lifestyle. However, possible confounding variables that are known and measured can be adjusted for in the statistical analysis. An advantage of prospective cohort studies is that they study disease experience related to participants' actual diets, typically over a long period of time. This is the type of exposure of interest in a risk and benefit assessment. Additionally, participants in prospective cohort studies are healthy at the beginning of follow up, providing information on primary prevention of cardiovascular disease outcomes relevant for the general, healthy population. These five

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meta-analyses provided different types of quantitative estimates, as summarized in Table A-2 and outlined in the following subsections.

Pooled Relative Risk of Coronary Heart Disease or Stroke Associated with Some Fish Versus Little or No Fish Intake

Two analyses of coronary heart disease death, by He et al. (2004a) and Whelton et al. (2004), and an analysis of stroke by He et al. (2004b) reported pooled relative risks for some fish versus little or no fish intake. Each of these three analyses was based on nine to 13 prospective cohort study populations, including over 200,000 subjects, with follow up time averaging about 12 years in the He et al. (2004a and b) papers and ranging from five to 30 years in the Whelton et al. (2004) paper. The results for the pooled relative risks are shown in Table A-3. With some fish consumption compared with little or none, He et al. found a 15 percent decreased risk and Whelton et al found a 17 percent decreased risk of CHD death, which were statistically significant. Whelton et al. also found a 14 percent decreased risk of total CHD, also statistically significant. He et al found a 13 percent decreased risk of all strokes, which was statistically significant, and a 32 percent decreased risk of ischemic stroke, which was highly statistically significant, as seen from the confidence intervals in Table A-3. Not all papers provided separate results for ischemic and hemorrhagic strokes. Therefore, the meta-analysis of ischemic and hemorrhagic stroke was based on about 150,000 of the 200,000 total study participants. For hemorrhagic stroke, there were only 548 stroke events at all intake levels and this analysis showed a 21 percent increased risk, with wide confidence intervals which were not statistically significant (32 percent decreased risk to 85 percent increased risk).

Pooled Relative Risk of Coronary Heart Disease or Stroke by Category of Fish Intake

The meta-analyses by He et al. (2004a and b) also reported pooled relative risks for CHD death and stroke, respectively, for five categories of fish intake including the baseline, fish intake less than once per month (Table A-4). In the He et al. meta-analyses, the estimated amount of fish consumed at each frequency category was calculated in grams per day for each original study, using available portion size information corresponding to that study (or using default assumptions if portion size was not reported). The fish consumption categories in the overall meta-analysis were then standardized across studies so that the frequency categories corresponded to quantitative estimates of intake in grams per day.

At the highest intake category, fish intake five or more times per week, there was a 38 percent decreased risk of CHD death, a 31 percent decreased risk of total stroke and a 35 percent decreased risk of ischemic stroke, all statistically significant. At the two highest intake categories (fish intake two to four times per week or five or more times per week), there was a decreased risk of hemorrhagic stroke, 11 percent and 20 percent, respectively, but these were not statistically significant. These analyses provided categorical information on a dose-response relationship between fish intake and CHD death or

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stroke. The pooled relative risk for CHD death decreased monotonically for each category of greater fish intake, and the dose-response was further analyzed in a meta-regression, as discussed below. The pooled relative risk for total stroke also decreased monotonically for each category of greater fish intake, but the test for trend was not statistically significant, $p = 0.06$. For ischemic stroke, there was no indication of a change in relative risk with higher fish intake categories, and the test for trend was not significant, $p = 0.24$.

Meta-Regression Dose-Response for Fish Consumption and Pooled Relative Risk of Coronary Heart Disease or Stroke

In a meta-regression analysis, He et al. (2004a) used a weighted linear regression to model the natural logarithms of the relative risks at each intake category from the individual studies (Table A-5). The result showed a seven percent decrease in relative risk with each 20 gram per day (about 2/3 ounce) increment of fish intake; the 95 percent CI was one percent to 13 percent decreased risk, p for trend = 0.03.

Five papers describing a quantitative risk-benefit analysis of fish consumption were published together in 2005 by a group led by Cohen and Gray at the Harvard Center for Risk Analysis. The coauthors were an interdisciplinary expert panel assembled by the lead authors. Two papers reported quantitative dose-response relationships between fish consumption and CHD death (König et al., 2005) and stroke (Bouzan et al., 2005).

The meta-regression of CHD death included seven cohort studies with over 150,000 subjects and follow-up time from six to 30 years (Table A-2). The analysis of stroke included four cohort studies with almost 130,000 subjects and 12 to 30 years follow-up time, and also included a case control study of over 800 subjects. The number of included studies and subjects was lower than for the other analyses in Table A-2 because additional exclusion criteria were used. For example, these meta-regressions excluded studies with quality rated “C” (significant bias that may invalidate the results) by Wang et al. (2004), and studies in which the subjects were special populations such as vegetarians or smokers. For studies that reported fish consumption as grams per day, these meta-regressions assumed that 100 grams of fish is equivalent to one serving.

For CHD death, the meta regression by König et al. (2005) found a 17 percent decreased risk for a low level of fish consumption compared with no fish consumption (intercept parameter) (Table A-5). The model also predicted a 3.9 percent further decrease in risk for each serving per week increase in fish consumption (slope parameter). Both model parameters were statistically significant.

For nonfatal heart attack, the slope parameter was not statistically significant, and the point estimate was for increased (rather than decreased) risk with each additional serving of fish per week. Therefore, the final model was the intercept-only model. This predicted a 25 percent decreased risk of nonfatal heart attack with a low level of fish intake

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compared with little or no intake, and the intercept was statistically significant. The authors considered this intercept-only model for nonfatal heart attack to have questionable biological plausibility, because the biological mechanism consistent with this model would be a decrease in arrhythmia. Such a decreased tendency for arrhythmia would be expected to decrease the risk of sudden death and fatal heart attack, but not the risk of nonfatal heart attack. Therefore, the authors concluded that the effect of fish consumption on nonfatal heart attack is very uncertain.

For stroke, the model by Bouzan et al. (2005) found a statistically significant 12 percent decreased risk for a low level of fish consumption compared with no fish consumption (intercept parameter) (Table A-5). The stroke model also predicted a two percent further decrease in risk for each serving per week increase in fish consumption (slope parameter). Both parameters were statistically significant.

König et al. (2005) stated that their findings for CHD mortality risk corresponds to a 5.5 percent decrease in relative risk per 20 grams of fish/day (assuming that one serving per week, that is, 100 grams of fish/week, corresponds to approximately 14 grams of fish/day). König et al. (2005) stated that this was similar to the results reported by He et al. (2004a), a seven percent decreased risk of CHD mortality for each 20 grams/day increase in fish consumption.

Quantitative Risk Assessment Published “What if” Scenarios

The quantitative risk-benefit analysis of fish consumption by the Cohen and Gray group used a probabilistic model to predict changes in CHD mortality and stroke in the U.S. that would result from specific changes in fish intake (Cohen et al., 2005a). The risk assessment model used an FDA exposure model (Carrington & Bolger, 2002; Carrington et al., 2004) to describe the population distribution of U.S. fish consumption and to estimate changes in fish consumption under specific scenarios. The model used the dose-response parameters of König et al. (2005) and Bouzan et al. (2005) to predict changes in CHD mortality and stroke that would result from changes in fish intake. The dose-response parameters were reported as servings per week (Table A-5), and the Cohen et al. (2005a) risk assessment apparently assumed that one serving of fish corresponds to 100 grams of fish, the same assumption used by König et al. (2005) and Bouzan et al. (2005). The analysis assumed no change in fish intake in the 15 percent of the population who consume no fish.

The risk assessment developed five “What if” scenarios to examine hypothetical population responses to the FDA/EPA joint fish consumption advisory (FDA/EPA 2004). Three of these scenarios (Scenarios Three, Four, and Five) involved changes in fish consumption by adult men and by women age 45 and older, with predicted changes in CHD mortality and stroke.

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The authors stated that their analysis does not consider the nutritional impacts of other food sources of energy and protein that may be consumed if fish intake rates drop because of concern about contaminants in fish (e.g., carbohydrates and other types of meat). For example, the authors noted that an increase in the consumption of foods higher in saturated fat may increase cardiovascular disease risks.

- In Scenario Three, everyone in the general population decrease their overall fish consumption by 17 percent, or 3.9 g/day based on average intake of 23.1 grams/day. This decrease was predicted to result in an additional 6,700 deaths from CHD, 1,200 deaths from stroke and 1,500 nonfatal strokes per year.
- In Scenario Four, everyone in the general population except for women of childbearing age increase their overall fish consumption by 50 percent, or 11.6 grams/day based on average intake of 23.1 grams/day. This increase was predicted to result in a decrease of 16,000 CHD deaths, 2,900 stroke deaths and 3,200 nonfatal strokes per year.
- In Scenario Five, everyone in the general population including women of childbearing age increase their overall fish consumption by 50 percent, or 11.6 grams/day based on average intake of 23.1 grams/day. This increase was predicted to result in the same decrease of 16,000 CHD deaths, a slightly greater decrease of 3,000 stroke deaths, and a slightly greater decrease of 3,400 non-fatal strokes per year. These small additional decreases involve women of childbearing age. For men and for women not of childbearing age, Scenario Five was similar to Scenario Four.

Meta-Analysis of Combined Observational Studies and Randomized Clinical Trials

Mozaffarian and Rimm (2006) published a clinical review to address the seemingly conflicting reports on the risks and benefits of fish intake and the role of fish consumption in a healthy diet. The review considered the scientific evidence for adverse and beneficial health effects of fish consumption. For cardiovascular benefits of fish intake, the review identified five randomized controlled trials and 15 prospective cohort studies of fish or fish oil intake and CHD death. There were four studies in populations with high CHD mortality rates (1,000 to 3,000 per 100,000 person years), seven studies in populations with intermediate rates (150 to 1,200 per 100,000) and nine studies in populations with low rates (less than 150 per 100,000). Graphs of CHD mortality rate by intake of EPA plus DHA for each group showed that modest EPA/DHA consumption (250 to 500 mg/day) decreased relative risk by about 25 percent or more. The graphs showed that higher intake did not provide additional lowering of CHD mortality, suggesting a threshold effect. This was confirmed in a pooled dose-response analysis of all 20 studies (Table A-6). At EPA/DHA intakes up to 250 mg/day, there was a 14.6 percent decreased risk of CHD death for each 100 mg/day intake (95 percent confidence interval, eight percent to 21 percent). This gave a total 36 percent reduction in risk for intake of 250 mg/day EPA/DHA compared with no intake (95 percent confidence interval, 20 percent to 50 percent). At intakes above 250 mg/day, there was little additional risk reduction (Table A-6).

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The authors considered the effect of the estimated 36 percent decreased risk of CHD death in populations with different proportions of total mortality from CHD. In middle aged populations, about one quarter of deaths are from CHD and in populations with existing CHD, about half of deaths are from CHD. If total mortality were reduced through a 36 percent decrease in CHD deaths, this would reduce total mortality by roughly nine percent to 18 percent in the respective populations, or about 14 percent in a mixed population. (Note that 36 percent x 25 percent = nine percent and 36 percent x 50 percent = 18 percent.) The authors noted that the estimated 14 percent decrease in total mortality is consistent with the nonsignificant 14 percent decreased relative risk of total mortality found in meta-analysis of randomized clinical trials (95 percent confidence interval, -30 percent to +4 percent) (Hooper et al., 2006). The authors conducted a meta-analysis of fish or fish oil intake and total mortality by combining 15 randomized clinical trials of marine sources of omega-3 fatty acids: 12 studies used by Hooper et al. plus three studies of patients with cardiac defibrillators, summarized above and published since 2003. The meta-analysis results showed a significant, 17 percent reduction in total mortality for high versus low intake of fish or fish oil (95 percent confidence interval, -32 percent to 0 percent, $p = 0.046$). The authors commented that this reduction compares favorably with a 15 percent reduction of total mortality by statins in a meta-analysis of randomized trials (95 percent confidence interval, -21 percent to -8 percent).

The authors concluded that, in the general population, 250 mg/day of EPA and DHA is a reasonable target intake to reduce CHD mortality, corresponding to a weekly intake of one 6-oz serving/wk of wild salmon or similar oily fish, or more frequent intake of smaller or less n-3 PUFA-rich servings. The authors noted that, for individuals with CHD, their analysis suggests that lower doses of EPA and DHA may be sufficient, compared with the 1000 mg/day recommended by AHA and the European Society of Cardiology. However, because of this population's higher risk and because most of the data are from primary prevention studies, the authors stated that a target intake of 500 to 1,000 mg/day appears reasonable to reduce CHD mortality for individuals with CHD. This is consistent with the large GISSI trial and corresponds to one 6-oz serving/wk of fish richest in n-3 PUFAs (e.g., farmed salmon, anchovies, and herring), more frequent consumption of other fish, or supplements.

Comparative Meta-Analyses of Drug and Dietary Lipid-Lowering Interventions on Mortality

Studer et al. (2005) assessed the efficacy and safety of different lipid-lowering interventions based on mortality data. A systematic search was conducted of randomized controlled trials published up to June 2003, comparing any lipid-lowering intervention with placebo or usual diet with respect to mortality. Outcome measures were mortality from all, cardiac, and noncardiovascular causes.

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RESULTS: A total of 97 studies met eligibility criteria, with 137,140 individuals in intervention and 138,976 individuals in control groups.

Compared with control groups, risk ratios for overall mortality were:

1.00; fibrates	(95% CI, 0.91-1.11),
0.97; diet	(95% CI, 0.91-1.04).
0.96; niacin	(95% CI, 0.86-1.08),
0.87; statins	(95% CI, 0.81-0.94),
0.84; resins	(95% CI, 0.66-1.08),
0.77; n-3 LC PUFA	(95% CI, 0.63-0.94)

Compared with control groups, risk ratios for cardiac mortality indicated benefit from

0.78; statins	(95% CI, 0.72-0.84),
0.70; resins	(95% CI, 0.50-0.99) and
0.68; n-3 LC PUFA	(95% CI, 0.52-0.90).

The authors concluded that statins and n-3 fatty acids are the most favorable lipid-lowering interventions with reduced risks of overall and cardiac mortality. Evaluation of lipid lowering drug therapy is beyond the scope of this paper. However, the comparable magnitude of decreased risk of cardiac mortality for statin and resin drugs and for n-3 LC PUFA provides context for the quantitative dose-response information reviewed in this paper.

(d) Conclusions of Some Recent Reviews of Possible Cardiovascular Risk from Methylmercury in Fish

In contrast to the large, recognized body of science reporting the cardiovascular health benefits of fish consumption, a few studies have suggested that methylmercury in fish may increase cardiovascular risk. Studies examining a possible association between methylmercury and cardiovascular risk are addressed briefly in the FDA draft risk and benefit report.

As described in the reports and reviews summarized in this section, many of the studies that found that fish consumption was associated with decreased CHD risk focused on fish consumption itself, rather than on n-3 fatty acids or other nutrients or components in fish. Thus, the cardiovascular benefits associated with fish consumption in these studies are the net result of beneficial components in fish together with possible risk from harmful components such as methylmercury. To provide context between the reported cardiovascular benefits of fish consumption and possible cardiovascular risk of methylmercury in fish, this section will also briefly summarize the research on the possible cardiovascular risk of methylmercury in fish.

The possible association of mercury exposure with clinical cardiovascular outcomes has been investigated in five study populations (Table A-7). An association of mercury in

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fish with increased cardiovascular risk was supported by results in two study populations: a cohort study of 1,871 men in Eastern Finland and a case-control study of about 1,400 heart attack patients and controls in eight European countries and Israel. A large cohort study of U.S. male health professionals did not find an association, nor did a case-control study in Northern Sweden or a cohort study of Swedish women. As outlined in Table AC-8, most, but not all, reviews and reports have concluded that the studies are inconsistent, and that there is not sufficient evidence to conclude that methylmercury in fish increases cardiovascular risk.

The available studies have several limitations. The case control study in Northern Sweden had a small sample size, and found no consistent association between blood mercury and heart attack risk. The cohort study of Swedish women used serum mercury levels, whereas most methylmercury is found in red blood cells. Interestingly, there was a negative correlation between serum mercury and heart attack that was not statistically significant, but suggested that the direction of any effect was decreased risk with higher serum mercury. The cohort study of U.S. male health professionals was large, but the five year follow up time was relatively short, giving only 470 CHD cases for the nested case control study. Over half of the subjects were dentists, and had occupational exposure to elemental mercury from amalgam fillings. The mean toenail mercury in the dentists was about twice that in the nondentists. There was no association between toenail mercury and heart disease risk after adjusting for age, smoking and heart disease risk factors. This did not change with additional adjustment for dietary omega-3 fatty acids, DHA plus EPA (Table A-7). When the dentists were removed from the analysis, there was a weak positive association (multivariate Relative Risk = 1.27) between toenail mercury and CHD risk which was not statistically significant (95 percent CI 0.62 to 2.59, p for trend 0.43). With additional adjustment for DHA, the result was still not statistically significant, p for trend 0.41. The authors concluded that their findings do not support an association between total mercury exposure and CHD risk, but a weak relation cannot be ruled out. Note that the lack of association between toenail mercury and CHD risk when the dentists were included indicates absence of association between elemental mercury and CHD risk.

The cohort study in Eastern Finland found increased risk of heart attack and CHD death in men with higher levels of hair mercury. The study cohort consumed lean fish from highly polluted lakes, and had a diet low in selenium due to the low soil selenium in the region. The increased risk was mainly associated with the upper third of hair mercury levels, greater than two micrograms mercury per gram of hair (parts per million, ppm) and averaging about 10 ppm. (This compares with U.S. levels of hair mercury that average about 1.0 ppm.) Interestingly, in the paper by Virtanen et al. (2005), those in the middle third of hair mercury had lower risk of CHD death than those in the lowest third, RR = 0.61, not statistically significant (95 percent CI, 0.34 to 1.10). The higher risk of CHD death, RR = 1.21, in the upper third of hair mercury compared with the lower third, was also not statistically significant (Table A-7). The authors then combined the lower two-thirds of hair mercury as a reference group. They found increased risk of CHD death

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in the upper third compared with the lower two-thirds, RR=1.56 (95 percent CI, 0.99 to 2.46), nearly statistically significant.

In the Euramic study, a multicenter case control study of men in several European countries and Israel, the levels of toenail mercury were much higher in the two Spanish centers, Malaga and Granada, and the ratio of case/control mean toenail mercury was much higher in Malaga (Guallar et al., 2002). Although formal statistical tests found no effect modification (interaction) between mercury level and center, this does not rule out the possibility that results from Malaga or from both Spanish centers may have been influential in the reported positive association between toenail mercury and heart attack risk, and led to an incorrect association for the full study population. A previous report from the Euramic case control study (Aro et al., 1995) failed to find an association between trans fat level in adipose tissue and heart attack risk. This contradicted the consensus about trans fat intake and heart disease risk that was developing in the 1990's. However, when results from two influential centers were removed, the association had similar magnitude to other studies although marginally statistically significant, removing the contradiction. Thus, the positive association of toenail mercury with cardiovascular risk in this study may relate to subpopulations in one or two centers, and may not be generalizable either to the other subpopulations in the study, or to population groups outside the study.

Additionally, SACN (2004) and Mozaffarian & Rimm (2006) noted that, even in the Eastern Finland cohort and the Euramic study, the two studies that observed higher cardiovascular risk with higher mercury levels, the net effect of fish consumption was still beneficial: greater mercury exposure lessened the benefit associated with consumption of fish or n-3 LC PUFAs but did not increase overall risk (Rissanen et al., 2000; Guallar et al., 2002; Virtanen et al., 2005).

In summary, the association of mercury exposure and clinical cardiovascular outcomes has been examined in only a few studies. The available studies have limitations and the results are inconsistent. Most reviews and reports have concluded that, overall, these studies are inconsistent and that there is not sufficient evidence to conclude that methylmercury in fish increases cardiovascular risk. Mozaffarian and Rimm (2006) suggest that the principal question may be not whether consumption of mercury-containing fish increases cardiovascular risk but whether consumption of such fish would decrease risk even further if mercury were not present. Although this is an important public health issue, they conclude that this should not obscure evidence for net cardiovascular benefits of fish consumption, particularly fish richer in n-3 LC PUFAs.

(e) Discussion and Conclusions

Consensus of Scientific Panels: As summarized above, reviews by scientific panels, including those from AHA, the Dietary Guidelines Advisory Committee, SACN, EFSA, WHO, ISSFAL and the European Society of Cardiology have recommended increased

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consumption of fish to decrease CHD risk in the general population. The systematic review by AHRQ also concluded that increased consumption of omega-3 fatty acids from fish or fish-oil reduces the rates of all-cause mortality, cardiac and sudden death, and possibly stroke. Strong support for these conclusions and recommendations came from a large body of high-quality, primary prevention observational studies, especially prospective cohort studies of men and women in which fish consumption was associated with decreased CHD risk. The large secondary prevention trial, GISSI, showed that fish oil supplements reduced the risk of CHD mortality in men who previously had a heart attack, supporting the role of fish and fish oil in this CHD prevention setting. In general, the panels gave little weight to one poor quality secondary prevention trial which found that fish oil supplements or fish advice increased heart disease risk in men with angina (Burr et al., 2003). The scientific panels noted that a limitation of the evidence is the lack of intervention trials for primary prevention and identified this as an area for further research.

Quantitative Dose-Response from Cohort Studies: The feasibility of estimating quantitative dose-response relationships for the association between fish consumption and change in cardiovascular risk is shown by the published meta-analyses summarized above and in Tables A-2 through 5. Table A-4 shows pooled relative risks by category of fish intake and Table A-5 shows changes in relative risk with changes in fish intake based on meta-regression. These estimates are based on the large body of high-quality, primary prevention observational studies of the association between fish consumption and CHD risk which provided strong support for the consensus fish intake recommendations of many scientific panels. The results of the meta-analyses estimate the cardiovascular health benefits of fish consumption, regardless of whether the benefits are due to omega-3 fatty acids or to other nutrients in fish. Additionally, the results of these analyses integrate the cardiovascular benefits of fish consumption with any cardiovascular risks from fish or contaminants in fish.

Differences between the cohort study meta-analyses, as well as the overall consistency of their results, were discussed in published correspondence. He and coauthors noted that their analysis included additional usable studies because they obtained original data from certain authors and because they pooled relative risks for studies that reported results only by subcategories (He et al., 2006). Additionally, He et al. (2004a, 2004b, 2006) emphasized the importance of systematically standardizing the fish consumption categories across the original studies, basing portion sizes or absolute amount of fish consumption on the published information when available. In contrast, König et al. (2005) and Bouzan et al. (2005) used a default estimate of 100 grams of fish per serving. In response to these comments, Cohen and coauthors (2006) acknowledged the value of the inclusion of additional studies and the standardization of fish consumption categories in the He et al. meta-analyses.

However, Cohen et al. (2006) also noted the value of their intercept and slope analysis, compared with the He et al. slope only analysis for CHD risk and categorical analysis for

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stroke (see Tables A-4 and 5). The intercept term quantifies the benefit of consuming some fish versus little or no fish and allows for the possibility that further fish consumption may not provide additional health benefits. Cohen et al. (2006) stated that their slope term for CHD mortality corresponds to a 5.5 percent reduction in risk for each additional 20 grams of fish consumption, in addition to the 17 percent risk reduction for some fish compared with little or no fish (König et al., 2005, Cohen et al., 2006). Because some of the decreased risk is already accounted for by the 17 percent intercept term, this slope is slightly less than the seven percent decreased risk per 20 grams of fish consumption found by He et al. (2004a). Cohen et al. (2006) emphasized that, in spite of differences in methodological details, the findings of the cohort study meta-analyses are remarkably similar. They concluded that this similarity supports the robustness of the findings of both groups: that increased fish consumption decreases risk of CHD and stroke, and that the data are sufficient to quantify the magnitude of these benefits (Cohen et al., 2006).

Quantitative Dose Response Estimates and Measurement Error. There is inherent error in measuring dietary intake of individuals, and this includes measuring intake of fish or n-3 LC PUFA in observational studies. Random or nondifferential error in measurement of exposure variables, such as dietary intake, results in nondifferential misclassification of exposure status of study participants. In general, nondifferential error in exposure measurement attenuates the apparent magnitude of the association between exposure and health outcome. That is, nondifferential misclassification obscures an existing association; it produces bias towards “no association” or towards the null value (such as an odds ratio of 1.0) but it does not generate an apparent association when none exists, nor does it accentuate an existing positive or inverse association (Boffetta and Trichopoulos 2008). For example, if 20 percent of exposed individuals are classified as unexposed, and 20 percent of unexposed classified as exposed, for those both with and without a particular health outcome, and if the true odds ratio for the association of exposure and outcome is 3.0, the observed odds ratio would be reduced (attenuated) to 1.91 (Marshall 2003). For 20 percent nondifferential exposure misclassification with an inverse association, a true odds ratio of 0.33 would be observed as 0.52, an attenuation towards the null. Similarly, with 20 percent nondifferential exposure misclassification, a true odds ratio of 10 would be observed as 3.63 and a true odds ratio of 0.1 would be attenuated to 0.275.

Measurement of nutritional biochemical markers, or biomarkers, can have less error than dietary assessment of intake (Potischman 2003, Kaaks 1997). However, biomarkers of dietary exposure, particularly concentration biomarkers, may also involve measurement error. A concentration biomarker is based on the concentration of a compound, such as a fatty acid, in a biological sample at a given point in time. Concentration biomarkers may not have the same quantitative relationship with dietary intake for every individual in a study population; they do not directly correspond to absolute intake levels per day but they do correlate with dietary intake levels (Kaaks 2002). Individual variation in concentration biomarkers may be determined not only by dietary intake but by variations

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in digestion and absorption, distribution over body compartments, the body's own synthesis and metabolism of the biomarker compound, and excretion. As described previously, an AHRQ report by Balk et al (2004) estimated dose response relationships in clinical trials of supplements of DHA plus EPA and concentrations of DHA plus EPA in phospholipids in plasma/serum, platelets and red blood cell membranes.

Supplementation of one gram of EPA and/or DHA corresponded to approximately a one percent increase in EPA plus DHA. EPA plus DHA supplements accounted for between 39 percent and 72 percent of the variation in EPA plus DHA concentration in phospholipids. Balk and coworkers did not report the standard deviation or 95 percent confidence intervals of the dose-response slopes (regression coefficients). Kaaks (2002) states that, in contrast with concentration biomarkers, "Recovery-based markers are based on precise and quantitative knowledge of the physiological balance between intake and output of a compound or chemical element." An example of a recovery biomarker is 24-hour urinary nitrogen excretion, which equals 80 percent of nitrogen intake for individuals in energy and nitrogen balance. Unfortunately, recovery-based biomarkers are available for only a few nutrients. Therefore, exposure measurement for nutrition epidemiology relies upon dietary intake assessment or concentration biomarkers, both of which involve measurement error.

A limitation of the quantitative dose-response relationships from the meta-analyses, summarized in Tables A-2 through A-5, is that they may have been attenuated by measurement error of dietary intake and therefore may underestimate the true inverse association between fish intake and cardiovascular disease risk. Methods have been developed to determine and correct for nondifferential measurement error in associations of dietary exposure and health outcomes (Willett 1999, Kaaks 1997, 2002). Error correction methods involve dietary exposure measurement by two or three independent methods and detailed analysis of statistical error structure (Fraser et al 2005, McNaughton et al 2005). In the absence of a detailed error structure analysis, the possible attenuation of quantitative dose-response relationships can be considered by comparing the strength of the association estimated by dietary assessment and by EPA and DHA biomarkers.

In a case-control study, individuals in the highest quartile of dietary intake of n-3 LC PUFA had a decreased risk of primary cardiac arrest compared with men in the lowest quartile, adjusted OR 0.4 (95 percent CI, 0.2 to 0.7) (Siscovick et al., 1995). In a subset of individuals with biomarker measurements of red blood cell membrane n-3 LC PUFA, those in the highest quintile of n-3 fatty acid concentration had decreased risk with an adjusted OR of 0.1 (95 percent CI, 0.1 to 0.4). Thus, the 90 percent decreased risk with exposure measurement by n-3 fatty acid biomarker concentration was apparently attenuated to about 60 percent decreased risk with exposure measurement by dietary intake assessment. Note that this comparison is not exact because the dietary intake analysis used five intake groups (quintiles) and the biomarker analysis used four groups (quartiles), and the biomarker analysis was in a subset of participants. Additionally, the spouses of participants answered the dietary intake questionnaires, rather than the

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participants themselves (some of the cardiac arrests were fatal) and this likely increased the measurement error. Nevertheless, the example illustrates substantial attenuation of strength of association apparently due to measurement error of dietary intake compared with n-3 fatty acid blood concentrations. In an 11-year follow-up of the U.S. Physicians Health Study, men who consumed fish five or more times per week at baseline had decreased risk of sudden death compared with men who consumed fish less than once per month, adjusted relative risk 0.39 (95 percent CI, 0.15 to 0.96) (Albert et al., 1998). In a 17-year follow-up of the same study, a nested case control was conducted measuring exposure by whole blood concentrations of n-3 LC PUFA at baseline (Albert et al., 2002). Men in the highest quartile of n-3 fatty acid concentrations had decreased risk of sudden death compared with men in the lowest quartile, adjusted relative risk 0.10 (95 percent CI, 0.02 to 0.48). In the two analyses by Albert and coworkers, the 90 percent decreased risk with exposure measurement by n-3 fatty acid biomarkers was apparently attenuated to about 61 percent decreased risk with exposure measurement by dietary intake assessment. Again, the comparison is not exact because the dietary exposure analysis grouped the men by quintiles and the biomarker exposure analysis grouped them by quartiles, and the length of follow-up was different in the two analyses. Nevertheless, this example is consistent with the previous example in illustrating substantial attenuation of strength of association due to apparent measurement error of dietary intake compared with n-3 fatty acid blood concentrations.

Therefore, the quantitative dose response relationships (Tables A-2 through A-5) between fish intake and cardiovascular disease risk are likely to be attenuated (weakened) due to dietary intake measurement error and this limitation should be recognized. In particular, this should be considered in comparing associations based on dietary intake assessment of exposure with associations based on biomarker assessments of exposure — either nutritional n-3 LC PUFA concentrations such as the above examples, or biomarkers of potential harm such as methylmercury. In general, compared with the true association, the observed associations based on dietary intake may be attenuated (weakened) to a greater degree due to measurement error than the associations based on biomarkers.

Overview of Scientific Evidence Base. The science reviewed in this section includes published reports on the relationship between fish or n-3 LC PUFA consumption and coronary heart disease (CHD) risk:

- Secondary prevention, randomized clinical trials of fish or fish oil consumption. The large, well-conducted secondary prevention trial, GISSI, included over 10,000 men and found a 15 percent decrease in all deaths plus nonfatal heart attacks and strokes, a 26 percent decrease in cardiovascular deaths plus nonfatal heart attacks and strokes and a 45 percent decrease in sudden death, all significant (GISSI, 1999; Marchioli et al., 2002). Results of the DART1 study were consistent with GISSI, but results differed for the poor quality DART2 study (Burr et al., 1999, 2003).
- Primary and secondary prevention, randomized clinical trial of fish oil consumption. The recently published JELIS trial from Japan included over 18,000 men and women. Almost 15,000 participants had no record of coronary artery disease (primary

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prevention). Results showed a 19 percent decrease in major coronary events (fatal plus nonfatal) for all subjects, a 19 percent decrease for secondary prevention subjects and a 18 percent decrease for primary prevention subjects. The decrease in risk was similar in magnitude for primary and secondary prevention, but was not statistically significant for primary prevention alone ($p = 0.13$). For the full study and for both subgroups, there was no significant decrease in sudden cardiac death or coronary death alone, probably reflecting that the high baseline fish intake in Japan is above a possible threshold for effect on risk of sudden death or CHD death.

- Meta-analyses of randomized controlled trials of fish or fish oil consumption. The Hooper et al. (2004, 2006) meta-analysis of 12 randomized controlled trials, including the poor quality DART2 study, with more than 19,000 subjects in the meta-analysis, found a 14 percent decrease in total mortality with fish or fish oil consumption, but the results were not statistically significant, $p = 0.12$ (Table A-9). When three recent RCTs were included by Mozaffarian and Rimm (2006), there was a significant, 17 percent decrease in total mortality. This became more highly significant with removal of two studies of questionable quality including DART2 (Table A-9). With addition of the newer, JELIS study, giving a total of more than 35,000 subjects, the decreased mortality risk was moderated to 13 percent and remained significant. (Note that the JELIS population baseline fish intake was likely above the threshold for effect on decrease in risk of sudden death or CHD death.)
- Observational studies of blood levels of n-3 LC PUFA and CHD risk. As summarized by SACN (2004) and others, additional evidence for the cardiovascular benefits of fish and fish oil consumption is provided by several cohort or case control studies that found decreased CHD risk associated with higher blood levels of DHA and EPA. SACN stated that, “Taken together, these data support the hypothesis that LC n-3 PUFA are responsible for the observed inverse association between fish consumption and sudden cardiac death.”
- Observational studies of fish consumption and risk of cardiovascular disease. In observational studies, subjects consume their usual diets; and dietary intake of fish or fish oil is not randomly assigned by the investigator. Therefore, an observational study cannot conclusively demonstrate a cause and effect relationship. However, possible confounding variables that are known and measured can be adjusted for in the statistical analysis. Prospective cohort studies examine disease experience related to participants’ actual diets, typically over a long period of time. Additionally, participants in prospective cohort studies are healthy at the beginning of follow up, providing information on primary prevention of cardiovascular disease outcomes relevant for the general, healthy population. Numerous observational studies of fish consumption and risk of cardiovascular disease, especially CHD and stroke, have been reported in the literature, and have been summarized and evaluated by expert panels, systematic reviews and meta-analyses.
- Meta-analyses of observational studies of fish consumption and risk of cardiovascular disease. There are several meta-analyses of observational studies of fish consumption and risk of CHD or stroke (Tables A-2 through A-5), with fairly consistent results among the meta-analyses. The prospective studies of CHD death included more than

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200,000 men and women and the prospective studies of stroke also included more than 200,000 men and women. For example, the meta-analyses of He et al (2004a,b) found a 15 percent decreased risk of CHD death and a 13 percent decreased risk of stroke associated with fish intake once per week compared with less than once per month, both significant (Table A-3).

- Meta-analysis of observational studies and randomized clinical trials of fish or fish oil intake. Mozaffarian and Rimm (2006) conducted a meta-analysis including five randomized controlled trials and 15 prospective cohort studies of fish or fish oil intake and CHD death: four studies in populations with high CHD mortality rates seven in populations with intermediate rates and nine in populations with low rates. They showed graphically that modest EPA/DHA consumption (250 to 500 mg/day) decreased relative risk by about 25 percent or more. The graphs also showed a threshold effect; higher intake did not provide additional lowering of CHD mortality. This was confirmed in a pooled dose-response analysis of all 20 studies (Table A-6). At EPA/DHA intakes up to 250 mg/day, there was a significant 14.6 percent decreased risk of CHD death for each 100 mg/day intake, giving a total 36 percent reduction in risk for intake of 250 mg/day EPA/DHA. At intakes above 250 mg/day, there was little additional risk reduction (Table A-6).
- A meta-analysis (Studer et al., 2005) with 137,140 individuals in intervention and 138,976 individuals indicated that the benefits of n-3 LC PUFAs were comparable to (or greater than) the benefits of statins for overall mortality.

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Table A-2: Meta-analyses of observational studies of fish consumption and coronary heart disease or stroke

Study	Health Outcome	Number of study populations	Number of subjects	Follow-up time	Pooled relative risk reported			
					Intake Yes or No	Dose-response		
						Intake categories	Meta regression slope	Meta regression intercept
	Coronary Heart Disease							
He et al., 2004a	Coronary heart disease death	13*	222,364	Average 11.8 years	Yes	Yes	Yes	
Whelton et al., 2004	Coronary heart disease death	13	215,705	Range 5 to 30 years	Yes	**		
	Total coronary heart disease				yes	**		
	Cohorts	7	190,262	Range 5 to 19 years				
	Case control	5	4,964	Not applicable				
König et al., 2005	Coronary heart disease death	7	157,835	Range 6 to 30 years			Yes	Yes
	Stroke							
He et al., 2004b	Stroke	9	200,575	Average 12.8 years	Yes	Yes		
Bouzan et al., 2005	Stroke						Yes	Yes
	Cohorts	4	129,767	Range 12 to 30 years				
	Case control	1	823	Not applicable				

Included studies are cohort studies unless otherwise noted.

*11 independent studies

** Intake categories for subsets of studies

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Table A-3: Pooled relative risk of coronary heart disease or stroke associated with some fish intake, from observational studies

Study	Health Outcome	Number of Participants	Number of Events	Intake Comparison	Pooled Relative Risk	95% Confidence Interval
	Coronary Heart Disease					
He et al., 2004a	coronary heart disease death	222,364	3,032	once per wk vs less than once per month	0.85	0.76, 0.96
Whelton et al., 2004	coronary heart disease death	215,075	3,273	any vs little or none	0.83	0.76, 0.90
	total coronary heart disease	195,226	10,533	any vs little or none	0.86	0.81, 0.92
	Stroke					
He et al., 2004b	Stroke	200,575	3,491	once per wk vs less than once per month	0.87	0.77, 0.98
	ischemic stroke	154,337	1,138	once per wk vs less than once per month	0.68	0.52, 0.88
	hemorrhagic stroke	154,337	548	once per wk vs less than once per month	1.21	0.78, 1.85

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Table A-4: Pooled relative risk of coronary heart disease or stroke by fish intake category, from cohort studies*

Health Outcome	Number of participants	Number of events	Pooled Relative Risks (RR) and 95% Confidence Intervals (CI)									p for trend
			less than once per month	1 to 3 times per month		once per week		2 to 4 times per week		5 or more times per week		
			RR	RR	CI	RR	CI	RR	CI	RR	CI	
Coronary heart disease death**	222,364	3,032	1	0.89	0.79, 1.01	0.85	0.76, 0.96	0.77	0.66, 0.89	0.62	0.46, 0.82	***
Stroke****	200,575	3,491	1	0.91	0.79, 0.98	0.87	0.77, 0.98	0.82	0.72, 0.94	0.69	0.54, 0.88	0.06
Ischemic stroke	154,337	1,138	1	0.69	0.48, 0.99	0.68	0.52, 0.88	0.66	0.51, 0.87	0.65	0.46, 0.93	0.24
Hemorrhagic stroke	154,337	548	1	1.47	0.81, 2.69	1.21	0.78, 1.85	0.89	0.56, 1.40	0.80	0.44, 1.47	0.31

*Fish consumption categories were standardized across studies to correspond to quantitative estimates of intake in grams per day.

**He et al., 2004a

***See meta regression in Table A-5

****He et al., 2004b

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Table A-5: Meta regression dose-response for fish consumption and pooled relative risk of coronary heart disease or stroke from observational studies.

Study	Health Outcome	number of subjects	Change in intake	dose-response meta regression intercept		Change in intake	dose-response meta regression slope		p for trend
				change in relative risk	confidence interval		change in relative risk	confidence interval	
	Coronary Heart Disease								
He et al., 2004a	coronary heart disease death	222,364		None (slope only analysis)		per 20 g/d fish intake	-0.07	-0.01, -0.13	0.03
König et al., 2005	coronary heart disease death	157,835	some vs little or none	-0.17	-0.088, -0.25	per 1 serving/wk fish intake	-0.039	-0.011, -0.066	
	nonfatal heart attack	133,493	some vs little or none	-0.27	-0.21, -0.34	per 1 serving/wk fish intake	0.0083	0.028, -0.012	
	nonfatal heart attack	133,493	some vs little or none	-0.25	-0.22, -0.29		None (intercept only analysis)		
	Stroke								
Bouzan et al., 2005	stroke	130,590	some vs little or none	-0.12	0.01, -0.25	per 1 serving/wk fish intake	-0.02	0.027,-0.066	

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Table A-6: Non-parametric dose-response analysis for fish or fish oil consumption and pooled relative risk of coronary heart disease death from prospective cohort studies and randomized clinical trials.

dose-response slope non-parametric analysis						
Study	Health Outcome	number of studies	Intake Range	Change in intake	change in relative risk	confidence interval
Mozaffarian & Rimm, 2006	coronary heart disease death	20*	0 to 250 mg/day EPA plus DHA	per 100 mg/day EPA plus DHA	-0.146**	-0.08, -0.21
			More than 250 mg/day EPA plus DHA	per 100 mg/day EPA plus DHA	none	-0.009, 0.008; p = 0.94

*15 cohort studies and 5 randomized trials, primary and secondary prevention.

**Total risk reduction for intakes up to 250 mg per day EPA plus DHA = 36 percent (95 percent confidence interval 20 to 50 percent, p < 0.001).

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Table A-8: Reviews and reports addressing the possible cardiovascular risk of mercury in fish.

Publication	Type and Purpose.	Author's/Institution's Conclusion
Chan & Egeland (2004)	Narrative review Possible cardiovascular risk of mercury in fish	There is currently highly contradictory and insufficient evidence to suggest that mercury is associated with CHD risk.
Stern (2005a)	Narrative review Possible cardiovascular risk of mercury in fish	Overall, the epidemiologic studies, including analyses of deaths from Minamata, suggest an association between MeHg exposure and heart disease, including (but possibly not limited to) MI.
SACN (2004)	Report Recommendations on benefits and risks of fish consumption.	Overall, the prospective cohort studies suggest that those who consume fish have a lower risk of CHD than those who do not.
Kris-Etherton (2004)	Report Recommendations on fish consumption and cardiovascular disease	Finally, another explanation for the discordant results of epidemiological studies pertains to the hypothesized adverse effects of methylmercury, an environmental contaminant found in certain fish that may diminish the health benefits of omega-3 fatty acids. Recent studies have produced conflicting results with regard to the effects of methylmercury on CHD risk. Thus, the extent to which methylmercury in fish may mask the beneficial effects of omega-3 fatty acids requires further study.
EFSA (2005)	Report Scientific opinion on contaminants and safety of wild and farmed fish.	High mercury content in fish was reported to attenuate the beneficial effect associated with fish consumption (Rissanen et al., 2000) or high levels of DHA in adipose tissue (Guallar et al., 2002) on mortality from coronary heart disease. A high mercury content in hair attenuated the protective

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		effects of high serum DHA and DPA concentration (Virtanen et al., 2005). This study suggests that failure to adjust for contaminant exposures may explain some of the apparent differences between studies [of beneficial cardiovascular effects of fish consumption].
FDA (2004)	Letter Qualified health claim on omega-3 fatty acids and reduced risk of CHD	Thus, these observational studies showed inconsistent results regarding the relationship between mercury and CHD. FDA believes that whether mercury has any role in CHD risk is an unanswered scientific question. Consequently, it is not possible to determine whether mercury counteracts the cardio-protective effects of EPA and DHA omega-3 fatty acids from fish.
EPA (2005)	Cost benefit analysis of regulation of mercury emissions	As the science on the impact of methylmercury on the risk of cardiovascular events remains uncertain, and the weight of the evidence, in fact, supports a positive association between fish consumption and potential cardiovascular benefits, the impacts of methylmercury from fish consumption are only discussed qualitatively. (Appendix C) For one of these health effects [of mercury], cardiovascular disease, the Agency conducted a critical review of the available literature and determined that while some studies show that the effect may exist, it is premature to include analysis of cardiovascular effects in our benefit analysis. Studies investigating the relationship between methylmercury exposure and cardiovascular impacts have reached different conclusions. The findings to date and the plausible biologic mechanisms warrant additional research in this area. (Chapter 10)
Rice & Hammitt (2005)	Cost benefit analysis of regulation of mercury emissions	nitively show whether methylmercury exposures increase the e evidence that supports the cardioprotective effects of fish questions are whether co-exposure to methylmercury attenuates

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		<p>and, if so, under what conditions and by how much. We have evaluated the relationship between fish consumption and [beneficial] effect. When compared to the body of evidence, fish consumption may reduce the risk of myocardial events, ...the relationship between methylmercury exposures and cardiovascular effects in these subjects and only three independent cohorts.</p> <p>The predicted myocardial risks associated with methylmercury exposures should be interpreted with caution. Most of the evidence of such risks is based on observations from a single cohort. Additionally, a great deal of evidence indicates that fish consumption in general protects individuals from incurring adverse cardiac events.</p>
König (2005) Cohen (2005a)	Systematic review, meta-analysis and quantitative risk benefit analysis of fish consumption	<p>Because of their design and the type of outcomes investigated, we judged it to be inappropriate to use these studies to quantify the extent to which mercury attenuates the relationships described above.</p> <p>Qualitatively, the studies...are ambiguous. Results from eastern Finland and the results reported by Guallar et al. support the hypothesis that MeHg exposure increases the risk of CHD events. On the other hand, results reported by Yoshizawa et al. and by Hallgren et al. revealed no consistent association. Ahlqwist et al. found a negative association between MeHg exposure and MI risk.</p>
Hooper (2004)	Systematic review and meta-analysis of omega-3 fatty acids for prevention and treatment of cardiovascular disease	<p>Perhaps in DART 2 the cumulative harmful effects of the PCBs, dioxins and/or mercury, contained within oily fish and fish oils had time to develop and be expressed as illness. This interpretation has been supported by several cohort studies which have assessed relationships between oily fish, contaminants and cardiovascular disease (zATBC Pietinen 1997; zKuopio Rissanen 00; Salonen 1995).</p>
IOM (2006)	Report	Observational studies in adult men from the general

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	<p>Examine relationships between benefits and risks associated with seafood to help consumers make informed choices</p>	<p>population have produced mixed results regarding the associations between fish consumption, mercury level, and cardiovascular health. Overall, the data considered suggests an increased risk of myocardial infarction among men with higher hair mercury levels.</p> <p>Increased methylmercury exposure might be a risk factor for adult cardiovascular toxicity, although the data available are not extensive and uncertainties remain.</p>
<p>Mozaffarian & Rimm (2006)</p>	<p>Clinical review Scientific evidence for adverse and beneficial effects of fish consumption; includes quantitative meta analyses.</p>	<p>Meta analysis of the 5 cohort studies in Table A-7 showed a pooled relative risk = 1.12 (95% Confidence Interval 0.71 to 1.75, p = 0.62) for incidence of CHD in highest compared with lowest category of mercury exposure.</p> <p>The conflicting results provide inconclusive evidence for cardiovascular toxicity of mercury. Notably, in the 2 studies observing higher risk with higher mercury levels, the net effect of fish consumption was still beneficial: greater mercury exposure lessened the benefit associated with consumption of fish or n-3 PUFAs but did not increase overall risk (146,148,150). Thus, the principal question may not be whether consumption of mercury-containing fish increases cardiovascular risk but whether consumption of such fish would decrease risk even further if mercury were not present. This would be most true for oily fish species containing higher amounts of n-3 PUFAs (i.e., most mercury-containing ocean fish), compared with lean freshwater fish. This is an important public health issue, which requires balancing potentially attenuated benefits of fish intake due to presence of mercury with the costs and practicality of reducing mercury contamination in fish species. Nevertheless, this should not obscure evidence for net cardiovascular benefits of fish consumption, particularly fish richer in n-3 PUFAs.</p>

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Table A-9: Pooled relative risk of total mortality due to fish or fish oil consumption in randomized clinical trials

Meta-analysis	Studies included	Number of Participants	Number of Participants	Number of Deaths	Number of Deaths	Pooled Relative Risk	95% Confidence Interval	P value for effect	P value for heterogeneity
		Fish or fish oil group	Control group	Fish or fish oil group	Control group				#
Hooper et al, 2004, 2006	12 RCTs*	9,656	9,486	888	967	0.86	0.70-1.04	0.12	0.05
Mozaffarian and Rimm, 2006	Add 3 RCTs**	10,229	10,061	913	1,003	0.83	0.68-1.00	0.046	0.04
	Omit 2 studies with methodology problems***	8,536	8,459	616	748	0.83	0.74-0.92	0.001	0.75
	Add JELIS study****	17,862	17,778	902	1,013	0.87	0.76-0.99	0.048	0.29

* Systematic literature review through February, 2002

** Added 3 RCTs in patients with cardiac defibrillators (Raitt et al 2005, Leaf et al 2005, Brouwer et al 2006).

*** Omitted DART2 study (Burr et al., 2003) and study by Singh et al. (1997).

**** Added Japanese EPA Lipid Intervention Study, JELIS (Yokoyama et al., 2007; reported in proceedings in 2006). Added only in secondary analysis because of high fish intake in reference group, estimated EPA plus DHA intake 900 mg/day. This would be expected to obviate the benefits of additional fish oil intake on heart disease death or total mortality.

A significant test for statistical heterogeneity ($p < 0.05$) indicates lack of comparability among studies, resulting from clinical features of the population studied or study methodology or both. A pooled relative risk should be viewed with caution if there is heterogeneity.

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SECTION B

OVERVIEW OF SCIENTIFIC INFORMATION ON THE EFFECT OF FISH CONSUMPTION AND OMEGA-3 FATTY ACIDS ON NEURODEVELOPMENTAL HEALTH BENEFITS

(a) Introduction

There is a large volume of original scientific studies on the health benefits associated with consumption of fish. Overall, there is a body of data on the relationship between fish consumption and neurodevelopment. This section provides an overview of this body of data, and also identifies reports of quantitative dose-response relationships with potential for use in risk and benefit assessment modeling.

Fish is a source of easily digestible protein of high biological value, and provides micronutrients including vitamins A and D, the minerals iodine and selenium and the amino acids taurine, arginine and glutamine (EFSA 2005 p 30, He and Daviglus 2005). Additionally, many fish provide a unique food source of omega-3 (also called n-3) long-chain polyunsaturated fatty acids (LC PUFA), and a large body of scientific literature addresses the health benefits of these lipids.

The n-3 LC PUFA, docosahexaenoic acid (DHA) has been shown to be essential for development of the central nervous system. Consequently, there is interest in knowing whether there is an association between fetal, infant, and child neurodevelopment and maternal intake of fish or n-3 LC PUFA during pregnancy and lactation (SACN 2004). A related research question is whether consumption of fish or n-3 LC PUFA by adults is associated with prevention of neuropsychiatric disorders including depressive symptoms, psychosis, aggression, suicide, mild cognitive decline with aging, or overt dementia (Schachter 2005).

The n-3 long chain polyunsaturated fatty acids found in fish and fish oil, EPA and DHA, are 20-carbon and 22-carbon fatty acids, respectively. Another n-3 fatty acid, found in plant foods and vegetable oil, is alpha-linolenic acid (ALA), an 18-carbon fatty acid. ALA cannot be synthesized by humans, but must be supplied by dietary means and is therefore considered an essential nutrient (IOM 2002). Additionally, humans can use ALA as a starting material to synthesize the n-3 long-chain fatty acids, EPA and DHA. Preterm and term infants are able to convert ALA to DHA, but it is not known whether this conversion can meet the needs of the developing brain for DHA (IOM 2002).

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Studies of the neurodevelopmental health benefits of fish consumption include studies of the effects on fetal, infant and child development of maternal consumption of fish or supplemental DHA (such as from fish oil) during pregnancy or lactation. The major neurodevelopmental outcomes studied are visual development and cognitive development. Related studies looked at effects on fetal, infant and child neurodevelopment of varying levels of DHA in breast milk, reflecting the DHA in maternal diet. Additionally, there is a body of literature examining the effect on infant and child development of infant formula supplemented with DHA. Although supplementation of infant formula with DHA does not fall in the category of fish consumption, results of these studies may help to answer scientific questions regarding possible neurodevelopmental benefits of DHA during infancy.

The table below lists a number of recent reports and reviews on the neurodevelopmental health benefits of fish or n-3 LC PUFA consumption. These include reports and recommendations from national and international expert groups. Also listed are a number of review articles, including systematic reviews, meta-analyses and risk assessments. This report will summarize the purpose and conclusions of the reports and reviews listed in Table B-1, followed by an overview of selected key studies, and a brief synthesis and discussion.

Table B-1. Recent reports and reviews on the neurodevelopmental health benefits of fish or n-3 LC PUFA consumption

Recent Reports and Recommendations

- WHO (World Health Organization), 1994
- NIH/ISSFAL Workshop Statement on the Essentiality of and Recommended Dietary Intakes for Omega-6 and Omega-3 Fatty Acids, 2000
- Child Health Foundation Report of Workshop. LC-PUFA and Perinatal Development, 2001
- IOM (Institute of Medicine) Dietary Reference Intakes, 2002
- SACN (Scientific Advisory Committee on Nutrition), United Kingdom, 2004
- EFSA (European Food Safety Authority) 2005
- AHRQ (Agency for Health Care Research and Quality), DHHS, Omega-3 Fatty Acids, Effects on Maternal and Child Health , 2005
- IOM Seafood Choices, 2006
- ISSFAL (International Society for the Study of Fatty Acids and Lipids), expected, 2008

Other Recent Systematic Reviews, Meta-Analyses and Risk Assessments

- Willatts and Forsyth., Prostaglandins 2000, premature and term, cognitive
- SanGiovanni et al., Early Human Development, 2000; premature infants, clinical trials, vision
- SanGiovanni et al., Pediatrics, 2000; term infants, clinical trials, vision
- Simmer, Cochrane Systematic Reviews, term infants, 2001
- Lauritzen et al., Progress in Lipids Research, 2001; comprehensive review, 94 pages
- Uauy et al., Journal of Pediatrics, 2003; term infants, clinical trials, vision
- Simmer and Patole, Cochrane Systematic Reviews, preterm infants, 2004
- Harvard Risk/Benefit Papers, American Journal of Preventive Medicine 2005

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- Cohen et al. (2005c) meta-analysis of n-3 fatty acid supplements and cognitive development in infants
- Cohen et al. (2005a) assessment of risk and benefit of fish consumption
- Fleith, 2005, narrative review
- Mozaffarian and Rimm, JAMA, 2006; risk benefit assessment
- Eilander et al., Prostaglandins 2007, narrative review
- Smithers et al., American Journal of Clinical Nutrition 2008, preterm infants, cognitive
- Simmer et al. (2008a). Cochrane Systematic Reviews, preterm infants
- Simmer et al. (2008b). Cochrane Systematic Reviews, term infants

See reference list for full citations for Table B-1.

[Note regarding citations: Complete citations for selected references are found in the Reference Section. For the complete citation for the remaining references marked “#,” please refer to the respective report or review.]

(b) Conclusions and Recommendations from Some Recent Reports

Food and Agriculture Organization/World Health Organization (FAO/WHO)

A Joint FAO/WHO Expert Consultation on Fats and Oils in Human Nutrition was published in 1994. The experts reviewed the roles of dietary fats and oils in human nutrition, the intakes and health effects of different types of fats and oils, and the technical factors associated with production and utilization of edible fats and oils. The consultation made recommendations about dietary fats and oils to assist policy makers, health-care specialists, the food industry and consumers.

Minimum desirable intakes of fats and oils:

The consultation concluded that the consumption of an adequate amount of essential fatty acids is important for normal growth and development, that AA and DHA are particularly important for brain development, and that breast milk is a good source of these acids. (The 20-carbon n-6 fatty acid, arachidonic acid, is abbreviated AA.) The experts noted potential problems for preterm infants who had an insufficient intra-uterine supply of AA and DHA and are born with low fat reserves. The consultation recommended that infants should be fed breast milk if at all possible, and that the fatty acid composition of infant formulas should correspond to that of breast milk. Young children from weaning to at least two years of age should consume 30 to 40 percent of energy from fat, and the fat composition should provide similar levels of essential fatty acids as are found in breast milk.

Essential fatty acids:

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The consultation discussed the role of n-6 and n-3 fatty acids in membrane structures and as precursors of biologically active eicosanoid molecules. The experts noted that essential fatty acids are especially important for normal fetal and infant growth and development, especially for brain development and visual acuity. In well-nourished women, approximately 2.2 grams of essential fatty acids are deposited in maternal and fetal tissues each day during pregnancy. The consultation recommended that

“Particular attention must be paid to promoting adequate maternal intakes of essential fatty acids throughout pregnancy and lactation to meet the requirement of fetal and infant development.”

NIH/ISSFAL Workshop Statement on the Essentiality of, and Recommended Dietary Intakes for, Omega-6 and Omega-3 Fatty Acids

A workshop on omega-6 and omega-3 fatty acids was co-sponsored in 1999 by the National Institutes of Health (NIH), the International Society for the Study of Fatty Acids and Lipids (ISSFAL) and several industry groups. The workshop statement made recommendations for adequate intakes (AIs) for adults for various fatty acids (Simopolous et al., 2000). The Workshop AIs were given for a 2,000 calorie diet. The AI for ALA was 2.22 grams per day (one percent of energy), for DHA plus EPA the AI was 0.65 grams per day (0.3 percent of energy), with at least 0.22 grams per day (0.1 percent of energy) for DHA and the same for EPA. In addition, the workshop recommended that pregnant and lactating women should ensure a DHA intake of 300 milligrams per day (0.3 grams per day). The workshop statement also recommended AIs for infant formula as percent of fatty acids. The Workshop AI for ALA was 1.5 percent of fatty acids, and the AI for DHA was 0.35 percent of fatty acids. An upper limit for EPA in infant formula was given as 0.1 percent of fatty acids. The workshop statement noted that the views expressed in the statement were not an official position of the U.S. Department of Health and Human Services. (Note that the “AIs” from the NIH/ISSFAL Workshop are different recommendations from the AIs and other Dietary Reference Intakes from the Institute of Medicine, described below.)

Several individual reflections and commentaries were published together with the workshop statement. One commentary summarized the strengths of the statement and also noted some limitations (Cunnane 2000). The commentary by Cunnane observed that the workshop statement is too brief and is not referenced. The commentary also stated that, although in principle it seems reasonable to recommend a certain amount of DHA intake during pregnancy, there is little experimental or epidemiologic research to support the specific intake recommendation. Therefore, the commentary concluded that, although the Workshop recommendations for fatty acid intake deserve serious consideration, a clearer, referenced description of the rationale for the proposals is needed.

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Child Health Foundation. Report of Workshop. LC-PUFA and Perinatal Development

A scientific workshop on the role of LC-PUFA in pregnancy, lactation and early life was organized and funded by the Child Health Foundation, Munich, Germany. Participants were the leading researchers who conducted randomized trials of LC-PUFA status and function in pregnancy and lactation and in preterm and term infants. The workshop, which was closed to the public, was held in Munich and resulted in a consensus statement (Koletzko et al., 2001). Only studies published in full or in abstract form were used to provide information for the consensus statement. The workshop statement briefly reviewed the scientific knowledge base regarding LC-PUFA and perinatal development, citing 48 references. The consensus statement recommended breastfeeding, which supplies preformed LC-PUFA, as the preferred feeding method for healthy infants. The statement recommended that formulas for term infants should contain at least 0.2 percent of total fatty acids as DHA and 0.35 percent as AA. These levels were stated to be prudent because they are at the lower end of the range of human milk DHA worldwide. Formulas for preterm infants were recommended to contain at least 0.35 percent of total fatty acids as DHA and 0.4 percent as AA. It was stated that higher levels of LC-PUFA might provide additional benefits, and further study was recommended in order to define optimal intakes for term and preterm infants.

The consensus statement found an absence of published studies showing direct functional benefits of supplementation of LC-PUFA for pregnant and lactating women, and therefore did not recommend supplementation. In the meantime, the statement recommended that

“It seems prudent for pregnant and lactating women to include some food sources of DHA in their diet in view of the assumed increase in LC-PUFA demand in these physiological conditions and the relationship between maternal and foetal DHA status.”

Institute of Medicine of the National Academies. Dietary Reference Intakes

The Institute of Medicine (IOM) considered recommendations for intake of n-3 fatty acids in its 2002 report on Dietary Reference Intakes, called the IOM Macronutrient Report (IOM 2002). The IOM Macronutrient Report covered first, the metabolic requirements for specific nutrients and second, quantitative guidance on proportions of energy sources (such as protein, carbohydrate and fat) to decrease chronic disease risk.

Metabolic Requirements for N-3 Fatty Acids:

The IOM reviewed scientific evidence showing that the n-3 PUFA, ALA, which is an 18-carbon fatty acid, is an essential nutrient. Specifically, ALA cannot be synthesized by

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humans, and must be supplied by dietary means. There was not sufficient data for the IOM Macronutrient Committee to determine an Estimated Average Requirement (EAR) or a Recommended Dietary Allowance (RDA) for ALA. Instead, the IOM set a level for Adequate Intake (AI) of ALA, based on median intake of the population. For adults age 19 and older, the AI for ALA was set at 1.6 g/d for men and 1.1 g/d for women.

Additionally, humans can use the 18-carbon n-3 fatty acid, ALA, as a starting material to synthesize the long chain n-3 fatty acids, EPA and DHA, which are 20-carbon and 22-carbon fatty acids, respectively. Thus, the IOM stated that small amounts of EPA and DHA can contribute towards reversing a deficiency of n-3 fatty acids. EPA and DHA contribute approximately 10 percent of the total n-3 fatty acid requirement, and therefore this percent contributes toward the AI for ALA.

The IOM Macronutrient Report noted that the membrane lipids of brain gray matter and the retina contain very high concentrations of DHA. The developing brain accumulates DHA during prenatal and postnatal development, continuing through the first two years after birth. The essential role of ALA seems to be as a precursor for EPA and DHA, and the developing brain is more sensitive to n-3 fatty acid deficiency than the mature brain. Studies have confirmed that preterm and term infants are able to convert ALA to DHA, but the studies do not provide quantitative information on whether the conversion can meet the needs of the developing brain for DHA.

Adequate Intakes for Infants:

The IOM Macronutrient Report tabulated clinical trials comparing term infants fed formula with and without added DHA regarding growth and measures of visual, motor and mental development. Based on studies from 1995 through 2000, the Committee stated:

“In conclusion, randomized clinical studies on growth or neural development with term infants fed formulas currently yield conflicting results on the requirements for n-3 fatty acids in young infants, but do raise concern over supplementation with long-chain n-3 fatty acids without arachidonic acid. For these reasons, growth and neural development could not be used to set an EAR.”

Therefore, the Committee based the AI for infants from birth through six months on the amount of n-3 fatty acids, total fat and energy provided by human milk from women in the U.S. and Canada. The AI for infants ages seven through 12 months was based on the average intake from human milk and complementary foods. For both age groups of infants, the AI for total n-3 polyunsaturated fatty acids is 0.50 grams per day. For birth through 6 months of age, this corresponds to approximately one percent of energy (calories). For ages seven through 12 months, this corresponds to approximately 0.67 percent of energy (calories).

Adequate Intakes for Pregnancy and Lactation:

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The Committee noted that, during pregnancy, the demand for n-3 polyunsaturated fatty acids for incorporation into placental tissue and for the developing fetus must be met from maternal tissues or through dietary intake. The demand for n-3 fatty acids for secretion in milk during lactation must also be met from maternal tissues or diet. There is some data showing lower plasma and red blood cell DHA levels during pregnancy and lactation, compared with nonpregnant, nonlactating women. However, the Committee stated that this may be a normal physiological change of pregnancy. Additionally, studies show that supplementation with fish oil during pregnancy increases DHA in the mother and newborn infant and that fish oil supplementation during lactation increases DHA in breast milk and in the infant's blood. However, the Committee concluded that: "Evidence is not available to show that increasing intakes of DHA in pregnant and lactating women consuming diets that meet requirements for n-6 and n-3 fatty acids have any physiologically significant benefit to the infant."

Therefore, the Committee set AIs for pregnant and lactating women based on the median intake of ALA in the United States, where a deficiency is basically nonexistent in noninstitutionalized populations. The AI for pregnant women for ALA is 1.4 grams per day, based on the median intake of 81 pregnant women studied in the 1994-1996 Continuing Survey of Food Intake of Individuals (CSFII). The AI for lactating women for ALA is 1.3 grams per day, based on the median intake of 44 lactating women in CSFII 1994-1996. The Committee stated that small amounts of DHA and EPA can contribute towards reversing an n-3 fatty acid deficiency and therefore contribute toward the AI for ALA.

Quantitative Guidance on Proportions of Energy Sources to Decrease Chronic Disease Risk:

The 2002 Macronutrient Report reviewed the scientific evidence on macronutrients and chronic disease, and described Acceptable Macronutrient Distribution Ranges (AMDRs). The AMDR represents intakes that are associated with reduced risk of chronic disease, intakes at which essential nutrients can be consumed at adequate levels and to maintain energy balance.

The IOM report discussed the scientific evidence regarding the association of n-3 fatty acid intake with decreased risk of heart disease and stroke. The committee concluded that EPA and DHA may provide beneficial health effects when consumed at moderate levels. The AMDR for ALA was estimated by using the AI for ALA as the lower end of the intake range. For adults, the ALA AI of 1.1 g/d for women and 1.6 g/d for men would correspond to about 0.6 percent of energy (calories). The highest intake of ALA from food in North America corresponds to around 1.2 percent of energy. This was set as the upper end of the AMDR intake range. Thus the AMDR for ALA was set at 0.6 to 1.2 percent of energy. The committee stated, "Approximately 10 percent of the AMDR for

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n-3 fatty acids (linolenic acid) can be consumed as EPA and/or DHA (0.06 to 0.12 percent of energy).”

Scientific Advisory Committee on Nutrition, United Kingdom

As described in Section A, the Food Standards Agency (FSA) of the United Kingdom requested advice on the benefits and risks of fish consumption, particularly oily fish, from the Scientific Advisory Committee on Nutrition (SACN) and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT). A report, *Advice on Fish Consumption: Benefits and Risks*, was published in 2004 by the joint SACN/COT Subgroup (SACN 2004). The report had the purpose of combining the nutritional considerations of fish consumption from SACN and the toxicological considerations of contaminants in fish from COT; weighing the nutritional benefits against possible risks; and developing dietary advice for the public.

The SACN report reviewed two aspects of health benefits of fish consumption: the effects of LC PUFA on early human growth and cognitive function and the relationship of fish consumption and cardiovascular disease (discussed in Section A).

DHA Requirements in Pregnancy and Lactation:

A background paper in an Annex to the SACN report reviewed the knowledge base on DHA requirements in pregnancy and lactation. The Annex estimated that during pregnancy at least 10 grams of DHA must be accreted, including about six to seven grams accreted by fetal brain development over the last trimester. In addition, about 2 grams DHA would be deposited in fetal adipose tissue, and additional DHA would be deposited in the placenta. The deposition of 10 grams DHA in the last trimester would correspond to a need for about 100 mg DHA per day above nonpregnant DHA intake. During lactation, about 70 to 80 mg DHA per day would be needed for milk formation, with a total of about 12 grams DHA needed during six months of lactation. For a non-pregnant woman, a typical DHA intake would be about 100 mg per day, corresponding to a total intake of nine grams DHA in the last trimester and 18 grams DHA during six months of lactation. Thus, if a non-pregnant woman was in balance when consuming 100 mg DHA per day, she would need to double her DHA intake during the last trimester of pregnancy and increase DHA intake by about 70 percent during lactation. However, there is no evidence that such an increased intake occurs.

Thus, it seemed that many women would not meet the additional need for DHA for pregnancy and lactation from their typical daily intake. Additionally, there was little evidence that this need would be met by metabolic conservation of LC-PUFA, by mobilization of DHA from adipose tissue stores, or by increased maternal formation of DHA from the precursor ALA. The background paper concluded that there is some evidence that many women may have marginal status for n-3 LC-PUFA during

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pregnancy and lactation. However, the full SACN report noted that the data currently available from which to draw this conclusion are limited.

Studies of Maternal or Infant LC N-3 PUFA Intake and Infant Neurodevelopment and Growth:

The SACN report tabulated and summarized the results of available studies of maternal or infant intake of n-3 LC PUFA, grouping the studies in three general categories: observational studies of maternal n-3 LC PUFA intake and infant development; clinical trials of infant formula supplements; clinical trials of maternal n-3 LC PUFA supplements.

Observational Studies of Maternal N-3 LC PUFA Intake and Infant Development

Two of the observational studies considered visual development. (These two studies are discussed later in this section):

- Williams et al. (2001) found better visual stereoacuity (depth perception) at age 3.5 years in children whose mothers ate oily fish during pregnancy, in a prospective cohort study of 435 children.
- Jørgensen et al. (2001) found a positive association between visual acuity at four months of age and DHA level in mothers' milk in a cross-sectional study of 39 breastfed infants.

Two studies considered cognitive development. These prospective studies found no significant association between cord blood levels of DHA and cognitive function in 128 four year olds (Ghys et al., 2002) and 306 seven year olds (Bakker et al., 2003). One study suggested that higher DHA and EPA intake from fish among Faroe Islanders compared with Danes accounted for longer gestation time among Faroe Islanders (Olsen et al., 1995). Olsen and Secher (2002) found increased risk of preterm delivery and low birth weight in women with low fish consumption in a prospective study of 8,729 pregnant women.

Clinical Trials of Infant Formula Supplements:

The available clinical trials were for visual or cognitive function in preterm or term infants.

- Visual function, preterm infants (SACN Table 2.1). Nine of the 10 tabulated studies, published from 1990 to 2002, showed improved visual function in experimental groups fed infant formula supplemented with sources of n-3 LC PUFA. The report stated that these trials support the efficacy of LC PUFA intake on early development of the visual system, consistent with a meta-analysis by San Giovanni et al. (2000) and with a Cochrane review (Simmer 2000#).
- Visual function, term infants (SACN Table 2.2). Four of the nine tabulated studies, published from 1995 to 2003, showed improved visual function in groups supplemented with n-3 LC PUFA.

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- Visual function, breastfed infants weaned to supplemented formula (SACN Table 2.3). Two studies, published in 2002 and 2003, both showed improved visual function, measured by sweep visual evoked potential, in groups of infants weaned to formula supplemented with n-3 LC PUFA. Positive effects in one study were measured at four, six, and 12 months of age and in the other study at 12 months of age.
- Behavioral development, preterm infants (SACN Table 2.4). Four studies, published in 2001 and 2002, showed little or no effect on behavioral development, measured by the Bayley Mental Development Index and other instruments, in groups supplemented with n-3 LC PUFA.
- Behavioral development, term infants (SACN Table 2.5). Three of the ten studies, published from 1995 to 2003, showed some positive effect on behavioral development in groups supplemented with n-3 LC PUFA.

In summarizing the results, the report found that the clinical trials on visual function in preterm infants consistently demonstrate a short-term beneficial effect on visual evoked potential. The results in term infants were less consistent, with six of 10 trials showing a beneficial effect, especially measuring visual evoked potential, but others found no effect, including the largest trial. Eleven of the 14 trials of behavioral measures in both preterm and term infants found no effect.

Clinical Trials of Maternal N-3 LC PUFA Supplements:

- Infant neurodevelopment. Two supplement trials of pregnant women considered neurodevelopmental function in their children. (These studies will be discussed further in a later section):
 - Helland et al (2003) found higher intelligence test scores at age four among 48 children whose mothers had received fish oil supplements during pregnancy compared with 36 children whose mothers received placebo. The supplemented women received fish oil containing two grams per day of n-3 LC PUFA from 18 weeks of pregnancy to three months postpartum. The SACN report noted that the children studied in this follow up were only a small subgroup of the 590 pregnant women in the original supplement trial.
 - Malcolm et al (2003a and b) found no effect on visual function shortly after birth or at 50 or 66 weeks corrected age among 28 children whose mothers received fish oil supplements during pregnancy compared with 27 control children. The supplemented women received fish oil containing 0.2 grams per day of n-3 LC PUFA from 15 weeks of pregnancy until delivery.
- Birth weight and length of gestation (SACN Table 2.7). Three of five studies, published from 1992 to 2003, found a beneficial effect of n-3 LC PUFA supplements on gestational length (two studies) or recurrence of preterm delivery (one study).

Thus, the SACN report found that there is some evidence that increased n-3 LC PUFA was beneficial, especially in lower birth weight populations, and this may be more

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relevant in populations that tended to have a lower background intake of n-3 LC PUFA. There were no adverse effects, even at relatively high doses of n-3 LC PUFA. In the trials that found no effect of maternal n-3 LC PUFA supplements on birth weight or gestation length, the infants of nonsupplemented mothers had birth weights greater than 3,600 grams (about 7.9 pounds).

This finding was reflected in the overall SACN advice on fish consumption:

“In pregnancy and lactation there is a demand on the mother to supply the fetus and infant with LC n-3 PUFA, which are required for the development of the central nervous system. There is some evidence that increased maternal LC n-3 PUFA intake produces beneficial effects, especially in lower birth weight populations, and this may be more relevant in populations that tend to have a lower background intake of LC n-3 PUFA, i.e. where fish intake is low. No adverse effects of maternal LC n-3 PUFA supplementation have been observed, even at relatively high doses.”

“The dose-response relationship is derived from the cardiovascular evidence, as the evidence for maternal intake and pregnancy outcome is insufficient for this.”

“SACN, therefore, endorsed the population recommendation to eat at least two portions of fish per week, of which one should be oily, and agreed that this recommendation should also apply to pregnant women. Two portions of fish per week, one white and one oily, contain approximately 0.45g/d LC n-3 PUFA.”

“An increase in population oily fish consumption to one portion a week, from the current levels of about a third of a portion a week, would confer significant public health benefits in terms of reduced risk of CVD. There is also evidence that increased fish consumption might have beneficial effects on fetal development.”

The SACN emphasized that this was a minimal achievable objective, considering the low background fish consumption in the United Kingdom. Although the Committee found that it may be beneficial for individuals to consume more fish than this guideline, they were unable to identify a level for increased consumption. They stated it would be inappropriate to discourage fish consumption at higher levels than the recommendation, unless there was an upper limit beyond which people should not consume.

The SACN advice took into account the toxicological considerations related to methylmercury and dioxins and dioxin-like PCBs in fish.

Regarding methylmercury, the SACN advice stated:

“On the basis of the COT opinion, the FSA has advised that pregnant women, women intending to become pregnant and children under 16 should avoid eating shark, marlin and swordfish. One weekly portion of these fish would not be

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harmful for other adults. Pregnant women and women intending to become pregnant may eat to up to four medium-size cans or two tuna steaks a week. Children and other adults do not need to restrict the amount of tuna they eat.”

Regarding dioxins and dioxin-like PCBs, the SACN advice stated:

“Women of reproductive age and girls should aim to consume within the range of one to two portions of oily fish a week, based on maintaining consumption of dioxins and dioxin-like PCBs below the TDI of 2 pg WHO-TEQ/kg bodyweight per day.”

“Women past reproductive age, boys and men should aim to consume within the range of one to four portions of oily fish a week, based on maintaining consumption of dioxins and dioxin-like PCBs below the guideline value of 8 pg WHO-TEQ/kg bodyweight per day.”

European Food Safety Authority

As described previously in Section A, the European Food Safety Authority (EFSA) formed an Interpanel working group to respond to a request from the European Parliament for a scientific assessment of the human health risks related to consumption of wild and farmed fish. In June, 2005, the EFSA published its report, “Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the European Parliament Related to the Safety Assessment of Wild and Farmed Fish.” The Summary of the report stated

“There is evidence that fish consumption, especially of fatty fish (one to two servings a week) benefits the cardiovascular system and is suitable for secondary prevention in manifest coronary heart disease. There may also be benefits in foetal development, but an optimal intake has not been established.”

Metabolism, Function and Physiological Requirement for N-3 LC PUFA in Humans:

In an appendix, the EFSA report reviewed the metabolism and function and the physiological requirement for n-3 LC PUFA in humans (EFSA Annex 2). This review noted that there is a dietary requirement for n-3 ALA and stated that there is no consensus on the need for an intake of preformed DHA in adults, although data indicated that DHA is conditionally indispensable for preterm infants. The report noted that the Scientific Committee on Food (SCF) of the European Commission in 1993 set population reference intakes for total n-3 fatty acids at 0.5 percent of energy intake (calories) for adults and recommended that total n-3 fatty acid intake not exceed five percent of energy intake. Regarding the need for n-3 LC PUFA during pregnancy and lactation, the review summarized the accretion of n-3 LC PUFA in the fetus and placenta during pregnancy and the DHA secreted in breast milk during lactation. The EFSA report noted the estimate of the SACN report (2004) that women may need to increase DHA intake to

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about 0.2 grams per day during pregnancy and about 0.16 to 0.17 grams per day during lactation. This may be particularly important in successive pregnancies.

The review noted the lack of association between blood levels of DHA at birth and later cognitive performance in two studies (Ghys et al, 2002; Bakker et al, 2003). However, DHA status at birth was significantly related to better movement quality and visual acuity and behavior at seven to eight years of age in another study (Hornstra 2005). The review stated that:

“This underlines the importance of an adequate maternal DHA intake during pregnancy as a condition for an ample supply of the fetus and its importance for cognitive, motor, visual and behavioural development of the infant and child. This adequate maternal intake during pregnancy and lactation can be reached by a higher intake of fatty fish (or of fish oil).”

Effect of Fish or LC N-3 PUFA in Pregnancy on Outcome

The EFSA report noted that, for pregnant and breastfeeding women, the DHA level in blood phospholipids and in breast milk is determined by maternal fish consumption or LC n-3 PUFA intake. The report summarized research studies of associations between maternal fish intake, LC n-3 PUFA intake, LC n-3 PUFA blood levels or LC n-3 PUFA breast milk levels and health outcomes in mothers or offspring.

- **Gestational length and birth weight.** In an observational study in the Faroe Islands, higher fish consumption was associated with longer gestational duration. An observational study in Denmark showed no association between intake of LC n-3 PUFA and gestational length, birth weight or birth length, but fish intake was associated with lower risk of preterm delivery and of low birth weight. A randomized trial of cod liver oil supplementation in pregnant women showed no differences in gestational duration or birth weight between supplemented and placebo groups. However, among all neonates, there was a positive association between DHA levels in cord blood phospholipids and gestational duration. Another trial of DHA supplements in eggs during pregnancy showed a statistically significant increase of gestational duration by six days for the supplemented group compared with placebo.
- **Pregnancy-induced hypertension (PIH).** Observational studies in Inuit women suggested an association between LC n-3 PUFA and decreased risk of PIH. However, a clinical trial of fish oil supplementation in high risk pregnant women showed no difference in PIH risk with supplementation compared with placebo.
- **Visual function and cognitive development.**
 - A neurological exam of apparently healthy term infants at 10-14 days of age showed the neurologic optimality score was positively associated with indices of DHA level in umbilical artery and vein. Neurologically abnormal infants had lower DHA indices (Dijck-Brouwer 2005).

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- In the randomized trial of cod liver oil supplementation in pregnant women, there was no difference in electroencephalogram (EEG) scores at age two days and three months or novelty preference (Fagan test) at age six and nine months between supplemented and placebo groups. However, among all neonates, there was a positive association between DHA levels in cord blood phospholipids and maturity of EEG scores at age two days (Helland 2001). In a subset of children tested at four years of age, children of supplemented mothers scored higher on an intelligence test, the Kaufman Assessment Battery for Children (Helland 2003).
- In a group of healthy breastfed infants, length of breastfeeding was positively associated with IQ at age 6 ½ years. Length of gestation, duration of breastfeeding and the ratio of DHA to AA in colostrum (early breast milk) explained 76 percent of the total variation in IQ (Gustafsson 2004).
- Visual function development has been used as a marker for neurodevelopment. In some but not all studies, breastfed infants were shown to have better visual acuity, more advanced retinal development and more advanced visual function up to 3 ½ years of age.
- In a group of pregnant women and their infants in the U.S., higher maternal fish consumption was associated with higher infant scores for visual memory recognition at six months of age. In the same study, visual memory recognition scores were negatively associated with maternal hair mercury concentration (Oken et al., 2005).
- In a prospective study, stereoacuity (depth perception) in children at age 3½ years was associated with breastfeeding and with maternal consumption of fatty fish during pregnancy (Williams et al., 2001).
- In another randomized trial of fish oil supplements in pregnant women, there was no difference in visual evoked potential (VEP) shortly after birth between supplemented group and placebo. Neither was there an effect of supplementation on gestation length, birth weight or on DHA concentration in umbilical cord red blood cells. However, among all infants, there was a significant correlation between infant DHA blood levels and maturity of the retina as assessed by electroretinography within the first week of life and with the maturity of the pattern-reversal VEP at 50 and 66 weeks postconceptional age (Malcolm et al., 2003a,b).

The Summary and Conclusions of the report stated:

“Fish is an important source of proteins of high biological value, LC n-3 PUFA, essential minerals, especially iodine, selenium and calcium, and vitamins, especially vitamins A and D and B12. LC n-3 PUFA are not essential in human nutrition beyond the foetal and neonatal period, but may be conditionally essential in immature and young infants.”

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“There is an increased demand for LC n-3 PUFA of the foetus with advancing pregnancy. This has to be satisfied predominantly by the mother by enhanced synthesis from the precursor LNA, by mobilisation of tissue stores or by dietary intake. Fish consumption corresponding to at least 0.2 g DHA/day can satisfy both the demands of the foetus and maternal requirements. However, both the intake of high amounts of LC n-3 PUFA (> 2 g/day) and of LNA (> 3 g/day) can decrease the AA status of the infant, which is undesirable. Therefore, both the requirement of LC n-3 PUFA and the relationship between n-3 and n-6 fatty acids and AA content in the maternal diet are of concern and the optimal mixture needs to be identified. The Scientific Committee on Food (2003) considered the available evidence insufficient for setting a mandatory minimum content of LC PUFA for infant formula intended for healthy mature infants.”

The EFSA report concluded that there is evidence that fish consumption or fish oil benefits the cardiovascular system and is suitable for secondary prevention in manifest coronary artery disease. The report further stated:

“Nevertheless, results from both epidemiologic and interventional studies suggest that health benefits are associated with the consumption of certain levels of EPA/DHA from fish and fish oils also in the healthy population. The expected benefits include a decrease in the risk of cardiovascular disease and stroke and improved neurodevelopmental and perinatal growth in infants.”

U.S. Agency for Health Care Research and Quality. Effects of Omega-3 Fatty Acids on Maternal and Child Health.

In 2005, the U.S. Agency for Health Care Research and Quality (AHRQ) published the results of a systematic review of the scientific literature regarding the human evidence for the effects of omega-3 fatty acids on maternal and child health. The review was conducted by the Evidence Based Practice Center at the University of Ottawa, Canada (Lewin 2005).

Key Questions:

The AHRQ review addressed a range of key research questions, involving both maternal and child populations and several types of outcome data, both clinical pregnancy outcomes and clinical child development outcomes. As outlined in the Summary of the AHRQ review, the key questions included:

“Maternal population, pregnancy outcomes/biomarkers associations:

- What is the evidence that intake of omega-3 fatty acids influences
 - duration of gestation?
 - incidence of births of human infants small for gestational age (SGA)?

“Child population, growth patterns, neurological, visual or cognitive developmental outcomes/biomarkers associations:

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- What is the evidence that maternal intake of omega-3 fatty acids
 - during pregnancy influences any of the clinical outcomes in term or preterm human infants?
 - within maternal breast milk, infant formula, both and/or other sources (i.e., diet) influences any of the clinical outcomes in term or preterm human infants?
- What is the evidence that term or preterm human infants' clinical outcomes are associated with the omega-3 or omega-6/omega-3 fatty acids content of
 - maternal or fetal biomarkers during pregnancy?
 - child biomarkers?"

Systematic Review:

The systematic review identified 2,049 records for initial screening. Of the 191 reports reviewed, 117 reports, describing 89 unique studies, met the inclusion criteria. These included 63 randomized controlled trials and 26 observational studies. If at least two randomized controlled trials (RCTs) were identified for a population and outcome, no other types of design were considered. However, if there were too few RCTs available, then non-RCT clinical trials (such as trials without random allocation) and observational studies (such as cohort, case control or cross sectional studies) were included. Descriptive study designs were not included.

Meta-analyses:

The full AHRQ report considered the evidence for each key research question individually. Meta-analyses were conducted for many of the research questions regarding the effect of omega 3 fatty acid supplements on pregnancy outcomes and child growth and development, including neurological and cognitive development of term infants and visual function in both term and preterm infants. The results of specific AHRQ meta-analyses will be summarized in this section under **Other Recent Systematic Reviews, Meta-Analyses and Risk Assessments**.

Conclusions:

In its overall conclusion, the AHRQ report found an absence of reports of moderate to severe adverse events associated with omega-3 fatty acids in maternal and child health. Regarding beneficial maternal and child outcomes, the report found that:

“Pregnancy outcomes were either unaffected by omega-3 fatty acid supplementation, or the results were inconclusive.... regarding evaluations of the duration of gestation, some discrepancies were observed, although most of the studies failed to detect a statistically significant effect. Biomarker data failed to clarify patterns in pregnancy outcome data.”

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“Results concerning the impact of the intake of omega-3 fatty acids on the development of infants are primarily, although not uniformly, inconclusive. The inconsistencies in study results may be attributable to numerous factors.”

“In addition, making clear sense of the absolute or relative effects of individual omega-3 fatty acids, or even omega-3 fatty acid combinations, on child outcomes is complicated or precluded by the following problem. Studies typically employed interventions that involved various cointerventional or background constituents (e.g., omega-6 fatty acids), yet whose metabolic interactions with the omega-3 fatty acid(s) were not taken into account in interpreting the results. The dynamic interplay among these fatty acid contents (e.g., competition for enzymes), and how this interplay may influence outcomes, may differ in important ways depending on whether DHA or olive oil is added to the combination of cointerventional or background constituents, particularly in the maternal population. This strategy prevented the isolation of the exact effects relating to the omega-3 fatty acid content. It is thus very difficult to reliably ascribe definite child outcome-related benefits, or the absence thereof, to specific omega-3 fatty acids. Biomarker data failed to clarify patterns in child outcome data.”

“Future research should likely consider investigating the impact of specific omega-6/omega-3 fatty acid intake ratios, in no small part to control for the possible metabolic interactions involving these types of fatty acids. To produce results that are applicable to the North American population, populations consuming high omega-6/omega-3 fatty acid intake ratios should likely be randomized into trials also exhibiting better control of confounding variables than was observed, especially in the present collection of studies of child outcomes.”

Institute of Medicine of the National Academies. “Seafood Choices. Balancing Benefits and Risks”

In 2006, the Institute of Medicine (IOM) of The National Academies released a report, titled “Seafood Choices. Balancing Benefits and Risks,” which examined relationships between benefits and risks associated with seafood to help consumers make informed choices. The report used a qualitative approach to balancing benefits and risks of seafood intake by population groups.

As part of its task to analyze and balance the benefits and risks of seafood consumption, the IOM report reviewed and evaluated the scientific literature on the benefits associated with nutrients from seafood. In Section A we summarized the IOM’s review and evaluation of the literature regarding CHD and stroke. Here we summarize the IOM’s review and evaluation regarding neurodevelopmental health benefits.

In an appendix, the report tabulated the studies reviewed, noting the author, study type, subjects, exposure, exposure timing, exposure amount and results. The table also

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classified the conclusion of each study by designating the conclusion as: B, evidence of benefit; N, evidence of no association or no clear association; A, evidence of an adverse effect; and N/A, conclusion not available, data presented for background only.

Benefits to Women During and After Pregnancy:

Postpartum depression. The report tabulated nine studies that addressed the question of whether low DHA levels in the brain in late pregnancy and early postpartum period may contribute to the emergence of postpartum depression. The nine studies included one review, one randomized controlled trial, one open trial, five cohort studies and five cross cultural (ecologic) study. The ecologic study compared 41 population groups and found a positive association between seafood consumption and higher DHA levels in breast milk, and this was associated with lower prevalence of postpartum depression (Hibbeln 2002). However, a clinical trial of DHA supplements in lactating women found no difference between supplemented and control groups for measures of postpartum depression. The report noted that there are no randomized clinical trials of omega-3 fatty acids supplementation in pregnancy and risk of postpartum depression. The committee found that the existing evidence was insufficient to draw a conclusion about the possible association of EPA/DHA intake and postpartum depression.

Since the 2005 IOM report, a large epidemiological study and a randomized controlled trial have provided additional information regarding depression during pregnancy.

Epidemiological study (Golding et al., in press): This study used a net risk and benefits of fish consumption approach to assess relative risk of high levels of depressive symptoms among approximately 9,000 women in the third trimester of pregnancy in the ALSPAC cohort in the United Kingdom. Statistical analysis took social and lifestyle factors into account. Results: Unadjusted and adjusted analyses showed lower maternal intake of omega-3 from seafood was associated with high levels of depressive symptoms. Compared to women consuming >1.5g omega-3 from seafood per week, those consuming none were more likely to have of high levels of depressive symptoms at 32 weeks gestation (AOR 1.54, 95% CI 1.25, 1.89). The authors concluded that a woman needs to consume at least three portions of seafood per week to maintain her mental health during pregnancy. They reported that since the risk of depressive symptoms was lowest among those consuming >1.5g of omega-3 from seafood per week (three or more portions of seafood per week), it was likely that limiting intake below this amount would increase the risk of maternal depressive symptoms during pregnancy.

Randomized Controlled Trial (Su et al., 2008): This randomized controlled trial compared 3.5 g/d of n-LC PUFAs compared to placebo among n=33 pregnant women with major depressive disorder severe enough to meet ICD –9 criteria. Twenty four women completed the study. At six weeks the group randomized to omega-3's higher rates of clinical response (62%, n-3 LC PUFA to 27% Placebo, p<0.03). At eight weeks the n-3 LC PUFA group had significantly lower depressive symptoms on the EPDS and

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Beck depression inventory rating scales. The n-3 LC PUFAS were well tolerated with no adverse effects for mother or infant.

Benefits to Infants and Children Associated with Prenatal Omega-3 Fatty Acid Intake:

The report noted that the level of maternal DHA intake influences DHA levels in both maternal blood and milk. DHA is selectively transported across the placenta, therefore increased maternal blood DHA in pregnancy may enhance placental DHA transfer to the fetus. This could influence the DHA supply available to the fetal brain and to other organs and tissues. Brain DHA accumulates rapidly from about 22 weeks of gestation to at least 2 years of age.

Duration of gestation and birth weight. The report tabulated 16 studies of the association of fish oil supplementation, seafood intake or other food sources of DHA with duration of gestation and birth weight. There were 10 randomized controlled trials, five cohort studies and one case control study. The report found that “observational studies suggest and several of the experimental studies support that EPA/DHA supplementation or higher seafood intake is associated with an increased duration of gestation.”

Development in infants and children. The report tabulated 34 studies addressing the association of infant and child development with maternal n-3 LC PUFA intake. There were four reviews, six randomized controlled trials, 15 cohort studies, two case control studies, three cross sectional studies and four animal studies.

Visual acuity. In a prospective observational study, stereoacuity (depth perception) at 3 ½ years of age was associated with breastfeeding, greater maternal age, and maternal consumption of fatty fish during pregnancy in the Avon Longitudinal Study of Parents and Children (ALSPAC Study) (Williams et al., 2001). In a prospective observational study of breastfed infants, breast milk DHA levels were positively associated with visual acuity and speech perception at two months of age (Innis 2001).

Cognitive development. In a randomized controlled trial of cod liver oil supplementation in pregnant women in Norway, children of supplemented mothers had higher Mental Processing Composite scores at four years of age (Helland 2001, 2003). A randomized controlled trial of DHA supplementation in lactating women found a higher Bayley Psychomotor Development Index (PDI) in children of supplemented mothers at 30 months of age (Jensen 2005). In this study, there were no effects of supplementation on visual acuity at four or eight months or on developmental indexes at 12 months. In a preliminary report, children of supplemented mothers also had longer sustained attention at five years of age (Jensen 2004). The committee noted that these two trials found benefits from maternal supplementation not in infancy but in early childhood and suggested that other trials that did not continue developmental follow-up after infancy may have missed benefits to children of improving maternal omega-3 fatty acid intake.

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A prospective observational study found positive association of maternal seafood intake with visual memory recognition score at six months of age. The study also found an inverse association of infant visual memory recognition score at six months with level of mercury in maternal hair during pregnancy (Oken et al., 2005). A prospective observational study of children in the ALSPAC cohort found a positive association between maternal fish intake during pregnancy and scores on the Denver Developmental Screening Test and on the MacArthur Communicative Development Inventory (Daniels et al., 2004). Cord tissue mercury levels were not associated with developmental scores.

Overall, regarding maternal intake of seafood or n-3 LC PUFA, the report found that:

“The strongest evidence of benefit for higher maternal seafood or EPA/DHA intake is an increase in gestation duration, with anticipated benefits to the newborn. Populations or subgroups within populations who have the lowest baseline consumption of seafood may show the greatest benefit in duration of gestation with higher EPA/DHA intake. Observational and experimental studies offer evidence that maternal DHA intake can benefit development of the offspring; however, there are large gaps in knowledge that need to be filled by experimental studies.”

“The average EPA/DHA intake among U.S. women is considerably below that of most other populations in the world and the majority of the data on benefits to infants and children from increased DHA levels come from populations outside the U.S. and/or from studies using supplementation rather than seafood consumption.”

Benefits to Infants from Postnatal Supplementation through Formula:

The committee reviewed evidence related to DHA-supplemented infant formulas to consider whether these data support the findings on benefits associated with seafood consumption or fish oil supplementation in pregnant and lactating women.

Visual Acuity. The report tabulated 23 studies considering DHA supplementation of infants and visual acuity. There were four reviews (including one Cochrane review), two meta analyses, 12 randomized controlled trials, three cohort studies, one cross sectional study and one animal study. Overall, the report found that all but one of the randomized controlled trials of preterm infants and about half of the trials of term infants found higher visual acuity at some age in infants consuming DHA supplemented formula. A review by Uauy et al. (2001) and meta-analyses by SanGiovanni et al. (2000a, b) concluded that DHA supplementation resulted in improved visual acuity in both term and preterm infants. However, a Cochrane systematic review concluded that there was no association between DHA supplementation and increased visual acuity or general development in term infants (Simmer 2001).

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Cognitive and Motor Development. The report tabulated 32 studies considering DHA supplementation of infants and cognitive and motor development. There were nine reviews (including two Cochrane reviews), 18 randomized controlled trials, two cohort studies, and five animal studies. The report summarized some of the inconsistent results regarding cognitive and motor development in DHA supplement trials of preterm and term infants. The committee noted that global tests such as the Bayley Scales of Infant Development and the Brunet-Lezine administered in infancy may be less related to performance on cognitive tests in childhood than more specific tests of attention and problem solving. There is limited evidence from global tests of infant development to support cognitive benefit of DHA supplementation of infants. For specific tests more strongly related to developmental parameters, the evidence is mixed. Overall, the report stated:

“At most, specific outcomes have been measured in only one or two individual trials and these have been measured at different ages. Even though numerous developmental outcomes have been identified that collectively suggest there are benefits associated with EPA/DHA supplementation, it is difficult to subject the studies in total to a systematic review, because of the differences in experimental design among the studies. The benefits of postnatal DHA supplementation for cognitive development need further study because of the heavy reliance on global assessments as outcomes and the limited employment of more specific developmental outcomes. Furthermore, the majority of trials stopped looking at development well before children reached school age, when more sophisticated measures of cognitive function may be employed.”

Overall, regarding visual acuity and cognitive and motor development with supplemented infant formula, the report stated:

“The strongest evidence of benefit for postnatal DHA supplementation in formula-fed preterm and term infants is higher visual acuity, an outcome that has been measured repeatedly in clinical trials. In addition, some positive effects have been found on cognitive function in infancy and childhood in both experimental and observational studies and in relation to both pre- and postnatal DHA intake. Reviews that take into account all lines of evidence have concluded that omega-3 fatty acid can be beneficial to cognitive development (Cohen 2005c, McCann and Ames 2005), whereas reviews that rely strictly on published results from experimental trials limited to global assessments of cognitive development, e.g., the MDI [Bayley Mental Development Index], do not offer strong support (Simmer and Patole 2005, Simmer 2005).” [Note these citations correspond to Simmer and Patole (2004) and Simmer (2001).]

Primary Findings:

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The Primary Findings of the report regarding health benefits of seafood in women, infants and young children were stated as:

1. Seafood is a nutrient-rich food that makes a positive contribution to a healthful diet. It is a good source of protein, and relative to other protein foods, e.g. meat, poultry, and eggs is generally lower in saturated fatty acids and higher in the omega-3 fatty acids EPA and DHA and selenium;
2. The evidence to support benefits to pregnancy outcome in females who consume seafood or fish oil supplements as part of their diet during pregnancy is derived largely from observational studies. Clinical trials and epidemiological studies have also shown an association between increased duration of gestation and intake of seafood or fish oil supplements. Evidence that the infants and children of mothers who consume seafood or EPA/DHA supplements during pregnancy and/or lactation may have improved developmental outcomes is also supported largely by observational studies;
3. Increased EPA/DHA intake by pregnant and lactating women is associated with increased transfer to the fetus and breast-fed infant.
 - a. A number of observational studies show a positive association between maternal blood or breast milk DHA levels and a range of developmental outcomes in infants and children.
 - b. Two experimental studies of maternal EPA/DHA supplementation found cognitive benefits for the children when they were four or five years of age.
 - c. Because these two studies differed dramatically in timing of EPA/DHA supplementation (pre- and postnatally or postnatally), source (cod-liver oil or algal DHA), and amount (2.0 grams or 0.20 grams EPA/DHA) and, likely in usual seafood intake (Norway or U.S. residents), insufficient data are available to define an ideal level of EPA/DHA intake from seafood in pregnant and lactating women.
4. A large number of experimental trials have provided DHA directly to human infants through infant formula and have found benefits for infant and child neurological development. These trials offer the best evidence that infants/children would benefit from increased DHA in breast milk and increased maternal seafood intake.
 - a. Visual acuity has been measured in the most trials and is increased by DHA supplementation, with preterm infants more likely to benefit than term infants.
 - b. Cognitive benefits of postnatal DHA supplementation with formula have also been found in infancy and early childhood. However, the number of trials has been limited and the specific outcomes varied, precluding a systematic review.
5. At present, there is no convincing evidence that ADHD [attention deficit hyperactivity disorder], other behavioral disorders, and asthma can be prevented or treated in children with seafood or EPA/DHA consumption.

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Research Gaps and Recommendations:

The Committee made the following research recommendations related to its review of health benefits for women, infants and children:

Pregnant and Lactating Women

Recommendation 1:

Better data are needed to determine if outcomes of increasing consumption of seafood or increasing EPA/DHA intake levels in U.S. women would be comparable to outcomes of populations in other countries. Such studies should be encouraged to include populations of high fish-consumers outside the continental United States to determine if there are differences in risks for these populations compared to U.S. populations.

Recommendation 2:

Dose-response studies of EPA/DHA in pregnant and lactating women are needed. This information will help determine if higher intakes can further increase gestation duration, reduce premature births, and benefit infant development. Other studies should include assessing whether DHA alone can act independent of EPA to increase duration of gestation.

Infants and Toddlers

Recommendation 3:

Research is needed to determine if cognitive and developmental outcomes in infants are correlated with performance later in childhood. This should include:

- Evaluating preschool and school-age children exposed to EPA/DHA *in utero* and postnatally, at ages beginning around four years when executive function is more developed and;
- Evaluating development of school-age children exposed to variable EPA/DHA *in utero* and postnatally with measures of distractibility, disruptive behavior and oppositional defiant behavior, as well as more commonly assessed cognitive outcomes and more sophisticated tests of visual function.

Recommendation 4:

Additional data is needed to better define optimum intake levels of EPA/DHA for infants and toddlers.

Children

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Recommendation 5:

Better-designed studies about EPA/DHA supplementation in children with behavioral disorders are needed.

Balancing Risks and Benefits:

Because of scientific uncertainties, the committee found that it was not feasible to quantify the health benefits of seafood consumption, the health risks of potential contaminants for all population subgroups, or the benefit-risk interactions. Therefore, rather than presenting a quantitative benefit-risk assessment and balancing, the committee used its expert judgment to develop a qualitative scientific benefit risk analysis and balancing of the benefits and risks of seafood consumption. Based on its analysis and balancing, the committee developed specific guidance for healthy consumption for population subgroups. The consumption guidance for women and children is:

1. Females who are or may become pregnant or are breastfeeding:

- a. May benefit from consuming seafood, especially those with relatively higher concentrations of EPA and DHA;
- b. A reasonable intake would be two three-ounce (cooked) servings but can safely consume 12 ounces per week;
- c. Can consume up to six ounces of white (albacore) tuna per week;
- d. Should avoid large predatory fish such as shark, swordfish, tilefish, or king mackerel.

2. Children up to age 12:

- a. May benefit from consuming seafood, especially those with relatively higher concentrations of EPA and DHA;
- b. A reasonable intake would be two three-ounce (cooked), or age-appropriate, servings but can safely consume 12 ounces per week;
- c. Can consume up to six ounces of white (albacore) tuna per week;
- d. Should avoid large predatory fish such as shark, swordfish, tilefish, or king mackerel.

(c) Other Recent Reviews, Meta-Analyses and Risk Assessments

Controlled Clinical Trials of Omega-3 Fatty Acid Supplementation of Infant Formula

A number of clinical trials of omega-3 fatty acid supplementation of infant formula have examined possible risks and benefits of formula supplements for infant development. As noted above, although supplementation of infant formula with DHA does not fall in the category of fish consumption, results of these studies may help to answer scientific questions regarding possible neurodevelopmental benefits of DHA during infancy. Table B-2 lists several reports and reviews of this body of literature. Three of the reports were

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summarized in the previous subsection: the IOM Macronutrient Report, the United Kingdom SACN report and the IOM Seafood Choices report. This subsection will summarize the remaining reviews in Table B-2, including specific meta-analyses from the AHRQ report that was summarized in general in the previous subsection (Lewin 2005).

Willatts and Forsyth (2000)

Willatts P, Forsyth JS. The role of long-chain polyunsaturated fatty acids in infant cognitive development. *Prostaglandins Leukot Essent Fatty Acids*. 2000 Jul-Aug;63(1-2):95-100.

Developmental Outcomes: cognitive development in premature and term infants (narrative review with tabulated studies).

The authors noted an inconsistent pattern of results of randomized studies of the effects of infant formula supplemented with n-3 LCPUFA on cognitive development in both preterm and term infants. The studies reviewed used various types of behavioral assessments: psychomotor development, visual attention, problem solving and language development. Criteria for study inclusion and exclusion were not stated for this narrative review. According to the reference citations several of the studies reviewed were reported in abstract form.

- **Psychomotor development.** Two studies of preterm infants found significantly higher scores for infants fed supplemented formula using the Mental Development Index (MDI) of the Bayley Scales of Infant Development at corrected ages of six months and 12 months, respectively. A third study found no difference in MDI scores in supplemented and unsupplemented preterm infants at corrected age 12 months. For term infants, several studies showed no differences between formula groups on various global developmental tests. However, one study showed significantly higher Bayley MDI scores for supplemented infants at age 18 months, as well as a positive correlation between 18 month MDI scores and four month plasma and red blood cell DHA levels across study groups (Birch 2000).
- **Visual information processing.** Two studies of preterm infants showed no differences between supplemented and unsupplemented infants in visual recognition memory, but supplemented infants showed shorter duration of looking at familiar versus novel test stimuli, indicating more efficient information processing compared with controls. One study of term infants found significantly higher visual recognition memory scores in supplemented infants at nine months of age and another study found shorter look duration in supplemented term infants at three months of age compared with controls.
- **Infant problem solving.** In one study, supplemented term infants showed higher problem-solving scores on a three-step problem at 10 months of age compared with controls. The same clinical trial found higher problem solving scores on a two-step problem compared with controls at age nine months in a

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subgroup of supplemented infants who had lower birth weight and poorer attention control at three months.

- **Infant language development.** In one study, term infants supplemented with DHA alone (without arachidonic acid, AA) had marginally lower scores at age 14 months for vocabulary production on the MacArthur Communicative Development Inventory, compared with control infants or infants supplemented with DHA plus AA. There were no differences among groups for the vocabulary comprehension or communicative gesture scores. Also, there was a negative correlation between red blood cell DHA at age four months and both vocabulary production and comprehension scores. In a follow up at age 39 months, there were no differences between groups in measures of IQ and vocabulary.

The authors noted that, despite the inconsistent results of the clinical trials, it would be premature to conclude that n-3 LC PUFA has no effect on cognitive development. Tests of psychomotor development have limitations in assessment of infant cognitive ability, and global tests such as the Bayley Scales may be relatively insensitive in detecting specific but subtle effects on cognition. The authors observed that studies of infant visual attention and problem solving were more consistent in showing effects of n-3 LC PUFA supplementation. These measures also correlate with cognitive scores in later childhood. The authors also noted that in the clinical trials reviewed there were no adverse effects of infant formulas supplemented with DHA plus AA. Only one study reported a negative effect of supplementation and this was for a supplement of DHA without AA.

SanGiovanni et al. (2000a)

SanGiovanni JP, Parra-Cabrera S, Colditz GA, Berkey CS, Dwyer JT. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. *Pediatrics*. 2000 Jun;105(6):1292-8.

Developmental Outcomes: vision in premature infants (meta-analysis).

Study Characteristics: The authors conducted a systematic review of peer-reviewed and published reports of clinical trials of n-3 LC PUFA supplementation and visual acuity in healthy preterm infants from 1965 through July, 1999. Four clinical trials, published in 1992 through 1996, met the inclusion criteria for the meta-analysis (Carlson et al., 1993#; Carlson et al., 1996#; Birch et al., 1992#; Birch et al., 1993#). Other studies were excluded because they had no measure of visual resolution acuity, had no comparison group that received no DHA or had results published only in abstract form. Visual acuity testing was behaviorally based or electrophysiologically based. For the meta-analysis, results of visual acuity measurements were reported in cycles per degree (cy/deg) of visual angle and expressed on an octave scale, where a one-octave difference is a doubling or halving of the number of cy/deg resolved. The review tabulated the fatty acid composition of the test formulas and the design, analytic and experiment-based

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characteristics of the included studies. Comparison groups fed test formulas without DHA were compared with test groups fed DHA-supplemented formula (randomized comparison) or with a group of breast fed infants (nonrandomized comparison) or both. The two studies from Carlson et al. (1993#, 1996#) were randomized comparisons; Birch et al. (1993#) was a nonrandomized comparison; and Birch et al. (1992#) used both types of comparisons. In Carlson et al. (1996#), the DHA test formula was fed until two months corrected age and then changed to a formula without DHA but with a higher level of ALA. Notably, in these relatively early studies, none of the test or comparison formulas contained AA. The Carlson et al. (1993#, 1996#) trials used behaviorally based acuity testing at zero, two, four, six, nine and 12 months corrected ages. The Birch et al. (1992, 1993) trials used both behaviorally based and electrophysiologically based acuity testing at four months corrected age and Birch et al. (1992#) also used electrophysiologically based testing at zero months corrected age.

Study results:

- **Behaviorally based tests.**
 - **Randomized comparisons.**
 - **Two months corrected age.** Results showed significantly better visual acuity for DHA test formulas (Carlson et al., 1993, 1996).
 - **Four months corrected age.** There was significantly better visual acuity for DHA test formulas than for the comparison formula in Carlson et al. (1993#) and than for the corn oil based comparison formula in Birch et al. (1992#). There was also better visual acuity for the DHA formula than for the soy oil based comparison formula in Birch et al. (1992#), but the difference was not statistically significant. (The soy oil formula was higher in ALA than the corn oil formula.) Visual acuity was slightly but not significantly lower for infants who received the DHA test formula until 2 months corrected age than for infants who received the comparison formula in Carlson et al. (1996#).
 - **Zero, six, nine and 12 months corrected age.** At these ages, there were no significant differences in visual acuity between DHA and comparison formulas (Carlson et al., 1993#, 1996#).
 - **Nonrandomized comparisons.**
 - **Four months corrected age.** Breastfed infants had better visual acuity than infants given the comparison formula without DHA in Birch et al. (1993#) and than infants given either the corn oil or soy oil comparison formula without DHA in Birch et al. (1992#), but for the soy oil comparison formula the difference was not statistically significant.
- **Electrophysiologically based tests.**

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- **Randomized comparisons.**
 - **Zero months corrected age.** There was better visual acuity for DHA test formulas than for either the corn oil or soy oil comparison formula in Birch et al. (1992#), but for the soy oil comparison formula the difference was not statistically significant.
 - **Four months corrected age.** There was significantly better visual acuity for DHA test formulas than for either the corn oil based or soy oil based comparison formula in Birch et al. (1992#).
- **Nonrandomized comparisons.**
 - **Four months corrected age.** Results were parallel to the results for behaviorally based tests. Breastfed infants had better visual acuity than infants given the comparison formula without DHA in Birch et al. (1993#) and than infants given either the corn oil or soy oil comparison formula without DHA in Birch et al. (1992#), but for the soy oil comparison formula the difference was not statistically significant.

Meta-analysis results

- **Behaviorally based tests.**
 - **Randomized comparisons.**
 - **Two months corrected age.**
 - Better acuity for infants in the DHA test formula groups.
 - Combined estimated difference in visual resolution acuity:
 - 0.47 octaves of cy/deg (95 percent CI 0.21 to 0.74), $p < 0.001$
 - based on two studies, two comparisons above
 - 48 infants in DHA groups, 49 infants in DHA-free groups
 - **Four months corrected age.**
 - Better acuity for infants in the DHA test formula groups.
 - Combined estimated difference in visual resolution acuity:
 - 0.28 octaves of cy/deg (95 percent CI 0.14 to 0.43), $p < 0.001$
 - significant heterogeneity between studies
 - based on 3 studies, 4 comparisons above
 - 70 infants in DHA groups, 56 infants in DHA-free groups
 - **Zero, six, nine and 12 months corrected age.**

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- At these ages, there was no significant difference in visual acuity between test formula groups
- The combined estimated differences in visual resolution acuity were not significantly different from zero
 - based on two studies, two comparisons above
- **Randomized and nonrandomized comparisons.**
 - **Four months corrected age.**
 - When the randomized and nonrandomized comparisons above were combined, there was better acuity for infants in the breastfed or DHA test formula groups rather than in the groups with comparison formulas without DHA
 - combined estimated difference in visual resolution acuity
 - 0.35 octaves of cy/deg (95% CI 0.21 to 0.49), $p < 0.001$
 - significant heterogeneity between studies
 - based on four studies, seven comparisons above
 - 80 infants in DHA groups, 87 infants in DHA-free groups
- **Electrophysiologically based tests.**
 - **Randomized comparisons.**
 - **Zero months corrected age.**
 - combined estimated difference in visual resolution acuity not reported
 - **Four months corrected age.**
 - better acuity for infants in the DHA test formula group
 - combined estimated difference in visual resolution acuity
 - 0.83 octaves of cy/deg (Standard error of the mean (SEM), +/- 0.08), $p < 0.001$
 - results of heterogeneity test not reported
 - based on one study, two comparisons above
 - 13 infants in DHA groups, 28 infants in DHA-free groups
 - **Randomized and nonrandomized comparisons.**
 - **Four months corrected age.**
 - When the randomized and nonrandomized comparisons above were combined, there was better