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Scientific Advisory Committee on Nutrition

Advice on fish consumption: benefits & risks

2004

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Preface

The aim of this report is to bring together the nutritional considerations from the Scientific Advisory Committee on Nutrition (SACN) on fish consumption and the toxicological considerations from the Committee on Toxicity (COT) on the contaminants in fish. An Inter-Committee Subgroup was established to conduct the risk assessment. The Subgroup weighed the nutritional benefits against possible risks and developed coherent dietary advice for the public on the consumption of fish, with particular reference to oily fish.

This is the first time that SACN and COT have worked so closely together on an issue and I should like to thank the Inter-Committee Subgroup members for their participation in this successful collaboration.

A large body of evidence suggests that fish consumption, particularly of oily fish, reduces cardiovascular disease risk; furthermore, there is also evidence that increased fish consumption might have beneficial effects on fetal development. Balanced against this, however, are the possible detrimental effects associated with the contaminants found in fish.

Interested parties have commented that mixed messages are being given to consumers and so this review aims to bring these views together in order to allow the Food Standards Agency to provide clear and helpful advice to consumers.

An important consideration in this assessment is dose: both for the beneficial effects as well as the risks. Overall, the UK population should be encouraged to eat more fish, especially oily fish. 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 without appreciable risk from the contaminants in fish.

Now that the Inter-Committee Subgroup has completed its risk assessment, it is for the Food Standards Agency to explain these complex issues to the public in a manner that is easily understood. I should like to emphasize the

need to encourage fish consumption, particularly oily fish, and the need to communicate to consumers the important messages in plain, clear English.

I should like to thank the Inter-Committee Subgroup members for their commitment and enthusiasm. I should also like to thank the Secretariat for their contribution to the risk assessment and the production of this report.

Professor Alan Jackson
Chair of the Inter-Committee Subgroup

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Membership of Scientific Advisory Committee on Nutrition: SACN/COT Fish Inter-Committee Subgroup

Chairman

Professor Alan Jackson	Professor of Human Nutrition, School of Medicine, University of Southampton (SACN)

Members

Dr Timothy Key	Reader in Epidemiology, University of Oxford Cancer Research UK Epidemiology Unit, Radcliffe Infirmary, Oxford (SACN)
Professor Christine Williams	Professor of Human Nutrition, University of Reading (SACN)
Professor Ieuan Hughes	Professor and Head of Department of Paediatrics, University of Cambridge (COT)
Professor Kevin Chipman	Professor of Cell Toxicology, University of Birmingham (COT)
Professor Ian Rowland	Professor of Human Nutrition and Director of Northern Ireland Centre for Diet and Health, University of Ulster (COT)

For members' declaration of interests please refer to the Committee Annual Reports.

Secretariat for the Fish Inter-Committee Subgroup

Food Standards Agency
Dr Alison Tedstone (SACN – Scientific)
Dr Diane Benford (COT – Scientific)
Dr Peter Sanderson (SACN – Scientific)
Mr Jeff Allder (SACN – Administrative)

Department of Health
Dr Sheela Reddy (SACN – Scientific)

Membership of Scientific Advisory Committee on Nutrition (SACN)

Chair

Professor Alan Jackson	Professor of Human Nutrition, University of Southampton

Members

Professor Peter Aggett	Head of School, Lancashire School of Health and Medicine, Professor of Child Health and Nutrition, University of Central Lancashire
Professor Annie Anderson	Professor of Food Choice, Centre for Public Health Nutrition Research, University of Dundee
Professor Sheila Bingham	Deputy Director, Medical Research Council's Dunn Human Nutrition Unit, Cambridge
Professor John Cummings	Professor in Experimental Gastroenterology, Department of Pathology and Neuroscience, University of Dundee
Miss Gill Fine	Head of Food and Health, Sainsbury's Supermarkets Ltd
Mrs Christine Gratus	Retired Director and International Vice-President of J Walter Thompson Advertising Agency (lay member)

Dr Timothy Key	Reader in Epidemiology, University of Oxford Cancer Research UK Epidemiology Unit, Radcliffe Infirmary, Oxford
Professor Peter Kopelman	Professor of Clinical Medicine, Vice-Principal/Deputy Warden (Education), Barts and The London, Queen Mary's School of Medicine and Dentistry, University of London
Dr Ann Prentice	Director, Medical Research Council Human Nutrition Research, Cambridge
Professor Emeritus Andrew Rugg-Gunn	University Clinical Professor, Newcastle University Dental School
Dr Anita Thomas	Associate Medical Director / Consultant Physician in General (Internal) and Geriatric Medicine, Derriford Hospital, Plymouth Hospitals NHS Trust Clinical Sub Dean, Peninsula Medical School, Universities of Exeter and Plymouth
Mrs Stella Walsh	Senior Lecturer, Leeds Metropolitan University
Dr Anthony Williams	Senior Lecturer and Consultant in Neonatal Paediatrics, St George's Hospital, London
Professor Christine Williams	Professor of Human Nutrition, University of Reading

Observers

Mr Tom Murray	Food Standards Agency
Ms Imogen Sharp	Department of Health

Dr Lesley Wilkie	Scottish Executive, Health Department
Mrs Maureen Howell	The Welsh Assembly, Health Promotion Division
Dr Naresh Chada	Department of Health, Social Services and Public Safety, Northern Ireland

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Membership of Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT)

Chair

Professor Ieuan Hughes — Professor and Head of Department of Paediatrics, University of Cambridge

Members

Dr David Bell — Reader in Molecular Toxicology, University of Nottingham

Professor Alan Boobis — Professor of Biochemical Pharmacology, Imperial College, London

Dr Philip Carthew — Senior Pathologist, SEAC Toxicology Unit, Unilever

Dr Rebecca Dearman — Head of Immunology, Syngenta

Dr Joy Hinson — Reader in Molecular and Cellular Endocrinology, Barts and the London Queen Mary School of Medicine and Dentistry, University of London

Dr Peter Jackson — Reader in Clinical Pharmacology and Therapeutics, University of Sheffield

Professor Joseph Lunec — Director of Clinical Pathology, University of Leicester

Professor David Ray — Head of Applied Neuroscience Group, University of Nottingham Medical School

Professor Ian Rowland	Professor of Human Nutrition and Director of Northern Ireland Centre for Diet and Health (NICHE), University of Ulster
Dr Lesley Rushton	Head of Epidemiology, Medical Research Council, Institute for Environment and Health, University of Leicester
Dr Lesley Stanley	Head of Operations, CXR Biosciences
Professor Stephen Strobel	Director of Post Graduate Clinical Education, Peninsula Medical School, Plymouth
Miss Alison Ward	Public Interest Representative
Mrs Alma Williams	Public Interest Representative

1   Advice on fish consumption – overview

1.1   The Food Standards Agency (FSA) sought advice from the Scientific Advisory Committee on Nutrition (SACN) and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) on the benefits and risks of fish consumption, with particular reference to oily fish. A joint SACN/COT Subgroup was convened to consider the matter. The aims of the Inter-Committee Subgroup were to: bring together the nutritional considerations from SACN on fish consumption and the toxicological considerations of the contaminants in fish from COT; and weigh the nutritional benefits against possible risks and develop coherent dietary advice for the public on consumption of fish, with particular reference to oily fish.

The nutritional considerations

1.2   For detail see the nutritional considerations section. SACN reviewed the evidence on the health benefits of fish and fish oil consumption, with specific reference to cardiovascular disease risk and pregnancy outcome. The UK recommendations on fish consumption and long chain n-3 polyunsaturated fatty acid (LC n-3 PUFA) intake (Department of Health, 1994) were considered in light of the evidence that had arisen since they were made. The reported benefits of fish consumption on the development of some cancers and other aspects of brain function (e.g. cognitive decline) were not considered due to the paucity of data.

1.3   Evidence suggests that fish consumption, particularly that of oily fish, decreases the risk of cardiovascular disease (CVD); this is thought to be due to their LC n-3 PUFA content. The recommendation made by the Committee on Medical Aspects of Food Policy (COMA) to 'eat at least two portions of fish, of which one should be oily, weekly' (Department of Health, 1994) was based on a review of scientific evidence that related fish consumption (especially oily fish and fish oils) inversely to coronary heart disease (CHD). As most people in the UK consume considerably less than

one portion of oily fish per week, COMA concluded that CHD reductions would be gained by increasing levels of consumption.

1.4   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.

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

1.6   Randomized controlled trials (RCT) with subjects who had previously experienced a myocardial infarction (MI) have only used one dose of 0.9g/d LC-n-3 PUFA, which is equivalent to two-three portions of oily fish per week. These trials provide evidence that increased fish consumption, or fish oil supplementation, decreases mortality among patients who have suffered a MI. The most probable mechanism for the effect of 0.9g/d LC n-3 PUFA on secondary CHD prevention is the stabilization of arrhythmias. One randomized trial in angina patients found an adverse effect of fish advice on cardiac mortality.

1.7   The prospective epidemiological evidence is suggestive of a plateau effect in high-risk populations, at intakes of about 0.9g/d; however, where fatty acid composition analyses of blood or blood compartments have been determined, a positive relationship, with no plateau, is observed.

1.8   A number of studies have examined the mechanism by which fish oil improves cardiovascular health. Such studies have shown that a higher dose, of at least 1.5 g/d LC n-3 PUFA, is required for demonstrable beneficial effects on cardiovascular risk factors such as a reduction of

plasma triacylglycerol levels, blood pressure, platelet aggregation and the inflammatory response.

1.9 The evidence provided by RCTs is suggestive of beneficial effects occurring within a short time scale, from a few months to 2 years. Prospective studies, however, suggest a longer time-course before a beneficial effect is observed which might be due to a combination of statistical and biological considerations. The dose-response relationship between fish consumption and risk of CVD may vary in populations with different risks of CVD. Relative to other countries, the UK population is at high risk of CVD; however, sub-populations within the UK may exhibit different risk.

1.10 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.

1.11 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.

1.12 SACN emphasized that this recommendation represents a minimal and achievable average population goal and does not correspond to the level of fish consumption required for maximum nutritional benefit. The evidence to support benefit at higher levels of consumption is insufficient to enable accurate quantification.

1.13 It would be inappropriate to discourage fish consumption at levels higher than the dietary recommendation unless there was an upper limit beyond which people should not consume.

The toxicological considerations

1.14   The current key concerns relate to the dioxins and dioxin-like polychlorinated biphenyls (PCBs) and to methylmercury. In addition, there is a need to keep under review the concentrations in fish of other persistent organic pollutants such as the brominated flame retardants (BFRs).

1.15   It should be noted that the dioxins and dioxin-like PCBs and the BFRs of concern are persistent lipophilic compounds that accumulate in lipid. They are therefore particularly likely to be present in oily fish. In contrast, methylmercury is not specifically found in oily fish. It is present in the marine environment and accumulates up the food chain in fish, with levels being highest in large predatory species.

1.16   Tolerable Daily or Weekly Intakes are established to protect consumers from the adverse effects associated with chemical contaminants in food. The tolerable intake is set to protect against the most sensitive toxic effects in the most susceptible subgroups of the population, taking into account human variability, and is defined as an amount that can be consumed daily over an entire lifetime without appreciable risk to health. It is not a threshold for risk and there is uncertainty about the degree of risk above the tolerable intake. The most sensitive individuals may be at risk from a small exceedance, whereas many individuals will not be. Any risk is likely to increase with the degree and duration of exceedance of the tolerable intake, but COT has not considered it possible to quantify the risk.

1.17   There is currently no established methodology for risk-benefit analysis that can be applied to fish. This report therefore focuses on whether separate intake guidelines can be developed for different population groups. Such an approach would support dietary advice to consumers that would allow individuals at lesser risk of the toxic effects to maximize the nutritional benefits.

1.18   The most sensitive effects of chemical contaminants in fish relate to developmental changes in the unborn child, resulting from maternal exposure. On the basis that it takes about 5 half-lives to reach steady state body burden, for cumulative contaminants a woman's exposure before

pregnancy is likely to be more important for the total body burden than intake during pregnancy.

Methylmercury

1.19   The half-life of methylmercury is about 70 days in humans; fetal exposure is therefore likely to be determined by maternal exposure in the year leading up to pregnancy.

1.20   In December 2003, COT considered levels of mercury in fish (see Annex 3) and concluded:

- a methylmercury intake of 3.3μg/kg bodyweight per week may be used as a guideline to protect against non-developmental adverse effects.

- the 2003 JECFA PTWI[1] of 1.6μg/kg bodyweight per week is sufficient to protect against neurodevelopmental effects in the fetus. This PTWI should be used in assessing the dietary exposure to methylmercury of women who are pregnant, and who may become pregnant within the following year.

- a guideline of 3.3μg/kg bodyweight per week is appropriate in considering intakes by breastfeeding mothers as the intake of the breast-fed infant would be within the new PTWI of 1.6μg/kg bodyweight per week.

- consuming one weekly 140g portion of either shark, swordfish or marlin would result in a dietary methylmercury exposure close to or above 3.3μg/kg bodyweight per week in all age groups. We *consider* that this consumption could be harmful to the fetus of women who are pregnant or become pregnant within a year, but would not be expected to result in adverse effects in other adults.

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1 Provisional Tolerable Weekly Intake established by the Joint FAO/WHO Expert Committee on Food
   Additives (JECFA)

- the mercury content of tuna is lower than that of shark, swordfish or marlin, but higher than that of other commonly consumed fish. We *consider* that consumption of two 140g portions of fresh tuna, or four 140g portions of canned tuna, per week, before or during pregnancy would not be expected to result in adverse effects on the developing fetus.

1.21 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 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.

Dioxins and dioxin-like PCBs

1.22 In 2001, COT set a tolerable daily intake (TDI) of 2 pg WHO-TEQ/kg bw per day[2], to protect against effects on the developing male reproductive system resulting from the maternal body burden of dioxins (see Annex 5). This TDI was considered adequate to protect against other possible effects of dioxins, such as cancer and cardiovascular effects.

1.23 The Inter-Committee Subgroup established a guideline level to protect against non-developmental effects of dioxins and dioxin-like PCBs, in line with the approach taken by COT for methylmercury.

- A guideline level of 8 pg TEQ/kg bodyweight per day is appropriate in considering intakes in relation to the most sensitive and relevant non-development effect of dioxins – increased cancer risk.

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2 Toxic Equivalency Factors (TEFs) allow concentrations of the less toxic dioxin-like compounds to be expressed as a concentration equivalent to the most toxic dioxin - 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). These toxicity-weighted concentrations are then summed to give a single value, which is expressed as a Toxic Equivalent (TEQ). The system of TEFs used in the UK and a number of other countries is that set by the World Health Organization (WHO), and the resulting overall concentrations are referred to as WHO-TEQs

1.24   Because the dioxins and dioxin-like PCBs have half-lives of several years in humans, exposure throughout life up to time of pregnancy will determine the exposure to the fetus. The Inter-Committee Subgroup, therefore, agreed that the TDI should be used in considering dietary exposure to dioxins of females up to and including reproductive age. The guideline level could be used for older women and for males, and, because it is derived from a lifetime study, it also applies to young males.

1.25   The Subgroup also noted that an intake of twice the TDI for up to 12 months had a minimal effect on the body burden and was therefore not expected to result in adverse effects.

1.26   The Inter-Committee Subgroup were provided with estimates of dietary intake of dioxins and dioxin-like PCBs by an average 60kg adult from a range of oily fish together with intake from the rest of the diet. Overall, these data indicated that consumption of about two portions of oily fish per week could be consumed without appreciable exceedance of the TDI. Four portions of oily fish could be consumed per week without exceeding the guideline level. Fish containing higher concentrations of dioxins, such as herring, should be consumed less frequently than fish with lower amounts, such as trout. Salmon and mackerel have intermediate dioxin content.

1.27   The Subgroup noted uncertainty with respect to the effects of obesity, or of rapid weight loss during dieting, on the body burden, although it recognized that these factors would influence circulating blood concentrations of dioxins and dioxin-like PCBs. There is a need for information on whether the uncertainty factor incorporated into the TDI and guideline level is adequate to allow for this aspect of human variability.

Conclusions

1.28   The majority of the UK population does not consume enough fish, particularly oily fish, and should be encouraged to increase consumption. The Inter-Committee Subgroup endorsed the COMA population guideline recommendation that people should eat at least two portions of fish a week,

of which one should be oily. Consumption of this amount would probably confer significant public health benefits to the UK population in terms of reducing CVD risk. There may also be beneficial effects on fetal development.

1.29   The Inter-Committee Subgroup stated that this recommendation should also apply to pregnant and lactating women, subject to the restrictions on certain fish – marlin, swordfish, shark and, to a lesser extent, tuna – due to methylmercury contamination.

1.30   With regard to high levels of oily fish consumption and the dioxins and dioxin-like PCB contaminants therein, the evidence base is insufficient to conduct a quantitative risk-benefit analysis. Separate intake guidelines were, therefore, developed for different population groups.

1.31   The Inter-Committee Subgroup noted that it might be beneficial for some subgroups to consume more than the guideline recommendation, but was unable to identify a precise level. It was decided that a guideline range for oily fish consumption, based on the nutritional and toxicological considerations (levels at which there would be clear benefits without undue risk), should be recommended.

1.32   The guideline ranges for oily fish consumption were for:

•   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.

1.33   It was noted that consumers would need to be provided with information on the levels of dioxins and dioxin-like PCBs present in different species

of commonly consumed fish. This would enable consumers to make informed choices on the number and type of fish consumed per week.

1.34 The Inter-Committee Subgroup emphasized that exceeding the designated ranges over the short-term was not deleterious, but long-term exceedances could have deleterious effects in sensitive individuals. In the case of pregnant and lactating women, for example, a woman who had not consistently exceeded the guideline range previously, could increase her oily fish consumption throughout pregnancy and lactation above the guideline range (e.g. to 2-3 portions of oily fish a week) without detrimental effects.

2   Nutritional considerations

2.1   The Committee reviewed the evidence on the health benefits of fish and fish oil consumption with specific reference to cardiovascular disease risk and pregnancy outcome. The recommendations on fish consumption and long chain n-3 polyunsaturated fatty acid intake by the COMA (Department of Health, 1994) were considered, with regard to the evidence that had arisen since.

Current UK recommendations:

- That people eat at least two portions of fish, of which one should be oily

- An increase in the population average consumption of long-chain n-3 PUFA from about 0.1g/day to about 0.2g/day

2.2   The Committee recognized that groups of the population do not eat fish (e.g. vegetarians and vegans), but considered the evidence base insufficient to conduct a risk assessment on this issue, and did not, therefore, make specific recommendations for this group.

Fish consumption in the UK

2.3   White fish have flesh that is very low in fat as these fish accumulate fat in their livers, e.g. cod. Oily, or fatty, fish have fat in their flesh – the amount is related to their breeding cycle; after breeding, the fat content falls considerably. Oily fish are 5-20% fat compared with 1-2% fat for white fish. Oily fish include, sardines, salmon, pilchards, mackerel, herring and trout, whether fresh, frozen or canned. Fresh tuna is also included; however, unlike other canned oily fish, canned tuna is not regarded as oily, as processing of tuna during canning reduces the fat content of the fish to a low level. White fish include cod, haddock, turbot, bream, bass etc. Please see Annex 1, which lists in Table 4.1 oily and white fish and in Table 4.2 details of the commonly consumed fish in the UK taken from the 2000/2001 National Diet and Nutrition Survey (NDNS).

2.4   The UK population average total fish and fish products consumption was 143 grams/person/week in 2000 (National Food Survey). Of this total 36 grams was fresh, frozen or processed white fish and 20 grams was fresh and processed (other than canned) oily fish. Consumption of canned salmon was 6 grams/per person/week and other canned or bottled fish (including tuna) 26 grams. The remainder of the total is accounted for by cooked fish and fish products and shellfish.

2.5   The latest NDNS (Henderson *et al.,* 2002) of adults aged 19-64 years shows that mean consumption of white fish (including products and dishes) was 103g/week and for oily fish (excluding canned tuna) 50g/week. Correspondingly, mean consumption of white fish and oily fish by consumers was 221g/week and 194g/week respectively. Details are given in Annex 1, Tables 4.3-4.6. Most people in the UK consume very little fish. For example, during the period of the NDNS survey 74% of the participants did not consume oily fish (excluding canned tuna), 65% did not consume coated and/or fried white fish and 82% did not consume other white fish and dishes (Henderson *et al.*, 2002).

2.6   Comparison of the National Food Survey data from 1979 and 1999 shows that consumption of total fish and fish products increased by 13% between 1979 and 1999. Within the total, consumption of fresh oily fish more than doubled since 1979 while processed canned and shellfish increased by over 60% and fish products by over 40%.

2.7   The average fish portion size for adults is 140g. Details of other age groups given in Annex 1, Table 4.7.

2.8   The mean consumption of oily fish by adults (Henderson *et al.,* 2002) has increased from 34g to 53g/week (from about a quarter to a third of a portion) since the last survey of this age group in 1986/87. The mean consumption of oily fish (excluding tinned tuna) by consumers has increased correspondingly from 134g/week to 194g/week. This is mainly due to an increase in consumption by women, particularly older women. Increased salmon consumption largely accounts for the increase.

2.9   Long chain polyunsaturated fatty acids (LC PUFA) are defined as those fatty acids that comprise 20 or 22 carbon atoms. Eicosapentaenoic acid (20:5n-3; EPA) and docosapentaenoic acid (22:5n-3; DPA) and docosahexaenoic acids (22:6n-3; DHA) are collectively referred to as LC n-3 PUFA. The estimate used for LC n-3 PUFA content of an average oily fish is 2g/100g and for an average white fish 0.3g/100g (see Annex 1 Table 4.2). The LC n-3 PUFA content of shellfish based on the average consumption is about 0.4g/100g (derived from the National Food Survey).

Defining LC n-3 PUFA status

2.10   The LC n-3 PUFA content of different cell membranes and plasma constituents, as well as adipose stores, can be modified by dietary intake; however, the exact relationship between LC n-3 PUFA dietary intake and changes in the content of these different pools is not understood. Different pools may vary in their responsiveness to changes in dietary LC n-3 PUFA intake (Vidgren *et al.,* 1997). Homeostatic regulation may diminish sensitivity to dietary intake, e.g. membrane fluidity maybe maintained for functional reasons in red blood cells, membrane LC n-3 PUFA content may affect lymphocyte cell signalling.

2.11   As there is no agreed definition of what is meant by LC n-3 PUFA status, or how it is best measured or characterized. The term will only be used in the most general sense in this paper. It would be inappropriate to use the term where what is really meant is blood concentration, or some other single measure, which might or might not be taken to reflect overall status.

The effects of LC PUFA on early human growth and cognitive function

Background

2.12   DHA and arachidonic acid (AA) are essential for the development of the central nervous system in mammals. There is a growth spurt in the human fetal brain during the last trimester of pregnancy and the first postnatal months when a large increase in the cerebral and retinal content of AA and DHA occurs. These two LC PUFA can be synthesized from precursor essential fatty acids by chain elongation and desaturation: AA from linoleic

acid of the n-6 series, and DHA from alpha-linolenic acid (ALA) of the n-3 series. The same enzymes are utilized by the different series, resulting in competition between the n-6 and n-3 fatty acids. AA and DHA are preferentially incorporated in the cell membranes of neuronal cells, where they modulate the structure, fluidity and function of the membrane. DHA acyl chains promote the function of the G-protein-coupled system in photoreceptor cell membranes and enhance the signalling pathways of metarhodopsin II (see Larque *et al.,* 2002).

2.13   Although glial cells, astrocytes and cerebral endothelium may elongate and desaturate the precursor essential fatty acids, the main source of the DHA and AA that accumulates in the brain is drawn from the maternal circulation. Neither the fetal retina nor brain initially synthesizes LC PUFA and the capacity of the fetal brain to synthesize LC PUFA is a function of gestational age (Clandinin 1999), making placental transfer of LC PUFA crucial. During the last trimester of pregnancy fetal requirements for DHA and AA are especially high because of the rapid synthesis of brain tissue (Clandinin *et al.,* 1980; Martinez, 1992). The fetus and newborn infant are dependent on a maternal supply of DHA and AA.

2.14   As a consequence of the increased demand on maternal DHA supplies, it has been hypothesized that depletion of maternal DHA stores occurs over pregnancy, and successive pregnancies and periods of lactation may reduce levels further (Al *et al.,* 2000; Otto *et al.,* 2001).

2.15   In humans, the fetal and infant brain DHA content appears to be more affected by diet than AA content, suggesting that the endogenous metabolic regulation of AA content is more effective than that of DHA (Makrides *et al.,* 1994). In human breast milk the AA content is maintained within narrow limits; whereas, more than four-fold differences have been observed in the content of the n-3 PUFA series (ALA and DHA) (Rodriguez-Palmero *et al.,* 1999).

2.16   Markers of maternal LC n-3 PUFA status vary with fish and/or LC n-3 PUFA consumption during pregnancy. Regular consumption of oily fish (Olsen *et al.,* 1991; Sanjurjo *et al.,* 1995) or supplementation with LC n-3 PUFA (van Houwelingen *et al.,* 1995; Connor *et al.,* 1996) resulted in

increased circulating maternal DHA during pregnancy and at term. Maternal supplementation with ALA, however, was not shown to increase maternal and neonatal DHA plasma concentrations, despite increasing EPA and DPA concentrations (de Groot *et al.,* 2004); likewise, although ALA supplementation resulted in increased ALA and EPA content of human breast milk, DHA levels were unaffected (Francois *et al.,* 2003). A dose-dependent increase in the DHA content of human breast milk was observed, however, with fish oil supplementation (Harris *et al.,* 1984).

Maternal DHA requirements in pregnancy and lactation

2.17   Using the information available an assessment was conducted of the demands placed on a mother during pregnancy and lactation for n-3 PUFA, and her likely ability to meet these demands, either from her dietary intake, from her tissue reserves, or from *de novo* formation from precursors taken in the diet or mobilized from tissue reserves (see Annex 2 for details). Conservative estimates were used, based upon the limited data available, and it was assumed that all other nutrients were available in adequate amounts and no other factors operated to limit normal metabolic inter-conversions. The assessment concluded that for a significant proportion of women it is very likely that the demands of pregnancy and lactation are greater than can be readily achieved from the sum of current levels of dietary consumption, endogenous mobilization and *de novo* formation. This would suggest that a significant proportion of women are potentially at risk of inadequate LC n-3 PUFA status; however, the data currently available from which to draw this conclusion are limited.

Maternal LC n-3 PUFA dietary intake and infant neurodevelopment and growth

2.18   Williams et al (2001) observed in a prospective cohort study of 435 children that those children whose mothers ate oily fish during pregnancy, compared with those who did not, tended to have better visual function (stereoacuity) when assessed at age 3.5 years.

2.19   A cross-sectional study of 39 four month old breast-fed term infants (Jorgensen *et al.,* 2001) suggested a positive association between infant human milk DHA intake and visual acuity.

2.20 Two recent prospective cohort studies have investigated the relationship between umbilical venous plasma DHA and AA levels and cognitive function in 128 four year olds (Ghys *et al.,* 2002) and 306 seven year olds (Bakker *et al.,* 2003); however, no significant association was found.

2.21 Olsen et al (1995) suggested that higher DHA and EPA intake from fish in Faroe Islanders compared with Danes was the reason for longer gestation in Faroe Islanders. A more recent prospective cohort study (Olsen & Secher, 2002) of 8729 pregnant women found that low consumption of fish was a strong risk factor for preterm delivery and low birth weight. This relation was strongest below an estimated daily intake of 0.15g/d LC n-3 PUFA or 15g/d fish.

Infant formula supplementation with LC PUFA

2.22 In contrast to human milk, conventional milk infant formulas with fat derived from vegetable oils do not provide appreciable amounts of LC PUFA. A decrease in plasma and red blood cell AA and DHA content was observed in infant formula fed as compared with breast-fed infants (Makrides *et al.,* 1995). Moreover, the proportion of DHA in the brain cortex of breast-fed infants was higher compared to those fed infant formula without LC-PUFA (Makrides *et al.,* 1994).

2.23 Many studies have been undertaken to assess whether increasing LC PUFA dietary intake affects visual and cognitive functions in preterm and full-term infants. These are difficult studies since factors influencing brain development are complex and multi-factorial, and potential confounders include birth weight, parental education and socio-economic status, smoking, variability in the infants DHA levels at birth, different PUFA ratios among the infant formulas studied, samples size and different test methodology. These studies used doses of LC PUFA that were comparable with the concentrations found in human milk.

2.24 Possible adverse effects of supplementing infant formulas with LC PUFA have also been described. In preterm infants, postnatal growth was reduced by the feeding of infant formulas supplemented with fish oil rich in the LC n-3 PUFA eicosapentaenoic acid (EPA), but no appreciable amount of AA, thus inducing a reduction of plasma AA concentrations (Carlson *et al.,*

1993b). In these studies plasma AA concentrations were positively correlated with postnatal growth.

2.25   Similarly, a high dietary supply of ALA, associated with a low dietary ratio of n-6:n-3 PUFA, concomitantly reduced both plasma AA and weight gain until the age of 120 days in healthy term infants (Jensen *et al.,* 1997). In contrast, the provision of infant formulas with an adequate and balanced supply of dietary AA and DHA has been shown not to have adverse effects on growth (Koletzko *et al.,* 2001).

Visual function

2.26   Many studies investigating the effect of nutritional factors on neurodevelopment have used visual functions as outcome measures because of the well documented increases in visual functions in the first years of life (Teller, 1997). Visual acuity tests measure the integrity of the neural pathway from the retina to the occipital cortex and provide a surrogate marker of central nervous system function; however, the long-term significance of improved retinal and visual function on later neurodevelopment has yet to be shown. For preterm infants various studies have shown that those who were breast-fed had better visual acuity at 2-4 months of age and more advanced retinal development than those who were infant formula fed (Birch et al 1992a, 1992b). In full-term infants, some evidence suggests that breast-feeding is associated with enhanced visual function at age 3.5 years (Williams *et al.,* 2001), and children whose mothers ate oily fish during pregnancy, as compared with those who did not, tended to have better visual function.

Effects of LC PUFA on visual function

2.27   Preterm infants with birth weight of <1500g have a limited fat stores at birth, a possible insufficiency in the elongation/desaturation enzymatic pathways and an inadequate intake of LC PUFA provided by infant formula (Uauy *et al.,* 2001). Randomized controlled trials (RCT) that have included infant formula feeding with or without LC PUFA and assessed visual function in preterm and full-term infants are summarized in Tables 2.1 and 2.2 respectively.

Table 2.1: Effects of LC PUFA on visual function in preterm infants

Reference	Experimental group (n)	Post-conceptional age assessment (wk)	Measure	Outcome
Uauy et al., 1990	10-12	36	ERG	Marine oil infant formula and breast milk improved VF
Birch et al., 1992a	Further follow-up	57	Teller, VEP	Marine oil infant formula and breast milk improved VF
Birch et al., 1992b	9-16	36 & 57	VEP, FPL	Marine oil infant formula and breast milk improved VF
Carlson et al., 1993	33	38, 48, 57, 68, 79 & 92	Teller	Marine oil infant formula improved VF upto 48 wk; VF was associated with DHA concentrations upto 48 wk
Carlson & Werkman, 1996a	33-34	68, 79 & 92	Fagan	Marine oil infant formula improved VF upto 48 wk
Werkman & Carlson, 1996	26-33	39, 48, 57, 68, 79 & 92	Teller	Marine oil infant formula improved VF upto 48 wk
Carlson et al., 1996b	12-15	92	Fagan	Marine oil infant formula improved VF
Fadella et al., 1996	12-25	52	Flash VEP, ERG, BAEP	Marine oil infant formula and breast milk improved VEP only
O'Connor et al., 2001	140-143	8, 16, 26, 36, 52	Teller, Fagan, sweep VEP,	Marine oil infant formula with either fungal or egg DHA infant formula improved VEP only
van Wezel-Meijler et al., 2002	22	23, 36, 62, 114	Teller, Flash VEP	No effect; although. marine oil infant formula group showed non significant improvement in VF at 23 wk

VF, visual function; Teller, Teller acuity cards; FPL, forced-choice preferential looking; Fagan, Fagan novelty preference; VEP, visual evoked potentials; ERG, electroretinography; BAEP, brainstem acoustic evoked potentials.

2.28   These trials support the efficacy of LC PUFA intake on the early development of the visual system, which was not achieved to similar extents with infant formulas providing the precursor PUFA: linoleic acid or ALA. A meta-analysis by San Giovanni et al (2000) concluded that LC PUFA supplemented infant formulas showed significant differences at two

and four months of age. Similarly, a Cochrane review concluded that there is evidence that LC PUFA supplemented infant formula increases the early rate of visual maturation in preterm infants, although this did not take into account trials later than 1998 (Simmer, 2002).

Table 2.2: Effects of LC PUFA on visual function in full-term infants

Reference	Experimental group (n)	Assessment age (mth)	Measure	Outcome
Makrides et al., 1995	13-23	4, 7	VEP	Marine oil infant formula and breast milk improved VF
Carlson et al., 1996c	19-20	2, 4, 6, 9 & 12	Teller	Marine oil infant formula improved VF at 2 mth only
Auestad et al., 1997	26-28	2, 4, 6, 9 & 12	Sweep VEP, FPL	No effect
Jorgensen et al., 1998	11-25	4	Sweep VEP	Only breast milk improved VF; although. marine oil infant formula group showed non significant improvement
Birch et al., 1998	22-23	1.5, 4, 6, 12	Sweep VEP, FPL	Marine oil infant formula and breast milk improved VEP only
Hoffman et al., 2000	29	1.5, 4, 12	ERG, VEP	Marine oil infant formula and breast milk improved VF
Makrides et al., 2000	21-46	4, 8	Flash VEP	No effect of marine infant formula, but breast-fed infants had better VEP acuity at 34 weeks of age, but not at 16 weeks.
Auestad et al., 2001	119-120	12	VEP, Teller	No effect of either breast-feeding or Marine oil infant formula
Auestad et al., 2003	Follow-up	39	VMF, Teller	No effect of either breast-feeding or Marine oil infant formula

VMF, visual-motor function.

2.29   Some of the trials in healthy term infants show that LC PUFA improved visual acuity during the first year of life, but others found no significant effect. None of the trials reported negative effects on visual acuity. Differences among the results may be due to differences in the methodology and in supplementation strategies (Larque *et al.,* 2002).

2.30   Two recent RCTs where the infants were weaned from breast-feeding at 1.5 and 4-6 months respectively are summarized in Table 2.3.

Table 2.3: Effects of LC PUFA on visual function in full-term infants post weaning

Reference	Experimental group n number	Assessment age (mth)	Measure	Outcome
Birch et al., 2002	32-33	1.5, 4, 6, 12	Sweep VEP, stereoacuity	Marine oil infant formula improved VEP only at 4, 6 and 12 mth
Hoffman et al., 2003	30-31	12	Sweep VEP, stereoacuity	Marine oil infant formula improved VEP

2.31   Beneficial effects of LC-PUFA supplementation on visual function were observed. The first of these two trials (Birch *et al.,* 2002) provide evidence for a continued need for DHA in the infant diet beyond six weeks, while the latter (Hoffman *et al.,* 2003) extends this age to beyond four months.

Effects of LC PUFA on behavioural development

2.32   Different tests have been used to examine the effects of postnatal dietary LC-PUFA on neurodevelopment (Carlson, 2000). At present, it remains unclear which tests are most sensitive to detect any potential effects of LC PUFA. RCTs that have included infant formula feeding with or without LC PUFA and assessed behavioural development in preterm and full-term infants are summarized in Tables 2.4 and 2.5 respectively.

2.33   Overall, the results are equivocal, with some trials showing an effect of LC PUFA supplementation on the tests of behaviour employed while others do not. In nearly all trials that observed no effect of marine oil infant formula, no effect of breast-feeding was observed. While Agostoni et al observed no effect of marine oil infant formula (1997), developmental quotients were positively correlated with both AA and DHA levels at 4 months.

Table 2.4: Effects of LC PUFA on behavioural development in preterm infants

Reference	Experimental group n number *	Post-conceptional age assessment (wk)	Measure	Outcome
O'Connor et al., 2001	140-143	36, 52, 78	BMD, MacA	No overall effect of marine oil infant formula; however in infants with birth weight < 1250g marine oil infant formula group showed higher PDI for BMD
Lucas et al., 2001	65-116	49, 88	KP&S, BMD	No effect of either breast milk or marine oil infant formula
Fewtrell et al., 2002	78-81	49, 88	BMD PDI	Breast milk, but not marine oil infant formula improved scores
Wezel-Meijler et al., 2002	22	23, 36, 62, 114	BMD, PDI	No effect of marine oil infant formula

BMD, Bayley Mental Development Index; MacA, MacArthur Communicative Development Inventory; PDI, psychomotor developmental index; KP&S, Knobloch, Passamanick and Sherrards' developmental screening inventory. * the range of n numbers in the experimental and control groups is given.

2.34   A follow-up of the Willatts et al trial (1998), which also included other centres that participated in the original safety and tolerance studies, examined blood pressure at age six in relation to the trial interventions (Forsyth *et al.,* 2003). Children who had received either breast milk or LC PUFA supplemented infant formula had significantly lower blood pressure than those who received the non-supplemented infant formula.

Summary of the infant formula LC PUFA supplementation trials

2.35   The trials investigating an effect of LC n-3 PUFA on visual function in preterm infants consistently demonstrate a short-term beneficial effect on VEP.

2.36   The trials investigating an effect of LC n-3 PUFA on visual function in term infants are less consistent: six out of ten trials demonstrate a beneficial effect, particularly on VEP, but others, including the largest trial (Auestad *et al.,* 2001, 2003), failed to demonstrate an effect.

Table 2.5: Effects of LC PUFA on behavioural development in full-term infants

Reference	Experimental group n number	age (mth)	Measure	Outcome
Agostoni et al., 1995	27-30	4	B-L	Marine oil infant formula and breast milk improved
Agostoni et al., 1997	25-30	24	B-L	No effect of either breast-feeding or marine oil infant formula
Willatts et al., 1998	21-22	10	Problem solving	Marine oil infant formula improved problem solving
Scott et al., 1998	33-60	12, 14	MacA BMD	No effect of either breast-feeding or marine oil infant formula
Lucas et al., 1999	138-155		BMD	No effect of either breast-feeding or marine oil infant formula
Birch et al., 2000	17-20	18	BMD	No effect of marine oil infant formula
Makrides et al., 2000	21-46	4, 8	BMD	No effect of either breast-feeding or marine oil infant formula
Auestad et al., 2001	119-120	12	MacA BMD	No effect of either breast-feeding or marine oil infant formula
Auestad et al., 2003	Follow-up	39	IQ, Peabody	No effect of either breast-feeding or marine oil infant formula
Bouwstra et al., 2003	119-131	3	GM	Marine oil infant formula and, more so, breast-feeding improved general movements

B-L, Brunet-Lézine test; IQ, Stanford Binet IQ; Peabody, Peabody picture vocabulary test-revised; GM, general movements.

2.37   Eleven out of fourteen trials investigating an effect of LC n-3 PUFA on behavioural measures in both preterm and term infants failed to demonstrate an effect.

2.38   No adverse effect was observed in any of the trials investigating an effect of a balanced intake of LC n-3 PUFA on behavioural and visual function measures in preterm and term infants.

Effects of maternal LC n-3 PUFA dietary intake on infant neurodevelopment and growth

2.39   RCTs that have supplemented pregnant women with LC n-3 PUFA and assessed infant neurodevelopment are summarized in Table 2.6. It should be noted that those studied by Helland et al (2003) represent only a small subgroup of offspring from the 590 pregnancies recruited.

Table 2.6: Effects of maternal LC n-3 PUFA supplementation on infant neurodevelopment

Reference	Population	Dose* (g/d)	Start ^ (wk)	Outcome
Helland et al., 2003	Fish oil 48 Control 36	2	18 until 3 months post-partum	Marine oil supplementation improved mental processing composite of the K-ABC tests a four years of age (106 [7.4] vs 102.3 [11.3]; P=0.049); an association for higher scores for the sequential processing scale, simultaneous processing scale and non-verbal scale was also observed
Malcolmet al., 2003a & b	Fish oil 28 Control 27	0.2	15 until birth	No effect on VEP or ERG
^ week of pregnancy supplement started; * LC n-3 PUFA.				

2.40   RCTs that have supplemented pregnant women with LC n-3 PUFA during the third trimester and assessed gestation length and infant growth are summarized in Table 2.7.

2.41   Olsen et al (2000) also examined the effect of maternal LC n-3 PUFA supplementation on women who had previously experienced intrauterine growth retardation or pregnancy induced hypertension respectively; however, no effect was observed. Another trial, also on women who had previously experienced intrauterine growth retardation or pregnancy induced hypertension, also observed no effect of fish oil supplementation (Onwude *et al.,* 1995).

Table 2.7: Effects of maternal LC n-3 PUFA supplementation on gestation length and fetal growth

Reference	Population		Dose* (g/d)	Start ^	Birth weight (g)	Gestation length (d)	Outcome
Olsen et al., 1992	Intervention	266	2.7	30	3571 (528)	283.3 (11.1)	Fish oil increased gestation length
	Control	136			3445 (510)	279.4 (13.1)	
	Control (no oil)	131			3504 (531)	281.7 (11.6)	
Olsen et al., 2000	Intervention	110	2.7	20	3169 (674)	269.2 (19.7)	Fish oil reduced recurrence of preterm delivery
	Control	122			2960 (707)	260.7 (29.5)	
	Women who had previous preterm deliveries						
Helland et al., 2001	Intervention	175	2.0	18	3609 (493)	279.6 (9.2)	No effect
	Control	166			3618 (527)	279.2 (9.3)	
Malcolm et al., 2003a	Intervention	31	0.2	15	3507 (500)	279.7 (9.5)	No effect
	Control	29			3645 (495)	279.6 (8.5)	
Smuts et al., 2003	Intervention	142	0.13[†] DHA only	26	3209 (533)	274.1 (13.5)	High-DHA eggs increased gestation length #
	Control	149			3106 (551)	271.6 (15.6)	

Mean (SD); ^ week of pregnancy supplement started; * LC n-3 PUFA; [†] from high-DHA eggs; # after controlling for maternal BMI and number of previous pregnancies (276.5d versus 270.5d).

Summary of the trails investigating maternal LC n-3 PUFA dietary intake on infant neurodevelopment and growth

2.42   There is some evidence that increased maternal LC n-3 PUFA supply produced beneficial effects, especially in lower birth weight populations, and this may be more relevant in populations that tended to have a lower background intake of LC n-3 PUFA (Smuts at al, 2003). No adverse effects of maternal LC n-3 PUFA supplementation were observed, even at relatively high doses.

2.43   In the trials that observed no effect of maternal LC n-3 PUFA supplementation on infant birth weight or gestation length the control group had birth weights greater then 3600g.

Fish consumption and cardiovascular disease

2.44   The type of evidence presented in this paper has been restricted to human population studies with disease end points – essentially, prospective cohort studies and RCTs, although some case-control studies have also been included (see Tables 2.8 and 2.9). A large body of literature exists investigating the effects of fish, fish oils and LC n-3 PUFA consumption on CVD risk factors and much mechanistic work, including cell culture, animal and human studies, have been undertaken. These will not, however, be discussed in detail here. This paper is based on the advice sought by the FSA on the benefits of oily fish and fish oil consumption from SACN (http://www.sacn.gov.uk/sacn0212.pdf).

2.45   Several prospective cohort studies have investigated the relationship between fish consumption and the incidence of thrombotic stroke (Keli *et al.,* 1994; Morris *et al.,* 1995; Gillum *et al.,* 1996; Orencia *et al.,* 1996; Iso *et al.,* 2001; He *et al.,* 2002); however, the evidence remains equivocal and for the purposes of this paper the relationship between fish consumption and the incidence of CHD will be discussed. All studies observed no significant association between consumption of fish or fish oil and haemorrhagic stroke.

Prospective epidemiological studies

2.46   An inverse relationship between fish consumption, and LC n-3 PUFA intake, and CHD mortality has been reported in several (Mozaffarian *et al.,* 2003; Hu *et al.,* 2002; Yuan *et al.,* 2001; Kromhout *et al.,* 1995, 1985; Rodriguez *et al.,* 1996, Dolecek *et al.,* 1991), although not all (Osler *et al.,* 2003; Gillum *et al.,* 2000; Albert *et al.,* 1998; Kromhout *et al.,* 1996; Ascherio *et al.,* 1995; Morris *et al.,* 1995), prospective cohort studies.

2.47   In the Health Professionals Follow-up Study, (Ascherio *et al.,* 1995) a non-significant trend (RR, 0.74 95% CI 0.44 – 1.23) for a lower risk of fatal CHD with increasing fish consumption was observed; and although no association with CHD mortality was observed in the US Physicians' Health Study (Albert *et al.,* 1998) there was a significant reduction in sudden cardiac death with increasing fish consumption – this association was not observed after the initial follow-up period (Morris *et al.,* 1995). In

the Seven Countries Study (Kromhout *et al.,* 1996) fish intakes were inversely related to 25-year mortality from CHD in univariate analyses, but these associations became non-significant when the confounding effects of saturated fatty acids, flavonoids (a confounder not considered in many earlier studies) and smoking were taken into account.

2.48   A study in middle-aged Danish adults, however, found no inverse association between fish consumption and risk of CHD mortality or overall mortality (Osler *et al.,* 2003). Also, despite an inverse association between fish consumption and all cause mortality being observed in the NHANES I epidemiological follow-up, no association with CHD mortality was observed (Gillum *et al.,* 2000).

2.49   A systematic review of 11 prospective cohort studies by Marckmann and Grønbæk (1999) concluded that populations at high risk of CHD benefited most from increased consumption of fish. However, more recent studies have demonstrated an association of fish consumption with a reduced risk of CHD in populations with a lower CHD incidence, e.g. Shanghai, China (Yuan *et al.,* 2001). The risk of CHD increases markedly with age, as does the prevalence of risk factors such as hypertension and hypercholesterolaemia. Where an association between fish intake or n-3 fatty acids derived from fish has been reported, this has been in middle-aged and elderly subjects. The potential for CHD risk reduction, therefore, is likely to be greatest for those at highest risk; however a small risk reduction for the whole population could have a large public health benefit.

2.50   Furthermore some of the initial cohort reports showing no association on initial follow up have produced positive findings for fish consumption in the longer term. In the Honolulu Heart Program the initial report (Curb and Reed, 1985) suggested no relationship between fish intake and CHD risk and this led to the view at the time that a protective effect was only seen in populations with low fish intake. Later analysis, however, from the Honolulu Heart Program showed that fish intake and LC n-3 PUFA derived from fish were associated with a significantly reduced risk of CHD mortality (Rodriguez *et al.,* 1996).

2.51   The Nurses' Health Study (Hu *et al.,* 2002) recently reported an inverse association between fish intake and LC n-3 PUFA and CHD mortality in women. Compared with women who rarely ate fish (less than once per month), the risk for CHD death was 21%, 29%, 31%, and 34% lower for fish consumption 1 to 3 times per month, once per week, 2 to 4 times per week, and >5 times per week, respectively (*P* for trend<0.001). Comparing the extreme quintiles of fish intake, the reduction in risk for CHD deaths seemed to be stronger for CHD death than for nonfatal myocardial infarction (MI) (RR 0.55 versus 0.73).

2.52   In the Kuopio Ischaemic Heart Disease Risk Factor Study, a prospective population study in Eastern Finland (Rissanen *et al.,* 2000), an observed beneficial association of fish consumption on CHD mortality was shown to be attenuated by high mercury content in fish. More recently, the European Multi-Centre Case-Control Study (EURAMIC) (Guallar *et al.,* 2002) reported that DHA adipose levels (a measure of long-term fish consumption) were inversely, but not significantly, associated with risk of myocardial infarction, but that this inverse relation became stronger and statistically significant after adjustment for mercury levels. A previous study by this group showed no association between DHA adipose levels and risk of recurrence of myocardial infarction. However, levels of mercury contamination were not determined (Guallar *et al.,*1999). Although an association between mercury levels and CHD was observed in the EURAMIC analysis this was not observed in a nested case-control study of the US Heath Professionals' cohort (Yoshizawa *et al.,* 2002). Also, the studies suggesting that mercury attenuated the beneficial association of LC n-3 PUFA, still reported a positive association between fish consumption and CHD (Rissanen *et al.,* 2000; Guallar *et al.,*2002).

2.53   Overall, the prospective cohort studies suggest that those who consume fish have a lower risk of CHD than those who do not; and in high risk populations there appears to be a dose-dependent benefit of increasing fish consumption of up to 40-60g/d mixed type (corresponding to about 0.9g/d LC n-3 PUFA) (Marckmann and Grønbæk, 1999). This is borne out in a recent prospective cohort study in subjects aged 65 years or older, but with no known cardiovascular disease at entry to the study (Mozaffarian *et al.,* 2003). Five doses of fish consumption were assessed: less than once a

month; once to thrice a month; once a week; twice a week; and more than three times a week (estimated at 0, 0.13, 0.27, 0.55 and 0.92 g/d LC n-3 PUFA respectively). Total CHD deaths, and especially arrhythmic CHD deaths, were sequentially reduced with increasing fish intake; there was a 49% and a 58% lower risk of total CHD and arrhythmic CHD respectively with fish consumption more than three times a week compared with less than once per month.

2.54   More evidence for the benefits of fish consumption comes from studies that have explored the relationship between intermediary markers of fish consumption and CHD in men. These studies have measured the fatty acid composition of cell membranes and blood.

2.55   In the first follow up in the Physicians' Health Study, (Guallar, 1995) concentrations of DHA and EPA in plasma cholesterol esters and phospholipids did not differ between subjects with CHD and controls; however, in a more recent analysis of the same cohort (Albert *et al.,* 2002) whole blood levels of EPA and DHA were found to be lower at baseline in 94 men who subsequently died of sudden cardiac arrest, than in 184 controls matched for age and smoking (Albert et al. 2002). The relative risk of sudden death in subjects with levels of long chain LC n-3 PUFA in the highest quartile (ave. 6.87% total fatty acids) was 10% of those in the lowest quartile (ave. 3.58% total fatty acids) (P<0.001). A threshold effect that was observed in a prospective cohort study for protection against sudden death in relation to increased fish consumption (Albert *et al.,* 1998) was not seen in a nested case-control study within this cohort for blood levels of LC n-3 PUFA. A prospective nested case-control analysis of the Multiple Risk Factor Intervention Trial (Simon *et al.,* 1995) observed that serum DHA levels were inversely associated with CHD risk in 94 men with incident CHD and 94 men without incident CHD.

2.56   These prospective findings are very similar to those reported in a population-based case–control study involving 82 cases of sudden cardiac arrest (Siscovick *et al.,* 1995). That study found a strong inverse association between red blood cell LC n–3 PUFA composition at the time of the arrest and the risk of sudden cardiac arrest among subjects with no history of clinically recognized cardiac disease (i.e., 5.5 g of LC n-3

PUFA/month, equivalent to two fatty fish meals per week, was associated with a 50% reduced risk of primary cardiac arrest). 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.

Randomized controlled trials

2.57   There are no completed primary RCTs linking fish consumption or fish oil supplementation with primary prevention of CHD, although a number are on going or planned. The subjects in these trials will be healthy, but with increased risk of CHD. The earliest any of these trials will report is 2004.

2.58   Three secondary prevention trials – the Diet and Reinfarction Trial (DART) (Burr *et al.,* 1989, 1994), Singh *et al.,* 1997 and the GISSI-Prevenzione trial (GISSI-Prevenzione Investigators, 1999) – have shown that fish consumption or fish oil supplementation reduces coronary mortality among patients after MI. In the DART, which included 2033 men allocated to 3 dietary interventions, patients who received advice to eat more fish had a significantly lower (29%) total mortality during 2 years of follow-up. There was also a non-significant trend toward a reduction in recurrent ischemic heart disease events with increased fatty fish consumption. A smaller trial (Singh *et al.,* 1997), which included 240 MI patients, also demonstrated a significant reduction in all cause mortality when patients were supplemented with 2g/d LC n-3 PUFA.

2.59   In the more recent GISSI-Prevenzione trial, which included 11 324 MI patients (primarily men), daily supplementation (1 g/d) of LC n-3 PUFA for 2 years reduced occurrence of the main cardiovascular end points (cardiovascular death, nonfatal MI, and stroke) by 20%, cardiovascular death (including coronary or cardiac deaths and sudden deaths) by 30%, and all fatal events by 20%. Survival curves for LC n-3 PUFA treatment diverged early after randomization: total mortality was significantly lower after three months and risk of sudden death was significantly reduced after four months. This early effect of LC n-3 PUFA supports the hypothesis that the likely mechanism of action is the stabilization of arrhythmias (Marchioli *et al.* 2002).

2.60 A recent RCT (Burr *et al.,* 2003) investigating the effect in men with angina of dietary advice to increase fish consumption (MaxEPA fish oil was given to those men who found fish unpalatable) found an adverse effect of fish consumption (particularly for those given fish oil supplements) on cardiac mortality. The trial design, however, suffered from an interruption, due to funding problems; also, the assessment of compliance was only conducted in a very small subgroup.

2.61 The secondary prevention trials, therefore, provide evidence that increased fish consumption or fish oil supplementation would decrease mortality among patients who have suffered a myocardial infarction. Extrapolating evidence to a 'healthy' population is difficult e.g. dose levels may not be appropriate. This was previously recognized by COMA (Department of Health, 1994).

2.62 The UK population, however, is a 'high risk' population with regard to CHD: almost 30% of the English population have some form of cardiovascular disease (Department of Health, 1999).

The dose-response effect

2.63 The beneficial effect observed in the secondary prevention trials is observed in the order of 1g/d LC n-3 PUFA. It is not known whether doses above this level have any greater benefit. The prospective epidemiological evidence is suggestive of a plateau effect, in high-risk populations, at levels of about 0.9g/d; however, where fatty acid composition analyses of blood or blood compartments are determined, a positive relationship, with no plateau, is observed.

2.64 The dose of LC n-3 PUFA required for a demonstrable effect on cardiovascular risk factors, such as a reduction of plasma triacylglycerol levels (Sacks & Katan, 2002), blood pressure (Geleijnse *et al.,* 2002), platelet aggregation (Hornstra, 2001) and the inflammatory response (Calder, 2001) is greater then 1g/d. At least 1.5 g/d LC n-3 PUFA supplementation is required to produce beneficial effects on these factors. For example, to achieve increases in bleeding time, due to reductions in platelet aggregation, subjects need to be supplemented with 3g/d LC n-3 PUFA. These levels of intake have also been shown to raise low density

lipoprotein cholesterol levels in approximately 20% of subjects (Harris, 1997). The most probable mechanism for the effect of 1g/d LC n-3 PUFA on secondary CHD prevention is the stabilization of arrhythmias (Marchioli *et al.* 2002).

2.65   The nature of the evidence provided by the RCTs is suggestive of beneficial effects occurring within a short time scale, with benefit becoming apparent within a few months to 2 years. Prospective studies, however, suggest a longer time-course before a beneficial effect is observed. This difference maybe due to a combination of statistical and biological considerations. The dose-response nature of the relationship between fish consumption and risk of CVD may be different in populations of differing risk of CVD, and although the UK population is, relative to other countries, at high risk of CVD, sub-populations within the UK may exhibit different risk.

Summary of trials investigating the effect of fish and/or LC n-3 PUFA dietary intake on CHD

2.66   The majority of the evidence base suggests that fish consumption and the dietary intake of LC n-3 PUFA reduce risk from CVD. Three out of four of the RCTs conducted demonstrate a beneficial effect in people at risk of CVD. The largest of these, the GISSI-Prevenzione trial, which included 11 324 MI patients, confirmed the effect of LC n-3 PUFA in reducing risk from CVD. The recently published DART 2 (Burr *et al.,* 2003) reported an adverse effect of fish consumption on CVD risk; however, this is not consistent with other forms of evidence.

Conclusions

2.67   The Committee endorsed the COMA population guideline recommendation that people should eat at least two portions of fish a week of which one should be oily. It was noted that this signified a minimal achievable objective, against the low background UK population average fish intake.

2.68   The Committee stated that this should apply to pregnant and lactating women as it does to the rest of the population.

2.69   The Committee noted that it maybe beneficial for individuals to consume more then the guideline recommendation, but it was unable to identify a precise level.

2.70   The Committee revised the previous COMA population guideline recommendation concerning LC n-3 PUFA, to make it consistent with the recommendation for fish consumption by raising it to 0.45g/d from 0.2g/d.

Research recommendations

2.71   For dose-response studies to examine the response of different body pools to LC n-3 PUFA dietary intake – sufficient doses and fat stores/cells/plasma constituents should be examined.

2.72   To determine to what extend maternal LC n-3 PUFA dietary intake affects pregnancy outcomes, e.g. gestation length and birth weight, and follow-up measures.

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Table 2.8: Fish consumption, LC n-3 PUFA and CHD prospective cohort and specific case-control studies disease outcome

Reference	Type of Study	Intake* [EPA+DHA or FO or *n*-3 FA or Fish — g/d] (Source of estimated intake)	Study Duration[+]	Population[∀] [no & characteristics]	Disease Outcome[♦]
Osler *et al.,* 2003 Danish cohorts	Prospective cohort	Fish <1 serving/mo to ≥2 serving/wk, (FFQ)	5-18 yr	7540 General• aged 30-70 yr	No association observed
Mozaffarian *et al.,* 2003 Cardiovascular Health Study	Prospective cohort	Fish <1 serving/mo to ≥3 serving/wk, (FFQ)	9 yr	3910 General aged ≥ 65 yr	↓ CHD mortality, especially ↓ arrhythmic IHD death
Guallar, *et al.,* 2002 EURAMIC Study	Case-control	(DHA in adipose tissue) toenail mercury levels		684 cases, 724 controls M	↓ first MI – high mercury content in fish attenuated this protective effect
Hu, *et al.,* 2002 Nurses' Health Study	Prospective cohort	Fish (0.03- 0.24 % energy/d n-3 FA) 0 to 5 serving/wk, (FFQ)	16 yr	84 688 F General	↓ CHD mortality
Albert, *et al.,* 2002 Physicians' Heath Study	Nested, Case-control	(Blood samples EPA+DHA)	17 yr	94 cases and 184 controls, among 14916, M General	↓ sudden cardiac death
Yuan, *et al.,* 2001 Shanghai, China	Prospective cohort	Fish 50 →> 200 g/wk, (FFQ, n-3 FA)	10 yr	18244 M General	↓ fatal MI
Gillum *et al.,* 2000 NHANES I epidemiological follow-up	Prospective cohort	Fish (0Æ >1x/wk) FFQ	18.8 yr	8825 General	↓ all cause mortality but not association with CHD mortality
Oomen, *et al.,* 2000 Seven Countries Study – Finnish, Italian & Dutch subset	Prospective cohort	Lean and fatty fish (FFQ)	20 yr	2738 General	↓ CHD mortality for fatty fish only – no association observed for total fish consumption.

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Advice on fish consumption

Table 2.8: Fish consumption, LC n-3 PUFA and CHD prospective cohort and specific case-control studies disease outcome – continued

Reference	Type of Study	Intake* [EPA+DHA or FO or *n*-3 FA or Fish — g/d] (Source of estimated intake)	Study Duration[+]	Population[∀] [no & characteristics]	Disease Outcome[♦]
Rissanen, *et al.,* 2000 Kuopio Ischaemic Heart Disease Risk Factor Study	Prospective cohort	serum DPA+DHA hair mercury levels	10 yr	1871, M General	↓ MI – high mercury content in fish attenuated this protective effect
Guallar, *et al.,* 1999 EURAMIC Study	Case control	(DHA in adipose tissue)		639 case, 700 control M	No association observed
Albert, *et al.,* 1998 Physicians' Heath Study	Prospective cohort	Fish (0→ 4x/wk) FFQ	12 yr	14916, M General	↓ sudden cardiac death, NS MI & CHD mortality
Daviglus, *et al.,* 1997 Western Electric	Prospective cohort	Fish 0 →> 35 g/d , FFQ [0; 1-17; 18-34; >35g/d]	30 yr	1822, M General	↓ non-sudden death from MI
Kromhout, *et al.,*1996 Seven Countries Study	Prospective Longitudinal Health survey	Fish (FFQ)	25 yr	12783, General	No association observed
Rodriguez, *et al.,*1996 Honolulu Heart	Prospective cohort	Fish (0→ >1x/d) (FFQ)	23 yr	8006, General	↓ CHD mortalityΦ High fish could attenuate this negative effect of smoking
Ascherio, *et al.,* 1995 US Health Professionals' Follow-up Study	Prospective cohort	Fish (0.07→0.58g/d, n-3 FA) 0 to 5 serving/wk , FFQ	6 yr	44895, M General	No association observed
Guallar, *et al.,* 1995 US Physicians' Health Study	Nested, Case-control	(Blood samples EPA+DHA)	5 yr	14916, M General	No association observed

Table 2.8: Fish consumption, LC n-3 PUFA and CHD prospective cohort and specific case-control studies disease outcome – continued

Reference	Type of Study	Intake* [EPA+DHA or FO or *n*-3 FA or Fish — g/d] (Source of estimated intake)	Study Duration[+]	Population[∀] [no & characteristics]	Disease Outcome[♦]
Kromhout, *et al.,* 1995 Rotterdam, the Netherlands	Prospective cohort	Fish (+/-) (diet record)	17 yr	272, MF General	↓ CHD death
Morris, *et al.,* 1995 US Physicians' Health Study	Prospective cohort	Fish (1→ >5x/wk)	4 yr	21185, M General	No association observed
Simon, *et al.,* 1995 Multiple Risk Factor Intervention Trial	Nested Case-control	(Blood, DHA & EPA)	3.5 yr	188, General	↓ CHD risk
Siscovick, *et al.,* 1995 Seattle, WA	Case-control	(Blood), (FFQ) (5.5 g/mo., *n*-3 FA)		334 case 493 control, General	↓ first MI
Dolecek, *et al.,* 1991 MRFIT	Prospective cohort	Multiple 24hr recalls	6-8 yr	6258, M General	↓ CHD mortality
Lapidus, *et al.,* 1986 Gothenburg, Sweden	Population	Fish FFQ	12 yr	1462 F General	No association observed
Kromhout, *et al.,*1985 Dutch subset of seven countries study	Prospective cohort	Fish 0 Æ> 30 g/d , FFQ	20 yr	852, General	↓ CHD mortality

* **Symbols for intake in g/d include:** FO - Fish Oil; *n*-3 - omega-3 fatty acids; FA - fatty acid; DHA - doscosahexaenoic acid; EPA- eicosapentaenoic acid; DHA + EPA (FO) - amount of DHA and EPA from fish oil; g - grams; d - day; FFQ - Food frequency questionnaire.

+ **Symbols for study duration include:** yr. - year, mo. - month.

∀ **Symbols for description of population at time of enrollment:** MI - Myocardial Infarction; CHD - Coronary Heart Disease; CVD - Cardiovascular Disease. ∞ General is defined as free of indications of CHD; M - male only, F – female only.

♦ **Symbol for intervention effect measures:** ↑- increase in risk of CHD or CVD; ↓-decrease in risk of CHD or CVD.

X Increase in risk CHD using multivariate analysis and highest level of intake of omega-3 fatty acids derived from fish.

Φ Decrease in risk of sudden cardiac death and/or CHD mortality associated with highest level of fish intake.

Table 2.9: Fish consumption, LC n-3 PUFA and CHD intervention studies disease outcome

Reference	Intake* [EPA+DHA or FO or *n*-3 FA- g/d]	Study duration+	Population [number and characteristics∀]	Number of events and relative risk (95% confidence interval)			Outcome and comments♦
				All deaths	Cardiac deaths	Sudden deaths	
Burr, *et al.,* 2003	3 g/d FO or 2 portions oily fish/wk	3-9yr	Intervention 1571 Control 1543 Men with angina	283; 1.1 (0.9-1.3) 242	180; 1.2 (1.0-1.5) 139	73; 1.5 (1.0-2.2) 47	↑ CHD deaths, especially with fish oil. F&V groups were combined with fish groups to give fish vs no fish groups, as very low compliance in F&V groups.
GISSI, *et al.,* 1999	0.85-0.88 g/d EPA+ DHA (Ethyl esters)	3.5 yr	Intervention 5666 Control 5658 MI	472; 0.8 (0.7-0.9) 545	214; 0.8 (0.7-0.9) 265	122; 0.7 (0.6-0.9) 164	↓ CHD deaths Relative risk for non fatal MI 0.9
Burr, *et al.,* 1989	3 g/d FO or 2 or 3 portions oily fish/wk	2 yr	Intervention 1015 Control 1018 MI	94; 0.7 (0.6-0.9) 130	78; 0.7 (0.5-0.9) 116		↓ CHD deaths Relative risk for non fatal MI 1.5
Singh, *et al.,* 1997	1.08g/d EPA 0.72 g/d DHA	1 yr	Intervention 122 Control 118 MI	14; 0.5 (0.3-0.9) 26	12; 0.6 (0.3-1.3) 18	2; 0.2 (0.1-1.1) 8	↓ CHD deaths.

The RCTs below investigated an effect of fish oil supplementation on coronary atherosclerosis regression in patients with extensive coronary atherosclerosis, and also recorded disease outcomes. These RCTs were not considered in the formal analysis of the outcome data because the small numbers of subjects involved precluded statistical analysis of the data. In most cases high doses of fish oils were used that achieved levels of intake of EPA and DHA that could not be achieved by normal diet. A recent meta-analysis of RCTs (Bucher *et al*. 2002), which included the trials below, concluded that dietary and supplemental intake of LC n-3 PUFA reduces overall mortality, mortality due to myocardial infarction, and sudden death in patients with CHD.

Table 2.9: Fish consumption, LC n-3 PUFA and CHD intervention studies disease outcome – continued

Leaf, *et al.,* 1994	4.1 g/d EPA 2.8 g/d DHA	6 mo	Intervention Control	253 250	0; 0.2 (0.0-5.4) 2	0; 0.5 (0.0-14.6) 1	0; 0.5 (0.0-14.6) 1	A supplement of 8 g/d of omega-3 fatty acids failed to prevent the usual high rate of restenosis after PTCA
Sacks, *et al.,* 1995	2.9 g/d EPA, 1.9 g/d DHA	2 yr	Intervention Control	31 28	0; 0.4 (0.0-12.5) 1	0; 0.4 (0.0-12.5) 1		No effect on the progression of coronary atherosclerosis
Johansen, *et al.,* 1999	2.7 g/d EPA, 2.3 g/d DHA	6 mo	Intervention Control	196 192	1; 0.3 (0.0-3.1) 3	0; 0.2 (0.0-5.4) 1	1; 1.0 (0.1-15.5) 1	No effect on the incidence of restenosis
Von Schacky	1.06 g/d EPA, 0.65 g/d DHA	2 yr	Intervention Control	111 112	1; 0.5 (0.0-5.5) 2	0; 0.5 (0.0-14.7) 1		A modest effect on the progression of coronary atherosclerosis was observed

* **Symbols for intake in g/d include:** FO - Fish Oil; FA - fatty acid; DHA - doscosahexaenoic acid; EPA - eicosapentaenoic acid; DHA + EPA (FO) - amount of DHA and EPA from fish oil; g - grams; d - day.

+ **Symbols for study durations include:** yr. - year, mo. - month; d - day.

∀ **Symbols for description of population at time of enrollment:** MI - Myocardial Infarction.

♦ **Symbols for intervention effect measures:** ↑ – increase in risk; ↓ - decrease in risk.

3   Toxicological considerations

General toxicological principles

3.1   For many chemical contaminants it is possible to set intake levels at which no harmful effects are expected to arise. At intakes below a certain point (the threshold) the contaminant either has no effect or its effects are reversed by the body's defence mechanisms. This is often referred to as the threshold approach to risk assessment. When this approach is valid it is possible to estimate the amount of a substance that can be ingested daily over a lifetime without appreciable health risk. There are several terms to describe this intake, those most commonly used for chemical contaminants are the Tolerable Daily Intake (TDI) in the UK/EU and the Reference Dose (RfD) in the USA. Although there are minor differences in the precise definitions of these terms, they are safety guidelines based on similar principles. Whilst absolute safety cannot be guaranteed, these safety guidelines represent an intake where there is essentially no risk, as far as can be judged from the available scientific evidence. They are expressed in relation to the bodyweight (bw) in order to allow for different body size, such as for children of different ages. Some advisory committees use terms with longer referencing periods, such as Provisional Tolerable Weekly Intake (PTWI), for chemical contaminants that accumulate in the body.

3.2   The usual practice is to base the safety guideline on the most sensitive and relevant study. Adequate human data would be the preferred basis, but are rarely available and it is often necessary to rely on data from animal studies. A safety guideline is normally set by identifying an exposure that has shown no harmful effect in the most relevant study, and dividing it by uncertainty factors to allow for possible differences between the experimental animals and humans, and between the average and most sensitive humans. These are sometimes referred to as safety factors, but uncertainty is a more appropriate term, because we generally do not know the extent of variability between species or between different people. The uncertainty factors relate to the fate of the substance within the body, and to sensitivity to the toxic effects of the substance. Additional uncertainty factors may be used to allow for gaps in the scientific evidence. Smaller factors may be used if specific human data are available.

3.3 A safety guideline, such as the TDI, represents an intake that is without appreciable risk, but gives no indication of the possible risk at intakes above the guideline. Exceeding the safety guideline does not necessarily result in harmful effects, even in the most sensitive people. Assessing risk associated with exceeding the safety guideline requires consideration of information relating to the toxicity of the substance, the way in which the safety guideline was derived and the length of time and amount by which it has been exceeded.

Organic contaminants

Dioxins and dioxin-like PCBs

3.4 Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are very persistent chemicals which are ubiquitous in the environment and are generally present in low concentrations in foods, especially fat-containing foods including milk, meat and fish. PCDDs and PCDFs are commonly referred to as dioxins and the co-planar PCBs, which exhibit a similar mechanism of toxicity, are referred to as the dioxin-like PCBs. There has been a significant reduction in emissions of dioxins into the environment during the past decade and dietary intake has fallen by about 85% since 1982 (MAFF 1997, FSIS 105;FSA 2000, FSIS 4/00, FSIS 38/03).

Dioxins and PCBs in fish

3.5 In August 1999 the Ministry of Agriculture, Fisheries and Food (MAFF) published a survey (MAFF 1999a, FSIS 184) for dioxins, furans and PCBs in 132 samples of cod, haddock, plaice, whiting, red fish, herring, mackerel, salmon and fish fingers. This work complemented an earlier survey for these chemicals in samples of farmed trout (MAFF 1998a, FSIS 145) and eels from commercial fisheries (MAFF 1997, FSIS 105). Concentrations of dioxins and PCBs on a fat basis were higher in herring, red fish and plaice than in the other species. Concentrations of dioxins and PCBs on a fat basis were lower in haddock and mackerel. Concentrations also showed significant seasonal variations. Table 3.1 shows concentrations of dioxins and PCBs expressed on a fresh weight basis.

**Table 3.1: Concentrations of dioxins and PCBs in edible tissue samples from marine fish,
collected in 1995/96 (ng WHO-TEQ/kg fresh weight)**

| Fish type | Concentrations (ng WHO-TEQ/kg fresh weight) | | | | | |
| | Dioxins | | PCBs | | Dioxins and PCBs | |
	Mean	Range	Mean	Range	Mean	Range
UK landed:						
Cod	0.04	0.01-0.08	0.08	0.01-0.30	0.12	0.03-0.38
Haddock	0.03	0.01-0.07	0.03	0.01-0.06	0.06	0.03-0.10
Plaice	0.28	0.06-0.52	0.47	0.17-0.84	0.75	0.23-1.27
Whiting	0.04	0.01-0.08	0.11	0.01-0.33	0.14	0.02-0.38
Herring	2.44	0.34-3.76	6.15	0.46-10.38	8.59	0.8-13.85
Mackerel	0.66	0.14-1.70	2.45	0.34-6.02	3.11	0.48-7.49
Salmon	0.82	0.62-0.99	2.38	1.28-2.99	3.20	2.15-3.95
Trout	0.27	0.07-0.74	0.86	0.22-2.35	1.13	0.30-3.09
Fish fingers	0.06	0.03-0.17	0.12	0.03-0.49	0.18	0.08-0.52
Imported:						
Cod	0.03	0.01-0.09	0.05	0.01-0.16	0.09	0.03-0.25
Haddock	0.03	0.01-0.05	0.03	0.01-0.08	0.06	0.02-0.13
Plaice	0.30	0.25-0.34	0.46	0.32-0.64	0.76	0.57-0.94
Salmon	0.57	–	2.03	–	2.60	–
Red fish	0.50	0.40 - 0.59	1.51	1.42 - 1.59	2.00	1.82 - 2.18

Notes:

Results are given to 2 significant figures. Total concentrations of dioxins and PCBs may not equal the sum of individual dioxins and PCBs values due to rounding, and because the highest and lowest concentrations of dioxins and PCBs were not always found in the same samples.

3.6   In general, concentrations of chemicals such as dioxins and PCBs in fish depend on their fat content, the extent to which the fish migrate, the number of times they spawn, and their ages, size and feeding habits (Larrson *et al.,* 1996). For example, plaice are bottom-feeding fish and therefore may be more exposed to dioxins and PCBs bound to sediment. Herring has a relatively high fat content and is non-migratory, which renders it more subject to localized contamination sources (Strandberg *et al.,* 1998). The Scottish Office has found that concentrations of total PCBs in herring from the River Clyde are higher than in those from the North Sea and River Forth (Kelly *et al*., 1994). A recent study of contaminants in salmon (Hites *et al*., 2004) reported similar levels in UK farmed salmon to those found in the MAFF study (MAFF 1998a).

3.7   The concentrations found in the MAFF survey were broadly similar to those found elsewhere. However, higher concentrations have been found in fish taken from the Baltic Sea, a sea that is known to be contaminated.

Long term monitoring of PCBs in herring from the Baltic Sea since 1978 has shown that concentrations of PCBs have fallen by 6.3-13% per year (Bignert *et al.,* 1998). There is also some evidence that concentrations of dioxins and PCBs in fish from the South Atlantic are lower (Brevik, 1990).

3.8   The FSA is currently carrying out a major survey of fish, particularly oily fish and farmed and wild salmon.

Effects of dioxins and dioxin-like PCBs

3.9   Dioxins and dioxin-like PCBs exhibit similar types of biological effect, primarily mediated through interaction with the Aromatic hydrocarbon (Ah) receptor. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD or TCDD) has been studied in most detail and is one of the most potent dioxins. The potency of other dioxins is expressed as fractions of the TCDD potency, called toxic equivalents, which have been agreed internationally based on a scheme proposed by the WHO (referred to as TEQs or WHO-TEQs).

3.10   The risks associated with dioxins have been assessed several times in the last 20 years by the UK independent expert advisory committees, the Committee on Toxicity (COT), Carcinogenicity (COC) and Mutagenicity (COM) of Chemicals in Food, Consumer Products and the Environment. COT and COC completed the most recent review of the available data in 2001 (COC 2001, COT 2001).

3.11   Reports of effects in humans mainly relate to workers in chemical plants or exposure resulting from accidental contamination of the environment (e.g. Seveso in Italy) or edible oils (e.g. Yusho in Taiwan), with much higher levels of dioxin exposure than the general public. These studies have shown that exposure to high levels of dioxins causes the skin condition chloracne. There is still some debate with respect to cancer; in 1997 the International Agency for Research on Cancer (IARC) concluded that TCDD should be considered as a definite human carcinogen, whereas the COC reconfirmed that it should be regarded as a probable human carcinogen, at its latest evaluation in 2001. Weaker evidence suggests increased risks of cardiovascular disease and reproductive effects. A

number of other effects have been observed in people exposed to dioxins, but there is insufficient information to draw conclusions.

3.12   A wide range of toxic effects has been observed in animal studies, including cancer and effects on the immune and reproductive systems. The effects occurring at the lowest dose levels were observed when dioxins were administered to pregnant rats. The most sensitive and consistent effect was on the developing reproductive system of the male offspring, particularly changes in sperm production and quality. These changes indicate decreased fertility of the male, resulting from dioxin exposure *in utero*, which is consistent with observations seen at higher doses in a multigeneration study in rats.

3.13   The COC and COT decided that, despite the evidence of carcinogenicity, there was sufficient information to assume a threshold existed for the effects of dioxins and hence a TDI could be established. This conclusion was based on the considerable evidence that dioxins do not directly damage the genetic material, and the understanding of the biological reactions by which dioxins cause harmful effects, and evidence that these reactions will not occur at sufficiently low levels of exposure.

3.14   The available human data could not be used as the basis for the TDI because:

a)   the exposure data were rough estimations and did not include all the dioxins and dioxin-like substances of concern.

b)   the studies did not adequately consider other possible causes of the observed effects.

c)   in all except a series of Dutch developmental studies, the patterns of exposure included periods of high level exposure rather than continual low level exposure from food.

d)   in the occupational studies, exposed workers were mostly male and therefore the wrong population for the critical effect seen in animal studies (effects on the fetus).

3.15   The COT considered several studies of developmental effects in animals. Of these, a study by Faqi *et al.* (1998) identified the lowest TCDD exposure that decreased sperm counts. This study had a number of limitations, but these were not sufficient to discount the results and so the Faqi study was considered to be the key study for deriving the TDI. The COT considered that, because of the long-term accumulation of dioxins in the body, the effects were related to the total body burden rather than to a daily dose. The dose used in the Faqi study was converted into a maternal body burden, making mathematical corrections to compare to continual low level exposure from the diet, based on the distribution studies of Hurst *et al.* (2000 a and b).

3.16   The data supported the use of chemical-specific uncertainty factors based on the following elements:

   a)   Body burdens are used to indicate the concentration of TCDD in the fetus and it is therefore not necessary to correct for differences in toxicokinetics in different species – uncertainty factor of 1.

   b)   Rats are generally more sensitive to the adverse effects of dioxins than humans, but it could not be discounted that the most sensitive humans could be as responsive as rats. It was therefore not necessary to correct for differences in sensitivity either between species, or within the human population – uncertainty factor of 1.

   c)   There may be variability between humans in accumulation of the different dioxins and dioxin-like PCBs. This was allowed for by a factor to account for the potential increased body burden of dioxins and dioxin-like PCBs in the most susceptible individuals – uncertainty factor of 3.2.

   d)   The key study (Faqi *et al*., 1998) did not identify a level without effect – uncertainty factor of 3 used for extrapolation from a lowest observed adverse effect level (LOAEL) to a no observed adverse effect level (NOAEL).

3.17   The maternal body burden calculated from the Faqi study was divided by these adjustment factors to derive a tolerable human maternal body burden, which was estimated to result from a long-term daily intake of about 2 pg TCDD/kg bw per day. Because long term exposure is important, other scientific advisory committees have recommended tolerable intakes related to periods of one week or one month, but the COT considered that a tolerable daily intake could be more readily compared with intakes expressed on a daily basis.

3.18   Taking into account the possible effects of other dioxins and dioxin-like substances, the COT recommended a TDI of 2 pg TEQ/kg bw per day. As this TDI is based on the most sensitive end-point, the COT concluded it would also protect against the risk of other adverse effects, including carcinogenicity.

Uncertainties in the TDI

3.19   There are a number of uncertainties involved in assessing the evidence, setting a TDI and comparing this to realistic exposures:

a)   The key studies were conducted on TCDD whereas exposure is to a mixture of dioxins, which is allowed for by the TEQ approach. Toxic equivalents are agreed international ratios of toxicity to the nearest half order of magnitude for different congeners based on various parameters (comparative toxicity and Ah receptor binding). They are usually rounded up, which is precautionary.

b)   The maternal body burden in the Faqi study was measured, and relied on the best estimates of TCDD kinetic parameters in the rat – these could be under- or over-estimates.

c)   Other studies did not find the same effects at such low exposure levels as those in the Faqi study, thus use of the Faqi study is precautionary.

d)   Uncertainty whether the adjustment factors are too high or too low.

3.20   The TDI represents the best scientific judgement, but on balance the approach is precautionary because of the uncertainties involved.

Evaluations in other countries

3.21   The approach to the COT evaluation and resultant TDI is consistent with the evaluations of the EU Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), although those committees used longer referencing periods (SCF: 14pg WHO TEQ/kg bw/week; JECFA: 70pg WHO TEQ /kg bw/month). In contrast the US Environmental Protection Agency (EPA) favours an extreme precautionary approach based on quantitative cancer risk assessment. The EPA began a reassessment of dioxins in 1991, and has still not completed it deliberations. However, its most recent estimate was of a cancer risk of 1 in 1000 at an intake of 1 pg/kg bw/day. The COC consider that these estimations were not appropriate.

Dietary exposure to dioxins and PCBs

3.22   Dietary exposure to chemical contaminants is estimated by determining the concentrations of chemicals in different foods, combined with information on consumption of those foods obtained from diet and nutrition surveys. The 1994-6 MAFF survey (MAFF 1999a, FSIS 184) was carried out to enable the calculation of dietary exposure to dioxins and PCBs for consumers of fish. Intake of dioxins and PCBs from the consumption of fish only using 1986 consumption data (Gregory *et al.,* 1990) was estimated to be 0.5 pg WHO-TEQ/kg bodyweight/day for an average UK adult consumer and 3.6 pg WHO-TEQ/kg bodyweight/day for a high level adult consumer (Table 3.2). The average concentrations of dioxins and dioxin-like PCBs in UK and imported samples of a given fish species were used. Intake calculations have been repeated using food consumption data from the 2000/01 adults survey (Henderson *et al.,* 2002). The slight increase in oily fish consumption is reflected in a slight increase in dietary intake of dioxins and dioxin-like PCBs. *Upper bound* intakes from fish only were estimated to be 0.6 pg WHO-TEQ/kg bodyweight/day for an average UK adult consumer and 3.9 pg WHO-TEQ/kg bodyweight/day for a high level adult consumer (Table 3.3). *Lower bound* intakes have also been estimated (Table 3.4) and after approximation do not differ from the *upper bound* intakes.*

•   *Upper bound* intakes are calculated by using the limit of detection for those values below the limit of detection. *Lower bound* intakes assume a value of zero for concentrations below the limit of detection.

3.23   Estimated adult intakes of dioxins and dioxin-like PCBs from the whole
        diet are reported in Table 3.5. These dietary intakes were calculated using
        the concentrations of dioxins and dioxin-like PCBs found in the 1982,
        1992, 1997 and 2001 TDS samples, including fish. These calculations refer
        to average and high level consumers of all food categories and not average
        and high level consumers of fish only as Tables 3.2, 3.3 and 3.4. For this
        reason the values in Tables 3.2, 3.3 and 3.4 are not directly comparable to
        the values in table 3.5.

3.24   In 2001, the COT referred to the intake estimates of 1.8 and 3.1 pg TEQ/kg
        bw/day (based on the results of the 1997 TDS) for the average and high
        level adult consumers, respectively and concluded:

        a)   There are no short-term measures that can be used to decrease the
              body burden of dioxins and dioxin-like PCBs in humans because of
              their long half-lives and widespread presence at low levels in food.

        b)   Similarly, because of the long half-life, short-term exceedances of the
              tolerable intake are not expected to result in adverse effects.
              Nevertheless, it is not possible to identify a duration and degree of
              exceedance at which adverse effects might occur.

3.25   Recalculation of the dietary exposure using the latest consumption data
        and the 1997 TDS results indicates a slight increase for high level
        consumers but not for average consumers (Table 3.5). However the intakes
        of dioxins and dioxin-like PCBs from the whole diet calculated using the
        latest TDS data (2001) (Table 3.5) have decreased considerably
        (approximately a half) compared to 1997.

Table 3.2: Estimated upper bound adult dietary intakes (pg WHO-TEQ/kg bodyweight/day) of dioxins and dioxin-like PCBs via individual species of fish (estimated using food consumption data from the adults survey 1986).

Fish type	Mean		High level	
	Consumption (g/day)	Estimated *upper bound* adult dietary intakes of dioxins and PCBs (pg TEQ/kg body weight/day)**	Consumption (g/day)	Estimated *upper bound* adult dietary intakes of dioxins and PCBs (pg TEQ/kg body weight/day)**
Cod	22.8	0.04	67.5	0.1
Haddock	18.1	0.02	40.4	0.04
Plaice*	22.7	0.3	46.2	0.6
Whiting*	18.4	0.04	36.3	0.09
Misc. white fish	13.7	0.09	41.8	0.3
Fish fingers/fish cakes	14.0	0.04	40.2	0.1
All white fish	26.3	0.1	72.7	0.4
Herring	23.4	3.4	46.9	6.7
Mackerel	15.1	0.8	48.2	2.5
Salmon	12.6	0.7	39.4	2.1
Trout*	22.2	0.4	35.5	0.7
Misc. oily fish	9.4	0.06	45.4	0.3
All oily fish ***	18.6	1.1	67.7	5.1
All fish ***	30.3	0.5	89.2	3.6

Notes:

Intakes were estimated from the average concentrations found in UK and imported samples of a given fish species

*   Fewer than 60 recorded consumers. High level intakes to be regarded with caution.

**   Estimates assume a typical adult bodyweight of 60 kg.

*** The total intakes of dioxins and PCBs by the high level consumer or by the mean average consumer for all fish types combined are not equal to the sum of the intakes from the individual fish species. They refer to the dietary intakes by a consumer of one or any combination of the fish species containing that chemical. These values are derived from a distribution of the consumers' consumption patterns with regard to the individual fish species.

There were no recorded consumers of red fish.

Table 3.3: Estimated upper bound adult dietary intakes (pg WHO-TEQ/kg bodyweight/day) of dioxins and dioxin-like PCBs via individual species of fish (Estimated using food consumption data from the adults survey 2000/1).

Fish type	Mean		High level	
	Consumption (g/day)	Estimated *upper bound* adult dietary intakes of dioxins and PCBs (pg TEQ/kg body weight/day)**	Consumption (g/day)	Estimated *upper bound* adult dietary intakes of dioxins and PCBs (pg TEQ/kg body weight/day)**
Cod	22.1	0.04	57.6	0.1
Haddock	19.8	0.02	42.6	0.04
Plaice*	22.4	0.3	58.3	0.7
Whiting**	29.9	0.07	35.1	0.08
Misc. white fish	17.3	0.1	59.3	0.4
Fish fingers/ fish cakes	10.9	0.03	36.9	0.1
All white fish	25.4	0.1	77.0	0.4
Herring	20.6	3.0	45.4	6.5
Mackerel	22.9	1.2	79.5	4.1
Salmon	19.1	1.0	60.8	3.2
Trout*	22.3	0.4	45.5	0.9
Misc. oily fish	13.4	0.09	27.4	0.2
All oily fish ****	24.4	1.3	81.7	4.6
All fish ****	31.8	0.6	90.1	3.9

Notes:

Intakes were estimated from the average concentrations found in UK and imported samples of a given fish species

*   Fewer than 60 recorded consumers. High level intakes to be regarded with caution.

**   Only two recorded consumers. High level intakes to be regarded with extreme caution.

***   Estimates assume a typical adult bodyweight of 60 kg. The estimates do not take account of recipes new since the young persons survey of 1998.

****   The total intakes of dioxins and PCBs by the high level consumer or by the mean average consumer for all fish types combined are not equal to the sum of the intakes from the individual fish species. They refer to the dietary intakes by a consumer of one or any combination of the fish species containing that chemical. These values are derived from a distribution of the consumers' consumption patterns with regard to the individual fish species. The same applies to the 97.5th percentile and mean consumption of fish.

There were no recorded consumers of red fish.

**Table 3.4: Estimated lower bound adult dietary intakes (pg WHO-TEQ/kg bodyweight/day)
of dioxins and dioxin-like PCBs via individual species of fish (Estimated using
food consumption data from the adults survey 2000/1.)**

Fish type	Mean		High level	
	Consumption (g/day)	Estimated *lower bound* adult dietary intakes of dioxins and PCBs (pg TEQ/kg body weight/day)**	Consumption (g/day)	Estimated *lower bound* adult dietary intakes of dioxins and PCBs (pg TEQ/kg body weight/day)**
Cod	22.1	0.03	57.6	0.09
Haddock	19.8	0.02	42.6	0.04
Plaice*	22.4	0.3	58.3	0.7
Whiting**	29.9	0.07	35.1	0.08
Misc. white fish	17.3	0.1	59.3	0.4
Fish fingers/fish cakes	10.9	0.02	36.9	0.08
All white fish	25.4	0.1	77.0	0.4
Herring	20.6	3.0	45.4	6.5
Mackerel	22.9	1.2	79.5	4.1
Salmon	19.1	1.0	60.8	3.2
Trout*	22.3	0.4	45.5	0.8
Misc. oily fish	13.4	0.1	27.4	0.2
All oily fish ****	24.4	1.3	81.7	4.6
All fish ****	31.8	0.6	90.1	3.9

Notes:

Intakes were estimated from the average concentrations found in UK and imported samples of a given fish species

*   Fewer than 60 recorded consumers. High level intakes to be regarded with caution.

**   Only two recorded consumers. High level intakes to be regarded with extreme caution.

***   Estimates assume a typical adult bodyweight of 60 kg. The estimates do not take account of recipes new since the young persons survey of 1998.

****   The total intakes of dioxins and PCBs by the high level consumer or by the mean average consumer for all fish types combined are not equal to the sum of the intakes from the individual fish species. They refer to the dietary intakes by a consumer of one or any combination of the fish species containing that chemical. These values are derived from a distribution of the consumers' consumption patterns with regard to the individual fish species. The same applies to the 97.5th percentile and mean consumption of fish.

There were no recorded consumers of red fish.

Table 3.5: Estimated adult intakes (pg WHO-TEQ/kg bodyweight/day) of dioxins and dioxin-like PCBs from the whole diet in 1982, 1992, 1997 and 2001

	1982	1992	Adults 1986* 1997 Lower	Upper	Adults 2000/1** 1997 Lower	Upper	2001 Lower	Upper
Average	7.2	2.5	1.5	1.8***	1.5	1.8	0.7	0.9
High level	13	4.3	2.7	3.1***	3.0	3.3	1.5	1.7

Note:

*   Food consumption data from the adults survey 1986 (Gregory t al, 1990)

**   Food consumption data from the adults survey 2000/1 (Henderson et al., 2002)

***   Intake estimates on which COT based its conclusion in 2001

These dietary intakes were estimated using the concentrations of dioxins and dioxin-like PCBs found in the 1982, 1992, 1997 and 2001 TDS samples. These values are not directly comparable to the values in tables 3.2, 3.3 and 3.4.

Fish oil supplements

3.26   In June 2002 the FSA published a survey for dioxins and dioxin-like PCBs in fish oil supplements (FSA 2002a, FSIS 26/02). The estimated upper bound intakes of adults of dioxins and dioxin-like PCBs were in the range <0.1 to 7.1 pg WHO-TEQ/kg bw/day. These estimated intakes did not take account of intakes from the whole diet. COT members agreed it is appropriate to consider the survey results in the light of the TDI of 2 pg TEQ/kg bw/day, in the context of intake of dioxins and dioxin-like PCBs from the UK diet. Some fish oil samples, if taken at the recommended dosage, would lead to a higher intake of dioxins than from dietary sources. The problem was particularly evident with two samples which would exceed twice the TDI (i.e. exceed 4.0 pg TEQ/kg bw/day) from intake of the oil alone in virtually all age groups (FSA, 2002b).

3.27   In the light of COT's advice the Agency asked manufacturers to withdraw the batches of products for which intakes from the oils alone would exceed twice the TDI.

Implications of exceeding the TDI

3.28   The TDI is set to protect the most sensitive individuals, and because it is based on effects on the fetus, it is assumed that pregnant women are the population at greatest risk. Dioxins accumulate gradually in the body over a period of about 30 – 40 years, after which the level of intake will be about

the same as the level of elimination from the body (steady state). Therefore the exposure to females up to the time of pregnancy is of greatest concern.

3.29   At steady state, the total body burden will be about 2000 times higher than the average daily intake. Thus, an intake of 10 times the TDI on a single day would result in a 0.5% increase in the body burden, which would not be sufficient to have any harmful effect. Occasionally consuming more than the TDI would not significantly increase the body burden and would not be expected to result in harmful effects, providing that the average intake over a prolonged period is within the TDI. Similarly, modification of the diet during pregnancy is unlikely to be sufficient to alter the body burden. Because of the way in which the TDI was derived, and the uncertainties in the risk assessment, it is not possible to identify a duration and extent of intake above the TDI at which effects on the developing fetus might occur.

3.30   The TDI is set to protect against the most sensitive effect of dioxins, which is considered to be impaired development of the fetal male reproductive system, caused by fetal exposure in utero and correlated with the maternal body burden. Other developmental effects have also been reported at higher doses in animal studies. Taking also into account that dioxins accumulate in the body over many years, the most susceptible subgroup is considered to be females up to the time of final pregnancy, by virtue of exposure of fetuses that they might bear. Other populations, particularly women past child-bearing age and men, are not at risk of the developmental effects and are likely to be less susceptible to dioxin toxicity; therefore an alternative safety guideline could be proposed for these groups.

3.31   The most sensitive and relevant non-developmental effect of dioxins that can be used for risk assessment is increased cancer risk. As described at paragraph 3.14 above, the COT has concluded that the human data were not adequate to identify safety guidelines. The COC noted that the excess cancer mortality reported in the heavily exposed industrial cohorts was small and any increased risk of cancer at background levels of exposure is likely to be extremely small and not measurable by current epidemiological methods. The data from experimental animals have

therefore been used to recommend a Guideline Level of daily intake over a lifetime that would not be associated with an appreciable risk of cancer. Prior to the recent evaluation, the COT derived the dioxin TDI from the rat carcinogenicity study of Kociba et al. (1978). From this lifetime feeding study, COT identified no effect levels for TCDD of 10 ng/kg bw/day for tumours, and 1 ng/kg bw/day for hepatocellular nodules. Since TCDD is considered to have a non-genotoxic mechanism of carcinogenicity, it is appropriate to base a safety assessment on the lesion seen at the lowest dose in the target tissue for tumourigenicity, i.e. the hepatocellular nodules. The interval between dose-levels was larger than is now considered appropriate for carcinogenicity studies, and therefore derivation of the Guideline Level from the LOAEL of 10 ng/kg bw/day is justifiable.

3.32   Assuming a bioavailability of 0.5 and a half-life of 21 days in rats the steady-state body burden at an intake of 10 ng/kg/day would be 152 ng/kg. In deriving the TDI, the COT applied an adjustment factor (3.2) for variability in accumulation of different dioxin-like compounds and a factor of 3 for the use of the LOAEL. Applying this combined adjustment factor of 9.6 (3 x 3.2) to the body burden of 152 ng/kg bodyweight results in a guideline body burden of 16 ng/kg bodyweight.

3.33   This guideline body burden can be converted into a Guideline Level for long term average intake of 8 pg TEQ/kg bodyweight per day, using the equation

$$\text{daily intake (pg/kg/day)} \quad = \quad \frac{\text{body burden (pg/kg bw)} \; \times \; \ln 2}{\text{bioavailability} \; \times \; \text{half-life in days}}$$

assuming a bioavailability of 0.5 and TCDD half-life of 2740 days (7.5 years) in humans

3.34   Table 3.6 shows estimates of intakes of dioxins and dioxin-like PCBs by UK consumers of oily fish based on the results of analyses made in a survey of dioxins and PCBs in marine fish, 1994-1995. The estimates include the contaminant intakes from one portion of cod per week and from the rest of the diet, and assume a portion size of 140g (70g for eels) and bodyweight of 60kg.

3.35   The estimated intakes have been compared with the Tolerable Daily Intake (TDI) of 2 pg WHO-TEQ/kg bw/day. Consumption of one 140g portion per week of herring, kipper or eels, by a 60kg adult, would result in a 44-78% exceedance of the TDI. Consumption of 2 portions of salmon would lead to a 40% exceedance of the TDI, whereas 3 portions of trout could be consumed per week before the TDI would be exceeded. Exceedances would be smaller if a larger average bodyweight was assumed for adults.

3.36   The Guideline Level of 8 pg WHO-TEQ/kg bw/day is used to indicate a long term average intake that would not be expected to be associated with an appreciable increase in cancer risk (analogous to the TDI definition). Table 3.6 indicates that 2 portions per week of herring or kipper, or 3 or more portions of mackerel, salmon or trout could be consumed without exceeding the guidance level.

3.37   The above calculations are based on long term intakes. However, some women may wish to modify their oily fish consumption during pregnancy, either because of the nutritional benefits or because of concern about risks associated with contaminants. The COT has previously noted (COT, 2001):

   •   *there are no short-term measures that can be used to decrease the body burden of dioxins and dioxin-like PCBs in humans because of their long half-lives and widespread presence at low levels in food.*

   •   *Similarly, short term exceedances of the TDI are not expected to result in adverse effects. Nevertheless, it is not possible to identify a duration and degree of exceedance at which adverse effects might occur.*

3.38   COT paper TOX/2004/10 (Toxicological opinion on the results of the SUREmilk project) cites a physiologically-based pharmacokinetic (PBPK) model of the effects of lactation on a woman's body burden of dioxins. This model indicates that breast-feeding a first baby would lower the mother's body burden, which suggests that the risk of exceeding a harmful body burden would be lower if oily fish consumption was increased in a subsequent pregnancy. Furthermore, a short time (e.g. 6-12 months) of

modifying the diet may not have a significant impact on the total body burden (see Figures 1-2, AR Renwick, unpublished).

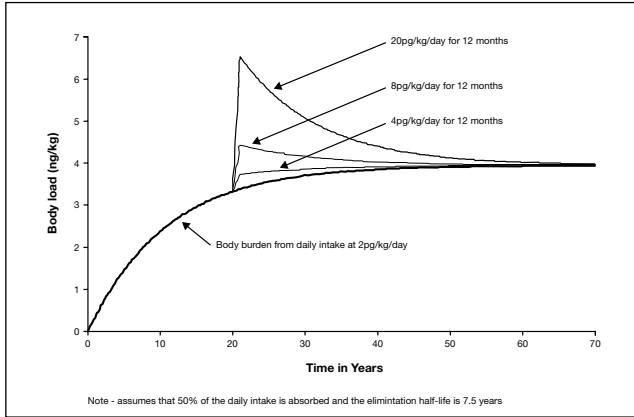**Figure 3.1: Influence of 12 months of high intake on body burden of dioxin (Renwick, unpublished)**

Figure 3.2: Influence of 6 months of high intake on body burden of dioxin (Renwick, unpublished)
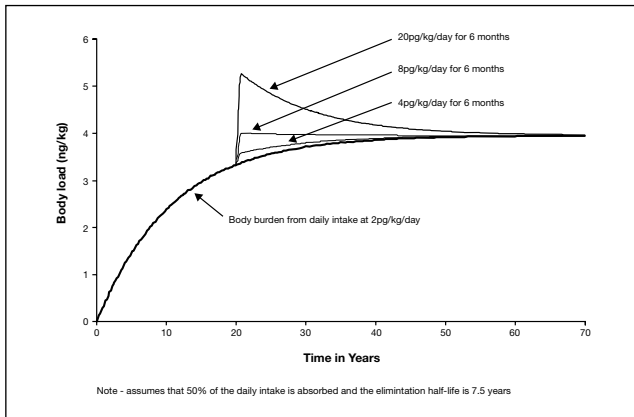
Table 3.6: Estimated dietary intake of dioxins and dioxin-like PCBs from oily fish and the rest of the diet for an adult of 60 kg bodyweight

	Herring	Kipper	Mackerel	Salmon	Trout	Eel
Mean concentration (& range)[a] (pg WHO-TEQ/g wet weight)	8.59 (0.8-13.85)	8.59[b] (0.8-13.85)	3.11 (0.48-7.49)	3.15 (2.15-3.95)	1.13 (0.30-3.09)	10.23
Intake from one portion fish per week [c] (pg WHO-TEQ/kg bw/day)	2.9	2.9	1.0	1.1	0.4	1.7
Intake from rest of the diet [d] (pg WHO-TEQ/kg bw/day)	0.7	0.7	0.7	0.7	0.7	0.7

						% TDI or guidance level						
Portions per week	Herring		Kipper		Mackerel		Salmon		Trout		Eel	
	%TDI [e]	%GL [f]	%TDI	%GL	%TDI	%GL	%TDI	%GL	%TDI	%GL	%TDI	%GL
1	178	45	178	45	87	22	88	22	54	13	120	30
2	321	80	321	80	139	35	140	35	73	18	205	51
3	465	116	465	116	191	48	193	48	92	23	290	73
4	608	152	608	152	242	61	245	61	110	28	376	94

a   Average concentrations in UK and imported fish species taken from MAFF (1999), FSIS 184, MAFF (1998A), FSIS 145 and .MAFF (1997), FSIS 105.

b   No concentration data were available for kipper, so it has been assumed that the concentration is the same as for herring.

c   Assumes 140g portion size for all fish except eels (70g).

d   Averaged daily intake of dioxins and dioxin-like PCBs from the non-fish part of the diet (0.7 pg WHO-TEQ/kg bw/day) and from one portion of cod per week (0.04 pg WHO-TEQ/kg bw/day).

e   TDI = 2 pg WHO-TEQ/kg bw/day

f   Guideline level for less susceptible subgroups = 8 pg WHO-TEQ/kg bw/day

Brominated Flame Retardants

3.39   Brominated flame retardants (BFRs) are widely used to reduce the risk of fire in plastics, electronic equipment and textiles. As a consequence, they have become widespread in the environment where they are bioaccumulative and are probably persistent.

Recent and current studies

3.40   The Centre for Environment, Fisheries and Aquaculture Science (CEFAS) has investigated polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) in fish and sediment from UK rivers and the North Sea. This work was carried out to assess the effects of BFRs on the fish (Allchin *et al.,* 1999 and 2001).

3.41   In addition to the work carried out by CEFAS, other researchers have found elevated concentrations of the chemicals in marine fish from the North Sea (Boon *et al.,* 2002). A market basket study was carried out in Sweden in which PBDE intake was investigated in samples of fish, meat, milk products, eggs, fats and oils and pastry food groups (i.e. the product groups assumed to contribute most to total intake) (Darnerud *et al.*, 2000). Almost half the total intake originated from fish products whilst approximately 15 per cent was accounted for by meat, milk products and fats and oils.

3.42   Following the report by CEFAS, the Agency analysed PBDEs and HBCDs in samples of trout and eels caught at various locations in the Skerne-Tees River system and in 2001 TDS samples (FSA 2004). The COT concluded:

- We *conclude* that the uncertainties and deficiencies in the toxicological databases for PBDEs and HBCD prevent establishment of tolerable daily intakes. A Margin of Exposure (MoE) approach has therefore been used in this risk assessment.

- We *consider* that the most sensitive endpoint for the PBDEs appears to be neurodevelopmental effects resulting from a single oral administration to neonatal mice at a developmental stage comparable to infants up to one month of age, and limited data indicate that HBCD

could also have this effect. It is reassuring that infants of this age do not eat fish and therefore are not directly exposed to PBDEs from this source.

- We *note* the uncertainty in the relevance of the neurodevelopmental effects for exposure to the fetus or breast-fed infant following maternal consumption of fish containing high levels of PBDEs or HBCD. This results from the lack of neurodevelopmental studies with exposure during pregnancy and the lack of information on concentrations in breast milk that could result from consumption of fish by the mother.

- We *note* that consumption of fish from the Skerne Tees is unlikely to be widespread since there are no commercial fisheries in the area. However given the variability in BFR levels observed in this limited survey, it is not possible to exclude higher intakes in a small number of anglers or others eating their fish.

- We *consider* that comparison of the worst case estimated intakes from consumption of a single portion of eels or trout per week from the Skerne Tees with the available toxicological data indicates that these intakes are unlikely to represent a risk to health. However, in view of the uncertainties surrounding the toxicological database and exposure assessments, this conclusion should be considered tentative.

- PentaBDE and octaBDE are being phased out in 2004, which offers some reassurance that exposure to these compounds is unlikely to increase significantly. Concentrations of deca-BDE and HBCD should continue to be monitored, particularly in fatty foods.

3.43   The FSA is currently conducting a survey of PBDEs, HBCD and the other major BFRs tetrabromobisphenol A (TBBPA) and the polybrominated biphenyls in fish. The COT will be invited to advise on the toxicological implications of the data when available.

Additives

3.44   Concerns have been expressed in the media about the use of additives in farmed fish. Use of these is controlled by regulatory measures that are set to ensure that safety guidelines are not exceeded.

Inorganic contaminants

Which inorganic contaminants are present and why

3.45   Metals and other elements are present in water both from natural sources, such as the rocks of the sea bed, and as a result of human activities, such as emissions from industrial processes. These elements are taken up by marine organisms and many tend to accumulate in organisms such as predatory fish which are higher up the food chain. As a result, the concentrations in fish of many elements, including mercury, arsenic, lead, and cadmium whose potential toxicity is of concern, can be relatively high compared with levels in other foods. For instance, in the most recently published survey of metals and other elements in the TDS (MAFF, 1999b), which is representative of the average UK diet, the composite fish group contributed 94% and 33% to the total population dietary exposure for arsenic and mercury respectively. Fish can therefore make a significant contribution to the dietary intakes of these elements.

Mercury

3.46   Mercury occurs naturally as a mineral and is widely distributed throughout the environment as a result of natural and human activities (Environment Agency, 2002a). The major natural sources are volatilization from marine and aquatic environments, volcanic emissions and degassing from geological materials. The major anthropogenic sources of mercury include combustion of fossil fuels (particularly in Asian countries including China, India, South and North Korea), and emissions and discharges from industrial processes such as cement production, production of non-ferrous metals and iron and steel, and disposal of waste containing mercury.

3.47   Mercury cycles between atmosphere, water and terrestrial compartments, undergoing a series of complex and physical transformations, not all of which are fully understood. Wet deposition is the main way in which

mercury is transported from the atmosphere to surface waters (US EPA, 1997). Mercury deposited on land can also be washed into water and it is also present naturally from mercury containing rocks of the sea bed. Thus the flux of mercury between atmosphere to land or water at any one location is comprised of contributions from the natural global mercury cycle, regional and local sources which also include direct discharges to water in addition to air emissions.

3.48   Mercury typically enters bodies of water as elemental mercury, or inorganic mercury salts. It may be adsorbed onto organic sediment particles and is likely to remain bound unless consumed by aquatic organisms. Ingestion of elemental or inorganic mercury by biological organisms, mainly sulphur-reducing forms of anaerobic bacteria and other organisms of the first trophic level, results in the biotransformation of mercury into methylmercury (Friberg *et al.,* 1986). Methylmercury is a fat soluble molecule that passes easily through cell membranes, and is readily taken up by aquatic organisms. It progressively accumulates in the tissues of fish and other aquatic /marine animals and is magnified up successive levels of the food chain. Thus longer lived, larger fish that feed on smaller fish accumulate the highest levels of methylmercury.

3.49   Methylmercury is the predominant form of mercury in fish. Studies have reported methylmercury percentages with respect to total mercury of 75 –100% in tuna (with an average of 91% -Storelli *et al*., 2002), greater than 85% in muscle of sardines (Joiris *et al.*, 1999), between 67% and 100% in swordfish muscle and canned tuna (Kamps and Miller, 1972) and 81 – 100% in shark (Storelli *et al.,* 2001).

Are some types of fish/geographical locations better/worse?

3.50   There are a number of variables that can influence mercury fish tissue concentrations, including the species of fish, the size/length/age of the fish and the proximity of the fish during its lifetime to sources of mercury. FSA surveys to date have measured mercury in a variety of different fish species that are consumed in the UK, but considerations of size/length or geographical location were not factored into these surveys. A brief review of some of the available scientific literature gives an indication (as

described below) as to the importance of these factors, but it should be borne in mind that this review was not extensive.

Species

3.51   As discussed above, predatory fish can contain relatively high levels of mercury because they are high up the food chain. Unlike dioxins and PCBs, mercury is not specifically associated with oily fish. This was apparent in the results of a recent FSA survey of mercury in imported fish and shellfish, UK farmed fish and their products (Table 3.6) (FSA 2002c), in which shark, swordfish and marlin were found to contain higher levels of mercury than other marine fish. Other fish, such as fresh tuna and orange roughy were in the middle of the range of concentrations found, containing on average 2 – 4 times less mercury than those fish containing the highest levels of mercury. Canned tuna was found on average to contain half the amount of mercury as fresh tuna. This is because different species and smaller more immature fish are used for canning. Levels of mercury in other fish were relatively low and similar to those found in a previous multi-element survey of the most commonly consumed marine fish. It must be stressed however that only a limited number of some species were sampled.

3.52   That some fish species accumulate higher levels of mercury is also reflected in EU legislation. EC Regulation 466/2001, as amended by EC regulation 221/2002 (Commission Regulation EC No 221/2002), sets a limit for total mercury of 0.5mg/kg in all fish, except for some species at a higher trophic level (such as shark, swordfish, marlin, pike and tuna) for which a higher maximum limit of 1 mg/kg applies. The vast majority of fish comply with these limits although occasionally samples of more exotic fish species such as shark and swordfish can contain higher levels. Increasing amounts of these fish can be found in shops, although the total amount of shark and swordfish (including marlin) imported and landed in the UK in 2001 is still minimal (around 1,500 tonnes), when compared with over 170, 000 tonnes of cod and haddock.

Table 3.7: Results of the 2002 survey of mercury in imported fish and shellfish, UK farmed fish and their products.

Fish	Samples	No of Levels of Hg (mg/kg) (adjusted for recovery)			
		Minimum	Maximum	Median	Mean
Fresh/Frozen Fish					
Halibut	8	0.04	0.62	0.29	0.29
Hoki	2	0.08	0.31	0.19	0.19
Monkfish	2	0.1	0.30	0.20	0.20
Orange Roughy	6	0.53	0.65	0.60	0.60
Other*	13	0.006	0.661	0.04	0.11
Pollack	4	0.007	0.02	0.01	0.01
Salmon	14	0.03	0.08	0.05	0.05
Sea Bass	4	0.03	0.09	0.07	0.07
Sea Bream	4	0.05	0.06	0.05	0.05
Shark	5	1.00	2.2	1.4	1.5
Swordfish	13	0.15	2.7	1.4	1.4
Marlin	7	0.41	2.2	0.88	1.1
Trout	14	0.01	0.1	0.05	0.06
Tuna	20	0.1	1.5	0.31	0.40
Processed Smoked					
Other **	9	0.01	0.93	0.23	0.31
Salmon	9	0.04	0.08	0.05	0.06
Trout	9	0.05	0.09	0.07	0.07
Processed Canned					
Anchovy	9	0.03	0.06	0.05	0.05
Other ***	9	0.003	0.08	0.01	0.02
Pilchard	9	0.005	0.05	0.01	0.02
Salmon (Pink)	19	0.008	0.04	0.03	0.03
Salmon (Red)	13	0.01	0.07	0.03	0.04
Sardine	9	0.01	0.10	0.03	0.04
Tuna	54	0.03	0.71	0.15	0.19

Notes:
1   Atlantic icefish
*   Fresh/frozen fish - 'Other' included samples of: hake, red tilapia, plaice, sardines, St Peter's fish, snapper, Mekong catfish, Antarctic ice fish, haddock and anchovies.
**   Processed, smoked 'Other' included samples of: haddock, halibut, eel, swordfish, tuna, marlin, mussels and oysters.
*** Processed canned 'Other' included samples of: lumpfish caviar, vongole, crab, clams, oysters, cockles, cod roe, shrimps and herring.

3.53   The last two FSA/MAFF surveys of fish have covered those fish which are most widely or frequently consumed in the UK. However, there is likely to be a number of species consumed in the UK that have the potential to accumulate relatively high levels of mercury and on which we have no data. These could include predatory species such as pike and bass. We also

have limited data on variation in fish families – for instance shark and tuna. In the FSA survey, fish labelled as shark were analysed, but other members of the shark family such as huss and dogfish – popular in fish and chip shops – were not sampled.

Size

3.54   A large number of studies in the scientific literature have reported a positive correlation between the size of fish and mercury levels reflecting an accumulation with age (Holsbeek *et al*., 1997; Polak-Juszczak and Sobolewska, 1999; Burger *et al*., 2001). This is not surprising as those fish species which live longer and grow to greater sizes have longer for methylmercury to build up in their bodies. It has previously been proposed that this correlation may be strong enough to be used as an approach to regulating mercury in fish, that is to establish fish size limits with safe heavy metal concentrations (Storelli *et al*., 2002).

Geographical location

3.55   Studies of fish from areas of known mercury pollution have found higher levels of mercury than in fish from less contaminated areas (Diaz *et al.,* 1994; Abreu *et al.*, 2000; Gerstenberger and Dellinger, 2002) and location is considered to be an important factor in predicting mercury fish tissue concentration (Qian *et al.,* 2001). This may reflect that the uptake of mercury can be faster by fish in contaminated areas and hence they can achieve a higher tissue mercury content in a shorter period of time (Castilhos *et al*., 2001).

3.56   However, it is often difficult to compare the results of different studies investigating the relationship between geographical location and mercury content because of the inconsistency in information provided in terms of sample size and fish length and weight, which are significant variables in themselves (Storelli *et al*., 2002). A number of studies have not demonstrated a significant difference in mercury fish muscle levels from different locations (Burger *et al*., 2001), which may reflect the movements or migration patterns of the fish (Polak-Juszczak, 1996).

Origin labelling

3.57   The Fish Labelling (England) Regulations came into force on 28th March 2003. The new Regulations require that certain fish products* must be labelled with:

    a)   A commercial designation (i.e., an agreed name for that species)

    b)   The production method (i.e., whether it is farmed, or caught in the wild)

    c)   The catch area (i.e., an area of the ocean in the case of sea caught fish; the member state or country of origin in the case of farmed fish or fish caught in inland waters.).

Effects of mercury

3.58   Humans may be exposed to three forms of mercury: elemental, inorganic and organic. There is a common mechanism of mercury toxicity, binding of mercuric ions to thiol groups in proteins leading to alterations in cell function and cell death. However, the organs affected vary as a result of differences in the physicochemical properties between the three forms. The mercury present in fish is methylmercury (organic form), whereas inorganic mercury is more likely to be present in other foods. Organic mercury is considered to be more toxic than other forms of mercury following ingestion. The threshold approach is considered to be appropriate.

3.59   Acute toxicity of methylmercury affects the kidneys and the central nervous system. The developing central nervous system of the fetus is particularly at risk. High exposure *in utero* has resulted in cerebral palsy or severe mental retardation in the neonate. Based on a number of poisoning incidents (Minamata, Niigata, Iraq), JECFA concluded that a minimum level of toxicity would be associated with exposures resulting in 200 μg/L

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*   The fish products covered are those included in Chapter 3 of the customs tarrif codes. Broadly speaking, this is fish and shellfish that has not been cooked / processed, and that does not contain any additional ingredients, e.g. fresh, frozen and chilled fish, smoked fish, boiled crustaceans and molluscs, shelled/peeled crustaceans and molluscs. Value added products such as fish fingers and ready meals are not covered.

mercury in blood or 50 µg/g mercury in hair. This association was used to derive a PTWI for methylmercury of 3.3 µg/kg bw/week (corresponding to a blood mercury level of 33 µg/L or hair mercury level of 8.25 µg/g in a 70kg adult). JECFA noted that pregnant women and nursing mothers may be at greater risk than the general population (WHO, 2000).

3.60   Exposure in pregnant women, at levels without effect in other adults, has been reported to cause subtle neurological defects such as delays in reaching milestones (walking, talking) and reduced learning capacity. A number of epidemiological studies have been conducted in an attempt to establish a threshold for the effects on the neurodevelopment of children, the major ones based in the Faroe Islands and Seychelles Islands (Grandjean *et al.*, 1997; Davidson *et al.,* 1998). These studies have investigate whether effects are occurring in populations with exposures associated with maternal hair mercury concentrations in the region of 10 µg/g, correlating with a dietary intake of about 0.7 µg/kg bw/day. However the epidemiological evidence is inconsistent, the Faroe Islands study has demonstrated detrimental effects, whereas that in the Seychelles has not. In contrast, higher scores (enhanced performance) in some measures were associated with higher mercury exposure in the Seychelles study, which the researchers considered to be possibly due to the assumed beneficial effects of fish consumption. Based on the data from the Faroe Islands, and earlier incidents in Iraq, the US Environmental Protection Agency (EPA) concluded that effects would be expected in 5% of the population with 12 µg mercury/g of maternal hair. This was used in setting a reference dose (RfD) for methylmercury of 0.1 µg/kg bw/day (NRC, 2000).

3.61   In June 2003, JECFA revised its PTWI to 1.6 µg/kg bw/week, in order to be protective of the developing fetus. This evaluation took into account new data from the Seychelles Child Development Study, re-analyses of the Faroes study, and additional epidemiological data, including inconclusive evidence of an association with cardiovascular disease.

Uncertainty in the TDI & RfD

3.62   There are a number of uncertainties involved in assessing the evidence, setting the safety guideline and comparing this to realistic exposures:

a) Inconsistencies in the epidemiological evidence lead to uncertainty over which data should be used in establishing a safety guideline

b) The EPA took a more precautionary approach than JECFA, basing its RfD on a minimal human effect level for the high risk groups, using an uncertainty factor of 10 for human variability. An uncertainty factor of 10 is normally used to extrapolate from the average to the most sensitive individuals and may be unnecessarily high to allow for variability within the high risk group.

c) There are differences between the affected populations and the UK population

Dietary exposures to mercury

3.63   The estimated dietary exposure to total mercury from the UK diet is 3.1 µg/day for an average adult consumer (equivalent to 0.044 µg/kg bw/day for a 70.1 kg adult) and 6.4 µg/day for a high-level consumer (equivalent to 0.09 µg/kg bw/day for a 70.1 kg adult). These results come from a survey of metals in the 1997 UK TDS (MAFF, 1999b) and are similar to estimates for other countries.

3.64   Dietary exposure of UK consumers of fish were estimated from the results of a survey of mercury and other metals in various commonly-consumed fish and shellfish and reported in May 1998 (MAFF, 1998b). In this survey, a high-level adult consumer of fish or shellfish had an estimated exposure to mercury of 11 µg/day (equivalent to 0.16 µg/kg bw /day assuming average bodyweight of 70.1 kg).

3.65   An FSA survey of mercury in exotic fish species including shark, swordfish and marlin, as well as other imported fish products like fresh and canned tuna, and UK farmed salmon and trout was recently carried out. The aim was to provide data on fish and shellfish on which we previously had limited or no information, and to keep our exposure estimates up-to-date. The following tables are the exposure estimates for fish where consumption data were available (Table 3.8), or for fish for which there is no consumption data in the NDNS, the averaged intakes arising from one

weekly portion of fish (canned tuna is included for comparative purposes) (Table 3.9).

Table 3.8: Estimated mean and high level dietary intakes of mercury from salmon, prawns, canned tuna and the whole diet.

Consumer group	Mercury Intake - µg/kg bw/day 1							
	Salmon [2]		Prawns [2]		Canned Tuna [2]		Whole Diet [3,4]	
	Mean	97.5%	Mean	97.5%	Mean	97.5%	Mean	97.5%
Infants	0.0014	0.0014	0.00	0.00	0.0057	0.019	0.0086	0.02
Toddlers	0.026	0.076	0.019	0.064	0.12	0.35	0.12	0.29
Young People aged 4–6	0.026	0.056	0.013	0.049	0.076	0.23	0.11	0.26
Young People aged 7–10	0.016	0.051	0.0086	0.021	0.056	0.18	0.089	0.20
Young People aged 11–14	0.013	0.033	0.0057	0.019	0.046	0.14	0.061	0.17
Young People aged 15–18	0.011	0.021	0.0057	0.016	0.039	0.097	0.051	0.12
Adults	0.0086	0.034	0.0057	0.02	0.036	0.089	0.05	0.12
Adults Women only	0.0086	0.026	0.0057	0.017	0.039	0.089	0.049	0.11

Notes:
1 Consumption data for salmon, prawns and tuna are taken from the following sources:
   • Dietary and Nutritional Surveys of British Adults (Gregory et al., 1990).
   • Food and Nutrient Intakes of British Infants Aged 6-12 Months (Mills and Tyler, 1992).
   • National Diet and Nutrition Surveys Children Aged 1.5 – 4.5 years (Gregory et al., 1995).
   • National Diet and Nutrition Survey: young people aged 4-18 years. Volume 1 report of the diet and nutrition survey (Gregory et al., 2000)
2 Mercury intake from eating the named fish only, for the mean and 97.5th percentile consumers.
3 Mercury intake from consumption of fresh salmon, prawns, canned tuna and the rest of the normal UK diet (based on the 1997 TDS) for consumers of fish 27. The total mercury intakes do not equal the sum of the mercury intakes from the named fish because the populations of consumers differ (for example not all fish consumers eat prawns).
4 The measurement of mercury does not distinguish between inorganic and organic mercury. Therefore although methylmercury is the major contributor to mercury intake from fish, the estimate of intake from the whole diet also includes inorganic mercury.

Table 3.9: Mercury intake from one portion of shark, swordfish, marlin, fresh tuna or canned tuna.

Age group (years)	Body Weight (kg)	Average Portion Size (g)	Weekly methylmercury intake assuming one portion of fish per week (µg/kg bw/day)				
			Shark	Swordfish	Marlin	Fresh Tuna	Canned Tuna
1.5 – 4.5	14.5	50	0.75	0.66	0.54	0.20	0.094
4 – 6	20.5	60	0.63	0.56	0.46	0.17	0.080
7 –10	30.9	85	0.60	0.53	0.43	0.16	0.074
11 – 14	48.0	140	0.63	0.56	0.46	0.17	0.079
15 – 18	63.8	105	0.36	0.32	0.26	0.094	0.044
Adults	70.1	140	0.43	0.38	0.31	0.11	0.054

Notes:
1   The average portion size that each age group of the population would consume at a single meal event for fish consumption, as recorded in the following National Diet and Nutrition Surveys (NDNS):
•1995 National Diet and Nutrition Survey: Children aged one-and-a-half to four-and-a-half years (Gregory et al., 1995).
•2000 National Diet and Nutrition Survey: young people aged 4 to 18 years (Gregory et al., 2000).
•1990 The Dietary and Nutritional Survey of British Adults (Gregory et al., 1990).
2   This intake estimate does not include the intake from the rest of the diet, which is estimated to be 0.052 µg/kg bw/day for a 60kg average consumer (0.36 µg/kg bw/week). However not all of this will be methylmercury.

COT assessment

3.66   The COT considered methylmercury toxicity in 2002 following the survey of mercury levels in imported and UK farmed fish (COT, 2002). COT concluded that because of the risk to the developing brain and nervous system of the fetus or neonate, pregnant women, women who may become pregnant within the next year and breast feeding mothers should be considered as high risk groups when considering methylmercury toxicity. Whilst the JECFA PTWI was sufficiently protective for the general population, the EPA reference dose would be more applicable for the high-risk groups since it is based on the effects on the developing fetus.

3.67   The COT concluded:

a)   Average and high-level dietary exposure to mercury is within the JECFA PTWI for methylmercury for all age groups.

b) Adult women who are high level consumers of fish may marginally exceed the EPA reference dose for methylmercury but this would be unlikely to result in adverse effects to the developing fetus.

c) Consumption by adults of one weekly portion of shark, swordfish or marlin would result in a dietary exposure close to or exceeding the JECFA PTWI. This consumption was not expected to result in adverse effects in the general population, but could be harmful to the fetus or breast-fed infant. For children less than 14 years of age, the occasional consumption of these fish would not be expected to result in adverse effects.

d) Consumption of one portion (140g) of fresh tuna or two medium-size cans of tuna a week (with a drained weight of about 140g per can) by pregnant women, women who may become pregnant within the next year and breast feeding mothers would not be expected to result in adverse effects on the developing fetus or neonate.

3.68   Following the revision of the JECFA PTWI in June 2003, the COT reviewed its opinion and issued an updated statement (COT, 2003). The updated COT conclusions were:

- We *note* that there has been no new information published to indicate that the 2000 PTWI of 3.3 µg/kg bw/week is not sufficiently protective of the general population. We therefore *consider* that a methylmercury intake of 3.3 µg/kg bw/week may be used as a guideline to protect against non-developmental adverse effects.

- We *conclude* that the 2003 JECFA PTWI of 1.6 µg/kg bw/week is sufficient to protect against neurodevelopmental effects in the fetus. This PTWI should be used in assessing the dietary exposure to methylmercury of women who are pregnant, and who may become pregnant within the following year.

- We *consider* that a guideline of 3.3 µg/kg bw/week is appropriate in considering intakes by breastfeeding mothers as the intake of the

breast-fed infant would be within the new PTWI of 1.6 µg/kg
bw/week.

- We *consider* the NDNS blood level data are reassuring with respect to
  average and high level consumption of fish. The adults surveyed had
  blood mercury levels indicating that 97.5% of the population had
  dietary intakes below 1.6 µg/kg bw/week.

- We *conclude* that average and high-level dietary exposure to
  methylmercury, resulting from the wide range of fish for which
  consumption data are available, is not likely to be associated with
  adverse effects in the developing fetus or at other life stages.

- We *note* that consuming one weekly 140 g portion of either shark,
  swordfish or marlin would result in a dietary methylmercury exposure
  close to or above 3.3 µg/kg bw/week in all age groups. We *consider*
  that this consumption could be harmful to the fetus of women who are
  pregnant or become pregnant within a year, but would not be expected
  to result in adverse effects in other adults.

- We *note* that the mercury content of tuna is lower than that of shark,
  swordfish or marlin, but higher than that of other commonly
  consumed fish. We *consider* that consumption of two 140g portions of
  fresh tuna, or four 140g portions of canned tuna, per week, before or
  during pregnancy would not be expected to result in adverse effects on
  the developing fetus.

- We *recommend* that further research should include development of
  analytical methodology to allow direct measurement of
  methylmercury, mechanistic studies to help elucidate population
  groups more at risk and research integrating the risks with nutritional
  benefits of fish consumption.

3.69   The Inter-Committee Subgroup has further reviewed estimated intakes of
        mercury from 1 to 3 portions of oily fish per week (Table 3.10). These
        values are compared with the PTWI and Guideline Level for
        methylmercury. Non-fish sources of mercury are not included in this

comparison, since they are likely to be inorganic forms, which are less well-absorbed and therefore less toxic via the oral route. Furthermore, not all mercury in fish is expected to be methylmercury, and therefore this comparison is precautionary.

3.70   These data demonstrate that commonly consumed oily fish, other than swordfish and fresh tuna, are unlikely to result in exceedance of the PTWI.

Mercury in fish oils

3.71   The levels of mercury measured in fish oil supplements were analysed in a survey of dietary supplements reported in 1998 (MAFF, 1998c) and were found to be lower than those found in fish. The COT considered these results and concluded that estimated dietary intakes of metals from the supplements analysed in addition to the rest of the diet were not a cause for concern.

Mercury in infant foods

3.72   The FSA has recently carried out a multi-element survey of a wide range of manufactured infant foods (FSA 2004). 189 samples of commercial baby foods (infant formulae, manufactured ready-to-eat and dried baby foods, desserts, rusks and infant drinks) were analysed for mercury and 11 other metals and elements. Infants consume a diet that is different in many ways from that of adults and of children old enough to eat conventional adult foods. Infants' diets are made up of a more restricted range of foods, particularly before and in the early stages of weaning when the diet is made up entirely or largely of breast milk and/or commercial infant formulae. On weaning, solids may be given, a large proportion of which may be commercially available baby foods. The composition of commercial infant formulae and baby foods can be very different from the foods that make up the diet of the general population.

3.73   Mercury was detected at concentrations at or above the Limit of Detection (LOD) in about one quarter of the samples in this survey, most of which are dried foods. The mean mercury concentration was 0.003 mg/kg, with a range of less than 0.0005 mg/kg to 0.02 mg/kg. Both the mean and maximum mercury concentrations are twice that of the last infant food

Table 3.10: Estimated dietary intake of mercury from oily fish and the rest of the diet for an adult of 60 kg bodyweight

	Herring	Mackerel	Salmon	Trout	Fresh Tuna	Swordfish
Concentration [a] (µg /g wet weight)	0.09	0.05	0.05	0.06	0.4	1.4
Intake from one portion fish per week [b] (µg/kg bw/week)	0.21	0.12	0.12	0.14	0.93	3.27
Intake from rest of the diet [c] (µg/kg bw/week)	0.21	0.21	0.21	0.21	0.21	0.21

	% PTWI or guidance level for methylmercury											
Portions per week	Herring		Mackerel		Salmon		Trout		Fresh Tuna		Swordfish	
	%PTWI [d]	%GL [e]	%PTWI	%GL	%PTWI	%GL	%PTWI	%GL	%PTWI	%GL	%PTWI	%GL
1	13	6	7	4	7	4	9	4	58	28	204	99
2	26	13	15	7	15	7	18	8	117	57	408	198
3	39	19	22	11	22	11	26	13	175	85	613	297
4	53	25	29	14	29	14	35	17	233	113	817	396

a   Concentrations in oily fish species and cod taken from surveys for mercury in marine fish 1995-97 (cod, herring and mackerel) and 2002 (salmon and trout). Predominantly but not exclusively in the form of methylmercury.

b   Assumes 140g portion size for all fish

c   Averaged weekly intake of mercury from the non-fish part of the diet (0.06 (mg/kg bw/week) and from one portion of cod per week (0.15 mg/kg bw/week). Provided for information, but not included in the comparison with the PTWI and guidance level

d   PTWI = 1.6 mg/kg bw/week for methylmercury

e   Guideline level for less susceptible subgroups = 3.3 mg/kg bw/week for methylmercury.

survey reported in 1998 (mean of 0.0014 mg/kg, range <0.0003 mg/kg to 0.01 mg/kg). However, the increase in the mean concentration of mercury is in part due to the slight decrease in sensitivity for mercury analyses in this survey. This resulted in increased LODs which will effect the upper bound mean values reported (compared to LODs in previous survey, the higher LODs have increased the mean by about one third).

3.74   In the absence of current consumption data for this age group (the most recent UK infant dietary survey is 1986), three approaches were taken to estimate infant exposure. The COT used two of these approaches (the third was not considered appropriate because of insufficient supporting data). Exposures were estimated using consumption data from the 1986 infant survey (6-12 month olds), and using manufacturers feeding recommendations (from birth to 12 months), thus providing a range of exposures that could be compared to relevant safety guidelines. The approach based on manufacturer's feeding recommendations is a worst case, as it does not take into account wastage/non-retention of food by the infant. A summary table of exposures is given below (Table 3.11).

3.75   The major contribution to dietary mercury exposure was from ready-to-feed manufactured meals. Fish containing meals (7 samples) made a significant contribution to the mean concentration of mercury in this food group, accounting for approximately half of the mercury reported (although they only accounted for a small proportion of the overall mean mercury concentration for all foods surveyed – about one thirtieth). Fish containing meals contributed about one fifth of the dietary exposure to mercury using this approach. However, since this survey was carried out, the manufacturers of the fish meals covered in this survey either no longer manufacture fish containing infant foods or have ceased to manufacture infant foods entirely.

3.76   Fish containing meals made less of an overall contribution to mercury exposures estimated using the other approach which was based on manufacturers feeding recommendations.

Table 3.11: Dietary exposures to mercury from manufactured infant foods using Approach 1 (6-12 month old UK infant survey) and Approach 2 (manufacturers feeding recommendations).

| | | Intake for each age range (mg/kg bw/day) | | | |
		0-3	4-6	7-9	10-12
Approach 1	Mean				0.04
	97.5%				0.11
Approach 2	Normal Diet	0.07	0.18	0.18	0.19
	Soya Diet	0.07	0.19	0.19	0.20

COT Assessment

3.77   The COT considered that the increase in the concentrations of mercury in infant foods could, in part, be accounted for by the number of fish meals and the higher limit of detection for mercury in this survey compared to the previous one.

3.78   In considering the exposure estimates the COT took a precautionary approach, assuming that all of the mercury was in the more toxic organic form and that the 2003 JECFA PTWI for methylmercury of 1.6 µg/kg bw/week (0.23 µg/kg bw/day) was the most appropriate safety guideline. The COT concluded that:

a)   The consumption of the infant foods sampled in the survey would not result in the intake of such quantities of any of the analysed elements such as would give concern for the health of infants.

b)   The levels of mercury in the foods should continue to be monitored to ensure that they are not rising.

Other inorganic contaminants that can accumulate in fish that are of toxicological concern.

Arsenic

3.79   Arsenic can enter the environment from natural sources, such as rocks and sediments, and as a result of human activities such as coal burning, copper smelting, dye production from tanneries and processing of mineral ores (National Academy of Sciences, 1977). Arsenic has a high ability to

accumulate in bottom sediments (Svobodova *et al.,* 2002), so arsenic levels are higher in the aquatic environment than on land. Therefore, marine and mainly marine crustaceans and molluscs strongly accumulate arsenic compounds.

Toxicology

3.80   Arsenic in drinking-water (primarily inorganic, as arsenate and to a lesser extent arsenite) is classed as "carcinogenic to humans" (Group 1) on the basis of "sufficient evidence" for an increased risk for cancer of the urinary bladder, lung and skin (IARC, 2002). Chronic exposure to arsenic in drinking water has also been associated with peripheral vascular diseases, cardiovascular diseases and possibly with diabetes and reproductive effects.

3.81   Most arsenic in fish (>90%) is in the form of arsenobetaine which is also the main form found in crustaceans and bi-valve molluscs, the remainder is arsenocholine and a small amount of inorganic arsenic (usually < 1%) (Kohlmeyer *et al.*, 2002). Fish is the main source of arsenic in the diet; arsenobetaine is therefore the main form of arsenic present in food.

3.82   The fate of organic arsenic has not been clearly defined in experimental animals or in humans. In general organoarsenicals are thought to be less extensively metabolized than inorganic arsenic and more rapidly excreted. Limited data indicate that organic arsenic compounds such as arsenobetaine and arsenocholine are not converted to inorganic arsenic *in vivo.* Despite the limited database, the organic forms of arsenic are generally assumed to be less toxic than the inorganic compounds. In contrast to mercury, there are no reports of toxicity in man or animals from the consumption of organoarsenicals in seafood. Limited data indicate that arsenobetaine and arsenocholine are not genotoxic in mammalian cells *in vitro*.

3.83   The COT has concluded that there are no relevant tolerable intakes or reference doses by which to assess safety of either inorganic or organic arsenic in the diet. Inorganic arsenic is genotoxic and a known human carcinogen, and therefore exposure should be as low as reasonably practicable (ALARP) (COT 2003).

Exposure

3.84   Fish generally contains relatively high levels of arsenic compared with other foods and is the most significant source of arsenic in the UK diet. In the 1997 UK TDS (MAFF, 1999b), the fish group contained the highest average concentrations of arsenic [4.4 mg/kg in comparison with other food groups which contained average arsenic concentrations ranging between 0.0004 and 0.007 mg/kg]. Fish consumption contributed 94% of the average population dietary exposure to arsenic in comparison to the next most significant dietary contributor of 2% (jointly, bread, miscellaneous cereals and beverages which each contributed 2%).

3.85   Inorganic arsenic was measured for the first time in the 1999 UK TDS. Again in this survey, the fish group contained the highest average level of total arsenic (3.2 mg/kg, 44 times greater than the poultry food group, which contained the second highest average level, 0.073mg/kg). Inorganic arsenic was only measurable in three out of the twenty food groups that make up the TDS - fish, poultry and miscellaneous cereals. This is because all other food groups contained levels of total arsenic below the LOD for inorganic arsenic. The highest average level of inorganic arsenic was recorded for the fish group (0.0159 mg/kg).

3.86   Fish consumption again contributed the major portion (almost 90%) of the average population dietary exposure to total arsenic. Most of this exposure is to organic species of arsenic as can be seen from consumer exposure estimates. For an average adult consumer, dietary exposure to total arsenic from fish is 1.63 μg/kg bw/day, whereas exposure to inorganic arsenic is 0.008 μg/kg bw/day. For a high level consumer, dietary exposure to total arsenic from fish is 4.64 μg/kg bw/day, whereas exposure to inorganic arsenic is 0.023 μg/kg bw/day.

COT Assessment

3.87   The COT noted that fish is a major contributor to dietary exposure to arsenic with the predominant form of arsenic in fish being organic. Members also noted that the general assumption that organic arsenic is less toxic than inorganic arsenic is based on an extremely limited database. However they considered that there is no evidence that exposure to organic

arsenic through high levels of fish consumption would result in harmful effects, and therefore concluded that the dietary exposure to organic arsenic identified in the survey was unlikely to constitute a hazard to health.

3.88   The COT were also reassured that the average population dietary exposure to total arsenic was lower than that estimated for previous years, indicating that dietary exposure to total arsenic through food is not increasing

Manufactured infant foods

3.89   As described in the above section on mercury, the FSA has recently completed a multi-element survey of infant foods.

3.90   Arsenic was detected in most samples but generally at very low concentrations (mean 0.023 mg/kg, range of less than 0.0002 to 0.78 mg/kg), with the highest concentrations found in products containing fish. Higher levels were also seen in manufactured meals containing poultry and rice, although these were lower than the levels measured in fish-containing dishes. These results are consistent with results obtained for the foods making up the adult diet and with other scientific literature. The mean value for all foods is similar to the mean of 0.016 mg/kg found in a 1998 MAFF survey of infant foods. (MAFF, 1998d)

3.91   Exposures to arsenic from manufactured infant foods were estimated as described in the section on mercury and are summarized in table 3.11 below.

Table 3.12: Dietary exposures to arsenic from manufactured infant foods using Approach 1 (6-12 month old UK infant survey) and Approach 2 (manufacturers feeding recommendations).

| | | Intake for each age range (µg/kg bw/day) | | | |
		0-3	4-6	7-9	10-12
Approach 1	Mean				0.25
	97.5%				0.87
Approach 2	Normal Diet	0.09	1.3	1.8	1.8
	Soya Diet	0.18	1.6	2.0	1.9

3.92   The COT considered that whilst the intakes of arsenic calculated using manufacturers feeding recommendations (approach 2) were high, those calculated using approach 1 are comparable to those seen in the previous survey and so it is unlikely that there has been an increase in exposure to arsenic. This was corroborated by the similar levels of arsenic found in infant foods compared to those seen in the previous survey. They concluded that:

a)   The consumption of the infant foods sampled in the survey would not result in the intake of such quantities of any of the analysed elements such as would give concern for the health of infants.

b)   There are no relevant tolerable intakes or reference doses by which to assess safety of either inorganic or organic arsenic in the diet. Inorganic arsenic is genotoxic and a known human carcinogen therefore exposure to inorganic arsenic should be as low as reasonably practicable (ALARP). However it was reassuring that since the previous survey arsenic intakes do not appear to have increased.

Lead and Cadmium

3.93   EC Regulation 466/2001, as amended by EC Regulation No 221/2002, sets limits for lead, cadmium, mercury and 3-monochloro propandiol (MCPD) in those foods which contribute significantly to dietary exposure. There are limits for both lead and cadmium in fish. However, in comparison to other food groups that make up the typical UK diet, fish is not one of the top 5 dietary contributors of either contaminant in the UK (MAFF, 1999b).

Cadmium

3.94   Cadmium occurs naturally in association with other metals and ores, and is widely distributed in the Earth's crust. It is also released by human activities and is a pollutant that is not degraded in the environment. Some cadmium enters the general environment from the natural weathering of minerals, from forest fires and volcanoes, but much larger amounts are released by human activities. These include production of non-ferrous metals and of iron and steel, combustion of fossil fuels, waste incineration,

and application of phosphate fertilizer and sewage sludges (Environment Agency, 2002b).

3.95 Some forms of cadmium can dissolve in water, either in rivers and ponds or in soil water. Cadmium is not very soluble and is found mainly in sediments and suspended particles. In general, cadmium enters the marine environment from atmospheric deposition and from effluent discharges. Aquatic animals, notably fish and invertebrates, absorb cadmium directly from the water, as well as from food. Cadmium is taken up directly from seawater by absorption through the cell membrane. Cadmium is reported in the literature at higher levels in fish detoxifying organs and in some species which can naturally accumulate higher levels of cadmium (Mason *et al.,* 2000). This is reflected in the EC Regulation that set a higher maximum limit for certain fish species, and only applies to muscle meat. Levels of cadmium reported in a multi-element survey of the most commonly consumed marine fish in the UK (MAFF, 1998b) were all below EC limits.

Exposure

3.96 Food and tobacco smoke constitute the most important routes for human exposure to cadmium (Friberg *et al.,* 1986). Cadmium is present at low concentrations in most foods, with those that are consumed in larger quantities making the greatest contribution to population dietary exposure. For example, from the 1997 TDS (MAFF, 1999b), cadmium concentrations in food were highest in the offal (0.077 mg/kg) and nuts (0.059 mg/kg) food groups, whereas the bread and potatoes food groups contributed the most to dietary exposure of the general population (i.e. each contributed 25% of the dietary exposure). The fish group contributed 2% of total dietary exposure to cadmium.

Lead

3.97 Lead occurs naturally in the silicate lattice of rocks and is released into the environment by a number of processes such as weathering of rocks, volcanic activity and its uptake, and subsequent release by plants. Forest fires, sea spray, plant uptake and release and windblown dusts redistribute lead in the environment (Environment Agency, 2002c).

3.98   Anthropogenic sources such as the effects of lead mining, smelting and processing, the burning of fossil fuels over many thousands of years and its long residence time in the environment have resulted in lead becoming an ubiquitous environmental pollutant. Currently, lead is used in paints, plastics, batteries, roofing materials, etc.

3.99   Like cadmium and some other inorganic chemical contaminants, lead associates with sediment. It is also labile and may be highly available for up-take by organisms. Some species of fish naturally accumulate higher levels of lead, and this is reflected by a higher limit for those species (EC Regulation 466/2001).

Exposure

3.100   Food is one of the major sources of lead exposure in the UK, the others being air and water. Dietary exposures of the general UK population have declined from 0.12mg/day estimated from the 1980 TDS to 0.026 mg/day from 1997 TDS (MAFF, 1999b). Although exposure has decreased over this period, the extent of the decrease is in part an artefact of the reduction in the limit of detection for lead over this period. The cause of this decrease is partly because of a lowering of the LOD for lead, but an actual decrease in exposure is also evident. This decrease in dietary exposure reflects the success of the measures taken by the UK and the EC to reduce lead exposure and contamination of food. Mean concentrations of lead in the 20 food groups analysed were all below 0.1 mg/kg, with the highest mean concentration of lead seen in the offals group (0.09 mg/kg). Beverages made the greatest contribution to the population dietary exposure (54%), because of the high levels of consumption of this food group. The fish group was reported with a mean concentration of 0.02 mg/kg, and fish consumption accounted for less than 1% of the overall population dietary exposure to lead.

3.101   A survey reported in 1998 (MAFF, 1998b) on the concentration of metals and other elements in commonly eaten marine fish, reported levels close or below the limit of detection for all samples, which were within the EC limit for lead of 0.2 mg/kg.

Other organic environmental contaminants

3.102   A large number of other contaminants may accumulate in fish, and there are varying amounts of information on the toxicity of these. Fish containing high levels of dioxins and PCBs are also likely to contain high levels of non-dioxin like PCBs. There is currently no agreed method of risk assessment for these compounds, although this is the subject of combined discussions of the European Food Safety Authority, US EPA and World Health Organization, scheduled for completion in December 2004.

Other inorganic environmental contaminants

3.103   In addition it should not be assumed that substances generally viewed as nutrients are necessarily safe when ingested in large amounts. For example, daily use of selenium supplements together with high level dietary exposure results in a selenium intake that is at the Safe Upper Level (SUL) recently proposed by the Expert Group on Vitamins and Minerals. High fish consumption could potentially result in the SUL being exceeded for an individual who is also taking supplements. The implications of this may depend on the simultaneous presence of methylmercury in fish as there is evidence that selenium counteracts the harmful effects of methylmercury.

Mixtures of chemicals

3.104   The evaluation of dioxins and dioxin-like PCBs allows for the combined additive effects of these substances, because they are considered to act by a common mechanism. The COT recently completed a comprehensive evaluation of the risk assessment of mixtures of pesticides and similar substances, the conclusions of which are also relevant to mixtures of other classes of chemicals. The COT concluded that when exposure levels of chemicals within a mixture are within the no-effect levels, and the components have different modes of toxic action, no additivity or potentiating interactions are found.

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Annex 1
Fish consumption in the UK

Table 4.1: List of oily and white fish

Oily/fatty fish	White fish	
Salmon	Cod	Parrot fish
Trout	Cod	Pollack
Mackerel	Haddock	Pomfret
Herring	Plaice	Red & grey mullet
Sardines	Coley	Red fish
Pilchards	Whiting	Red Snapper
Kipper	Lemon sole	Rohu
Eel	Skate	Sea bass
Whitebate	Halibut	Sea bream
Tuna (Fresh only)	Rock Salmon/Dogfish	Shark
Anchovies	Ayr	Tilapia
Swordfish	Cat fish	Turbot
Bloater	Dover sole	White fish
Cacha	Flounder	
Carp	Flying fish	
Hilsa	Hake	
Jack fish	Hoki	
Katla	John dory	
Orange roughy	Kalabasu	
Pangas	Ling	
Sprats	Monk fish	

Table 4.2: Commonly consumed oily and white fish in National Diet and Nutrition Survey of British adults aged 19 to 64 years 2000/01 with corresponding LC n-3 PUFA content [3].

OILY FISH Type of fish [1]	% Consumers during the survey week [2]	EPA (g/100g)	DPA (g/100g)	DHA (g/100g)	LC [n-3] PUFA (g/100g)
Fresh salmon [4]	9	1.2	0.2	1.3	2.7
Canned [3] and smoked salmon	8	0.55	0.14	0.85	1.54
Pickled, smoked and canned sardines and pilchards [3]	4	1.17	0.23	1.20	2.60
Canned sardines [3]		0.55	0.14	0.86	1.57
Canned and smoked mackerel	3	N/A	N/A	N/A	N/A
Fresh trout [3]	2	0.23	0.09	0.83	1.15
Pickled, smoked and canned herring, kipper and bloater	2				
Herring [3]		0.51	0.11	0.69	1.31
Kipper [3]		1.15	0.10	1.34	2.49
Fresh tuna [4]	2	0.3	0.1	1.1	1.5
Fresh mackerel [3]	1	0.71	0.12	1.10	1.93

N/A, data not available.

Taking into account the relative quantities of fish consumed by an average consumer 100g of an average oily fish contains approximately 2g (calculated to 1.99g); therefore, one portion contains about 2.8g.

WHITE FISH Type of fish [1]	% Consumers during the survey week [2]	EPA (g/100g)	DPA (g/100g)	DHA (g/100g)	LC n-3 PUFA (g/100g)
Canned tuna [3]	27	0.06	0.04	0.27	0.37
Fresh cod [3]	25	0.08	0.01	0.16	0.25
Fresh haddock [3]	9	0.05	0.01	0.10	0.16
Fresh plaice [3] and whiting	2	0.16	0.04	0.10	0.30
Smoked and salted haddock	2	N/A	N/A	N/A	
Fresh sole, including lemon sole [4] and Dover sole	2	0	0	0.1	0.1

Taking into account the relative quantities of fish consumed by an average consumer 100g of an average white fish contains approximately 0.3g (calculated to 0.28g); therefore, one portion contains about 0.4g.

[1] Includes consumption of fish in dishes.
[2] Percentage who consumed during the seven day dietary recording period.
[3] MAFF fatty acids supplement to McCance & Widdowson's The Composition of Foods, 1998.
[4] MAFF fish and fish products. Third supplement to McCance & Widdowson's The Composition of Foods, 1993.

Table 4.3: Consumption of total fish in British adults[3]

TOTAL FISH		Population mean[4]	Consumer mean	97.5%ile	Number of consumers
Consumption (g/week)	All males (766)	218	314	989	548
	All females (958)	216	295	891	697
	All (1724)	217	304	947	1245

Table 4.4: Consumption of white fish in British adults[1]

WHITE FISH		Population mean[2]	Consumer mean	97.5%ile	Number of consumers
Consumption (g/week)	All males (766)	114	239	651	366
	All females (958)	94	204	545	439
	All (1724)	104	221	610	805

Table 4.5: Consumption of oily fish (excluding canned tuna) by British adults[1]

OILY FISH (EXCLUDING CANNED TUNA)		Population mean[2]	Consumer mean	97.5%ile	Number of consumers
Consumption (g/week)	All males (766)	51	202	703	208
	All females (958)	51	188	601	260
	All (1724)	50	194	608	468

Table 4.6: Consumption shellfish by British adults[5]

SHELLFISH		Population mean[2]	Consumer mean	97.5%ile	Number of consumers
Consumption (g/week)	All males (766)	24	135	491	141
	All females (958)	31	151	504	198
	All (1724)	27	143	497	339

[1] National Diet and Nutrition Survey of British Adults aged 19-64 years 2000-01.
[2] Mean consumption of fish including non-consumers.
[3] National Diet and Nutrition Survey of British Adults aged 19-64 years 2000-01.
[4] Mean consumption of fish including non-consumers.
[5] Intake data on *cis* n-3 PUFA for British Adults is unpublished until June 2003.

Table 4.7: National Diet and Nutrition Survey fish portion sizes

Age (years)	Sex	Number	Portion size (grams)			% of sample consuming fish
			Minimum	Mean	Maximum	
1½-4½ [6]	M&F	1675	2	47	170	6
4-6 [7]	M	184	21	57	78	5
	F	171	8	68	162	10
7-10 [2]	M	256	40	85	178	8
	F	226	14	84	170	8
11-14 [2]	M	237	48	137	237	7
	F	238	13	137	196	6
15-18 [2]	M	179	49	114	354	8
	F	210	18	97	198	4
19-64 [8]	M	766	10	148	340	20
	F	958	4	143	350	21

Excludes fish coated in batter or breadcrumbs, canned fish, smoked fish, shellfish and fish in recipe dishes.

[6] National Diet and Nutrition Survey: children aged 1½-4½ years 1992-93.
[7] National Diet and Nutrition Survey: young people aged 4-18 years 1997.
[8] National Diet and Nutrition Survey of British Adults aged 19-64 years 2000-01.

Annex 2.
DHA requirements in pregnancy and lactation.

A background paper for discussion by S.A. Wootton and A.A. Jackson, Institute of Human Nutrition, University of Southampton.

Introduction

5.1   It is clear that there is a requirement for long chain polyunsaturated fatty acids (LCPUFA) for the normal development of the mammalian brain. For the human the extent of accumulation of LCPUFA during fetal life and early infancy has attracted attention and some controversy. It has been suggested that preformed LCPUFAs, particularly docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), have to be provided preformed in the diets of infants to meet the high demands of rapidly growing tissues and organs. The mother is the primary source of these essential fatty acids for the fetus and breast-fed infant. Relatively little consideration has been given to the ability of the mother to provide adequate amounts of EPA and DHA and the effect this is likely to have upon her own dietary requirements.

5.2   The current dietary intakes of DHA in the UK can be estimated to be around 100mg/d for adult women. For those individuals who do not consume fish, or are consuming a low-fat diet the intake is likely to be substantially less [1]. There is evidence that the capacity for DHA synthesis from αLNA may be limited. Therefore, it has been recommended by some that DHA itself should be considered to be an essential dietary constituent [2] and that pregnant women have the need to consume as much as 300 mg/d DHA [3].   We have reviewed evidence which provides information on the maternal requirement for LCPUFA, with a special emphasis on DHA and EPA. We sought to adress the question: whether there is sufficient evidence based on biochemical and functional outcomes to identify a specific dietary requirement during

pregnancy and lactation. In particular we have looked for evidence of the existence of a depleted state during pregnancy and/or lactation that would support the proposal of the need for a dietary recommendation for individual LCPUFA in women of child bearing age.

Measuring the demands for LCPUFA during in pregnancy and lactation

5.3　There is very little information either on the LCPUFA requirements of non-pregnant women, or the extent to which the current dietary intakes are sufficient to meet their needs. In part this may be attributed to a lack of satisfactory, agreed markers which are suitable, sensitive and specific for defining LCPUFA status, or measures which can be taken to characterize an inadequacy.

5.4　Those markers of status that are usually reported relate to concentrations of fatty acids within the circulation. These may be expressed as total fatty acids or related to their concentrations in different circulating pools. The functional significance of these measures in characterising any aspect of functional status is uncertain. Similar measures have been measured during pregnancy and taken to mark LCPUFA status, such as concentrations of fatty acids in maternal or umbilical blood. These measures may have been related to dietary intake in the mother or associated with measures of the outcome for the pregnancy, such as the duration of pregnancy, infant size at birth, or measures of growth and functional development.

5.5　During pregnancy, in addition to meeting her own usual needs for EPA and DHA, a woman must also meet the additional demands associated with the accretion of maternal, placental and fetal tissues. The timing of the changed demands may vary at different stages of the pregnancy. The evidence suggests that the mother may accumulate increased adipose tissue as a reserve from very early in pregnancy, which can then be drawn on at later stages. For the fetus the demands are likely to be particularly high during the last trimester of pregnancy. The additional demands for LCPUFA or specific fatty acids during a normal pregnancy have not been adequately defined.

5.6   Estimates of LUCPFA accretion in fetal and placental tissues during a normal pregnancy have been made, based upon information from postmortem material. It has been estimated that the fetus accumulates about 60 to 70 mg n-3 LCPUFA/d during the last trimester of pregnancy, mostly in the form of DHA [4,5]. This may be a significant under-estimate as it does not take into account the LCPUFA associated with placental tissue, nor the extent to which LCPUFA accumulate in fetal adipose tissue as a reserve for early postnatal life. A more reasonable estimate for the net accretion by the fetus during the pregnancy would be at least 10 g DHA. Of this about 6 to 7g would represent fetal accretion over the last trimester mainly for brain development. A further 2 g DHA would be deposited within about 1 kg of fetal adipose tissue.

5.7   The delivery of nutrients to the infant through human milk draws upon nutrients taken in the diet, and also the maternal reserves deposited during pregnancy. It is recommended that infants should be fed on human milk exclusively, for the first six months of life. It has been estimated that during lactation the mother has to make available about 70 to -80 mg DHA/d for milk formation, in addition to her own basal requirements [6,7]. There may be some saving of DHA through a reduction in losses associated with menstruation during lactational amenorrhea, but these are likely to be small. Thus, if a woman is to have sufficient DHA available in reserves to cover the requirements of 6 months of lactation, she would need to accumulate about 12 g DHA during pregnancy as an integral part of her adipose tissue reserves.

5.8   Taken together, an estimate of the DHA which a woman would have to accumulate during pregnancy, to meet the increased needs of her pregnancy and lactation, would be of the order of 22 – 25 g. This would be in addition to that required by the woman to satisfy her own intrinsic requirements for DHA. A typical diet for a non-pregnant woman would provide about 100 mg/d DHA, that is about 9 g DHA over the last trimester of the pregnancy and about 18 g DHA during the 6 months of lactation. If the pattern and amount of DHA in the diet was unchanged over pregnancy and lactation, and if the woman had been in balance while consuming 100mg DHA/d prior to pregnancy, then to meet the increased needs by changing consumption would require a doubling of maternal intake during

the last trimester of pregnancy and a 60-70% increase in maternal intake during lactation. There is no evidence that women selectively increase their consumption of DHA during pregnancy or lactation.

Meeting increased demands in pregnancy

5.9   If the dietary supply of DHA is marginally adequate, and the intake is not changed during pregnancy and lactation, then the extent to which the increased requirement can be met will depend on:

i)   the extent to which LCPUFA may be conserved by reducing its rate of oxidized or loss through other routes,

ii)   the amount of pre-formed EPA and DHA present in adipose tissue stores reserves that can be effectively mobilized when needed,

iii) the ability to increase the formation of DHA from precursors such as αLNA.

5.10   Amenorrhea during pregnancy will conserve some nutrients and there will be a decrease in the loss of LCPUFA through this route, however, the impact on DHA requirements is likely to be modest. The usual diet consumed in the UK may be relatively rich in n-6 fatty acids such as linoleic acid, but is likely to be poor in n-3 fatty acids such as αLNA, EPA or DHA. Thus, most women are likely to enter pregnancy with marginal or poor n-3 PUFA status [8,9]. The dietary supply of preformed DHA is generally low at any time, and a woman's ability to access n-3 PUFA contained in adipose tissue might be critical when demands are increased. For n-3 PUFA derived from the diet, or mobilized from adipose reserves, the predominant fatty acid is likely to be αLNA and a woman's ability to effectively convert this into other fatty acids, such as DHA is likely to play an increasingly important role in satisfying the fetal demands for DHA. In circumstances where the maternal reserves are low, poorly mobilized or synthesis constrained, then it is likely that the effective supply of DHA to the fetus may be compromised unless the dietary intake of DHA during pregnancy is adequate.

5.11   There is direct evidence, for adults, term and preterm infants, to show that there is conversion of αLNA to DHA. However, the conversion appears tightly regulated with a limited capacity under many situations and in amounts which are so limited that it is unlikely to be sufficient to meet a substantial increase in the demands for DHA [10-13]. However, for non-pregnant women of reproductive age, the capacity is several orders of magnitude greater [14], possibly due to oestrogen-mediated up-regulation of the second Δ6-desaturation and final peroxisomal β-oxidation reaction [15]. There is the possibility that the fetus contributed to its own needs by synthesising DHA from LCPUFA.

5.12   There are no measurements of the magnitude of maternal DHA synthesis in pregnancy. Based, on information from tracer studies it is unlikely that more that 5% of available αLNA would be converted to DHA. It remains to be determined whether conversion at this rate is adequate to meet the increased needs of pregnancy and lactation, although the indications are that it is unlikely to be sufficient. Large dietary supplements aLNA to pregnant women do not appear to improve either their DHA status or that of their offspring [16]. An important consideration is that even when diets rich in aLNA are consumed, the ability to synthesize adequate amounts of DHA may be constrained by excess intake of the n-6 PUFA competing for the same synthetic pathway [17] or a limited availability of micronutrients which serve as co-factors in the synthesis of DHA. Diets limiting in iron [18], magnesium [19], zinc [20], calcium [21], riboflavin [22], pyridoxine [23] and B12 [24] have each been associated with constraint in LCPUFA desaturation and chain elongation. Furthermore, animal studies show that the consumption of imbalanced diets, low in protein [25] or high in sucrose [26], or modest consumption of ethanol [27] impairs LCPUFA status whilst acute or chronic inflammation may also increase demands on LCPUFA metabolism, altering the availability of circulating fatty acids [28].

5.13   In theory a poor dietary supply or limited synthesis may be ameliorated by mobilization of body reserves. The DHA in adipose tissue reserves of a woman as she enters pregnancy or accumulates during her pregnancy are potentially available if they can be mobilized. Crude estimates would suggest that for every 10kg of body fat that could be mobilized, might

yield about 15-18 g of DHA. This assumes that within the adipose tissue DHA comprises about 0.2% of total fatty acids, the same as observed in adult men [29] as there is no information on the fatty acid composition of adipose tissue in pregnant or non-pregnant women of child bearing age. The net accumulation of adipose tissue during pregnancy, makes it unlikely that mobilization of adipose tissue can make a substantial contribution to meeting the needs of the pregnancy itself, but may be an potential source of DHA during lactation. Maternal obesity is associated with insulin resistance, impairing mobilization of LCPUFA from adipose tissue reserves, whereas thin mothers with little body fat reserve may well have less to mobilize. In either case, there would be a greater need for endogenous DHA synthesis or a greater dependency on dietary DHA to satisfy the demands. There are no studies which have specifically examined the effect of maternal fatness on LCPUFA status during pregnancy.

Pregnancy and Lactation and Maternal DHA Depletion

5.14   In cross-sectional and prospective studies it has been demonstrated that pregnancy is associated with an increase in circulating concentrations of DHA in plasma phospholipids, by about 50% at 10 weeks compared with non-pregnant values. Some studies indicate that DHA remains elevated until term [8], whereas others report lower values at term than at 28 weeks [30]. The extent to which these changes reflect a general increase in phospholipid concentration associated with an increase in circulating lipoproteins (pregnancy-related hypertriglyceridaemia) or a specific mobilization of DHA from adipose tissue is unclear. There is some indication that women may track for plasma DHA concentration throughout pregnancy [Burdge unpublished]. Greater increases in the concentration of Mead acid (assumed to be a general marker of LCPUFA status) and Osbond acid (assumed to be a specific marker of DHA status) in plasma phospholipids during pregnancy have been seen by Hornstra and colleagues and taken to be indicative of a reduction in the functional LCPUFA status [31].

5.15   The magnitude of the increase in plasma DHA, and decline in 'functional DHA status' reported by Hornstra, differs markedly between women, but

the basis of these differences have not been explored in detail.. There is conflicting evidence on the effect of parity on the changes in DHA. If repeated pregnancies were to lead to poorer DHA status because of progressive depletion and incomplete recovery between pregnancies, it would be expected that there would be an inverse relationship between the DHA status of pregnant women and the number of completed pregnancies. Although an inverse relationship for plasma phospholipids DHA has been reported [32], the same group were unable to identify any relationship in a later study of non-pregnant women from the same population [33]. They concluded that this might in part be related to the interpregnancy interval, whereby if the interval was too short then there would incomplete replenishment of maternal DHA stores.

5.16 Maternal DHA concentration decreases by about 30% during the post-partum period [6], but is not necessarily immediate after parturition, but may be prolonged in duration. The decrease is observed in all mothers irrespective of whether they chooses to breast feed or not, suggesting that this may be related to changes in endocrine status or increased utilization of maternal DHA reserves, independent of lactation. However, the change in plasma phospholipids DHA concentration appears greater in lactating women and may be enhanced when the period of lactation is extended [34]. These observations would support the view that prolonged and extensive lactation may be causally related to maternal DHA depletion particularly when associated with multiple pregnancies. Numerous factors affect the DHA content of breast milk, but maternal DHA intake appears to be a major determinant. Whilst DHA supplementation has been shown to increase plasma and breast milk DHA concentration of lactating women [7], it remains is not known whether increased DHA intake would ameliorate maternal DHA depletion over successive pregnancies and periods of lactation.

Effects of supplementation on maternal status and outcome

5.17 The maternal supply of LCPUFA may be derived preformed from the diet or from body reserves, or synthesized within the body either from dietary constituents or from body reserves. For n-3 PUFA, diets rich in cold water

fish (or fish oils or marine lipids) can provide large amounts of EPA and DHA. Maternal PUFA status varies with fish and/or n-3 PUFA consumption in both the non-pregnant and pregnant state [9, 35]. Regular consumption of oily fish is associated with higher circulating DHA levels [36, 37], and supplementation with EPA and DHA increases circulating DHA levels during pregnancy and at term [38]. During a pregnancy in which the mother consumes fish or suitable supplements, there are higher concentrations of DHA and lower concentrations of n-6 PUFA in cord blood samples which in turn correlate with maternal blood DHA, and also maternal dietary n-3 PUFA intake [40]. The evidence linking n-3 PUFA intakes and changes in maternal n-3 PUFA status with alterations in duration of pregnancy length and fetal development is strong although the mechanisms underlying these relationships is unclear. In general, higher intakes during pregnancy of n-3 PUFA, in the form of oily fish, are associated with longer gestational length, greater birth length and weight and lower risks of intra-uterine growth retardation, small size for gestation age and premature birth [6, 40]. Some studies in which the dietary intervention has been EPA/DHA have shown such similar effects, whereas others have not demonstrated any change in the outcomes of pregnancy although there has been an increase in the circulating concentrations of DHA in the mother [31]. It may be that in part this difference in outcome reflects a difference in the background pattern of habitual fish consumption in the diet before and during pregnancy which would alter the background pattern and balance of LCPUFA that a mother brings to her pregnancy. Any effect of an increase in EPA/DHA consumption during pregnancy needs to be assessed against the background condition.

Conclusion

5.18   There is some evidence that for many women there is a marginal status for n-3 PUFA during pregnancy and lactation. Although, the formation of DHA and DHA status appear tightly regulated, a marginal state for many women during pregnancy and lactation can not be excluded. The extent of dietary dependence on increased levels of consumption of n-3 PUFA, or specifically of DHA, to improve pregnancy outcome needs to be demonstrated. The possible effects of the status for other nutrients, or stressful conditions in modulating DHA status needs to be determined.

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Annex 3.
Updated COT statement on a survey of mercury in fish and shellfish

Introduction

6.1 In 2002, the Committee reviewed the results of a Food Standards Agency (FSA) survey of the mercury levels in imported fish and shellfish and UK farmed fish and their products [1] and the provisional results of blood mercury levels in UK adults [2].

6.2 The Committee concluded that the Provisional Tolerable Weekly Intake (PTWI) of 3.3 µg/kg bw/week could be used in assessing methylmercury intakes by the general population. This PTWI was initially established by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) in 1972 and confirmed on a number of occasions up to the year 2000, However, the 2000 JECFA PTWI was not considered adequate to protect against neurodevelopmental effects. The EPA reference dose of 0.1 µg/kg bw/day (0.7 µg/kg bw/week) was therefore applied for women who are pregnant, or who may become pregnant within the following year, or for breast-feeding mothers. The COT also noted that its conclusions should be reviewed following the JECFA evaluation of methylmercury in 2003. [3]

6.3 In June 2003, JECFA recommended that the PTWI for methylmercury should be reduced from 3.3 µg/kg bw/week to 1.6 µg/kg bw/week. The Committee has therefore reviewed its previous evaluation in the light of the new JECFA PTWI, also taking into account more recent data on fish consumption by adults. This statement on mercury in fish and shellfish supersedes COT statement 2002-04.

6.4 The FSA has asked a subgroup of members of the COT and the Scientific Advisory Committee on Nutrition (SACN) to provide combined advice on

the risks and benefits associated with fish consumption. The advice expressed in this COT statement therefore aims to protect the populations who are most susceptible to the risks of methylmercury, without being over-protective of individuals at lesser risk.

Background

6.5   The toxicity of mercury is dependent on whether it is inorganic, elemental or organic (e.g. methylmercury). Methylmercury affects the kidneys and also the central nervous system, particularly during development, as it crosses both the blood-brain barrier and the placenta [4]. Both neuro- and nephrotoxicity have been associated with acute methylmercury poisoning incidents in humans, and neurotoxicity, particularly in the developing fetus, has been associated with lower level chronic exposures.

6.6   Exposure of the general population to mercury can occur via inhalation of mercury vapour from dental amalgam fillings (elemental), or through the diet (methylmercury and inorganic mercury) [5]. Methylmercury in fish makes the most significant contribution to dietary exposure to mercury, although smaller amounts of inorganic mercury are present in other food sources. All forms of mercury entering the aquatic environment, as a result of man's activities or from geological sources, are converted into methylmercury by microorganisms and subsequently concentrated in fish and other aquatic species. Fish may concentrate the methylmercury either directly from the water or through consuming other components of the food chain. Methylmercury has a half-life of approximately 2 years in fish; thus, large older fish, particularly predatory species, will have accumulated considerably more methylmercury than small younger fish.

Previous COT evaluation

6.7   The COT previously considered the results of a survey of metals and other elements in marine fish and shellfish [6] published by the Ministry of Agriculture, Fisheries and Food (MAFF) in 1998. The survey examined a number of fish and shellfish species landed in the UK or imported from overseas ports including cod, haddock, herring, mackerel, lobster, mussels, crab and shrimps and samples of cod fish fingers. The survey also

produced estimates of the mean and 97.5th percentile dietary intakes of the elements surveyed.

6.8 The 1998 survey demonstrated that the levels of mercury in the fish and shellfish tested were low and that average and high level fish and shellfish consumers in the UK would not exceed the then current JECFA PTWI for methylmercury of 3.3 mg/kg bw/week, even assuming all the mercury in fish was in this form. The estimated mercury intake for the highest level consumer was 1.1 mg/kg bw/week including mercury intake from the rest of the diet. The main conclusion drawn from the survey was that "dietary intakes of the elements surveyed were below safe limits, where defined, and did not represent any known health risk even to consumers who eat large amounts of marine fish or shellfish".

International Safety Guidelines

Previous Joint FAO/WHO Expert Committee on Food Additives (JECFA) Evaluations

6.9 In 1972, JECFA established a PTWI of 5 mg/kg bw/week for total mercury, of which no more than two thirds (3.3 mg/kg bw/week) should be from methylmercury[7]. The PTWI of 3.3 mg/kg bw/week for methylmercury was subsequently confirmed in 1989 and 2000[8,9]. The PTWI was derived from toxicity data resulting from poisoning incidents at Minamata and Niigata in Japan. In these incidents the lowest mercury levels associated with the onset of clinical disease in adults were reported to be 50 mg/g in hair and 200 mg/L in whole blood. Individuals displaying clinical effects, such as peripheral neuropathy, at these mercury levels were considered to be more sensitive than the general population, because there were a number of persons in Japan and other countries with higher mercury levels in hair or blood who did not experience such effects. However, the methods employed in determining the intake associated with toxicity, and the subsequent establishment of the PTWI are unclear.

6.10 In 1989, JECFA had noted that pregnant women and nursing mothers may be at greater risk than the general population to adverse effects from methylmercury. Therefore in its' 2000 re-evaluation of methylmercury, JECFA paid particular attention to possible effects of prenatal and

postnatal exposure, looking at large long-term prospective epidemiological studies conducted in the Seychelles Islands and the Faroe Islands. These studies attempted to identify the lowest dietary mercury exposure associated with subtle effects on the developing nervous system , , , . They followed the neurological development of the children by testing their learning and spatial abilities at a number of time-points during their childhood. A number of smaller studies were also considered.

6.11   JECFA compared the two main studies;

- The Faroe Islands cohort was tested up to the age of 7 years, whereas at the time of the JECFA evaluation, the Seychelles cohort had only been tested up to the age of 5.5 years.

- Exposure in the Seychelles was through consumption of a range of fish species with average mercury concentrations between 0.05 and 0.25 mg/kg. In the Faroe Islands, most of the population consumed fish at least three times a week and occasionally (approximately once per month) consumed pilot whale, which contains up to 3 mg/kg mercury. Pilot whale also contains high concentrations of polychlorinated biphenyls (PCBs), but a reanalysis of the data indicated that any effects seen in the Faroes cohort could not be attributed to confounding by the PCBs .

- The two studies used different methodology in assessing methylmercury exposure. The Seychelles study used maternal hair samples (approx. 9cm long), one taken shortly after birth to estimate methylmercury exposure during pregnancy and one taken 6 months later. The Faroe Islands study used cord blood and maternal hair (various lengths) taken at birth.

- The studies used different batches of tests to assess the effects of methylmercury on neurological development. The tests used in the Faroe Islands study examined specific domains in the brain (visual, auditory, etc.). The Seychelles study used tests of a more global nature, with each test examining a number of domains.

6.12   JECFA found that although the mean mercury exposures during pregnancy (assessed by maternal hair mercury) were similar[δ], the results of these two studies were conflicting. In the Faroes study, regression analysis showed an association between methylmercury exposure and impaired performance in neuropsychological tests, an association that remained even after excluding the results of children with exposures associated with greater than 10 µg/g maternal hair mercury. However in the Seychelles study regression analysis identified no adverse trends, but increased maternal hair mercury was associated with a small statistically significant improvement in test scores on several of the developmental outcomes. The investigators noted that this could be due to beneficial nutritional effects of fish. A secondary analysis was performed where the results were split into sub-groups based on the maternal hair mercury level. Test scores in children with the highest mercury exposures (12 - 27 µg/g maternal hair) were not significantly different from the test scores in children with lowest exposure (< 3µg/g maternal hair).

6.13   A smaller study carried out in New Zealand on 6 year-old children used a similar batch of tests to the Seychelles study and had similar exposure to methylmercury, yet found methylmercury related detrimental effects on behavioural test scores. However there were possible confounding factors that may have influenced the results of the New Zealand study, such as the ethnic group and social class of the children studied.

6.14   Having considered all of the epidemiological evidence, JECFA concluded that it did not provide consistent evidence of neurodevelopmental effects in children whose mothers had hair mercury levels of 20 µg/g or less. Since there was no clear indication of a consistent risk, JECFA did not revise its' PTWI, but recommended that methylmercury should be re-evaluated when the latest evaluation of the Seychelles study and other relevant data become available[9].

[δ] Seychelles: arithmetic mean 6.8 µg/g, range 0.5-26.7 µg/g;
      Faroes: geometric mean, 4.27 µg/g, the upper mercury level in maternal hair is not clear from
      the reported data but may be as high as 70 µg/g.

Environmental Protection Agency (EPA)

6.15   In 1997 the US EPA established a reference dose of 0.1 µg/kg bw/day for methylmercury . This was based on a peak maternal hair mercury level during pregnancy of 11 µg/g, which was associated with developmental effects (e.g. late walking, late talking, mental symptoms, seizures) in children exposed *in utero* during a poisoning incident in Iraq in 1971.

6.16   In 2000, the US National Research Council (NRC) published a review of this EPA reference dose[26]. Following analysis of the data resulting from the available epidemiological studies, the NRC identified a benchmark dose lower confidence limit of 12 µg/g in maternal hair (corresponding to 58 µg/L in cord blood, assuming a ratio of hair:cord blood of 200:1). This was the lower 5% confidence limit of the lowest dose considered to produce a sufficiently reliable neurological endpoint (a 5% increase in abnormal scores on the Boston Naming Test[**]) in the Faroe Islands study. The NRC made a number of assumptions in deriving an estimate of methylmercury intake and included a composite uncertainty factor of 10, to account for interindividual variability and database insufficiencies, concluding that the reference dose of 0.1 µg/kg bw/day, as had previously been used by the EPA, was scientifically justifiable.

2003 JECFA Evaluation

6.17   At its 61st meeting in June 2003 , JECFA reviewed the new data from the Seychelles Child Development Study[18], re-analyses of the Faroes and New Zealand studies, epidemiological data from a number of small scale cross-sectional studies, and additional epidemiological data on reproductive toxicity, immunotoxicity, cardiotoxicity and general medical status.

[**] The Boston Naming Test is a neuropsychological test that assesses an individual's ability to retrieve a word that appropriately expresses a particular concern, for example naming an object portrayed by a simple line drawing.

6.18   The 9-year neurodevelopmental evaluations from the Seychelles study were performed using neurodevelopmental tests which, in contrast to the earlier assessments, allowed a direct comparison with the results of the Faroes Islands Study. The new data from the Seychelles study were consistent with results obtained at younger ages and provided no evidence for an inverse relationship between maternal methylmercury exposure and neurodevelopmental performance in infants. Additional analyses carried out on the Seychelles data from younger ages did not alter the conclusion that in the Seychelles population of frequent fish-consumers, no adverse effects of prenatal methylmercury exposure have been detected.

6.19   No new data were available from the Faroes Islands study. New analyses of the existing data did not support a role of occasional exposure to higher levels of methylmercury or polychlorinated biphenyls (PCBs) from consumption of whale-meat, in accounting for the positive associations in this study [19,20,14,21]. The additional epidemiological data from smaller cross-sectional studies on neurodevelopmental effects of methylmercury were reviewed. Because of the cross-sectional design and because adult hair mercury levels do not accurately reflect previous exposure during the critical period for neurodevelopmental effects, JECFA did not consider that the results from these studies could be used to form the basis of a dose response assessment.

6.20   JECFA noted that despite additional evidence of immunotoxicity, cardiotoxicity, and reproductive toxicity, neurotoxicity was still considered to be the most sensitive endpoint, and concluded that the PTWI should be based on studies of this endpoint. It was uncertainty about the possibility that significant immunotoxicity or cardiovascular effects could occur at levels below the neurodevelopmental benchmark dose that had led to the inclusion of an additional safety factor for database insufficiencies in the composite factor of 10 recommended by the NRC.

6.21   JECFA based its evaluation on the Seychelles and Faroe Islands studies. In the absence of a dose response analysis of the latest Seychelles data, the analysis of the data from younger ages was used since it was consistent with the latest data. Exposure associated with a maternal hair concentration of 15.3 µg/g mercury was identified as the no observed adverse effect level

(NOAEL) for the Seychelles study[22]. A benchmark dose lower confidence limit (BMDL) of 12 µg/g mercury in maternal hair was determined from the Faroes data[23,24,25,26,27]. This was viewed as a surrogate for the NOAEL.

6.22   Averaging the NOAEL and the BMDL resulted in a composite maternal hair concentration of 14 µg/g mercury reflecting exposure that was without effects in these study populations. Dividing by the average hair:blood ratio of 250 allowed conversion of the 14 µg/g in hair to a maternal blood mercury level of 56 µg/L. A pharmacokinetic model appropriate to pregnancy was then used to convert the blood mercury level to a steady-state daily ingestion of methylmercury of 1.5 µg/kg bw/day, which would be without appreciable adverse effects in the offspring of the Seychelles and Faroe Islands study populations. The model assumed a maternal blood volume of 7 L (9% of body weight) whereas the EPA used a value of 5 L and the NRC 3.6 L.

6.23   JECFA then applied a data-specific adjustment factor of 2 to allow for inter-individual variability in the hair:blood ratio, and a default uncertainty factor of 3.2 to account for inter-individual variability in the association between blood mercury concentration and intake. This resulted in a PTWI of 1.6 µg/kg bw/week, which JECFA considered to be sufficiently protective of the developing fetus. A factor for inter-individual variability in toxicodynamics was not required because the PTWI was based on studies in the most sensitive subgroup.

6.24   In its review, JECFA found no additional information that would suggest that the general population is at risk of methylmercury toxicity at intakes up to the previous PTWI of 3.3 µg/kg bw/week.

Survey of the mercury levels in fish

6.25   The 2002 FSA survey complemented the previous MAFF survey since it examined a wider range of fish, including imported exotic species of fish that have become more widely available on the UK market. These included shark, swordfish, marlin, orange roughy, red snapper and monkfish, as well as UK farmed fish such as salmon and trout[1].

6.26   Of the fish species covered by the survey, all but 3 species had mean mercury levels falling within the range 0.01 –0.6 mg/kg of fish. This range is in line with the levels defined by European Commission Regulation 466/2001 as amended by European Commission Regulation 221/2002 (0.5 mg of mercury/kg for fish in general and 1.0 mg mercury/kg for certain larger predatory species of fish including shark, swordfish, marlin, tuna and orange roughy).

6.27   The 3 species with the highest mercury content were shark, swordfish and marlin. These fish had mean mercury levels of 1.52, 1.36, and 1.09 mg/kg respectively and were therefore above the levels defined in European Commission Regulation 221/2002. Fresh tuna contained mercury levels ranging from 0.141 to 1.50 mg/kg with a mean of 0.40 mg/kg (only one sample out of 20 exceeded 1 mg/kg, the maximum mercury concentration in the other 19 samples was 0.62 mg/kg), whereas canned tuna had a lower mean mercury level of 0.19 mg/kg.

Blood mercury levels in British adults

6.28   A report produced by the Medical Research Council Human Nutrition Research in March 2002 detailed the provisional blood total mercury data obtained from 1320 adults (aged 19-64 years) participating in the NDNS[2].

6.29   The mean and 97.5th percentile blood mercury levels in the survey were 1.6 and 5.88 µg mercury/L respectively. The highest blood mercury level found in the study was approximately 26 µg/L in an individual with a high fish intake. If the blood mercury level was at steady state, and assuming a body weight of 70 kg and a blood volume of 9% of the body weight, then using the same pharmacokinetic model employed by JECFA in its 2003 evaluation, this would correspond to a mercury intake of approximately 5.39 µg/kg bw/week (0.77µg/kg bw/day).

6.30   Of the population covered by the survey, 97.5% had blood mercury levels indicating that their mercury intakes were within the 2003 JECFA PTWI of 1.6 µg/kg bw/week.

COT evaluation

6.31 The Committee discussed the possible risks associated with dietary exposure to methylmercury, in the light of the new JECFA PTWI and the information on intakes from fish and on blood mercury levels in the UK population.

Toxicokinetic considerations

6.32 Following ingestion, approximately 95% of methylmercury is absorbed through the gastrointestinal tract, and it is subsequently distributed to all tissues in about 30 hours with approximately 5% found in blood and 10% in the brain. The methylmercury concentration in red blood cells is approximately 20 times higher than that in the plasma. Methylmercury readily crosses the placental barrier. Fetal brain mercury levels are approximately 5-7 times higher than in maternal blood. Methylmercury readily accumulates in hair and the ratio of hair mercury level (mg/g) to maternal blood mercury level (µg/L) is approximately 250:1. Based on comparisons to hair concentrations, cord blood concentrations are reported to be 25% higher than the concentrations in maternal blood[10].

6.33 The excretion process for methylmercury involves transfer of the glutathione-mercury complex into the bile, demethylation by gut microflora to the inorganic form, then elimination from the body in the faeces. The half-life of mercury in the body is approximately 70 days in adults, with steady state being reached in about one year. Significant amounts of methylmercury also pass into the breast milk of lactating women, resulting in a decreased mercury half-life of approximately 45 days[28].

6.34 Doherty and Gates[29] reported that the excretion rate of mercury in the suckling rodent is less than 1% of the adult excretion rate. Sundberg *et al*. reported a low elimination of mercury in suckling mice until lactational day[17]. This is probably because biliary secretion and demethylation by microflora (which lead to faecal excretion) do not occur in suckling animals. The role of these processes in suckling human infants is unknown[4].

6.35 The concentration of mercury in breast-milk is approximately 5% of the blood mercury concentration of the mother [28]. Amin-Zaki *et al*.[31] reported that in women exposed to high levels of methylmercury during the Iraqi poisoning incident, 60% of the mercury in breast-milk was in the form of methylmercury. Therefore it may be estimated that the concentration of methylmercury in the breast-milk is approximately 3% of the total mercury concentration in the blood. For an infant to be exposed to methylmercury at the new JECFA PTWI of 1.6 mg/kg bw/week, the mother would have to be exposed to the following methylmercury level:

Methylmercury intake of infant: = 0.23 μg/kg bw/day

Assuming a daily milk intake of 150 mL/kg bw
Concentration of methylmercury in milk = 1.53 μg/L

Assuming 3% methylmercury transfer from maternal blood to milk
Maternal blood mercury level = 51.1 μg/L

Using the pharmacokinetic model employed by JECFA in its 2003 evaluation, and assuming a maternal body weight of 65kg
Maternal methylmercury intake = 1.36 μg/kg bw/day (9.5 μg/kg bw/week)

Susceptible populations

6.36 In its 2003 evaluation of methylmercury, JECFA established a PTWI of 1.6 μg/kg bw/week in order to protect against neurodevelopmental effects but found no information to indicate that the previous PTWI of 3.3 μg/kg bw/week was not sufficiently protective for groups not susceptible to neurodevelopmental effects. The COT has been asked to advise on safety guidelines for methylmercury that could be used in assessing risks associated with fish consumption. The Committee concluded that the previous JECFA PTWI of 3.3 μg/kg bw/week could be used for the general population.

6.37 In its 2002 statement, the Committee had used the EPA reference dose of 0.1 μg/kg bw/day (0.7 μg/kg bw/week) in considering dietary exposure of the subpopulations at risk of neurodevelopmental effects. Members therefore discussed the differences between the 2003 JECFA PTWI and the

EPA reference dose. The major differences related to the use of default uncertainty factors in derivation of the EPA reference dose, whereas chemical-specific data had been incorporated into the JECFA PTWI. The 2003 JECFA evaluation also took into account data published since the EPA review. The Committee had previously noted that the EPA reference dose was precautionary and agreed that the 2003 JECFA PTWI of 1.6 µg/kg bw/week should be used to protect against neurodevelopmental effects in susceptible populations. This PTWI is only necessary for the neurodevelopmental endpoint and therefore does not apply to the general population.

6.38   Due to this approach of applying different guidelines for different population groups, the Committee has given particular consideration to determining which groups are at higher risk and can be considered to be susceptible populations.

6.39   The critical effect of methylmercury is on the developing central nervous system and therefore pregnant women are considered to be the most susceptible population because of the risk to the fetus. There have been no studies of the effects of exposure prior to becoming pregnant. However, because the half-life of methylmercury in the human body is approximately 70 days, steady state concentration is attained in approximately one year and a woman's blood mercury level at the time of becoming pregnant is dependent on the exposure to methylmercury during the preceding year. The Committee therefore agreed that women who may become pregnant within the next year should also be considered as a susceptible population.

6.40   The evidence regarding consideration of other susceptible populations is not conclusive. Animal experiments indicate that exposure via breast-milk has less serious consequences to the central nervous system than prenatal exposure. Spyker and Spyker [32] reported that the effects of prenatal exposure to methylmercury dicyandiamide on the survival and weight gain of the offspring were more severe than those seen with postnatal exposure, and were greatest when the methylmercury was administered late in the period of organogenesis. However, these results are not necessarily relevant to the health effects of concern in human exposure.

6.41   Data from a 5-year longitudinal study following the Iraq poisoning
        incident have suggested that some children exposed to methylmercury via
        breast-milk demonstrated delayed motor development [31]. The maternal
        blood mercury levels immediately following the incident were estimated
        by extrapolation to be in the range of approximately 100µg/L to 5000
        µg/L. Mothers who showed signs and symptoms of poisoning (ataxia,
        dysarthria, visual disturbance etc.) tended to have the higher blood levels
        (3000 to 5000 µg/L) although some women with levels in this range were
        asymptomatic.

6.42   The affected infants all had blood mercury levels above those associated
        with the 2000 JECFA PTWI of 3.3 µg/kg bw/week, and most of them had
        blood mercury levels higher than the minimum toxic level for adults of 200
        µg/L, defined by JECFA. There was no paralysis, ataxia, blindness or
        apparent sensory change and there were no cases of the severe mental
        destruction and cerebral palsy that had been seen in the prenatally exposed
        infants of Minamata. However, language and motor development of the
        children were delayed. The authors of the study concluded that breast-fed
        infants are at less risk than the fetus, since most of the brain development
        has already occurred and the effects seen in the breast-feeding infant are
        different from those seen in infants exposed prenatally and not as severe.

6.43   There is no evidence that chronic exposure to methylmercury via breast
        milk at levels below those observed in the Iraqi incident has any adverse
        effect on the neurophysiological/psychological development of the child.
        Data from the Faroe Islands study suggests that the beneficial effects of
        nursing on early motor development are sufficient to compensate for any
        adverse impact that prenatal exposure to low concentrations of
        methylmercury might have on these endpoints [33,34]. Grandjean *et al*. [33]
        looked at the relationship between seafood consumption and
        concentrations of contaminants in breast-milk in the Faroes Island
        population. Of 88 samples of breast-milk, three had a mercury level that
        would cause the infant to exceed the old PTWI for mercury.

6.44   There have been few studies of the effects of methylmercury on young
        children. Most information has come from the poisoning incidents in
        Minamata, Niigata and Iraq. In all of these cases the exposures were very

high, and in Iraq, the exposure was acute. Methylmercury is excreted by children as efficiently as by adults[4]. In the incidents where children were exposed to methylmercury directly rather than prenatally, the damage seen in the brain was similar to that seen in adults: focal lesions of necrosis. The damage seen when the fetus is exposed is much more widespread[4].

6.45   The longitudinal study in the Seychelles has attempted to examine the effects of postnatal exposure to methylmercury[12]. This is complicated by the facts that in the Seychelles, the children exposed to methylmercury postnatally are also exposed prenatally, and the study has been unable to demonstrate any mercury-related deficits in the neurological development of children. However higher postnatal methylmercury exposure had a positive association with test scores. It was suggested that this may be because a higher mercury level indicates a high fish intake and therefore a diet rich in n-3-polyunsaturated fatty acids and vitamin E, which have beneficial effects and may mask any subtle neurological deficits due to chronic low level exposure to methylmercury.

6.46   The risk is greater for women who are pregnant or likely to become pregnant within the following year because of the effects of methylmercury on the developing central nervous system of the fetus. There is uncertainty with respect to whether infants and young children are at greater risk of methylmercury toxicity whilst the central nervous system is still developing. The limited data available indicate that this is not the case for children but the possibility of increased sensitivity of infants cannot be discounted. Correlation of intakes by the breast-fed infant and the mother (paragraph 35) indicates that the methylmercury intake of the breast-fed infant is within the 2003 PTWI of 1.6 µg/kg bw/week if the mother's intake is within the 2000 PTWI of 3.3 µg/kg bw/week.

Assessment of dietary exposure estimates

6.47   Dietary exposure to mercury was estimated for those fish species for which reliable consumption data were available [35,36,37,38] (salmon, prawns and canned tuna) together with exposure from the rest of the diet. Dietary exposures to these fish were also calculated for adult women as this population group contains the most susceptible populations (Table 6.1). This table is a revised version of that which appears in the FSIS[1] as it

incorporates the most up-to-date consumption and occurrence data available for the rest of the diet from the TDS. Of these fish, canned tuna provided the largest contribution to dietary mercury exposure for high level consumers. Total fish consumption by the high level consumer was equivalent to approximately five portions per week (688g).

6.48   The estimates of average and high level total dietary exposure for almost all age groups, from fish for which consumption data are available, are within the 2003 JECFA PTWI for methylmercury of 1.6 μg/kg bw/week, and not expected to be harmful. The mercury exposure from the whole diet in toddlers and young people aged 4-6 years who are high level consumers exceeds the 2003 PTWI of 1.6 μg/kg bw/week by between 13 and 26% but are well within the 2000 PTWI. The estimated intakes of toddlers who are high level consumers of canned tuna exceeds the 2003 PTWI by 50%, but again are within the 2000 PTWI. Children of this age (1.5-4.5 years) are likely to be less susceptible to neurodevelopmental effects. Therefore this exceedance of the 2003 PTWI is not likely to result in harmful effects.

6.49   Estimates were also made of the methylmercury intake resulting from consumption of one portion of shark, marlin, swordfish or fresh tuna, for which consumption data are not available (Table 6.2), using portion sizes as recorded in the NDNS for fish consumption [36,37,38]. For comparative purposes similar estimates were made for canned tuna.

6.50   For adults, consumption of one weekly portion of shark, swordfish or marlin could result in a mercury intake in the range of 2.2 to 3.0 μg/kg bw/week, before considering intake from the rest of the diet (upper bound mean 0.28 μg mercury/kg bw/week, not all as methylmercury). Regular intake at this level during pregnancy, or in the year leading up to pregnancy could be associated with a risk of neurodevelopmental effects in the fetus. The methylmercury intake resulting from consumption of either two 140g portions of fresh tuna or four 140g portions of canned tuna would not be expected to result in neurodevelopmental effects.

6.51   Regular consumption of more than one portion of shark, swordfish or marlin per week could be associated with a risk of neurotoxicity in adults.

6.52   Dietary exposure of children is higher because their food intake is greater on a body weight basis. Regular consumption of one weekly portion of shark, swordfish or marlin per week by children under the age of 14 could result in a methylmercury intake in the range of 3.0 to 5.2 µg/kg bw/week, before considering intake from the rest of the diet. Consumption of two portions per week of fresh tuna, or 6 portions of canned tuna would not be expected to result in adverse effects in any of the age groups.

Conclusions

6.53   We *note* that there has been no new information published to indicate that the 2000 PTWI of 3.3 µg/kg bw/week is not sufficiently protective of the general population. We therefore *consider* that a methylmercury intake of 3.3 µg/kg bw/week may be used as a guideline to protect against non-developmental adverse effects.

6.54   We *conclude* that the 2003 JECFA PTWI of 1.6 µg/kg bw/week is sufficient to protect against neurodevelopmental effects in the fetus. This PTWI should be used in assessing the dietary exposure to methylmercury of women who are pregnant, and who may become pregnant within the following year.

6.55   We *consider* that a guideline of 3.3 µg/kg bw/week is appropriate in considering intakes by breastfeeding mothers as the intake of the breast-fed infant would be within the new PTWI of 1.6 µg/kg bw/week.

6.56   We *consider* the NDNS blood level data are reassuring with respect to average and high level consumption of fish. The adults surveyed had blood mercury levels indicating that 97.5% of the population had dietary intakes below 1.6 µg/kg bw/week.

6.57   We *conclude* that average and high-level dietary exposure to methylmercury, resulting from the wide range of fish for which consumption data are available, is not likely to be associated with adverse effects in the developing fetus or at other life stages.

6.58   We *note* that consuming one weekly 140 g portion of either shark, swordfish or marlin would result in a dietary methylmercury exposure close to or above 3.3 µg/kg bw/week in all age groups. We *consider* that this consumption could be harmful to the fetus of women who are pregnant or become pregnant within a year, but would not be expected to result in adverse effects in other adults.

6.59   We *note* that the mercury content of tuna is lower than that of shark, swordfish or marlin, but higher than that of other commonly consumed fish. We *consider* that consumption of two 140g portions of fresh tuna, or four 140g portions of canned tuna, per week, before or during pregnancy would not be expected to result in adverse effects on the developing fetus.

6.60   We *recommend* that further research should include development of analytical methodology to allow direct measurement of methylmercury, mechanistic studies to help elucidate population groups more at risk and research integrating the risks with nutritional benefits of fish consumption.

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Table 6.1: Estimated mean and high level dietary intakes of mercury from salmon, prawns, canned tuna and the whole diet.

Consumer group	Mercury Intake - µg/kg bw/week [a]							
	Salmon [b]		Prawns [b]		Canned Tuna [b]		Whole Diet [c,d]	
	Mean	97.5%	Mean	97.5%	Mean	97.5%	Mean	97.5%
Infants	0.01	0.01	- [e]	- [e]	0.04	0.13	0.04	0.13
Toddlers	0.18	0.53 [f]	0.13	0.45 [f]	0.84	2.45	0.56	2.17
Young People aged 4 – 6	0.18	0.39 [g]	0.09	0.34 [f]	0.53	1.61	0.55	1.82
Young People aged 7 – 10	0.11	0.36 [f]	0.06	0.15 [f]	0.39	1.26	0.41	1.40
Young People aged 11 – 14	0.09	0.23 [g]	0.04	0.13 [f]	0.32	0.98	0.29	1.05
Young People aged 15 – 18	0.08	0.15 [g]	0.04	0.11	0.27	0.68	0.25	0.84
Adults	0.10	0.32	0.04	0.14	0.30	1.05	0.31	1.19
Adults – Women only	0.11	0.32	0.05	0.16	0.34	1.19	0.34	1.19

a) Consumption data for salmon, prawns and tuna are taken from the following sources:
   •   2002 National Diet and Nutritional Survey: adults aged 19 to 64 years. [38]
   •   Food and Nutrient Intakes of British Infants Aged 6-12 Months. [35]
   •   National Diet and Nutrition Surveys Children Aged 1.5 – 4.5 years. [37]
   •   National Diet and Nutrition Survey: young people aged 4-18 years. Volume 1 report of the diet and nutrition survey. [36]
b) Mercury intake from eating the named fish only, for the mean and 97.5th percentile consumers.
c) Mercury exposure from the whole diet for individuals of the whole study population, including those that eat the named fish (taken from the 2000 Total Diet Study [39]). The whole diet mercury exposure does not equal the sum of the mercury exposures from the named fish and other foods in the typical UK diet.
d) The measurement of mercury does not distinguish between inorganic and organic mercury. Therefore although methylmercury is the major contributor to mercury intake from fish, the estimate of intake from the whole diet also includes inorganic mercury.
e) No infant consumption data were recorded for prawns in the Infant Survey.
f) Based on consumption data for fewer than 60 recorded consumers, therefore exposures to be regarded with caution.
g) Based on consumption data for fewer than 20 recorded consumers, therefore exposures to be regarded with extreme caution.
These estimates have been revised to incorporate up-to-date consumption and occurrence data for the rest of the diet from the TDS.

Table 6.2: Mercury intake from one weekly portion of shark, swordfish, marlin, fresh tuna or canned tuna.

Age group (years)	Body Weight (kg)	Av. Portion Size[a] (g)	Weekly mercury intake assuming one portion of fish per week[b] (µg/kg bw/week)				
			Shark	Swordfish	Marlin	Fresh Tuna	Canned Tuna
1.5 – 4.5	14.5	50	5.24	4.62	3.79	1.38	0.66
4 – 6	20.5	60	4.44	3.90	3.22	1.17	0.56
7 –10	30.9	85	4.17	3.69	3.04	1.10	0.52
11 – 14	48.0	140	4.44	3.92	3.21	1.17	0.55
15 – 18	63.8	105	2.51	2.21	1.82	0.66	0.31
Adults	70.1	140	3.04	2.68	2.20	0.80	0.38

a) The average portion size that each age group of the population would consume at a single meal event for fish consumption, as recorded in the following National Diet and Nutrition Surveys (NDNS):
- 1995 National Diet and Nutrition Survey: Children aged one-and-a-half to four-and-a-half years[37].
- 2000 National Diet and Nutrition Survey: young people aged 4 to 18 years[36].
- 1990 The Dietary and Nutritional Survey of British Adults38.

b) This intake estimate does not include the intake from the rest of the diet, which is estimated to be 0.04 µg/kg bw/day (0.28 µg/kg bw/week)[39].

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Annex 4
COT statement on brominated flame retardants in fish from the Skerne Tees rivers system

Introduction

7.1 The Food Standards Agency (FSA) has recently completed a survey to determine the concentrations of brominated flame-retardants (BFRs) in brown trout and eels from the Skerne-Tees river system. The Committee was asked to assess the toxicological properties of selected BFRs in order to advise on any health implications of the estimates of dietary exposure.

Background

7.2 Brominated flame-retardants (BFRs) are structurally diverse chemicals used in plastics, textiles and other materials to enhance their flame-retardant properties. Some BFRs, including polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are mixed into polymers rather than being chemically bound to them and can leach out of the products/materials in which they are used and into the environment.

7.3 PBDEs are produced by direct bromination of diphenyl ether. There are 209 individual PBDE congeners, each of which is identifiable by a unique congener number. Three commercial PBDE flame-retardants, pentabromodiphenyl ether (pentaBDE), octabromodiphenyl ether (octaBDE) and decabromodiphenyl ether (decaBDE) have been available in the UK. The commercial PBDEs are not pure products but a mixture of various diphenyl ethers with varying degrees of bromination.

7.4 The actual composition of the commercial products varies with supplier and is considered to be commercially sensitive information. However, example compositions of PBDEs have been published (see Table 7.1). These figures are broadly representative of the commercial products

currently supplied. The commercial products are usually named on the basis of the principal PBDE congener e.g. pentaBDE. Trade-name nomenclature may also incorporate a number, which is related to the performance characteristics (e.g. flame retardant properties) of the commercial mixture rather than the constituent congeners[1].

Table 7.1: Relative congener distribution for penta- and octaBDE

Commercial product	% congeners in commercial product						
	tetra	penta	hexa	hepta	octa	nona	deca
PentaBDE[1]	24-38	50-62	4-12	–	–	–	–
OctaBDE[2]	–	–	≤12	≤45	≤33	≤10	–
DecaBDE[3]	–	–	–	–	–	≤3	≤97

7.5   The commercial PBDEs have recently been evaluated under the EU Existing Substances Regulations. As a result of their potential to bioaccumulate in the environment, the EU has agreed to ban the marketing and use of penta- and octaBDE from 1 July 2004. However, for some time after this date there will still be existing PBDE-containing products in use.

7.6   There are limited data available on the potential of decaBDE to bioaccumulate in the environment and it is not currently included in the EU prohibition. In addition, it was not measured in the Skerne Tees survey. However, information on decaBDE has been included in this statement for completeness and as a basis for future evaluations because decaBDE will be the only PBDE product commercially available when the ban on penta- and octaBDE comes into force. The chemical structure of PBDEs is given in Figure 7.1.

Figure 7.1. Chemical structure of PBDEs
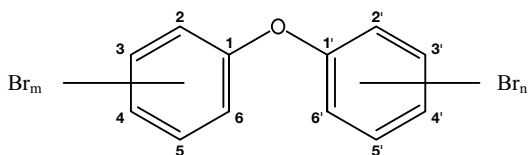
Where (m) plus (n) equal between 1 and 10 bromine atoms.

7.7   HBCD is synthesized through bromination of cyclododecatriene. It is commercially available in the UK as a mixture of three stereoisomers α, β and γ. There is currently no proposal to ban HBCD in the EU[4]. The chemical structure of HBCD is given in Figure 7.2.

Figure 7.2. Chemical structure of HBCD
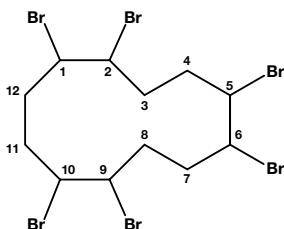
Toxicology of PBDEs and HBCD

7.8   Completed EU risk assessments are available for pentaBDE[1] and octaBDE[2], and draft risk assessments are available for decaBDE[3] and HBCD[4]. Unless otherwise indicated, the following summary is based on the information provided in these risk assessments.   The COT also considered new studies published subsequent to the final literature searches for the EU risk assessments. A more detailed summary of the toxicological data is available at http://www.food.gov.uk/multimedia/ pdfs/tox14.pdf. Throughout this statement the terms pentaBDE, octaBDE, decaBDE and HBCD refer to the commercial mixtures of brominated flame-retardants whereas individual congeners are denoted by inclusion of the specific congener number e.g. PBDE-99.

PBDEs

7.9   There is limited information on the toxicokinetics of penta-, octa- and decaBDE. Studies in laboratory animals have indicated that penta- and octaBDE are absorbed following oral administration however, the extent of absorption is unknown[1,2]. DecaBDE is not well absorbed after oral administration (<10%)[3].

7.10 The primary route of excretion for all PBDEs is considered to be the faeces, although it is unclear how much of the PBDE present in the faeces represents unabsorbed material. In rats, following oral administration, the majority of pentaBDE was detected unchanged in the faeces[1]. Limited information indicates that decaBDE is metabolized to lesser brominated phenolic products[3]. There is no information on the metabolism of octaBDE. Elimination of pentaBDE from rat adipose tissue is slow ($t_{1/2}$ = 25-47 days) indicating that it has the potential for bioaccumulation[1]. There is no information on the elimination of octa- or decaBDE in animals, or on the bioaccumulation or the route of elimination of PBDEs in humans.

7.11 There is no information on PBDE levels in adipose tissue from the UK. However, PBDE-47 (2, 2', 4, 4'-tetraBDE), PBDE-99 (2, 2', 4, 4', 5-pentaBDE), PBDE-100 (2,2',4,4',6-pentaBDE), PBDE-153 (2, 2', 4, 4', 5, 5'-hexaBDE) and PBDE-154 (2, 2', 4, 4', 5, 6'-hexaBDE) have been detected in human breast adipose tissue in the US. The sum of total PBDEs detected was 86 ng/g fat[5]. PBDE-47 has also been detected in adipose tissue in Sweden (1.0-98.2 ng/g lipid)[6].

7.12 In Sweden, there has been an increase in total PBDE levels in samples of human milk over a 25 year period from 1972-1997. The predominant congener was PDBE-47 (2,2',4,4'-tetraBDE) which accounted for 62% of the total (2.28 ng/g lipid). PBDE-99 (2, 2', 4, 4', 5-pentaBDE) accounted for 13% (0.48 ng/g lipid) and PBDE-153 (2, 2', 4, 4', 5, 5'-hexaBDE) accounted for a further 8% of the total (0.46 ng/g lipid). The remaining 17% was accounted for by other tri-, tetra-, penta- and hexa congeners[7]. Data from North America also indicate an increase in total PBDE levels in breast milk. In 1992, samples of breast milk from the Canadian milk bank contained less than 50 ng PBDE/g fat. In 1997, concentrations of approximately 150 ng PBDE/g fat were detected in samples from women in New York State and 200 ng PBDE/g fat was detected in samples from Austin and Denver in 2000[8]. A recent abstract reported a mean concentration of total PBDE of 6.6 ng/g lipid in breast milk sampled from women in the UK in 2001-3[9].

7.13 Repeat dose studies of commercial pentaBBDE in rodents have identified the liver as a key target organ, with effects seen at doses of 2 mg/kg bw/day

and greater. Based on a study in which a commercial pentaBDE product was administered to rats in the diet for 30-days (0-1 mg/kg bw/day) with no treatment related changes, the EU risk assessment concluded, the no observable adverse effect level (NOAEL) for pentaBDE was 0.45 mg/kg bw/day[1]. This value was derived based on the content of 50-62% pentaBDE in the commercial product and assuming a maximum oral absorption of 90%, by analogy with other polyhalogenated diaromatic compounds. Applying similar correction factors to the doses at which liver toxicity has been observed indicates a LOAEL of 0.9 mg/kg bw/day.

7.14   For octaBDE, a LOAEL of 7.2 mg/kg bw/day was identified for histopathological liver changes in the rat[2]. A NOAEL for liver changes following administration of octaBDE has not been established. The repeat dose toxicity of decaBDE is low. The EU risk assessment reported a NOAEL of 1,120 mg/kg bw/day for liver changes seen in the carcinogenicity study in rats (paragraph 19)[3].

7.15   In short term gavage studies in rats commercial mixtures of pentaBDE (10-300 mg/kg bw/day), PBDE-47 (2,2',4,4'-tetraBDE; 18 mg/kg bw/day) and a commercial mixture of octaBDE (10-100 mg/kg bw/day) have been shown to increase the metabolism of model substrates for drug metabolising enzymes, in a manner consistent with induction of cytochrome P450 isozymes of the CYP1A and CYP2B subfamilies and UDP-glucuronosyl transferase. This induction was associated with perturbation of thyroid hormones, liver enlargement and histopathological changes in the thyroid[1,10,11,12]. DecaBDE was not found to induce a similar spectrum of changes in CYP subfamilies and thyroid hormones[10,13]. In this study the effect of decaBDE was investigated in rats at doses up 100 mg/kg/day for 4 consecutive days. This dose is an order of magnitude below the LOAEL for lesions in the liver and thyroid in the carcinogenicity studies and as such, may have been insufficient to produce an effect in short-term studies.

7.16   Penta-BDE has not been shown to be mutagenic in four studies in *S. typhimurium* and one study in *S. cerevisiae*. A cytogenetic study in human peripheral blood lymphocytes also gave negative results. In a single study in *S. typhimurium*, a commercial pentaBDE referred to as Tardex 50 (10-

10000 µg/plate) was shown to increase point mutations by 3-fold at the highest concentration in the absence, but not in the presence, of metabolic activation. This single positive result was considered in the EU risk assessment to be a chance finding[1]. PentaBDE has not been tested for genotoxicity *in vivo*.

7.17   Commercial octaBDE preparations were not mutagenic in four studies in *S. typhimurium* and one in *S. cerevisiae* in the presence and absence of metabolic activation. One preparation referred to as Muster 82 showed weak mutagenic activity in *S. typhimurium* without activation. No information is available on the composition of this preparation. Commercial octaBDE did not induce unscheduled DNA synthesis in human fibroblasts, sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells or chromosomal aberrations in human peripheral blood lymphocytes. OctaBDE has not been tested for genotoxicity *in vivo*[2].

7.18   Commercial decaBDE preparations of known purity (97-98%) were not mutagenic in *S. typhimurium* or *E. coli* in the presence and absence of rat metabolic activation. Two preparations referred to as Muster 83 and Muster 88 had positive effects in strains TA 98, 100 and 1535 with and without activation, but not in strains TA 1537 or 1538. No information is available on the composition of these preparations. In studies reported by the NTP, decaBDE was not mutagenic in the mouse lymphoma assay and did not induce SCE or chromosomal aberrations in CHO cells. In a reproductive study with dietary administration of decaBDE (3-100 mg/kg/day), there was no increase in chromosomal aberrations in the bone marrow cells of parent rats or of the offspring at weaning[3].

7.19   There are no carcinogenicity data for pentaBDE and octaBDE. The EU risk assessment noted that the evidence for carcinogenicity of decaBDE was considered to be equivocal. In a 103-week feeding study in mice there was an increase in hepatocellular adenomas or carcinomas at the lowest dose (3200 mg/kg bw/day), but not at the higher dose (6650 mg/kg bw/day). Thyroid gland follicular cell adenomas were reported in male mice at both doses. There was no evidence of carcinogenicity in female mice. In a 103-week feeding study in rats there was a dose dependant increase in neoplastic liver nodules, which was significantly greater than

control in low dose males (1120 mg/kg bw/day) and in both sexes at the high dose (2240 mg/kg bw/day)[3].

7.20   In a developmental study in rats, oral administration of commercial pentaBDE on days 6-15 of gestation resulted in no adverse fetal effects at the doses up to 200 mg/kg bw/day[1]. Commercial preparations of octaBDE have been shown to cause fetal toxicity and malformations at doses below those causing maternal toxicity in rats and rabbits. The EU risk assessment identified 2 mg/kg bw/day as the lowest NOAEL for octaBDE, from a study in rabbits in which slight fetotoxicity was observed at 5 mg/kg bw/day[2]. A commercial preparation of decaBDE did not show developmental effects following dietary administration in the diet to rats at doses up to 100 mg/kg bw/day for 60 days prior to mating until weaning[3].

7.21   The PBDE congeners PBDE-47 (2,2',4,4'-tetraBDE) and PBDE-99 (2,2',4,4',5-pentaBDE) have been reported to cause neurobehavioural changes in NMRI mice following a single oral dose administered on post natal day (PND) 3, 10 or 19. Neurobehavioural effects were detected at 60 and 120 days of age[14,15,16]. In addition, perinatal exposure of CD1 mice to PBDE-99 resulted in neurobehavioural effects detected at 60 days[17]. The main neurobehavioural effect of PBDE-99 was delayed habituation behaviour, which was found to occur at the lowest doses tested (0.6 mg/kg[17]; and 0.8 mg/kg[15,16]).

7.22   Limited data on the interaction of a range of PBDE congeners with the arylhydrocarbon (Ah) receptor suggest that those measured have much lower potency than the dioxins and co-planar PCBs[18,19]. *In vitro* studies have suggested that a range of PBDEs have endocrine disrupting activity mediated via the oestrogen receptor[19,20].

HBCD

7.23   All toxicological studies with HBCD were conducted using the commercial mixture. Studies in laboratory animals have shown that, following oral administration, HBCD can be detected in the adipose tissue, liver and muscle. Longer-term exposure shows HBCD has the potential to bioaccumulate. The α-isomer has been found to accumulate more than the β- and γ-isomers. The extent of metabolism of the commercial HBCD is

unknown. Following oral administration, the majority of HBCD was detected unchanged in the faeces, although it is unclear how much of this was unabsorbed material. There is no information on the toxicokinetics of HBCD in humans[4].

7.24   Repeat dose studies of HBCD have identified the liver as a key target organ. Increased liver weights and disturbances in thyroid hormones were observed at 100 mg/kg bw/day which was the lowest dose tested[4].

7.25   HBCD was not mutagenic in one study using *S. typhimurium* and did not induce chromosomal aberrations in human peripheral blood lymphocytes in the presence and absence of metabolic activation. HBCD caused a slight but significant increase in somatic recombinations in a non-standard assay using two Chinese hamster cell lines containing duplication mutations in the *hprt* gene. The relevance of this is unclear. *In vivo*, there were no significant increases in the frequency of micronuclei in mouse bone marrow cells. In an 18-month lifetime study of dietary administration to mice (available to the EU rapporteur in summary form only) there were no treatment related increases in tumour incidence at HBCD doses of 13-1300 mg/kg bw/day)[4].

7.26   Administration of HBCD to pregnant rats at dietary doses up to 750 mg/kg bw/day, or gavage doses up to 1000 mg/kg bw/day did not result in fetal toxicity or teratogenicity[4]. HBCD has also been investigated for neurodevelopmental effects[21]. A single dose of HBCD resulted in changes in spontaneous behaviour. However, this information was only available as an abstract, the doses resulting in these effects were unclear and therefore the data could not be used in the risk assessment.

COT evaluation of the toxicological properties of PBDEs and HBCDs

7.27   There are deficiencies in the toxicological database of the PBDEs and HBCD and these uncertainties need to be reflected in the evaluation. The majority of studies reviewed were relatively old and, although conducted to the standards of the time would not meet current requirements for study design and reporting. In addition, the duration of the longest studies

undertaken with pentaBDE were similar to the reported half-life and the resulting tissue concentrations would only reach half of their maximal value by the end of the study.

7.28   The limited data on the toxicokinetics of the PBDEs suggest differences in absorption and excretion of individual compounds.   Data on the genotoxicity, carcinogenicity and reproductive toxicity of PBDEs and HBCD are also limited. As the EU is introducing prohibitions on the use of some PBDEs, it is unlikely that new studies will be undertaken to address the deficiencies in the toxicological databases.

7.29   The toxicity studies have generally been conducted using commercial mixtures of PBDEs and HBCD. The composition of the material used in many of the studies was unclear and likely to differ from the mixture of congeners measured in food and the environment.   Although a similar pattern of biological effects was reported for different mixtures, extrapolation of findings between mixtures should be treated with caution.

7.30   Whilst some Ah receptor mediated activity could be measured with PBDEs, the potency was low and the most sensitive end-points of toxicity were unlikely to be mediated by this mechanism.   It has also been suggested that PBDEs have endocrine disrupting activity mediated via the oestrogen receptor, although the current data are limited.

7.31   The liver is a target organ for the PBDEs, with pentaBDE being the most toxic, and decaBDE the least. OctaBDE has been found to exhibit reproductive toxicity, whereas pentaBDE and decaBDE did not produce adverse effects in routine developmental studies.

7.32   Non-routine studies suggest that the most sensitive endpoints are neurodevelopmental. Two of the congeners that are present in commercial pentaBDE, have been shown to cause neurobehavioural effects in adult mice following administration of a single postnatal oral dose. Octa- and decaBDE, and the congeners commonly found in them, have not been investigated using this protocol.

7.33   HBCD is also hepatotoxic. It has not shown evidence of developmental toxicity in routine studies. One study, available in abstract form only, indicates that it might produce neurodevelopmental effects but there is insufficient detail to use the data in risk assessment.

7.34   Table 7.2 shows the NOAELs and LOAELs for the key effects of the PBDEs and HBCD. Based on the available data, pentaBDE appears to be the most toxic of the PBDEs, with a NOAEL of 0.45 mg/kg bw/day for liver effects following repeat dosing and a LOAEL of 0.6 mg/kg bw/day for neurodevelopmental effects following a single post-natal dose. For HBCD, the LOAELs are 100 mg/kg bw/day for liver effects following repeat dosing and 0.9 mg/kg bw/day for neurodevelopmental effects following a single post-natal dose.

7.35   It is anticipated that the human perinatal blood-brain barrier would be as permeable to PBDE as that of the mouse neonatal blood-brain barrier. There is no single neurodevelopmental stage of the human brain that is directly comparable with the mouse, as different brain parts develop at different rates in the two species. These studies involved administration at postnatal days 3 and 10, at which age the mouse brain probably models the human brain from 1 month pre-natal to 1 month post-natal. There is a lack of data on levels in breast milk, which might be a major source at the critical time for neurodevelopmental toxicity.

Table 7.2: NOAELs and LOAELs for PBDEs or HBCD

BFR	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Target	Comments
Commercial pentaBDE[1]	0.45	0.9	Liver	No chronic studies available
PBDE-99 (2,2',4,4',5-pentaBDE)[15]		ND	0.6	Neurodevelopment
Commercial octaBDE[2]	2 ND	5 7.2	Reproduction Liver	No neurodevelopmental data
Commercial decaBDE[3]	ND	1,120	Liver	No neurodevelopmental data
Commercial HBCD[4]	ND	100	Liver	Chronic data seen in summary form only

ND: NOAEL was not determined in the study identifying the LOAEL

7.36   In view of the inadequacies in the toxicological database and the absence of identifiable no-effect levels, it was not possible to determine a tolerable daily intake (TDI). The Committee therefore decided to take a Margin of Exposure (MoE) approach in which the estimated human exposures are compared with the relevant NOAEL or LOAEL identified from the animal studies. Had it been possible to establish a TDI from a NOAEL, uncertainty factors (UFs) would be required to allow for inter-and intraspecies differences in toxicokinetics and toxicodynamics (100) and limitations in the database such as study duration and gaps in the data (up to 10). Combining these uncertainty factors suggests a target MoE of 1000 for liver toxicity of penta-BDE, above which risks to health would not be expected. A NOAEL has not been identified for the neurodevelopmental effects of penta-BDE and therefore an additional UF of 3-10 would be required for extrapolation from a LOAEL to a NOAEL. This suggests a target MoE of 3,000-10,000. Similarly, a NOAEL has not been identified for the hepatic effects of HBCD, indicating a target MoE of 3,000-10,000. The exposure is not expected to represent a risk to health if the calculated MoE exceeds the target MoE.

PBDEs and HBCDs in fish from the Skerne Tees survey and in the 2001 Total Diet Study (TDS)

7.37   In 1999, a study sponsored by the Department of the Environment, Transport and Regions measured the concentration of PBDEs in rivers and estuaries downsteam of potential sources. The maximum concentrations of PBDEs were detected in the livers of fish (1294 μg/kg wet wt) and sediment (239 μg/kg dry wt) from the Skerne-Tees river system and the Tees estuary at Newton Aycliffe, which is downstream from the Great Lakes Chemical Company[22]. The Great Lakes Chemical Company is known to have manufactured both penta- and octaBDE at this site until the late 1990's, and still produces HBCD. DecaBDE was never manufactured at the site but may have been distributed via this site. The concentrations detected in the fish livers and sediment were only measured at one time-point and it is important to note that the trout in this river are restocked annually indicating these figures may not accurately represent the actual levels of contamination in the river.

7.38   Following these reports, the Food Standards Agency conducted a survey to determine the concentrations of congeners known to be components of commercial penta- and octaBDE in brown trout and eels from the Skerne-Tees river system. The Great Lakes Chemical Company is also known to have manufactured HBCD and thus, it was included in the survey. The chemical formula of the each congener and the BFR to which each congener corresponds is given in Table 7.3. A preliminary assessment of dietary exposure based on analysis of food samples from the 2001 Total Diet Study (TDS) was also conducted.

Table 7.3: PBDE and HBCD congeners measured in the FSA survey

Congener	Chemical formula	Component
PBDE 28	2, 4, 4'-TriBDE	Possible component of commercial pentaBDE
PBDE 47	2, 2', 4, 4'-TetraBDE	Known substantial component of commercial pentaBDE
PBDE 99	2, 2', 4, 4', 5-PentaBDE	Known substantial component of commercial pentaBDE
PBDE 100	2, 2', 4, 4', 6-PentaBDE	Known substantial component of commercial pentaBDE
PBDE 153	2, 2', 4, 4', 5, 5'-HexaBDE	Possible component of commercial pentaBDE and known component of commercial octaBDE
PBDE 154	2, 2', 4, 4', 5, 6'-HexaBDE	Possible component of commercial pentaBDE and commercial octaBDE
α-HBCD	1R, 2S, 5R, 6R, 9S, 10S-HBCD	Known component of commercial HBCD
β-HBCD	1R, 2R, 5R, 6S, 9R, 10S-HBCD	Known component of commercial HBCD
γ-HBCD	1S, 2S, 5S, 6S, 9S, 10S-HBCD	Known component of commercial HBCD
R & S indicate the stereo position of the bromine on the HBCD molecule		

7.39   A survey was commenced in late 2001 to determine the concentrations of PBDE and HBCD in brown trout and eels from the Skerne-Tees river system. The samples examined included control samples of trout and eels from the Skerne and eels from the Tees. Samples were obtained from the river Skerne and river Tees at eight easily accessible locations that were upstream and downstream of the Great Lakes Chemical Company and at the confluence of the two rivers (see Figure 7.3). The PBDE congeners analysed in the survey were selected as a representative sample of those produced and used in the Skerne-Tees area. The chemical formula of each congener and the commercial PBDE to which each congener corresponds is given in Table 7.3.

7.40   At the time of this survey no data were available on other sources of dietary exposure to PBDEs and HBCDs in the UK. Therefore, a survey of the concentrations of PBDE congeners in food samples from the 2001 Total Diet Study (TDS) was commissioned in 2002. Single composite food group samples were formed by homogenising individual foods groups (excluding beverages) from 24 locations. These composite samples

were analysed for the same range of PBDE congeners as the fish survey. It had been planned to additionally analyse these samples for decaBDE, however since none of the congeners measured were detectable this analysis was not undertaken.

Analytical methodology

7.41   The analytical method involved solvent extraction of the fat component of composite samples from the TDS or a portion of flesh removed from fish or eels. The extracts contained BFRs and other compounds that were separated from the dissolved fat component by adsorption chromatography. The PBDEs were measured using gas chromatography coupled to a mass spectrometric detector (GC-MS).

7.42   For HBCD, a simplified clean-up stage was performed which consisted of shaking another aliquot of the crude extract with concentrated sulphuric acid. The acid destroys the fats but leaves HBCD compounds intact. The temperatures used to separate PBDEs during GC analysis can affect the structures of the isomers and result in conversion between the three HBCD isomers. Therefore HBCD was determined using a liquid chromatography mass spectrometry (LC-MS) method.

7.43   Due to their lipophilicity BFRs are most likely to be detected in fatty foods. The production of composite samples from the individual foods that comprise a particular food group in the TDS may result in dilution of the overall fat content and thus may reduce the ability to detect these substances.

Dietary exposure to PBDEs and HBCD from Skerne Tees trout and eels

7.44   Table 7.4 shows the concentration ranges of total PBDEs and HBCD in trout and eels taken from those test sites where the highest concentrations were found and from the control sites. The most contaminated trout were caught at the Haughton Road site, which was the closest river Skerne location downstream of the Great Lakes Chemical Company. No eels are caught at this site, and the most contaminated eels were caught from the next river Skerne downstream location at Oxenfield Bridge.The sum of the

concentrations of individual PBDE congeners detected in the edible portion varied from 12 to 14 μg/kg freshweight in trout and was 53 μg/kg freshweight in the eel at control sites. At test sites, the sum of the concentrations varied from 59 to 197 μg/kg freshweight in trout and 164 to 288 μg/kg freshweight in eels.

7.45   For HBCD the concentration in the edible portion of trout varied from 21-119μg/kg freshweight and was 159 μg/kg freshweight in the eel at control sites. At test sites, the sum of the concentrations was 159 to 6758 μg/kg freshweight in trout and 570 to 9432 μg/kg freshweight in eels. The trout population of the Skerne Tees River system is restocked annually and no information was available to ascertain the ages of the fish sampled and whether concentrations increased in older fish. Detailed results will be published in a Food Surveillance Information Sheet.

7.46   The estimated average intake from consumption of one weekly portion (120g) of trout at the maximum levels detected were 0.056 μg/kg bw/day of PBDEs and 1.9 μg/kg bw/day of HBCD. The estimated average intake from consumption of one weekly portion (20g) of eels, and 0.014 μg/kg bw/day of PBDEs and 0.45 μg/kg bw/day of HBCD for eels.

7.47   There are no commercial fisheries in the river and consumption would be limited to fish caught by anglers.

Total dietary exposure to PBDEs and HBCD

7.48   None of the congeners measured was present at concentrations exceeding the limit of detection (LOD) in any of the composite TDS samples analysed. Estimated intakes were therefore calculated from the upper bound concentrations (assuming that PBDE and HBCD were present at the LOD in all foods) together with consumption data from the 2000 National Diet and Nutrition Survey[24]. Dietary exposure for mean adult consumers was estimated to be ≤0.047 μg/kg bw/day for the sum of the PBDE congeners measured and ≤0.010 μg/kg bw/day for the sum of HBCD isomers. The actual intakes might be very much lower.

Table 7.4: Estimated average dietary intake of PBDE and HBCD following consumption of trout or eels from the Skerne-Tees river system

BFR and sampling location	Species	No of samples	Concentration range (μg/kg freshweight)	Maximum intake (μg/ portion) [c]	Maximum average intake (μg/kg bw/day) [d]
PBDE (Ricknall Grange)[a]	Trout	5	12-14	1.6	0.003
PDBE (Haughton Rd)[b]	Trout	7	59-197	24	0.056
PBDE (Ricknall Grange)[a]	Eels	1	53	1.1	0.0025
PBDE (Oxenfield Bridge) [b]	Eels	5	164-288	5.8	0.014
HBCD (Ricknall Grange)[a]	Trout	5	21-119	14	0.034
HBCD (Haughton Road) [b]	Trout	7	159-6758	810	1.9
HBCD (Ricknall Grange)[a]	Eels	1	159	3.2	0.0076
HBCD (Oxenfield Bridge) [b]	Eels	3	570-9432	190	0.45

a   Control site for Skerne River
b   Test site showing the highest concentration of PBDEs or HCBD in trout or eels
c   Portion sizes were assumed to be 120g trout or 20g eels, as cited in MAFF Food Portion Sizes[23]
d   Average daily intake was calculated assuming consumption of one portion of trout or eels per week and an adult bodyweight of 60kg

COT evaluation of dietary exposure to PBDEs and HBCD

7.49   The concentrations of PBDEs and HBCD detected varied widely in the fish sampled from the test sites and also from those sites considered to be control sites. In the absence of information to the contrary, it is assumed that the fish will move freely around the Skerne-Tees river system and therefore concentrations in the fish taken from one site are not representative. It is therefore not possible to identify an average concentration of PBDEs or HBCD which could be used to estimate intake for an individual regularly consuming fish caught from an individual site. Therefore worst-case intake estimations have been calculated from the

highest measured concentration at any site. It is unlikely that this worst case intake would be achieved on a regular basis.

7.50 The PBDE congeners measured in the survey were selected as major representative components of penta- and octaBDE. Toxicity data are not available for the individual congeners. Concentrations of the individual congeners have therefore been summed for comparison with the toxicity data on the commercial PBDE mixtures. Studies on the commercial PBDEs indicate that pentaBDE is the most toxic. Comparison of the estimated intakes of the sum of the measured PBDE congeners with the reported effect levels for pentaBDE provides a precautionary approach because some of the congeners are expected to be less toxic.

7.51 The most sensitive effect of pentaBDE and HBCD was considered to be neurodevelopmental. The LOAEL for pentaBDE (600 µg/kg bw/day) for this effect was obtained from studies in which the test material was administered by a single oral dose to mice on postnatal days 3 or 10. Limited evidence suggests that HBCD also has this effect. Human infants of comparable developmental stage (up to 1 month) would not eat fish and so would not be directly exposed to PBDE or HBCD from fish. Although pentaBDE is known to pass into breast-milk, the available data are not sufficient to estimate the potential exposure to the breast-fed infant resulting from consumption of contaminated fish by the mother. There is a need for data on levels of PBDEs and HBCD in breast-milk in the UK, in order to determine whether the breast-fed infant is at risk of neurodevelopmental effects arising from consumption of contaminated fish. The available studies on reproductive toxicity have not included investigation of neurobehavioural effects, and therefore there are no data of relevance to exposure by pregnant women. Overall, it was considered not possible to calculate a relevant MoE with respect to neurodevelopmental effects.

7.52 For older children and adults eating trout or eels contaminated with PBDEs and HBCD, the liver toxicity is the most relevant and sensitive effect on which to base a risk assessment.

7.53   The worst-case estimated intake of total PBDEs from consuming one portion of trout per week from the Skerne-Tees river system was 0.056 μg/kg bw/day indicating a MoE of approximately 10,000 compared with the NOAEL of 450 μg/kg bw/day for liver effects of pentaBDE in rats. The MoE for intake of PBDEs from consuming one portion of eels per week would be 40,000. Since these MoEs are larger than the target MoE of 1000 for pentaBDE, these intakes are unlikely to pose a health risk.

7.54   The worst case estimated intake of total HBCD from consuming one portion of trout per week was 1.9 μg/kg bw/day indicating a MoE of approximately 50,000 compared with the LOAEL of 100,000 μg/kg bw/day for liver effects of HBCD in rats. The MoE for intake of HBCD from consuming one portion of eels per week would be 200,000. Since these MoEs are larger than the target MoE of 3,000-10,000 for HBCD, these intakes are unlikely to pose a health risk.

Conclusions

7.55   We *conclude* that the uncertainties and deficiencies in the toxicological databases for PBDEs and HBCD prevent establishment of tolerable daily intakes. A Margin of Exposure (MoE) approach has therefore been used in this risk assessment.

7.56   We *consider* that the most sensitive endpoint for the PBDEs appears to be neurodevelopmental effects resulting from a single oral administration to neonatal mice at a developmental stage comparable to infants up to one month of age, and limited data indicate that HBCD could also have this effect. It is reassuring that infants of this age do not eat fish and therefore are not directly exposed to PBDEs from this source.

7.57   We *note* the uncertainty in the relevance of the neurodevelopmental effects for exposure to the fetus or breast-fed infant following maternal consumption of fish containing high levels of PBDEs or HBCD. This results from the lack of neurodevelopmental studies with exposure during pregnancy and the lack of information on concentrations in breast milk that could result from consumption of fish by the mother.

7.58   We *note* that consumption of fish from the Skerne Tees is unlikely to be widespread since there are no commercial fisheries in the area. However given the variability in BFR levels observed in this limited survey, it is not possible to exclude higher intakes in a small number of anglers or others eating their fish.

7.59   We *consider* that comparison of the worst case estimated intakes from consumption of a single portion of eels or trout per week from the Skerne Tees with the available toxicological data indicates that these intakes are unlikely to represent a risk to health. However, in view of the uncertainties surrounding the toxicological database and exposure assessments, this conclusion should be considered tentative.

7.60   PentaBDE and octaBDE are being phased out in 2004, which offers some reassurance that exposure to these compounds is unlikely to increase significantly. Concentrations of deca-BDE and HBCD should continue to be monitored, particularly in fatty foods.

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October 2003

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Figure 7.3: Sampling locations for the survey for brominated flame-retardants in fish from the Skerne-Tees River system
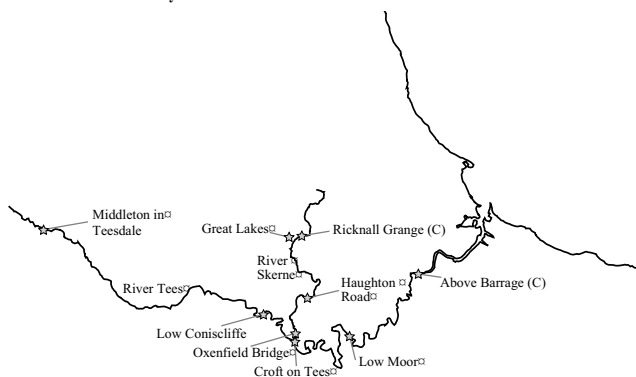
The sites shown on the map are the sites where samples of fish and eels were taken from. The sampling sites were upstream and downstream of the Great Lakes Chemical Company and at the confluence of the two rivers. Control samples (c) were obtained from the River Skerne at Ricknall Grange and from the River Tees at above the Tees Barrage. It was only possible to sample both trout and eels at the Oxenfield Bridge and Croft-on-Tees sampling sites.

Annex 5
COT statement on the tolerable daily intake for dioxins and dioxin-like polychlorinated biphenyls

Introduction

Dioxins and polychlorinated biphenyls (PCBs)

8.1   Dioxins are persistent organochlorine compounds that are widely dispersed environmental contaminants and which accumulate in fatty foods. The term "dioxins" is commonly used to refer to a group of 75 polychlorinated dibenzo-*p*-dioxin (PCDD) and 135 polychlorinated dibenzofuran (PCDF) congeners, of which less than 20 are considered to be biologically active. Dioxins are produced in a number of thermal reactions, including incineration of municipal waste, domestic fires and bonfires, forest fires and internal combustion in automobile engines. They are also generated as trace contaminants during the synthesis of many organochlorine compounds (e.g. chlorophenoxy herbicides such as hexachlorophene, chlorodiphenyl ether herbicides) and during some industrial processes (e.g. bleaching of pulp and paper with chlorine gas).

8.2   Polychlorinated biphenyls (PCBs) are environmentally stable, lipophilic chemicals that were widely manufactured for a range of industrial applications between the 1930s and 1970s. Use of PCBs for industrial purposes has been discontinued but these substances may still be released to the environment during disposal of materials and obsolete equipment. There are 209 theoretically possible PCB congeners, of which 12 non-*ortho* or mono-*ortho* compounds exhibit similar biological activity to PCDDs and PCDFs, and are therefore referred to as "dioxin-like PCBs".

8.3   There is continuing public concern about the health hazards of dioxins and related compounds. These compounds are persistent in the environment and tend to accumulate in biological systems. One of the most extensively studied PCDD congeners, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), exhibits a broad range of toxic effects in laboratory animals, some at very low doses.

8.4   Exposure of the general population to dioxins and dioxin-like PCBs is primarily from food. The estimated exposures from the UK Total Diet Study samples for all age groups have declined substantially. In 1982, average intakes for dioxins and dioxin-like PCBs were 7.2 and 18 pg TEQ/kg bw/day for adults and toddlers, respectively. In 1997 the averages had fallen to 1.8 and 4.6 pg TEQ/kg bw/day for adults and toddlers, respectively. [1] Over the same period, the intake estimates for high level (97.5th percentile) consumers have fallen from 13 and 28 pg TEQ/kg bw/day to 3.1 and 7.2 TEQ/kg bw/day, for adults and toddlers, respectively. Dioxins and PCBs are detectable in almost all types of food. Highest concentrations are found in meat, fish, eggs and dairy products. However, cereals, fats and oils contribute significant proportions of the total dioxin and PCB intake because they are major components of the diet. The decline has been attributed in part to controls on emissions to the environment and the discontinuation of production and use of dioxin-like PCBs. It is anticipated that exposures will continue to decline with present and planned environmental controls.

8.5   The highest dioxin exposures in humans have generally been associated with occupational exposure or accidental contamination of the environment or edible oils. Occupational exposure studies have been undertaken at plants in the USA, Germany, the Netherlands and the UK that manufacture chlorophenols and/or chlorophenoxy herbicides. Application of chlorophenoxy herbicides has been associated with much lower levels of exposure. Exposures to more highly chlorinated PCDDs have been estimated for workers exposed to pentachlorophenol and/or other chlorophenates at saw mills or manufacturing plants. In addition, an explosion in a chemical plant at Seveso in 1976, resulted in widespread release of TCDD to the environment and exposure of the local population. The ingestion of edible oils contaminated with high levels of

polychlorinated compounds including PCBs and PCDFs was associated with toxicity in food poisoning incidents in Yusho and Yu-Cheng, which we reviewed in 1997. [2]

Previous COT evaluations

8.6   The COT and our sister Committees on Carcinogenicity (COC) and Mutagenicity (COM) have considered dioxins and dioxin-like PCBs on several occasions in the past. [1-8] In 1989 we made a comprehensive statement about the health hazards of PCDDs and PCDFs. [3] We made a second statement in 1991 when UK exposure data on these compounds from food became available. [4] On that occasion, we endorsed the Tolerable Daily Intake (TDI) of 10 pg/kg bw/day 2,3,7,8-TCDD recommended by an expert group convened by the WHO Regional Office for Europe [9] and we recommended that, when considering mixtures of PCDDs and PCDFs, the TDI can be regarded as 10 pg/kg bw/day 2,3,7,8-TCDD Equivalents (TEQ). We further stated that, in view of the estimated long elimination half-lives of this class of compounds, it would be more appropriate to regard the TDI as a time-weighted average tolerable intake. We reviewed PCDD and PCDFs again in 1995, when we concluded that the new information available at that time did not necessitate the alteration of the previously agreed TDI. [6]

8.7   The Toxic Equivalency Factor (TEF) approach was initially used to facilitate risk assessment of PCDDs and PCDFs (i.e. dioxins). In 1997, we tentatively accepted that the TEF approach could be extended to include the dioxin-like PCB congeners [2] and in 1998 we endorsed the revised WHO-TEFs for dioxins and dioxin-like PCBs. [7]

Recent International evaluations

World Health Organization.

8.8   In 1998 the WHO European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS) conducted a re-evaluation of the TDI for dioxins and dioxin-like PCBs. The Executive Summary of this report was published in a special issue of Food Additives and Contaminants [10], devoted to the 1998 WHO-

ECEH/IPCS Consultation on Dioxins, allowing an evaluation of the basis on which the WHO consultation reached its conclusions.

8.9   The WHO consultation recommended a TDI for dioxins and dioxin-like PCBs of 1-4 pg WHO-TEQ/kg based on the NOAEL/LOAELs of those effects considered to be the most sensitive in experimental animals, namely endometriosis, developmental neurobehavioural effects, developmental reproductive effects and immunotoxicity.

Scientific Committee on Food

8.10   The Scientific Committee on Food (SCF) undertook a reassessment of the TDI for dioxins and dioxin-like PCBs for the European Union, adopting a temporary opinion in November 2000. This was revised in June 2001, in the light of newly published data allowing calculation of the total amount of dioxin in the fetus (the fetal body burden) associated with maternal exposure at steady state. [11] The SCF concluded that, because TCDD and related compounds have very long half-lives in the human body, the tolerable intake should be expressed on a weekly rather than a daily basis. Based on the LOAEL from a study showing developmental effects in male rat offspring following repeated subcutaneous administration of TCDD [12], the SCF established a tolerable weekly intake (TWI) of 14 pg WHO-TEQ/kg bw. [13]

Joint FAO/WHO Expert Committee on Food Additives

8.11   The Joint FAO/WHO Expert Committee on Food Additives (JECFA) also considered dioxins and dioxin-like PCBs in June 2001. The JECFA used a similar body burden approach to that used by the SCF and also took into account exposure from background contamination considered to be present in feed provided to laboratory animals. It proposed a provisional tolerable monthly intake (PTMI) of 70 pg WHO-TEQ/kg bw [14], based upon the lowest LOAEL and a NOAEL for developmental effects in male rat offspring. [12, 15]

Environmental Protection Agency

8.12   The U.S. Environmental Protection Agency (EPA) commenced a reassessment of dioxin exposure and human health effects entitled,

"Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds" in April 1991. In 1994, it released its initial external review draft describing health effects and exposures. In our 1995 statement[6], we welcomed the US EPA initiative to investigate further the health hazards of 2,3,7,8-TCDD and related compounds. The document provided a thorough review of the literature on the effects of these compounds on various biological systems. However, we did not consider it provided any new information or analysis that necessitated the alteration of the previously agreed TDI of 10pg TEQ/kg bw/day or of our previous advice.

8.13   Following public comment and advice from its Science Advisory Board, the EPA undertook an extensive revision of its review, particularly two key sections on Dose-Response Modelling and Integrated Summary and Risk Characterization.[16] These chapters were subject to public comment and further Science Advisory Board review during late 2000 and Spring 2001. The EPA's draft assessment considered that cancer was the most appropriate end-point for risk assessment and undertook an evaluation based on their risk assessment guidelines for carcinogens. The results of this evaluation are not yet available.

Evidence considered in the current evaluation.

8.14   In 2000, we were asked to review the risk assessments of dioxins carried out by the WHO, the SCF, and the US-EPA. We concluded that it was appropriate to conduct our own evaluation of the data, informed by these international assessments and other relevant information, before reconsidering the TDI for dioxins and dioxin-like PCBs. As part of this evaluation it was essential that the evidence concerning human cancer risks for dioxins be evaluated to determine whether or not it is appropriate to assume the existence of a threshold and hence whether a TDI could be established. We are grateful for the assistance provided by the COC and we have taken account of its conclusions [17] in completing our evaluation.

8.15   In undertaking our evaluation we have had access to the published assessments of all three international evaluations. The EPA documents provided the most detailed and comprehensive review of the published

literature. We have supplemented this by evaluation of the original publications identifying critical end-points and recently published data.

Mechanism(s) of action of TCDD

8.16   Most of the actions of TCDD and related compounds can be ascribed to the consequences of an initial binding to what has become known as the Ah or aryl hydrocarbon receptor (AHR), although this binding protein is now more properly termed a ligand activated transcription factor. This binding results in multiple changes in gene transcription leading to increases in biotransformation enzymes, modulation of cell cycling proteins and other responses. Inappropriate gene expression resulting from the high affinity binding and long term occupancy of the receptor may be the basis of the toxicity of TCDD. However, although the mechanisms of early molecular changes are well understood, the relationship between changes in gene regulation and observed toxicity is still unresolved.

8.17   It has become apparent that the sequence of events from TCDD binding to gene transcription involves other transcription factors, chaperones (such as HSP90) and regulatory proteins. The net result is the association of TCDD-AHR with another factor, the Ah receptor nuclear translocator (ARNT), in the nucleus followed by binding of the complex to 'dioxin' responsive regulatory elements (DREs) in enhancer regions upstream of particular genes. Downstream activation of promoter regions then occurs with production of mRNA from the genes. Most of the molecular events for transcription of the CYP1A1 gene have been elucidated. For other genes the sequence of events is far less clear but probably occurs in a similar manner[18] and the number of known AHR-regulated genes is still increasing.

8.18   Mechanistic studies on the role of the AHR in the toxicity of TCDD have shown that proteins similar to the AHR have been found in many organisms suggesting that this receptor has an essential biological function[19]. Sequencing studies on the AHR have shown that it is a member of a family of gene regulating proteins known as PAS (PER-ARNT-SIM)[20]. In mammals, these proteins (which include hypoxia inducible factor 1-α [HIF1α] and ARNT) regulate the transcription of specific genes.

Heterodimerization of the AHR with ARNT is apparently essential for the TCDD activated AHR to induce specific DNA binding and transactivation *in vitro*. However, heterodimerisation of ARNT can also occur with HIF1α and with a newly discovered factor AHR repressor (AHRR). [21] TCDD/AHR might act by competing against these, or even competing against the binding of a hypothetical normal endogenous ligand of the AHR that has yet to be found. Other studies have shown that levels of the AHR, AHRR, ARNT and HIF1α may be regulated by cell type and activation and by stages of growth and differentiation. [22]

8.19   A number of lines of evidence *in vivo* support the role of AHR in TCDD toxicity. For instance, polymorphism of the AHR, with varying affinities for TCDD, in mice correlates with variable susceptibility to toxic effects. [23] Different strains of mice that do not possess the functioning gene for the AHR (referred to as *Ahr* null or 'AHR knockout' mice) have been shown to be extremely resistant to very high doses of TCDD for a variety of toxic endpoints. Binding capabilities of dioxins and dioxin-like PCBs to the AHR, as shown by structure activity relationships, generally show similar ranked order to their elicitation of biochemical responses.

8.20   However, in seeking to understand the mechanisms of action in order to inform risk assessment, it might be inappropriate to place exclusive emphasis on the AHR. At very high doses of TCDD (in the *Ahr* null mouse) the chemical may have toxic actions which are not mediated by the receptor. Similarly, in some *in vitro* experiments, various effects of TCDD have been interpreted as non-AHR dependent.

8.21   In terms of binding to the AHR, some ligands may be competitors of TCDD-induced gene regulation. Conditional disruption of the *Arnt* gene has recently shown that ARNT is required for AHR-stimulated gene activation by TCDD in liver, but this association has yet to be extended to toxicity. [24] Other, as yet unidentified, AHR ligands may be present, or TCDD-AHR complex may participate in cell dysfunction by unknown routes not involving the regulation of gene expression via DREs and ARNT.   Although no endogenous ligand of AHR has been proven, a number of naturally derived chemicals are ligands.

8.22   Some data suggest that the binding affinity, and the effect of binding, of TCDD to the AHR, are much lower in humans than in rodents, even the resistant DBA/2 mouse. [25] This could contribute an extra safety margin but the difference in response may vary with endpoint. Some polymorphisms of the human AHR gene have been reported but the functional significance of these polymorphisms is still under investigation. [26] We note that, in view of this uncertainty, it is not possible to exclude the possibility that the most sensitive humans are as responsive as the most sensitive rodents. Overall, we agree that the evidence that toxicity is mediated via the AHR, and the limited evidence that dioxin/receptor interaction does not inevitably lead to a toxic response, are sufficient to consider a threshold approach to the risk assessment.

Toxicokinetics of dioxins

Absorption

8.23   The extent of gastrointestinal absorption of dioxins is reported to vary with the medium or vehicle of administration, and the lipophilicity of the individual congeners. The percentage absorption of TCDD is approximately 60% in rodents. [27] Similar absorption has been reported for other chlorinated dibenzodioxins and dibenzofurans, although the absorption of octachlorodibenzodioxin (OCDD) is less than 20%. PCDDs and PCDFs are incompletely absorbed because they are not in solution within the gut lumen, and absorption is dependent on the digestion and emulsification of the food matrix.

Distribution

8.24   Once absorbed, probably via chylomicrons, TCDD rapidly leaves the blood compartment with a distribution half-life of approximately 30 minutes in rats, after which time it is primarily found in adipose tissue, the liver, skin, muscle and other tissues. TCDD present within the blood is largely associated with lipoproteins. Studies in rats have shown that after initial rapid distribution there is a slower redistribution from muscle and other organs, primarily to the liver and adipose tissue, skin and thyroid gland; the concentrations in these organs show a slow increase over a period of about 4 days following a single intraperitoneal dose. [28] This

pattern of distribution is probably representative of distribution in humans and there is a high correlation between adipose tissue concentrations and the levels in serum.

8.25   The duration of the distribution phase is very short compared with the elimination phase. After tissue distribution, which takes about 4 days, *in vivo* elimination is adequately represented by a single mono-exponential decrease and half-life. The distribution phase is important in the interpretation of effects produced *in utero* in rats after a single oral dose given late in pregnancy, which are the basis for determining the tolerable intake.

Metabolism and elimination

8.26   Although early studies suggested that TCDD is not metabolized, it is now recognized that it is slowly converted to polar metabolites that are eliminated as glucuronides. The main metabolites of TCDD formed with rat hepatocytes *in vitro* are 1-hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-TCDD. Other metabolites have been identified in dog, including tri- and dichloro-hydroxy and dihydroxy- compounds. Oxidative metabolism does not appear to give rise to significant bioactivation or formation of DNA adducts and the limited available data indicate that the metabolites are less toxic than the parent compound.[29] A major route of elimination of the hydroxy- metabolites is as glucuronic acid conjugates in the bile. Unmetabolized PCDDs and PCDFs are not detected in bile but are excreted in the faeces and faecal fat by direct intestinal elimination.

8.27   The half-life of TCDD has been reported to be about 20 days in the rat, 12 days in mice, 90 days in the guinea pig and between 6 and 11 years in humans.[28,30-32] The elimination half-life in humans correlates positively with the percentage body fat, indicating slower elimination in individuals with higher fat composition. Consistent with this is evidence suggesting an age-related decrease in half-life in the elderly, as the fat stores are mobilized during redistribution from the subcutaneous to abdominal areas[33]. Mobilization of fat stores during lactation contributes to the presence of dioxins in breast milk[34], and this is associated with a decrease in the maternal body burden during breast-feeding.

Human data

Introduction

8.28   The human effects observed in one or more studies are summarized in Table 8.1. In assessing the effects of dioxins and dioxin-like PCBs in humans, we have selected those studies that provide the most information on the relationship between outcomes and exposure to TCDD-contaminated materials. Case reports were not reviewed and only cohort studies with calculated standardized mortality ratios (SMRs) or equivalent, were discussed in assessing mortality. Many of the studies reviewed are cross-sectional in design and there are inherent limitations of this type of study. We noted that the lack of adequate exposure data was a frequent limitation of the available epidemiology. Exposure is measured in different media and expressed in different units across the studies, which makes comparison difficult.   Some studies were only able to use indirect estimates of exposure, which cannot be directly related to dioxin levels. Development of a tolerable intake requires studies with quantitative assessment of exposure.

8.29   We focused our evaluation on studies in which exposure was assessed by measurement of dioxin concentrations in serum or body fat, which could be correlated with body burden and intake. The body burdens in human studies have been estimated in two ways. Firstly, the body burden may be calculated from the concentrations of dioxins in lipid and the percentage body composition as fat. This does not allow adequately for sequestration of dioxins within the liver, but this should produce only a minor error in the calculation of body burden. The second method is calculation of the body burden based on estimates of intake and half-life. In humans the intakes of dioxins will have varied historically and there is uncertainty about past exposures. In addition, little is known about the half-life of dioxins and dioxin-like PCBs at different life stages. Calculation of body burden based on daily intake has to allow for the bioavailability from the food matrix and the half-life or clearance from the body. A limitation to this method for considering mixtures of dioxins and dioxin-like PCBs is that reliable estimates of the half-life of TCDD and also its congeners are necessary.

8.30   We also focused on the relationship between exposure and response, particularly (although not exclusively, if other important health end points were investigated) for the health end points that are relevant/comparable to the results of animal studies. We noted that the EPA report identified six studies or series of studies in humans, which measured serum levels of TCDD and compared them with possible health effects. [35-43] We were also informed of an additional study in Dutch chemical workers, which provided exposure data [44] and a series of Dutch studies on cognitive development. [45-53]

8.31   With the exception of the series of Dutch studies on cognitive development, the studies reported the effects of high level occupational exposure or the results of accidental release. Occupational or accidental exposure would be associated with higher peak body burdens, followed by gradual elimination and were therefore difficult to compare with steady state conditions associated with background human exposure via the diet or with repeated exposure in animal studies. Also, the occupational studies have not addressed the reproductive effects that represent the most sensitive endpoints in the animal studies.

Studies of cognitive development

8.32   A series of Dutch studies involved cohorts in Rotterdam and Groningen, representing a highly industrialized region and a less industrialized, more rural area, respectively. The cohorts were sub-divided between breast-feeding for a minimum of six weeks and formula fed using a single batch of one commercial formula. Plasma from maternal and cord blood samples and milk samples were analysed for dioxins and PCBs, including some PCB congeners considered not to have dioxin-like properties. The infants were monitored at ages from 3 to 42 months, with assessments of motor and cognitive development, as well as indicators of thyroid function. [46-52] Similar studies were conducted on a smaller cohort in Amsterdam. [53] We invited additional expertise to ensure that these studies were reviewed adequately, particularly the relevance of the methodology, and we gratefully acknowledge the assistance provided.

Table 8.1: Effects associated with human exposure to dioxins.

Effect	Epidemiological evidence
Chloracne	Proven association No clear dose relationship
Gastrointestinal effects and liver enzymes	Transient increases in some liver enzymes
Cardiovascular diseases	Positive association in occupational studies, but not in airforce veterans exposed to herbicides in Vietnam (Operation Ranch Hand). Dose-response in some studies
Changes in lipid levels	Results not consistent
Diabetes	Overall results not consistent Increased risks of morbidity in Seveso and Ranch Hand study
Reproductive hormones	Inconsistent results
Reproductive outcomes	Change in sex ratio of offspring with highly exposed fathers in Seveso No data yet on possible effects such as endometriosis and fertility in women – Seveso endometriosis study on-going
Thyroid function	Results not entirely consistent. Some small differences reported in thyroid hormone uptake levels.
Neurological / psychological effects	Inconsistent findings. Some effects reported in Ranch Hand study and Seveso (polyneuropathies, abnormal co-ordination) No association with depression
Respiratory system	Inconsistent evidence Irritative effects and reduced lung function in some studies
Urinary system	No major renal or bladder dysfunctions observed.
Immunological effects	Inconsistent findings.
Neurobehavioural developmental effects	Some observed differences in on-going Dutch studies
Cancer	Regarded as a probable human carcinogen (based on human, animal and mechanistic data)

8.33   We noted that the measures used were standard for the age of children assessed, and the best available in the absence of an *a priori* hypothesis of specific effects. However, we were informed that in very young children it is only possible to perform crude tests which do not provide a clear distinction between motor and cognitive development.   Such tests therefore serve more as screening tests than definitive measures and thus

the interpretation of any observed change may be hard to assess. Tests of motor function had been conducted from shortly after birth until about 30 months of age. Tests of cognitive function were conducted from 3 months to about 42 months. Different patterns had been observed in the studies conducted at different ages and very little change was observed in the middle of the age range. We noted that Prechtl's neurological examination (as conducted on the Rotterdam and Groningen cohort [47,48]) was considered the most stringent, but with the disadvantage of generating a large number of false positives. For infants, the Bayley Scales, based on a very large sample and well standardized, is probably the best instrument. This scale is divided into a mental development index (MDI) that measures how motor tasks (e.g. control of hands) are applied and a psychomotor index (PSI) that measures gross movements (e.g. walking). However, the Bayley Scales provide a measure of timing of appearance of certain skills and not the quality with which they are carried out. We noted that the study used a 1969 version of the test whereas a more advanced version was published in 1993.

8.34   The paper of Patandin *et al*. [52] reported changes at 42 months using the Kaufman Assessment Battery. This was considered to be critical to our assessment, since cognitive function is stabilising at this age and becomes predictive of function in later life. In contrast, Bayley scales are used for younger age groups (3 months to 3 years) and have low predictivity. The Kaufman scores at 42 months suggested an effect of pre-natal dioxin exposure leading to an effect on cognitive development.

8.35   It was difficult to determine whether the effects were due to dioxin exposure or to confounding factors. Complex correlations were found between dioxin and PCB levels and confounding factors, such as breast-feeding, smoking and maternal education. Linear regression analysis had been used to assess the influence of confounding factors. It was not clear whether this was appropriate, and the data were insufficient to determine whether the statistical approach might result in over- or under-correction. Overall, if the effects were real, they were most likely to be due to pre-natal exposure. Breast-feeding ameliorated the effects. However concerns over the known and potential confounders made it impossible to reach firm conclusions.

8.36   We noted that distinction between pre-natal and post-natal exposure to dioxins and PCBs was an issue of concern relating to the Dutch studies. Prenatal exposure was based on analyses at 8 months gestation, and it was not clear whether these were fully representative of exposure throughout pregnancy.   However, when considering effects on thyroid function it should be noted that these effects may be confounded by changes in maternal thyroid hormone production prior to thyroid development in the fetus. None of the populations examined in the Dutch studies were considered to have exposures greater than the normal background range, differences were found between industrial and rural locations and there was a very large natural variation. In addition breast-feeding appeared to be a major confounder, with the highest proportion of breast fed infants having the highest dioxin concentration. Similarly level of maternal education appeared to correlate best with high exposure as did smoking. The paucity of information on the mathematical models used in the study made it impossible to determine whether effects were "real" or due to confounders.

8.37   We concluded that it was not possible to determine whether any cognitive changes represented temporarily delayed milestones of development or a persistent decrement and that follow-up studies were needed.   These should be carried out two to three years after the original study or during the teenage years, as increasingly sensitive measures can be used in older children. Decreased variability in older children also tends to make the tests more sensitive. In the absence of such studies we do not consider it possible to come to clearer conclusions about the outcome of dioxin exposure.

Sex ratio

8.38   A recent study has reported on the sex distribution of children born between 1 April 1977 and 31 December 1996, with one or both parents exposed to TCDD in the Seveso incident, for whom TCDD serum concentrations were available relating to the time of the incident [54]. The exposed individuals were between 3 and 45 years old in 1976. Compared with an unexposed population, there was a dose-related decrease in the proportion of male children born to TCDD-exposed fathers. We note that this difference was statistically significant at paternal serum TCDD

concentrations of 118 pg/kg or more. This could be estimated to correspond to a body burden of 24 ng/kg bw, which is equivalent to a daily intake of 12 pg/kg bw/day. However, the high exposure resulting from the Seveso incident is not comparable to steady state exposure, and the body burden derived from the peak serum concentrations in 1976 may not be the most appropriate dose surrogate for reproductive effects occurring in subsequent years.

Endometriosis

8.39   Eskenazi and co-workers published initial details of the Seveso Women's Health Study [55]. The primary objectives of this study are to investigate whether there is a relationship between TCDD exposure and the following end-points; endometriosis, menstrual cycle characteristics, age at menarche, birth outcomes of pregnancies conceived after 1976, time to conception, clinical infertility and age at menopause. Insufficient results are currently published to assess the effects of TCDD exposure in the Seveso incident on endometriosis and other reproductive end-points in women. We considered that further consideration of female reproductive outcomes should be deferred until further papers on the Seveso Women's Health Study become available.

Immunotoxicity

8.40   We noted that, compared with studies in experimental animals, there is much less information regarding immunotoxicity in humans. Nevertheless, there are suggestions that human immune function may be less susceptible to TCDD and dioxin-like PCBs that of rodents.

8.41   Evidence for immunotoxicity in humans resulting from occupational or accidental exposure to TCDD or related PCBs is inconsistent. However, a common feature of some investigations has been a modest exposure-related reduction in the frequency of peripheral CD4 T lymphocytes. The extent to which these effects represent an early indication of immunosuppression is unclear.

8.42   A recent paper has examined infectious and atopic diseases and immunological parameters in children with background levels of exposure

to PCBs. [56] These are the cohorts from Rotterdam discussed in paragraphs 32 to 37, above. A large number of analyses are reported, many of which are simply correlation coefficients and some of the statistically significant results are likely to have occurred by chance. The authors concluded that exposure to PCBs and dioxins might be associated with a greater susceptibility to infectious diseases and a lower prevalence of allergic diseases. However, we noted a number of contradictions in the reported results, in addition to the uncertainty over control for confounders as noted in paragraph 35. We concluded that the study did not provide convincing evidence of a causal relationship between pre-natal exposure or total body burden to PCBs and increased susceptibility to infectious diseases or decreased incidence of allergic disease.

Cardiovascular disease

8.43   Some studies have reported a positive association between exposure to TCDD, or to PCDDs and PCDFs, and the incidence of ischaemic heart disease.[39,40, 42,44]. These studies have indicated that a significant increase in ischaemic heart disease is associated with a body burden at or above 25 ng TCDD/kg bw, or 55 ng TEQ/kg bw for PCDDs and PCDFs. However, they did not adequately allow for confounding by other risk factors, such as smoking and diet. No studies included measurement of dioxin-like PCBs exposure or its contribution to the body burden.

Cancer

8.44   The Committee on Mutagenicity (COM) and the Committee on Carcinogenicity (COC) considered 2,3,7,8-TCDD in 1988/9 and concluded that this compound was carcinogenic in rodents but that this was unlikely to be due to a mutagenic mechanism. The COC gave further consideration to the carcinogenicity of 2,3,7,8-TCDD in 1993, when more epidemiological data were available. The Committee concluded that the new data strengthened the possibility of an epidemiological link between occupational exposure to 2,3,7,8-TCDD and an increase in total cancers in humans, although there was no consistent association with cancer at any specific anatomical site(s). It was considered that there was insufficient evidence for a clear causal link but it would be prudent at present to regard 2,3,7,8-TCDD as a possible human carcinogen [5].

8.45   The COC reviewed TCDD in 1998, following the publication of the IARC monograph which concluded that TCDD should be considered as a definite human carcinogen [57]. The COC agreed that TCDD is a potent carcinogen in laboratory animals, but that the information from the most heavily occupationally exposed cohorts suggested that there was, at most, only a weak carcinogenic effect in these individuals. It therefore concluded that there were insufficient epidemiological and toxicological data on TCDD to conclude a causal link with cancer in humans, but it would be prudent to consider TCDD as a "probable weak human carcinogen". [58]

8.46   The COC has reconsidered its 1998 statement in the light of recently published data on cancer epidemiology, including the twenty-year follow-up of the Seveso incident [59], and mechanisms of carcinogenicity. It agreed that TCDD should be regarded as a "probable human carcinogen" on the basis of all the available data. The COC agreed that although a precise mechanism for carcinogenesis in laboratory animals or humans could not be elucidated from the available information, the data (i.e. negative genotoxicity in standard assays, and evidence from studies of mechanisms) suggested that a threshold approach to risk assessment was likely to be appropriate. The COC did not consider it possible to quantify the margin-of-safety risk assessment in view of the difficulties in selecting the appropriate metric of exposures. However, it noted that the excess cancer mortality reported in the heavily exposed industrial cohorts was small and commented that any increased risk of cancer at background levels of exposure is likely to be extremely small and not detectable by current epidemiological methods. [17]

Animal data

8.47   There are few regulatory rodent toxicity studies and no regulatory non-rodent studies on the dioxins, and most of the available data relate to TCDD. Most of the regulatory toxicity studies were performed at least 20 years ago and cannot be considered adequate for the determination of NOAELs. The recent studies were conducted to non-standard protocols and many of the studies examining the most sensitive end-points also failed to identify NOAELs. We have reviewed the experimental toxicology

of TCDD, with particular consideration to those showing effects at the lowest doses.

Immunotoxicity

8.48 We noted that the available data presented a complicated picture, with diverse protocols, including the use of different species and strains; various routes and durations of exposure and a wide range of doses. Nevertheless, some general points could be made:

8.49 In rodent studies the most consistent effect is a reduction in antibody responses to sheep red blood cells (SRBC). The SRBC assay is primarily a measure of the integrity of humoral immunity. However, as initiation and maintenance of antibody responses to SRBC requires not only B lymphocytes, but also functional T lymphocytes and antigen processing/presenting cells, this assay provides something of an overall view of adaptive immunity.

8.50 The most sensitive adverse effect level resulting from exposure to TCDD in which an immune alteration has been implicated was reported by Burleson *et al* [60]. An increased mortality of mice following challenge with influenza A virus was found following a single exposure to 0.1, 0.05 or 0.01 μg/kg TCDD. However, there is no evidence that the observed increase in susceptibility to virus challenge was necessarily attributable to impaired immune function and mortality was not associated with increased titres of virus in the lungs of mice exposed to TCDD. Therefore it could not be concluded that the lowest dose in this study represents the LOAEL for TCDD-induced immunotoxicity in mice.

8.51 We concur with the conclusion of the WHO, EPA and SCF reviews in considering the studies of Gehrs and colleagues to be important in assessing the immune effects of dioxins. [61, 62] Pregnant rats (Fischer 344 strain) received a single oral dose (on gestational day 14) of 0.1, 0.3, 1.0 or 3.0 μg/kg TCDD. Exposure at all doses was associated with a persistent (up to 14 months) reduction in males of delayed-type hypersensitivity (DTH) responses to bovine serum albumin. Maternal doses of 0.3 μg/kg TCDD and above were required for persistent suppression of DTH reactions in female offspring. On the basis of these investigations it is

likely that 0.1 µg/kg TCDD should be regarded as the LOAEL for immune effects in young rats.

8.52   A second conclusion drawn from these studies was that maximal inhibition of immune function required both lactational and *in utero* exposure. This was more effective than lactational exposure alone, which was in turn more effective that *in utero* exposure only. It was noted that these differences in potency related to rats and might differ in humans.

Developmental and reproductive toxicity

8.53   The studies on developmental and reproductive effects in experimental animals mainly involved administration of TCDD alone, but there were comparative data for other congeners on teratogenicity and ovarian function. TCDD was able to elicit a number of different developmental effects although the sensitivity differed. The most sensitive and robust end-point was the effect on epididymal sperm count.

8.54   The EPA provided an excellent comprehensive review of the literature on developmental and reproductive toxicity, and although some new studies had emerged since it was written these did not have a major impact. The human sensitivity (based on *in vitro* data on embryonic AHR concentrations in different species) appeared to be in the middle of the range shown by experimental animals. Whilst the AHR was clearly implicated in the teratogenicity of TCDD, its role in other developmental effects was less clearly established. The reproductive effects were correlated with body burden at the critical stage of sexual differentiation (GD 15-16, as noted by SCF and JECFA [13,14]) and it appeared that equivalent fetal body burdens on day 16 of gestation were achieved by administration of different bolus doses on day 8 and day 15 of gestation.

8.55   We noted that the most sensitive end-points were observed following bolus administration and paid careful consideration to the relevance in deriving a tolerable intake. These studies are considered in detail in paragraphs 64-70. We noted that there was evidence to support an extrapolation from a bolus dose to a chronic exposure, as considered in paragraphs 71-74. The only multigeneration study was old [63] and was subjected to detailed evaluation in previous considerations by the Committee. [4] We considered

that the results from this multigeneration study supported the body burden estimates but that there were questions about the statistics which required further evaluation.

8.56   We were informed that data from animal developmental studies did not show differences in the sex ratio of offspring, as had been reported for humans in the Seveso region. However, we accepted the animal studies were not designed specifically to address this issue.

Endometriosis

8.57   In our 1995 statement we noted a study reporting an increased incidence of endometriosis in rhesus monkeys 10 years after completion of a study in which TCDD was administered in the diet for a period of about 4 years. [64] A recently published paper follows up the same group of monkeys 13 years after completion of the dietary study, reporting that the incidence of endometriosis correlated with serum levels of certain PCB congeners, but not TCDD. [65] Monkeys involved in a study in which lead was administered were also found to show an association between serum PCB levels and endometriosis. The authors could not account for the source of PCB exposure to these animals.

8.58   We noted that a number of aspects of this observational study undermined confidence in the results and in the earlier findings and concluded that it was not possible to draw reliable conclusions.

Acute, subchronic and chronic toxicity

8.59   TCDD causes a wide range of toxic responses after short and long term exposure with large differences in sensitivity between species/strains of animals to particular responses. Most of the reported toxic responses could be produced in every species provided an appropriate dose was given. The wide variability in sensitivity and the particular toxic response produced within and between species, makes it difficult to identify an appropriate endpoint for risk assessment. Lethality (as determined by LD50) varies with species from the highly sensitive guinea pig to the relatively insensitive hamster. There is also considerable variation within species. The value of these studies for risk assessment is doubtful given the age of

the various studies, and the use of different strains, dosing regimens, routes of administration and observation period. No single site of toxicity has been identified as the cause of lethality; each species has a different spectrum of organ toxicity with a wasting syndrome and hepatotoxicity as the most common features. The wasting effect occurs in several species, but no single explanation for this effect has been described. Hepatotoxicity includes a wide range of liver effects in many species with rats and mice at the sensitive end and guinea pigs and hamsters as the least sensitive species. There is considerable variation in response within different strains of rat. The chronic dietary administration studies of Kociba *et al.* [66] reported that the lowest dose of 0.001 µg/kg bw/day was a NOAEL for hepatocellular nodules, although low body weights were recorded at various times during the study and only animals surviving to the end of the study were necropsied. In this study, the tumour incidences were significantly increased at a number of sites at the 0.1 µg/kg bw/day dose level.

8.60   We noted that there was no adequate basis for decisions on acceptable risk levels in humans based on the standard toxicity studies. Two of the most sensitive endpoints across the species seemed to be induction of CYP 1A1 and oxidative stress. Although CYP 1A1 induction is not considered to be a toxic response, it could underlie toxicity resulting from disruption of various endogenous processes. However, we noted that induction of CYP isozymes does not always show a good correlation with responsiveness in different mouse strains, indicating that it cannot be directly linked to toxicity. Oxidative stress had been detected in mouse brain [67], although it was not clear whether this was related to CYP induction.

Overall assessment

Use of body burden as a dose surrogate.

8.61   We considered that the most appropriate measure of exposure for assessing the sensitive endpoints of TCDD toxicity were the associated tissue concentrations, rather than the administered dose. Ideally the concentration in the target tissue would be the most appropriate measure of dose for comparing effects in different species, but this is impracticable for humans. The tissue concentration is directly related to the body burden at steady-

state so that calculated body burdens are a valid surrogate. We therefore consider that the exposure/dose-response relationship for TCDD and related compounds should be based on body burden not external dose. The body burden approach allows for the massive interspecies differences in the half-life, and the potential for accumulation. An additional advantage of using body burdens, compared with previous dose-response assessments based on external dose, is that the body burdens can be estimated for occupational and accidental exposures, and body burden-response relationships assessed. We concur with the recent evaluations that, despite some limitations, the body burden provides the appropriate dose metric, and that there is sufficient scientific evidence to support the use of body burden.

Human daily intakes and body burden

8.62   Following dietary exposure to dioxins and dioxin-like PCBs, the body burden will be accumulated over a period of 15-30 years in humans, during which time the environmental concentrations of these substances have decreased. In consequence, the body is not truly at steady-state, and hence there will be errors in the daily intake when calculated from current concentrations in body lipids. A pharmacokinetic model that allows for decreasing environmental concentrations with time indicates that the simple steady-state assumption over-estimates daily intake by approximately 20%. Some equations relating daily intake to body burden (based on adipose levels) do not include a specific term for bioavailability, and this would need to be considered for each route/protocol for exposure. This analysis is particularly important in relation to interpretation of human epidemiology studies where the body burden and daily intake is based on analysis of adipose tissue concentrations.

8.63   Overall, the data indicate that dioxins and dioxin-like PCBs may be associated with a number of effects, including cancer and cardiovascular disease, but generally at body burdens at least 10-fold higher than those occurring in the general population. Most of the studies involve groups that have been exposed to very high levels of dioxins resulting from occupational or accidental exposure and the pattern of exposure does not reflect long-term dietary exposure.

Evaluation

Key studies

8.64　We conducted a detailed review of the human data linking health effects to dioxin exposure, and a summary of these data is available on the COT website (http://www.foodstandards.gov.uk/committees/cot/summary.htm). We concluded that the available human data did not provide a sufficiently rigorous basis for establishment of a tolerable intake. This was because:

- the epidemiological studies do not reflect the most sensitive population identified by animal studies,

- there are considerable uncertainties in the exposure assessments and inadequate allowance for confounding factors;

- the patterns of exposure did not reflect exposures experienced in the general UK population, which are mainly from diet.

We therefore found it necessary to base our evaluation on the data from studies conducted in experimental animals.

8.65　In accordance with the advice of the COC [17], we considered it appropriate to take a threshold approach to establishing a tolerable intake. This is based upon the negative genotoxicity in standard assays and evidence from studies of mechanisms.

8.66　Because a threshold-based approach was considered appropriate, we examined all of the toxicological effects, in addition to cancer, in order to identify the most sensitive end-points. We concluded that the most sensitive indicators of TCDD toxicity were the effects on the developing reproductive systems of male rat fetuses exposed *in utero*. These data were used despite inconsistencies in the findings reported, and the fact that none of the recent observations were made following sub-chronic or chronic dietary administration that would give constant (steady-state) maternal body burdens. We note that tolerable intakes were also derived from these endpoints in the recent SCF and JECFA evaluations. The key studies used different strains of rats and tended to give contradictory findings. A change

in urogenital distance was found after single oral doses given on day 15 of gestation (GD15) of 50ng/kg bw[15], 200ng/kg bw[68] and 1000ng/kg bw[69]. We considered that the data on ano-genital distance were not robust because of lack of correction for body weight or other means of normalization, and should be regarded as an intermediate marker with no functional significance. Decreases in sperm numbers, production, reserve or morphology were found after single oral doses of 50ng/kg bw and above (GD15) [68-70] and subcutaneous dosage to give a body burden of 25ng/kg bw [12], but not, in one study, at 800ng/kg bw (oral dose on GD15) [15]. Changes in the weight of the urogenital complex, including the ventral prostate were reported after an oral dose of 200ng/kg bw on GD15 [15] but not at 300ng/kg bw subcutaneously [12].

8.67   Despite some inconsistencies, we considered that the effects on sperm production and morphology represented the most sensitive effects. These were indicative of the functional adverse reproductive effects in the rat that were produced by long-term administration in the multigeneration study of Murray *et al* at doses resulting in a 10-fold higher body burden than those in the studies of sperm production [63]. We also note that the sperm reserve in the human male is much less than that in the rat, and therefore these changes are considered relevant. No NOAEL was available for these effects, but the study of Faqi [12] provided the lowest LOAEL. We noted limitations in this study but considered that the results could not be discounted and therefore, that this should be used as the basis for deriving the tolerable intake.

8.68   We considered that a tolerable intake based on these effects would also protect against any risk of carcinogenicity from dioxins and dioxin-like PCBs. This conclusion is based on the mode of action of dioxins and difference between the body burdens at background levels of exposure and those associated with increased cancer risk as observed by the COC [17].

8.69   Three of the studies [15,68,70] reported adverse effects in male rat offspring following a single oral dose of TCDD given on GD15, and one [12] following repeated weekly subcutaneous injections. In all cases the effects were observed postnatally and the pattern of both *in utero* and post-natal exposure would be different. Because of the long half-life of TCDD (21

days in rats), and its presence in milk, the male offspring would be exposed to decreasing concentrations until the time of measurements. The recent SCF and JECFA evaluations [13,14] used recently published toxicokinetic studies [11,27] that allow the fetal body burdens to be calculated on GD16, on the assumption that this is the appropriate site of action, and period of sensitivity.

8.70   We have adopted a similar approach to the SCF and the JECFA. However, in view of the numerous assumptions in this approach (described below), we have used a simplified calculation of fetal and maternal body burdens associated with these different dosage regimens and their conversion to the steady-state dietary intakes that would result in the same fetal body burdens. Calculation of a tolerable intake for humans is complex and requires a number of steps: calculation of the fetal body burden of rats under the experimental conditions; correction of the corresponding maternal body burden in rats to represent chronic daily intake *via* the diet; the use of uncertainty factors to give an equivalent tolerable human maternal body burden; and finally, derivation of a daily intake by humans that would result in the tolerable human maternal body burden.

Calculation of body burden

8.71   On the assumption that the critical period of exposure is GD16, the adverse effects following a single oral dose on GD15 would have been initiated at a time when the dose was undergoing tissue distribution. At this time, more of the maternal body burden would have been associated with well-perfused tissues, such as the liver, and the reproductive system and less with adipose tissue. It is possible to estimate the fetal exposure on GD16 by allowing for differences in the maternal dosage protocol using the toxicokinetic data of Hurst *et al.,* following a single oral bolus dose on GD15 [27] and following dietary administration of 1, 10 and 30ng/kg bw per day for 5 days per week from 13 weeks before mating [11].

8.72   A problem with the interpretation of the Hurst *et al* papers [11,27], which measured radioactivity after dosage with radioactive TCDD, is that the ratios of maternal to fetal body burdens on GD16 were not independent of dose, as would be predicted for such low doses. This non-linearity is difficult to explain on biological grounds and may have arisen as an

artefact of the low levels of radioactivity measured. The SCF evaluation used regression analysis with a power model forced through the origin to correct maternal dosage and derive a correction factor of 2.6 for the higher fetal body burdens when dosed on GD15 compared with daily treatment. [13] These regressions used the ratios of maternal:fetal body burdens in ng/kg bw after single doses of 50 and 200ng/kg bw on GD15 [27] (30:5.3 and 97.4:13.2, respectively) and after daily oral doses equivalent to 0.71, 7.1 and 21.3ng/kg bw/day [11] (20:1.4, 120:7.5 and 300:15.2 respectively). The JECFA evaluation confirmed the results of the power model but also used a linear model that gave a correction factor of 1.7, and the JECFA concluded that both models fitted equally well to the available data. [14] Although the power and linear models fitted equally well, they gave different correction factors, especially at very low body burdens. This resulted in a discrepancy (see JECFA, 2001) [14] when applied to the correction of the 5ng/kg bw subcutaneous maintenance dose used in the study of Faqi *et al* [12] (see below).

8.73   Because the correct mathematical model cannot be determined based on goodness of fit, and because the regressions are determined largely by body burdens higher than those relevant for derivation of a tolerable intake, we decided to adopt a simpler method of correction using the ratios calculated directly from the lowest doses in each of the studies by Hurst *et al.* [11,27] After a single oral dose of 50ng/kg bw on GD15, the fetal body burden on GD16 was 5.8-fold lower than the maternal body burden (5.3ng/kg bw compared with 30.6ng/kg bw). [27] After sub-chronic oral treatment with 1ng/kg bw/day for 5 days a week, which gave a maternal body burden of 19ng/kg bw, the fetal body burden on GD16 was 14.6-fold lower than the maternal body burden (1.3ng/kg bw compared with 19ng/kg bw). [11] Thus a bolus dose given on GD15 results in 2.5-fold higher fetal body burdens (14.6/5.8) on GD16, than would occur if the same maternal body burden had arisen as a result of sub-chronic treatment.

Derivation of the TDI

8.74   In order to derive a tolerable intake for humans, it was necessary to convert the subcutaneous dosage regimen used in the Faqi study [12] into a steady-state maternal body burden on GD16. The study involved a bolus dose of 25ng/kg bw, 14 days before mating, and subsequent weekly maintenance

doses of 5ng/kg bw. Assuming that the first day of mating corresponds to GD0, these weekly maintenance doses would have been given on GD-7, GD0, GD7, etc. By GD16, the doses given up to GD7 would have distributed to all tissues, representing steady-state distribution and resulting in a maternal body burden of 18.3ng/kg bw. This value is comprised of 9.3 + 2.3 + 3.0 + 3.7ng/kg bw remaining in the body from the doses given on GD-14, GD-7, GD0 and GD7, respectively, assuming a half-life of 21 days. The maternal body burden from the 5ng/kg bw maintenance dose given on GD14 would give a "non-equilibrium" maternal body burden of 4.5ng/kg bw on GD16. Using the correction factor described in paragraph 73, it can be estimated that a steady state maternal body burden of 2.5-fold higher (i.e. 11.3ng/kg bw) would be needed to produce the same fetal body burden as this "non-equilibrium" dose. Therefore the calculated total steady-state maternal body burden on GD16 arising from the subcutaneous dosing protocol at the LOAEL is approximately 30ng/kg bw, which would be about 33ng/kg bw after allowing for the TCDD intake from food.

8.75   Conversion of the calculated equivalent steady-state maternal body burdens from these studies in rats into an equivalent human body burden requires the use of uncertainty factors to allow for the use of a LOAEL and to allow for species differences and human variability. Both the SCF and the JECFA evaluations used a default factor of 3 to allow for the use of LOAEL, and an overall factor of 3.2 ($10^{0.5}$) to allow for species differences and inter-individual variability. [71,72] The latter factor is lower than the default of 100 normally used because it incorporates the following chemical-specific adjustment factors:

a)   inter-species differences in toxicokinetics: uncertainty factor of 1.0 because the body burden approach allows for toxicokinetic differences.

b)   inter-species differences and human variability in toxicodynamics: uncertainty factor of 1 to cover both of these aspects based on the assumption that in general, rats are more sensitive than humans, but the most susceptible humans might be as sensitive to TCDD as rats.

c) human variability in toxicokinetics: uncertainty factor of 3.2 to allow for potential increased accumulation, and hence body burden, of dioxins in the most susceptible individuals. This is only relevant for congeners with shorter half-lives than TCDD, because an individual with a 3.2-fold longer TCDD half-life would not reach steady-state body burden.

8.79   Applying the uncertainty factor of 9.6 (3 x 3.2) to the calculated maternal steady-state body burden from the study of Faqi *et al* (LOAEL=33ng/kg bw) gives a tolerable human equivalent maternal body burden of 3.4ng/kg bw.

8.80   Estimation of the daily intake of TCDD that would result in this body burden has to take into account the fraction absorbed (bioavailability) from the diet by humans (both the SCF and JECFA evaluations concluded that the bioavailability of TCDD in humans is 50%), and the very long half-life in humans (which the JECFA concluded was an average of 7.6 years, while the SCF used a figure of 7.5 years). The human equivalent body burdens can be converted into daily intakes by the equation:

$$\text{daily intake (pg/kg/day)} \quad = \quad \frac{\text{body burden (pg/kg bw)} \; \times \; \ln 2}{\text{bioavailability} \; \times \; \text{half-life in days}}$$

8.81   Using a bioavailability of 0.5 and a half-life of 2740 days (7.5 years), the tolerable human equivalent steady-state body burden from the study of Faqi *et al* [12] would be produced in humans by a daily intake of 1.7pg/kg bw/day. Given the imprecision and assumptions inherent in these calculations we concluded that the tolerable daily intake for dioxins and dioxin-like PCBs should be based on this value rounded to a single figure, i.e. 2pg WHO TEQ/kg bw per day. We note that SCF and JECFA have used longer averaging periods, but because intakes are usually expressed on a daily basis, we considered that establishment of a tolerable daily intake was more appropriate and transparent. This value is consistent with tolerable intakes derived recently using similar data (WHO: 1-4pg WHO TEQ /kg bw/day [10]; SCF: 14pg WHO TEQ /kg bw/week [13]; JECFA: 70pg WHO TEQ /kg bw/month[14]).

8.82   We note that the body burden is the most appropriate dose metric for establishment of a tolerable intake and, because of its long half-life, the body burden of TCDD at steady state is about 2000 fold higher the average daily intake. For example, an intake of 10 times the TDI on a single day would result in a 0.5% increase in the body burden. Therefore short term variation in intake does not significantly alter the body burden, and occasional exceedance of the TDI would not be expected to result in harmful effects, provided that intake averaged over a prolonged period is within the TDI.

Conclusions

8.83   We *conclude* that dioxins and dioxin-like PCBs have the potential to cause a wide range of adverse health effects. The health effects most likely to be associated with low levels of exposures relate to the developing embryo/fetus.

8.84   We *recommend* that a tolerable daily intake of 2 pg WHO-TEQ/kg bw per day is established, based upon effects on the developing male reproductive system mediated via the maternal body burden.

8.85   We *consider* that this TDI is adequate to protect against other possible effects, such as cancer and cardiovascular effects.

8.86   We *note* that the most recent intake estimates for the UK population are 1.8 pg/kg bw/day for the average consumer and 3.1 pg/kg bw/day for the 97.5 percentile consumer and that dietary intakes are decreasing.

8.87   There are no short-term measures that can be used to decrease the body burden of dioxins and dioxin-like PCBs in humans because of their long half-lives and widespread presence at low levels in food.

8.88   Similarly, because of the long half-life, short-term exceedances of the tolerable intake are not expected to result in adverse effects. Nevertheless, it is not possible to identify a duration and degree of exceedance at which adverse effects might occur.

8.89   Finally, we *confirm* our previous advice that, although intakes of dioxins and dioxin-like PCBs by breast-fed babies are higher than is desirable, encouragement of breast-feeding should continue on the basis of convincing evidence of the benefits of human milk to the overall health and development of the infant.

October 2001
COT/2001/07

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