
Decontamination of persistent organic pollutants in fishmeal and fish oil

Process optimization and modeling

Åge Oterhals



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Scientific environment

The research activity presented in this thesis has been performed at the Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima) Department Bergen under the supervision of Prof. Bjørn Kvamme at the University of Bergen, Department of Physics and Technology, and Prof. Ragnar Nortvedt, Department of Biology as a co-supervisor. Collaboration with NIFES has been established on the analysis of persistent organic pollutants (POPs) and minor components in fish oil. Other parts of the analytical work have been done at Nofima Department Bergen. The analytical laboratories at NIFES and Nofima are accredited according to ISO 17025. Experimental work on short-path distillation was performed in the pilot plant facility at UIC GmbH (Alzenau, Germany).

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Abstract

Persistent organic pollutants (POPs) are fat soluble chemical substances that persist in the environment and bioaccumulate in the food chain. Through long-range environmental transportation by air, water and migratory species they are transferred to new locations distant from the source of release and might cause adverse effects to human health and to the environment. POPs comprise pesticides, industrial chemicals and unwanted by-products. The European Commission has implemented food and feed legislations concerning maximum permitted levels (MPLs) of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), dioxin-like polychlorinated biphenyls (DL-PCBs), polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs). Fish and consequently fishmeal and fish oil has been identified as one of the most important contributors to the level of dioxins and DL-PCBs in food and feed products. Fish caught in some of the North-European fishing areas contain high dioxin and PCB levels resulting in fishmeal and oil with WHO-PCDD/F-PCB-TEQ levels above the maximum permitted. To meet the new industrial and social-economic challenges there is a need for development of cost-effective decontamination technologies.

Efficiency and limitations of alternative decontamination technologies to reduce the level of PCDD/Fs, DL-PCBs and polybrominated diphenyl ether flame retardants (PBDEs) in fishmeal (i.e., mechanical fat separation, organic solvent extraction and oil leaching) and fish oil (i.e., activated carbon (AC) adsorption and short-path distillation (SPD)) have been studied in this thesis. Factorial design experiments, response surface methodology and quantitative structure property relationships (QSPRs) have been utilized to model and optimize the fish oil decontamination alternatives. The trapping mechanisms for non- and mono-*ortho* PCBs on AC have been studied by use of molecular dynamics simulations.

Optimization of the existing mechanical fat separation steps in the fishmeal process is expected to be the most cost-effective way to reduce the POPs content. However, the obtainable effects are limited (estimated to maximum 20-30%) and have to be

combined with organic solvent extraction if high decontamination rates are needed. The feasible use of a new oil leaching process has been studied and found to give effects comparable to hexane and isopropanol extraction of fishmeal (i.e., > 75%).

AC adsorption of PCDD/Fs is highly effective with obtainable WHO-TEQ-reduction of 99%. Adsorption of DL-PCBs is less effective and dependent on ortho-substitution, i.e., non-*ortho*-PCBs are adsorbed more effectively than mono-*ortho*-PCBs, with maximum obtained levels of 87% and 21%, respectively. Compliance with present feed and food WHO-PCDD/F-PCB-TEQ legislations in fish oil can be achieved based on AC adsorption. The adsorption process had no effect on PBDEs. The selectivity can be explained based on dispersive electron interaction affected by sorbate planarity and steric effects and presence of electrostatic forces.

Within each of the studied homologue groups, the volatility and reduction after SPD of the individual congeners is linearly dependent on the number of chlorine or bromine substitutions and *ortho*-substitution. The QSPRs were combined with process parameters to establish decontamination models for each homologue group of POPs. High decontamination efficiency (> 90%) can be obtained by choice of favorable process conditions giving residual levels considerable below MPLs in feed and food.

AC and SPD did not have any negative effects on fish oil oxidation level. Oxidative stability of the oil after SPD was affected both negative and positive depending on processing conditions. SPD did not affect the level of polyunsaturated fatty acids (PUFA) nor induce geometrical isomerization.

Some co-evaporation and loss of vitamins, cholesterol and unsaponifiables can not be avoided during SPD. The retention level will depend on the applied process conditions and the concentration ratio and difference in vapor pressure between free and ester forms of the respective compounds. A high retention level (>80%) of volatile nutrients after SPD could be obtained by choice of process conditions giving residual WHO-TEQ-levels in accordance with present feed and food legislations and the voluntary industrial monograph of GOED.

List of publications

Papers

- I** Oterhals, Å. & Nygård, E. Reduction of persistent organic pollutants in fishmeal: A feasibility study. *J. Agric. Food Chem.* **2008**, 56, 2012-2020. DOI: 10.1021/jf072883k.
- II** Oterhals, Å., Solvang, M., Nortvedt, R. & Berntssen, M.H.G. Optimization of activated carbon-based decontamination of fish oil by response surface methodology. *Eur. J. Lipid. Sci. Technol.* **2007**, 109, 691-705. DOI: 10.1002/ejlt.200700083.
- III** Jensen, B., Kvamme, B., Kuznetsova, T. & Oterhals, Å. Molecular dynamics study of selective adsorption of PCB on activated carbon. Submitted to *Fluid Phase Equilib.* **2011**.
- IV** Oterhals, Å., Kvamme, B. & Berntssen, M.H.G. Modeling of a short-path distillation process to remove persistent organic pollutants in fish oil based on process parameters and quantitative structure properties relationships. *Chemosphere* **2010**, 80, 83-92. DOI: 10.1016/j.chemosphere.2010.04.016.
- V** Oterhals, Å. & Berntssen, M.H.G. Effects of refining and removal of persistent organic pollutants by short-path distillation on nutritional quality and oxidative stability of fish oil. *J. Agric. Food Chem.* **2010**, 58, 12250-12259. DOI: 10.1021/jf102660v.

Oral presentations

- I** Oterhals, Å. Dioxin – alternative rensemetoder for fiskemel/olje. SSF Industriseminar, December 10-11, 2002, Bergen, Norway.
- II** Oterhals, Å., Nygård, E., Solvang, M. Reduksjon av dioxin og PCB i fiskemel. Industriseminar for Fiskemel og Fôrindustri, December 9-10, 2003, Bergen, Norway.
- III** Oterhals, Å., Nygård, E., Solvang, M. Reduksjon av dioxin og PCB i fiskemel – A feasibility study. NFR Programkonferanse Havbruk, March 23-24, 2004, Gardermoen, Norway.
- IV** Oterhals, Å., Nygård, E., Solvang, M. Reduction of dioxin and PCB in fish meal – A feasibility study. 3rd European Federation for the Science and Technology of Lipids Congress, September 5-8, 2004, Edinburg, Scotland. Abstract available at http://va.gdch.de/wwwdata/abstracts/5852/5852_0209.pdf
- V** Oterhals, Å. Reduction of persistent organic pollutants (POPs) in fish oil and fish meal - Technical possibilities and perspectives. Workshop on The Possibilities of Decontamination of Fish Oil and Fish Meal for Dioxins, PCBs and other Persistent Organochlorine Compounds. European Commission, Health and Consumer Protection Directorate-General, Unit D3: Chemical and Physical Risks Surveillance, September 3, 2004, Brussels, Belgium.
- VI** Oterhals, Å., Solvang, M., Nygård, E., Berntssen, M.H.G. Nye grenseverdier og metoder for fjerning av miljøgifter i fiskemel og fiskeolje. Biomarint Industriseminar, December 7-8, 2004, Bergen, Norway.
- VII** Oterhals, Å. Metoder for fjerning av miljøgifter i fiskemel og fiskeolje. MARING Fagdag, May 31, 2005, Gardermoen, Norway.
- VIII** Oterhals, Å., Berntssen, M.G.H., Solvang, M., Nortvedt, R. Decontamination of fish oil by use of activated carbon adsorption. 23rd Nordic Lipid Symposium, June 1-4, 2005, Reykjavik, Iceland.
- IX** Oterhals, Å., Berntssen, M.H.G., Nygård, E., Nortvedt, R. Reduction of persistent organic pollutants in fish oil by short-path distillation. 35th WEFTA meeting, September 19-22, 2005, Antwerp, Belgium.
- X** Oterhals, Å. Miljøgifter i fiskemel/olje - nye grenseverdier og alternativer innen renseteknologi. Biomarint Industriseminar, December 6-7, 2005, Bergen, Norway.
- XI** Oterhals, Å., Berntssen, M.H.G., Solvang, M., Nortvedt, R. Reduksjon av dioxin i fiskeolje. Programkonferansen Havbruk, March 29-31, 2006, Bergen, Norway.

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- XII** Oterhals, Å. Rensing av fiskefôr – teknologiske muligheter og utfordringer. Norway Fish & Aqua, April 20-22, 2006, Bergen, Norway.
- XIII** Oterhals, Å. Reduction of persistent organic pollutants (POPs) in fish oil and fish meal. EWOS workshop on Cleaning Oils and Meals, August 28, 2006, Byrkjedalstunet, Norway.
- XIV** Oterhals, Å. Reduction of persistent organic pollutants in fish oil. UIC Seminar on Short Path and Thin Film Distillation, March 22, 2007, Bergen, Norway.
- XV** Oterhals, Å. Decontamination of fishmeal and fish oil – alternative technologies and challenges. Aquafeed Horizons, May 9-10, 2007, Utrecht, The Netherlands.
- XVI** Oterhals, Å., Kvamme, B., Berntssen, M.H.G. Reduction of persistent organic pollutants in fish oil by short path distillation – process optimization and effect on oil quality. 7th Euro Fed Lipid Congress, October 18-21, 2009, Graz, Austria.
- XVII** Oterhals, Å. Fjerning av miljøgifter i fiskeolje ved bruk av molekylærdestillasjon og effekt på oljekvalitet. Biomarint Industriseminar, December 1-2, 2009, Bergen, Norway.

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Abbreviations

AC	Activated carbon
ACP	Artic Contamination Potential
AhR	aryl hydrocarbon receptor
AV	Anisidine value
Bl&D	Chloroform-methanol extraction (Bligh and Dyer, 1959)
Bw	Body weight
CCD	Central composite design
DDD	1,1-dichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane (<i>p,p</i> -DDD)
DDE	1,1-dichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane (<i>p,p</i> -DDE)
DDT	1,1,1-trichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane (<i>p,p</i> -DDT)
DL-PCB	Dioxin-like PCB
ECHA	European Chemicals Agency
FFA	Free fatty acid
FHF	Fishery and Aquaculture Industry Research Fund
GOED	Global Organization for EPA and DHA Omega-3
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
Hp-	Hepta-
Hx-	Hexa-
IFFO	International Fishmeal and Fish Oil Organization
K _{AW}	Air-water partitioning coefficient
K _{OA}	Octanol-air partitioning coefficient
K _{OW}	Octanol-water partitioning coefficient
LOD	Level of detection
LOQ	Level of quantification
MD	Molecular dynamics
MLR	Multiple linear regression
MPL	Maximum permitted level
NDL-PCB	Non-dioxin-like PCB
O-	Octa-

OCP	Organochlorine pesticide
OPLS	Optimized potentials for liquid simulation
PAH	Polycyclic aromatic hydrocarbons
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo- <i>p</i> -dioxin
PCDD/F	Polychlorinated dibenzo- <i>p</i> -dioxin and polychlorinated dibenzofuran
PCDF	Polychlorinated dibenzofuran
PCN	Polychlorinated naphthalene
Pe-	Penta-
PFOS	Perfluorooctane sulfonate
PLSR	Partial least squares regression
POP	Persistent organic pollutant
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
QSPR	Quantitative structure property relationship
RMSEP	Root mean square error of prediction
SCAN	Scientific Committee on Animal Nutrition
SCF	Scientific Committee on Food
SFE	Supercritical fluid extraction
Soxhlet	Extraction performed in apparatus with continuous reflux of solvent
SPD	Short-path distillation
T-	Tetra-
TEF	Toxic equivalent factor
TEQ	Toxic equivalent
TWI	Tolerable weekly intake
UNECE	United Nations Economic Commission for Europe
UNEP	United Nations Environment Programme
USEPA	United States Environmental Protection Agency
WF	Working fluid
WHO	World Health Organization

1. Introduction

The research, surveillance and legislation focus on the existence and control of toxic organic pollutants in the environment and food chain goes back several decades but has been given increased attention after 1999 due to several severe contamination episodes (SCAN, 2000; SCF, 2000; Huwe, 2002; Burkow and Weber, 2003). My first encounter with the industrial challenges related to high content of dioxins and polychlorinated biphenyls (PCBs) in fish oil was during a research project exploring the extraction, refining and stabilization of herring and capelin oil for use in food products. The project was run at the Norwegian Herring Oil and Meal Industry Research Institute (SSF) over several years from 1986 to 1996. Levels of concern were observed in oil produced from herring with lower levels in capelin oil (Oterhals, 1990; Opstvedt et al., 1996). Also large seasonal variations were recognized linked to the fat content of the raw material. Initial processing studies also revealed that the polychlorinated dibenzo-*p*-dioxin and dibenzofuran level (PCDD/F) were only to a minor extent (10%) removed by steam deodorization at 180 °C. However, significant reduction of non dioxin-like PCB (NDL-PCB) (78%), DDT (72%) and toxaphene (51%) levels were obtained (Opstvedt et al., 1996). The possible combined use of activated carbon (AC) adsorption and deodorization to reduce the level of PCDD/Fs was identified based on literature search. Although the need for more processing studies related to decontamination technology was acknowledged, such research activity was not initiated at that time. Meanwhile, high level of dioxins found in cod liver oil put pressure on producers to evaluate the inclusion of a decontamination step in the refining process, either by use of AC adsorption, deodorization or SPD (Brevik et al., 1990).

After contamination of animal feeds with PCBs and dioxins in Belgium spring 1999 (Ashraf, 1999; Bernard et al., 1999) the European Commission asked the Scientific Committee on Animal Nutrition (SCAN) and the Scientific Committee on Food (SCF) to assess the level in animal feed and food products, including population intake and health risks. The systematization of existing data during autumn 1999

revealed a lack of quantitative data and an urgent need for initiation of surveillance programs. The International Fishmeal and Fish Oil Organization (IFFO) published in September 1999 an overview of existing data retrieved from different members of the organization (Anon, 1999). The overview clearly showed that levels in South East Pacific were lower than those found in European waters and North West Atlantic. The report was followed up by IFFO through a patent and literature search to identify available decontamination technology that could be used by the fishmeal and oil industry to reduce the levels. Being a member of the IFFO Scientific Committee, I attended a meeting September 6-8, 1999 in Hong Kong where the existing knowledge was presented and the dioxin issue discussed. The patent and literature search confirmed the limited availability of scientific information addressing the new industrial challenges. Identified public information on dioxin removal was limited to the use of extreme deodorization conditions (i.e., 250 °C, 10 hours; Mounts et al., 1976), AC adsorption (Mounts et al., 1976), and extraction using a perfluorocarbon fluid (Carr, 1998). Chlorinated pesticides and PCBs could be removed by commercially used deodorization conditions (process details not stated) or SPD (260 °C, 0.67 mbar) (Joseph, 1989), PCBs by supercritical fluid extraction (Krukoniš, 1989) and polycyclic aromatic hydrocarbons (PAHs) by adsorption to coconut charcoal (Stalling et al., 1977). Among the listed alternatives, AC adsorption was identified as most cost effective and feasible technology. However, available information regarding type, process conditions and needed amount of AC to achieve target levels was limited. The efficiency related to PCB reduction was also questioned. The industry officials acknowledged the need for more information but could not agree to establish a joint research project addressing these issues. The industry also acknowledged the lack of any economical viable technology for decontamination of fishmeal (Joas et al., 2001).

December 2002 I was contacted by Director Øyvind Lie at NIFES regarding an inquiry from the Fishery and Aquaculture Industry Research Fund (FHF) on defining of a research project exploring alternative technologies for decontamination of fish oil. The submitted project proposal was funded and initiated spring 2003 in collaboration with NIFES. Later also fishmeal decontamination technology was

included. The FHF project “Reduction of dioxins in fish oil” and follow up Norwegian Research Council project “Decontamination of persistent organic pollutants in fishmeal and fish oil” gave me the financial support to develop the knowledge presented in this PhD thesis. It is my hope that the included papers and others in preparation can be used by authorities and the processing industry to better understand the possibilities and limitations of alternative fishmeal and fish oil decontamination technologies and be of help in future decision making.

1.1 Objectives and thesis outline

The main objective of the research activity was to optimize and quantify the efficiency of alternative process technologies on reduction of POPs in fishmeal and fish oil, and to assess any negative effects on product quality. Studied technologies for fishmeal include increased fat separation, organic solvent extraction and oil leaching, and for fish oil AC adsorption and SPD.

Sub-goals:

- To study the effect of increased fat separation and organic solvent extraction on removal of POPs in fishmeal.
- To study the feasible use of a oil leaching process for removal of POPs in fishmeal.
- To optimize and model an AC adsorption process for reduction of PCDD/Fs, PCBs and PBDEs in fish oil.
- To optimize and model a SPD process for reduction of PCDD/Fs, PCBs and PBDEs in fish oil.
- To assess any negative effects on fishmeal and fish oil composition and quality after decontamination.

POPs are a diverse group of chemical compounds and it has been outside the scope of my research to study all. The papers included in this thesis primarily focus on the reduction of PCDD/Fs, dioxin-like PCBs (DL-PCBs) and polybrominated diphenyl ethers (PBDEs). However, some of the main effects described in the papers can be

generalized based on similarities and differences in physical and chemical properties and therefore included in the discussion part, e.g. NDL-PCBs, organochlorine pesticides (OCPs) and PAHs. Other POP groups are also mentioned in the background information and general discussion if appropriate to give the reader a broader picture of the complexity of the respective areas. Throughout the thesis the term dioxins are used as a synonym for PCDD/Fs. All referred PCDD/F decontamination rates are based on WHO₁₉₉₈ TEF values if not otherwise stated.

2. Background

2.1 The Geneva and Stockholm Conventions

POPs comprise a complex group of aromatic, fluorinated, chlorinated and brominated compounds with different physical and chemical properties. The term were originally defined by the Stockholm Convention on POPs (UNEP, 2001) based on a set of criteria (given in gray box on next page) including persistence, bio-accumulation, potential for long-range environmental transport, and adverse effects to human health or to the environment. The Stockholm Convention was adopted in 2001 and entered into force in 2004. Hitherto, 151 countries have signed the treaty, but several of these, including USA and the Soviet Union, have still not ratified the text. The document defines a set of 12 POPs (“dirty dozen” or “legacy POPs”): The organochlorine pesticides aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, toxaphene; the industrial chemicals hexachlorbenzene (HCB) and PCBs; and the unwanted byproducts, PCDDs and PCDFs (Table 1).

Challenges related to long-range transportation of air pollution was already addressed on a political level in the 1970s related to the acidification of lakes in the Scandinavian by SO_x and NO_x released from Central Europe. The negotiation agreements were expressed in the 1979 Geneva Convention on Long-range Transboundary Air Pollution (http://www.unece.org/env/lrtap/lrtap_h1.htm). The Convention went into force in 1983 and has been extended by 8 specific protocols, including one addressing discharge, emission and losses of POPs, referred to as the 1998 Aarhus Protocol on POPs (UNECE, 1998), which went into force in 2003. In addition to the 12 POPs included in the Stockholm Convention, chlordane, hexachlorocyclohexane (HCH), hexabromobiphenyl, and PAHs are included (Table 1).

The Stockholm Convention and Aarhus Protocol on POPs are administrated through the United Nations Environmental Program (UNEP; <http://www.unep.org/>) and the United Nations Economic Council for Europe (UNECE; <http://www.unece.org/>), respectively. Both agreements include mechanisms for adding new substances or groups of substances if certain criteria are met. The Aarhus Protocol was amended in

Stockholm Convention criteria for POPs (annex D of UNEP, 2001)**Persistence:**

- (i) Evidence that the half-life of the chemical in water is greater than two months, or that its half-life in soil is greater than six months, or that its half-life in sediment is greater than six months; or
- (ii) Evidence that the chemical is otherwise sufficiently persistent to justify its consideration within the scope of this Convention;

Bio-accumulation:

- (i) Evidence that the bio-concentration factor or bio-accumulation factor in aquatic species for the chemical is greater than 5,000 or, in the absence of such data, that the log Kow is greater than 5;
- (ii) Evidence that a chemical presents other reasons for concern, such as high bio-accumulation in other species, high toxicity or ecotoxicity; or
- (iii) Monitoring data in biota indicating that the bio-accumulation potential of the chemical is sufficient to justify its consideration within the scope of this Convention;

Potential for long-range environmental transport:

- (i) Measured levels of the chemical in locations distant from the sources of its release that are of potential concern;
- (ii) Monitoring data showing that long-range environmental transport of the chemical, with the potential for transfer to a receiving environment, may have occurred via air, water or migratory species; or
- (iii) Environmental fate properties and/or model results that demonstrate that the chemical has a potential for long-range environmental transport through air, water or migratory species, with the potential for transfer to a receiving environment in locations distant from the sources of its release. For a chemical that migrates significantly through the air, its half-life in air should be greater than two days; and

Adverse effects:

- (i) Evidence of adverse effects to human health or to the environment that justifies consideration of the chemical within the scope of this Convention; or
- (ii) Toxicity or ecotoxicity data that indicate the potential for damage to human health or to the environment.

2009 to include seven new substances: hexachlorobutadiene, octabromodiphenyl ether, pentachlorobenzene, pentabromodiphenyl ether, perfluorooctane sulfonates (PFOS), polychlorinated naphthalenes (PCNs) and short-chain chlorinated paraffins. However, these amendments have not yet entered into force for the parties that adopted them. Accordingly, the Stockholm Convention was amended in 2009 to include the following additional chemicals (UNEP, 2009): α -HCH, β -HCH, chlordecone, hexabromobiphenyl, hexa- and heptabromodiphenyl ether, lindane, pentachlorobenzene, perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride, and tetra- and pentabromodiphenyl ether. These amendments went into force on August 26, 2010. The ongoing evaluation of compounds for POP-like behavior can be expected to reveal new candidates to be added to future revised POP lists (Burkow and Weber, 2002; Lohmann et al., 2007). In addition to UNEP and UNECE such evaluation activity is carried out by the United States Environmental Protection Agency (USEPA), Environment Canada and the European Chemicals Agency (ECHA)



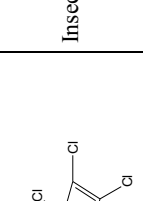
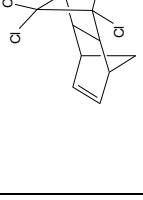

2.2 Production, emission and environmental fate of POPs

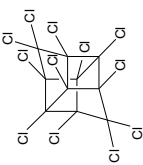
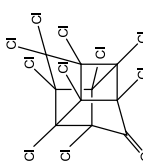
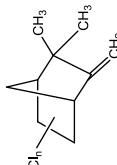
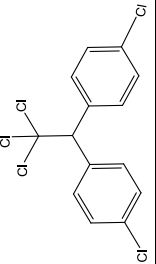
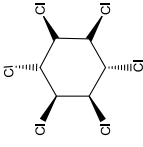
POPs can be grouped into industrial products, by-products and pesticides. The first group includes chemicals used in a variety of industrial and commercial applications including flame retardants, dielectric fluids, heat exchange fluids, paint additives, lubricants, textile impregnation, cosmetics etc. By-products are chemicals non-intentionally produced during the manufacturing of other industrial products, during incineration of wastes or from combustion processes (Kulkarni et al., 2008). Pesticides are substances intended for preventing, destroying, repelling, or mitigating any pest. Depending on use they can be divided into herbicides, fungicides, bactericides, insecticide, rodenticides (<http://www.epa.gov/pesticides/about/-index.htm>). Finally, incidents of large accidental release of dioxins have taken place, e.g. the explosion of a reactor in an herbicide production plant in Seveso, Italy (Pesatori et al., 2009) and the application of the dioxin contaminated defoliating

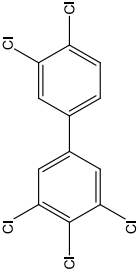
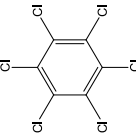
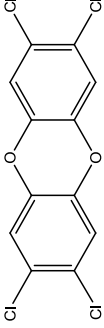
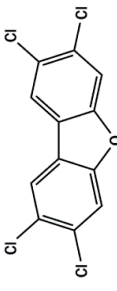
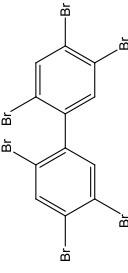
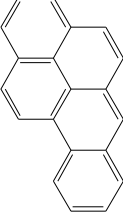
“Agent Orange” during the Vietnam war (Stellman et al., 2003). Forest fires and volcanoes can also be included in this category.

The main objective of the Stockholm convention is to establish international binding measures to eliminate or reduce the release of POPs into the environment. Compounds belonging to the 12 “legacy POPs” (Table 1) are either no longer produced or annual emission significantly reduced as a result of stringent emission standards and increased use of remediation technology (Kulkarni et al., 2008). Exact emission data is, however, in many cases difficult to obtain and the overview given in Table 1 is based on best available estimates. Once emitted to the environment, the further long range transportation and fate of the individual POPs depend on physical and chemical properties (Wania 2003, 2006; Lohmann et al., 2007), degradation half-life times (Sinkkonen and Paasivirta, 2000, Wania, 2006) and mass fluxes in the chemosphere, hydrosphere, geosphere and biosphere (Scheringer, 2009; Lohmann et al., 2007). Especially the long range transportation of POPs to the Arctic region has been given much attention (Burkow and Weber, 2002; Wania, 2003). Different transportation modes of POPs based on volatility and partitioning between air and water (K_{AW}) and octanol and air (K_{OA}) has been proposed to model their Arctic Contamination Potential (ACP) (Wania, 2003). Volatile chemicals (“flyers”, e.g., polyfluoro alcohols, cyclic siloxanes) are transported quickly and in high amounts via the air. Their high volatility also causes a very limited deposition in Arctic latitudes because of reduced temperature (“cold trap effect”). Chemical with a low Henry’s law constant or K_{AW} and persistency high enough to be transported by ocean currents are called “swimmers” (e.g., HCHs and perfluorinated compounds). Multiple hoppers are chemicals that are transported over a certain distance, deposited to the ground and re-volatilized during high summer temperatures so that another “hop” or long range transportation can take place. The classical POPs with known occurrence in the Arctic belong to this group, e.g., HCB, PCBs, chlordane and DDT. “Single hop” chemicals (e.g., PBDEs and PCBs) have such a high K_{OA} that they bind irreversible on particles which they are deposited and almost no re-volatilization take place.

Table 1 POPs controlled under the Stockholm Convention (UNEP, 2001) and the Aarhus Protocol on POPs (UNECE, 1998), including available estimates of global historical production or usage of some intentionally produced POPs or annual emissions (specific isomer given under chemical structure is stated in brackets under compound).

Compound	Chemical structure	Key source	Production period or reference years(s) for emission estimates	Cumulative global production/usage or annual emissions	Reference
Aldrin		Insecticide		500 kt	Burkow and Weber, 2003
Chlordane		Insecticide	1940s-	78 kt	Burkow and Weber, 2003
Dieldrin		Insecticide		34 kt	Burkow and Weber, 2003
Endrin		Insecticide	~1962	2-4 kt/year (USA)	van Esch and van Heemstra-Lequin, 1992
Heptachlor		Insecticide	1950s-		

Mirex		Insecticide, flame retardant					
Chlordecone ^a		Insecticide					
Toxaphene		Insecticide	1950-1993	1330 kt	Lohmann et al., 2007		
DDT (1,1,1-trichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane; <i>p,p'</i> -DDT)		Insecticide	1940s-present	4500 kt	Lohmann et al., 2007		
HCHs ^a		Insecticide	1948-1997	10 000 kt (technical grade)	Lohmann et al., 2007		
Lindane (γ -HCH)		Insecticide	1950-1993	720 kt	Lohmann et al., 2007		

PCB (3,3',4,4',5-PeCB; PCB 126)		Industrial chemical	1930-1993	1326 kt	Lohmann et al., 2007
HCB ^a		Fungicide, by-product	~1995	~23 t/year	Lohmann et al., 2007
PCDD (2,3,7,8-TCDD)		By-product	~1995	~9.9 kg TEQ/year (including PCDF)	Lohmann et al., 2007
PCDF (2,3,7,8-TCDF)		By-product			
Hexabromobiphenyl (2,2',4,4',5,5'-HxBB; PBB 153)		Flame retardant			
PAHs ^a (benzo[a]pyrene)		By-product	1966-1969	5 kt/year benzo[a]pyrene	Lohmann et al., 2007

^a Originally only regulated under the Aarhus Protocol (UNECE, 1998) but, except PAHs, included in the amended Stockholm Convention (UNEP, 2009).

Degradation of POPs in the environment is controlled by hydrolysis, biodegradation and photolysis (Sinkkonen and Paasivirta, 2000). The first mechanism is very slow at environmental conditions. Photodegradation in air is dominated by OH radical reactions and is proposed as the major degradation pathway (Sinkkonen and Paasivirta, 2000; Lohmann et al., 2007). In soil this mechanism is restricted to the top 1 mm layer and in water down to 2 m depth by the penetration ability of UV light. Atmospheric half-lives for POPs are in the range of years (Sinkkonen and Paasivirta, 2000). Biodegradation is the most important mechanism in soil, water and sediment compartments. However, reported values are very variable and range from days to years (Aronson et al., 2006).

Organic carbon plays a key role in the binding, transportation and cycling of POPs in water columns and soils (Cornelissen et al., 2005; Lohmann et al., 2007), and air (Götz et al., 2007). The interaction is linked to the content and adsorption to condensed, rigid, and aromatic structures typically found in unburned coal, kerogen, coke, cenosphere, and soot and charcoal (the remnants of incomplete burning, commonly termed “black carbon”). Two modes of POP fixation to black carbon have been proposed: Physical occlusion during black carbon formation and reversible adsorption on exterior and pore structures surfaces after black carbon formation (Koelmans et al., 2006). Particles containing black carbon are reported to be able to bind PCDD/Fs, PCBs, PBDEs, PAHs, chlorobenzenes and pesticides (Cornelissen et al., 2005). Adsorption to black carbon will also reduce the freely dissolved water concentration of these compounds and thereby also the biodegradation rate and uptake of POPs by living organisms. On a global scale the binding to black carbon has been proposed as key vectors for transport and partitioning of POPs in soil and marine environments (Cousins et al., 1999; Lohmann et al., 2007). Settling of black carbon containing particles in the ocean and deep lakes will contribute to remove the POPs from the environment but it is still unclear whether such deposition in sediments represents a permanent sink or not (Lohmann et al., 2007).

Once taken up in the biota POPs will accumulate in the food chain, representing a possible risk to human health. The congener specific bioaccumulation pattern differs between organisms. Only the 2,3,7,8-substituted PCDD/F congeners are retained in

the body of most species including fish, birds, monkey and humans. In guinea pig, crustaceans and mussels also otherwise substituted congeners are found (Oehme et al., 1989; Van den Berg et al., 1994).

3. Agro-industrial implications

3.1 Level of POPs in fish from North European oceans

One of the main objectives of the SCAN report (SCAN 2000) was to evaluate the level and contribution of different raw materials used in the agro industry as a source for the carry-over and bio-accumulation of PCDD/Fs and DL-PCBs in the food chain. POPs can in variable amounts be found in most feedingstuffs used in feed for domestic animals, poultry and fish. However, the SCAN report identified fishmeal and fish oil as the most heavily contaminated feed materials with products of European fish stocks more heavily contaminated than those from South Pacific stocks. Animal fat was next in order of dioxins concentration. All other feed materials of plant (roughages, cereals, legume seeds) and animal (milk by-products, meat and bone meal) origin were evaluated to contain low levels. To reduce the impact of the most contaminated feed materials, e.g. fishmeal and fish oil from Europe, on overall diet contamination they recommended substituting such materials by lesser contaminated sources, use of decontamination techniques to reduce their intrinsic level or replacement by none (less) contaminated alternatives. In case of fishmeal and fish oil this means increased use of products of South-American origin or plant and single cell alternatives.

High levels of PCDD/Fs and PCBs have been found in several fish species and consequently in fishmeal and fish oil produced from industrial fish and by-products (Anon, 1999; SCAN, 2000; Joas et al., 2001). The fish stocks of concern for the Northern European industry is sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) in the Baltic Sea and herring, sprat, sand eel (*Ammodytes tobianus* and *A. marinus*) and blue whiting (*Micromesistius poutassou*) in the North Sea (Joas et al., 2001; Mundell et al., 2003). Lower levels have been observed in the Norwegian Sea and Barents Sea (Mundell et al., 2003; Julshavn et al., 2004). Especially high levels have been found in herring caught in the Baltic Sea with a continuous increase of the dioxin concentration from west to east (Karl and Ruoff, 2007). In Norway the ongoing surveillance program on POPs in seafood is administrated by NIFES and the

main results available on a searchable seafood database (http://www.nifes.no/index.-php?page_id=137&lang_id=2).

3.2 Factors influencing the level of POPs in fishmeal and fish oil

The observed contamination levels in different fish and seafood products are mainly reflecting the general pollution level in the respective fishing areas (Anon, 1999; Julshavn et al., 2004; Karl and Ruoff, 2007). The levels are normally reported on a wet weight basis. This is most relevant related to seafood products as it enables the assessment of population dioxin intake based on consumption, but less informative for assessment of the expected level in fish oil and fishmeal. POPs are lipophilic compounds and will accumulate in the lipid phases (i.e., cell membranes, liver and adipose tissue) of the fish. Large seasonal variation in the total fat content, linked to feed intake and spawning, can be observed for many fish species (Mundell et al., 2003; Oterhals, 1995). Typically, the lowest fat level is observed early spring after spawning and the highest in fish caught late summer. This seasonal variation will give the highest POP levels on fat basis early spring as demonstrated for capelin during the winter 2000 season in Figure 1. In addition, age give rise to increased levels of POPs due to bioaccumulation (Mundell et al., 2003; Parmanne et al., 2006). A higher raw material fat content will dilute the POP concentrations in the lipid phase and give rise to reduced levels in fishmeal and fish oil. Fishmeal contains approx. 10% fat based on Soxhlet extraction. Consequently, a raw material containing <10% fat on wet weight basis will after fat separation and dewatering result in a fishmeal with a comparable higher dioxin level. Opposite, a raw material with >10% fat gives rise to a fishmeal with a lower dioxin level. In both cases the fish oil dioxin level will be higher and inversely proportional to the raw material fat content. A consequence of these relationships is the existence of low fat fish acceptable for food applications but above the maximum permitted levels (MPLs) if processed to fishmeal and fish oil (Paper I).

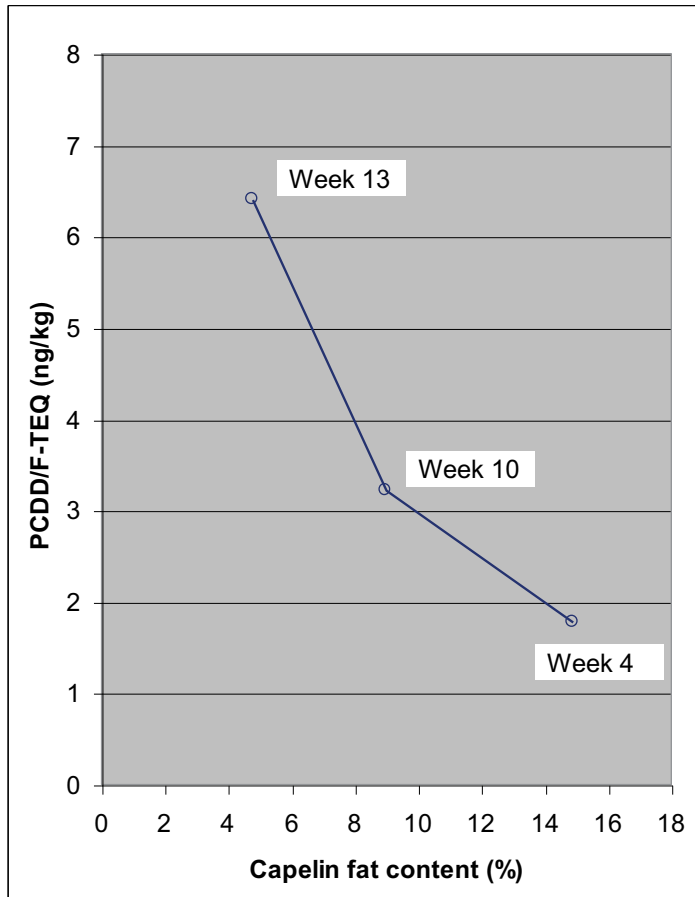


Figure 1. Variation in capelin oil PCDD/F-TEQ level during winter season 2000 depending on the raw material fat content (Oterhals, unpublished results). Fat level (i.e., ethyl acetate extractable lipids) based on the week average of capelin catches delivered to the Norwegian fishmeal and oil industry in the studied period.

Although only a minor part of the produced fish oil and fishmeal has a content of undesirable organic pollutants above the MPLs, the need for decontamination of the products to comply with the legislations will disfavor producers based on such raw material on a world basis. Approximately 20% of the industrial fish caught in EU waters might give fishmeal and fish oil dioxin levels above the MPLs and can not be used for feed without decontamination (Joas et al., 2001). The referred EU report was based on several assumptions including "comparable contamination level" if lack of

species specific data. However, a simple and effective action reducing the fraction of concern would be a change in fishing practice aiming at harvest of the conflict fish resources in periods of high fat content.

3.3 Toxicity and toxic equivalent factor

PCDD/Fs and DL-PCBs are highly toxic compounds and have been shown to be a risk factor in humans for a large range of clinical disorders, including cancer, immune deficiency, reproductive and developmental abnormalities, central and peripheral nervous system pathology etc. (Schechter et al., 2006) (<http://www.who.int/media-centre/factsheets/fs225/en/>). The toxic effect is mediated through high-affinity binding to the aryl hydrocarbon receptor (AhR), an intracellular ligand-activated transcription factor involved in regulation of the expression of a large number of genes (Schechter et al., 2006; Van den Berg et al., 2006).

The toxicity and persistency in humans of PCDD/Fs and DL-PCBs are linked to a lateral chlorine substitution in the 2, 3, 7 and 8 positions (Schechter et al., 2006). Although 75 different PCDD and 135 different PCDF congeners are possible, only 17 of these are considered toxic. Of the 209 possible PCBs only 12 have any dioxin-like toxicity. The structure and numbering of PCDD, PCDF and DL-PCB congeners are given in Figure 2.

The difference in toxicity of the 29 possible 2, 3, 7, 8-chlorine substituted congeners spans five orders of magnitude. The toxic equivalency (TEQ) concept was developed to establish a common basis for quantification of the toxicity of all possible mixtures of PCDD/Fs and DL-PCBs. The concept is based on the assignment of a toxic equivalency factor (TEF) to each of the 29 congeners relative to the most toxic congener TCDD (Table 2). The TEF value is multiplied with the chemical concentration of the individual congeners to give a TEQ value or a weighted toxicity relative to pure TCDD. All TEQ contributions in a given sample are summed to give a total TEQ value expressing the toxicity as if the sample were pure TCDD:

$$\text{TEQ} = \sum_{i=1}^n (C_i \times \text{TEF}_i) \quad (1)$$

Where C_i expresses the concentration of a congener $i = 1, \dots, n$, and its associated TEF_{*i*} value. A prerequisite for the use of this concept is that the combined effect of the different congeners are dose or concentration additive (Van den Berg et al., 1998).

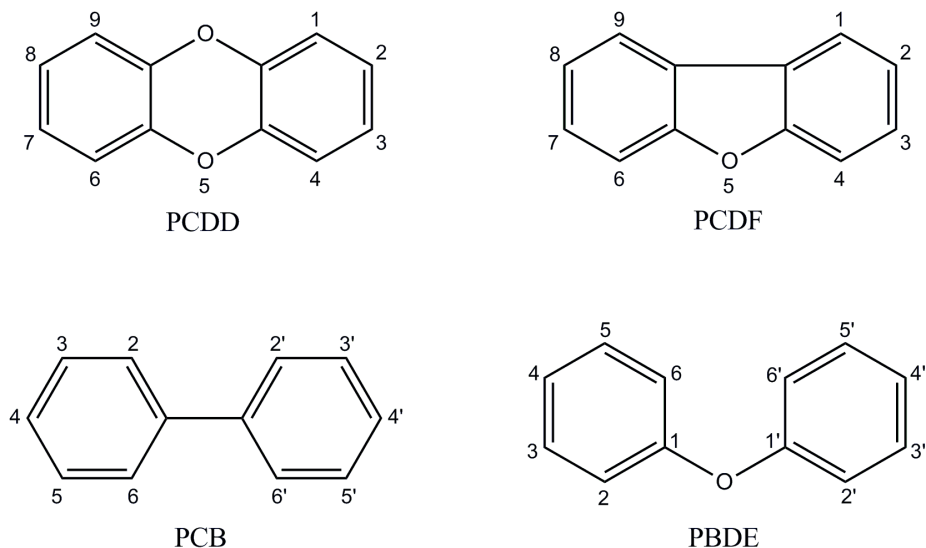


Figure 2. Numbering of the ring structure of PCDDs, PCDFs, PCBs and PBDEs.

The TEQ concept has been evaluated several times (Huwe, 2002). After a World Health Organization (WHO) expert meeting in Stockholm June 1997, the prior used TEF values were harmonized in a set of WHO₁₉₉₈ TEF values (TEF_{WHO98}; Table 2) (Van den Berg et al., 1998). The WHO TEF values were reevaluated during a WHO International Program on Chemical Safety expert meeting in Geneva June 2005 (Van den Berg et al., 2006). The new WHO₂₀₀₅ TEF (TEF_{WHO05}) values are given in Table 2.

The Geneva expert panel concluded that PBDEs did not have AhR agonist properties and should not be included in the TEF concept (Van den Berg et al., 2006). However, commercial mixtures of PBDEs can contain polybrominated dibenzo-*p*-dioxin (PBDDs) and polybrominated dibenzofurans (PBDFs), both shown to possess dioxin-

Table 2. Change in WHO toxicity equivalency factors (TEF_{WHO98} and TEF_{WHO05}) between assessments in 1998 and in 2005 with changes in bold.

Compound	TEF _{WHO98}	TEF _{WHO05}	Compound	TEF _{WHO98}	TEF _{WHO05}
Chlorinated dibenzo-<i>p</i>-dioxins			Non-<i>ortho</i> substituted PCBs		
2,3,7,8-TCDD	1	1	3,3',4,4'-TCB (#77)	0.0001	0.0001
1,2,3,7,8-PeCDD	1	1	3,4,4',5-TCB (#81)	0.0001	0.0003
1,2,3,4,7,8-HxCDD	0.1	0.1	3,3',4,4',5-PeCB (#126)	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1	3,3',4,4',5,5'-HxCB (#169)	0.01	0.03
1,2,3,7,8,9-HxCDD	0.1	0.1			
1,2,3,4,6,7,8-HpCDD	0.01	0.01			
OCDD	0.0001	0.0003			
Chlorinated dibenzofurans			Mono-<i>ortho</i> substituted PCBs		
2,3,7,8-TCDF	0.1	0.1	2,3,3',4,4'-PeCB (#105)	0.0001	0.00003
1,2,3,7,8-PeCDF	0.05	0.03	2,3,4,4',5-PeCB (#114)	0.0005	0.00003
2,3,4,7,8-PeCDF	0.5	0.3	2,3',4,4',5-PeCB (#118)	0.0001	0.00003
1,2,3,4,7,8-HxCDF	0.1	0.1	2',3,4,4',5-PeCB (#123)	0.0001	0.00003
1,2,3,6,7,8-HxCDF	0.1	0.1	2,3,3',4,4',5-HeCB (#156)	0.0005	0.00003
1,2,3,7,8,9-HxCDF	0.1	0.1	2,3,3',4,4',5'-HeCB (#157)	0.0005	0.00003
2,3,4,6,7,8-HxCDF	0.1	0.1	2,3',4,4',5,5'-HeCB (#167)	0.00001	0.00003
1,2,3,4,6,7,8-HpCDF	0.01	0.01	2,3,3',4,4',5,5'-HpCB (#189)	0.0001	0.00003
1,2,3,4,7,8,9-HpCDF	0.01	0.01			
OCDF	0.0001	0.0003			

like properties. Several other compounds were discussed for possible inclusion in the TEF scheme: PCB 37, mixed halogenated dibenzo-*p*-dioxin (PXCCDDs) and dibenzofurans (PXCDFs), HCB, PCNs, polybrominated naphthalenes (PBNs) and polybrominated biphenyls (PBBs) (Van den Berg et al., 2006).

With few exception of minor importance the WHO₁₉₉₈ TEF values have been reduced after the 2005 revision (Table 2). The effect on the level of TEQ in different feed and food groups is an overall reduction of 14% (EFSA, 2010). However, the degree of reduction varies greatly depending of the type of product due to major differences in congener composition. The reduction is mainly due to changes in TEFs for mono-*ortho* PCB and furan congeners with minor changes for dioxin and non-*ortho* PCB congeners (Table 2). Comparing the TEQ levels using TEF_{WHO98} and TEF_{WHO05} in marine oils for food and fish oil for feed applications, the mean level was reduced by 16% and 13%, respectively (EFSA, 2010). In feed for fur animals, pets and fish the reduction was 14%. The levels are in agreement with van den Berg et al. (2006) and calculations based on the fish oil used in Paper II, IV and V. Care should, however, be given in generalization of these values as the samples in most cases reflect targeted monitoring and not random selection (EFSA, 2010).

3.4 Population exposure and maximum permitted TEQ levels in food and feed

The Scientific Committee on Food (SCF) established in its risk assessment report November 2000 (SCF, 2000) a temporary tolerable weekly intake (t-TWI) of 7 WHO-PCDD/F-PCB-TEQ/kg body weight (bw). Based on new scientific information on the toxicity of dioxins and uncertainty factors this level was increased to 14 WHO-PCDD/F-PCB-TEQ/kg bw in an update report May 2001 (SCF, 2001). The Commission acknowledged at the same time that a considerable proportion of the European population has a dietary intake in excess of the tolerable intake (Commission Recommendation 2002/201/EC). Introduction of the new WHO₂₀₀₅ TEF values will also contribute to reduce the population fraction exceeding the TWI (VKM, 2007).

The average dietary intake of PCDD/Fs and DL-PCBs in the EU is in the range of 1.2-3 pg WHO-TEQ/kg bw and day (SCF, 2001). However, compared to a TWI of 7-14 pg/kg bw the level indicates that a considerable part of the European population exceeds recommended exposure levels. A positive trend has been observed the last decades and the dietary intake of dioxins in some European countries has decreased since the end of the 1980s by approximately 50% (SCF, 2000). More than 90% of the exposure derives from food products, with products of animal origin and fish giving the greatest contributions (SCF, 2000; Liem et al., 2000). Food consumption habits vary between countries and large differences in the relative contributions of different food groups to the total TEQ exposure can be expected. In a compilation of data from ten European countries (Belgium, Denmark, Finland, France, Germany, Italy, Netherlands, Norway, Sweden and United Kingdom) the following contribution ranges for different food groups were observed: milk and dairy products (16-39%), meat and meat products (6-32%), fish and fish products (2-63%) and other products, mainly of plant origin such as vegetables, cereals (6-45%) (SCOOP, 2000). The EU strategy is to reduce the average population intake to below 2 pg WHO-TEQ/kg bw and day (SCF, 2001).

To reduce the population exposure the European Union has developed an integrated approach to reduce the levels throughout the food chain, that is, from feed materials through food-producing animals to humans. An overview of the MPLs in selected products intended for animal feed is given in Table 3. All values are given on a 12% moisture basis to standardize the legislation levels across different types of products. Commission directive 2006/13/EC also gives a set of somewhat lower action levels, separate for PCDD/Fs and DL-PCBs since the sources are different. If exceeded the source of contamination shall be identified and appropriate measures taken to reduce or eliminate it. The EU legislations are based on the concept of “upper-bound” concentrations, i.e. if the concentration of a congener is below the limit of quantification (LOQ) the concentration is assumed to be equal to the LOQ and the TEQ contribution based on that value. Opposite, the concept of “lower-bound” requires using zero for the contribution of each non-quantified congener. A comparison of upper- and lower-bound results have shown that the former on average is 4% and 13% higher in food and feed samples, respectively, with large variations between groups (EFSA, 2010).

MPLs of PCDD/Fs and DL-PCBs in foodstuffs are given in Commission Regulation (EC) No 1881/2006 (Table 4). The TEQ levels are either given in pg/g fat or on a wet weight basis. A preferred fat extraction protocol is not given but has to be stated with the analytical results. In Norway ethyl acetate extraction is used (Amund Måge, NIFES, Bergen, Norway; personal comm.). A comparison of four commercially used extraction techniques (i.e., ethyl acetate, Bl&D, EC-method and Soxhlet) are given in Paper I. The tested fat extraction methods gave a systematic ranking of Bl&D > EC > Soxhlet. Ethyl acetate extraction showed a less systematic picture but was always below Bl&D extraction. The choice of fat extraction technique will especially have implications for low fat products where the MPLs are given on fat basis (Table 4). As for products intended for animal feed a set of action levels are also developed for foodstuffs (Commission Recommendation 2006/88/EC).

Table 3. Present and proposed maximum permitted levels of PCDD/Fs and DL-PCBs, and new proposed maximum permitted level of NDL-PCBs in selected feed material of plant and animal origin intended for feed, premixtures and compound feed (upper bound concentrations relative to a feedstuff with 12% moisture content).

	WHO-PCDD/F-TEQ (ng/kg)		WHO-PCDD/F-PCB-TEQ (ng/kg)		NDL-PCB ^d (µg/kg)
	Present ^a	Proposal ^b	Present ^a	Proposal ^b	
Feed material of plant origin with the exception of: - vegetable oils and their by-products	0.75 0.75	0.75 0.5	1.25 1.5	1.25 1.5	10 ---
Feed materials of animal origin: - Animal fat, including milk fat and egg fat - Other land animal products including milk and milk products and eggs and egg products - Fish oil - Fish, other aquatic animals, their products and by-products with exception of fish oil and fish protein hydrolysates containing more than 20% fat - Fish protein hydrolysates containing more than 20% fat	2.0 0.75 6.0 1.25 2.25	1.5 0.75 5.0 1.25 1.75	3.0 1.25 24.0 4.5 11.0	2.0 1.25 20.0 4.0 9.0	10 10 175 30 50
Binders, anti-caking agents, trace elements Trace elements and premixtures	0.75 1.0	0.75 0.75	1.5 1.5	1.5 1.5	10 10
Compound feed with the exception of: - compound feed for pet animals and fish	0.75 2.25	0.75 1.75	1.5 7.0	1.5 5.5	10 40

^a Commission directive 2006/13/EC (based on WHO₉₈-TEF)

^b SANCO/13513/2010 (based on WHO₀₅-TEF)

^c SANCO/13511/2010

^d NDL-PCBs (sum of PCB 28, 52, 101, 138, 153, 180; ICES 6)

Table 4. Present and proposed maximum permitted levels of PCDD/Fs and DL-PCBs, and new proposed maximum permitted level of NDL-PCBs in foodstuffs.

	Basis	WHO-PCDD/F-TEQ (ng/kg)		WHO-PCDD/F-PCB-TEQ (ng/kg)		NDL-PCB ^c (µg/kg)
		Present ^a	Proposal ^b	Present ^a	Proposal ^b	
Meat and meat products (excluding edible offal) of the following animals:						
- bovine animal and sheep	fat ^d	3.0	2.5	4.5	4.0	40
- poultry	fat ^d	2.0	1.75	4.0	3.0	40
- pigs	fat ^d	1.0	1.0	1.5	1.25	40
Liver of terrestrial animals, except sheep, and products thereof	fat ^d	6.0	4.5	12.0	10.0	40
Muscle meat of fish and fishery products and products thereof with the exception of wild caught eel, freshwater fish and Baltic region	w.w.	4.0	3.5	8.0	6.5	75
Muscle meat of wild caught fresh water fish, with the exception of diadromous fish species caught in fresh water, and products thereof	w.w.	---	3.5	---	6.5	125
Muscle meat of eel (<i>Anguilla anguilla</i>) and products thereof	w.w.	4.0	3.5	12.0	10.0	300
Fish liver	w.w.	---	---	25.0	20.0	200
Raw milk and dairy products, including butterfat	fat ^d	3.0	2.5	6.0	5.5	40
Hen eggs and egg products	fat ^d	3.0	2.5	6.0	5.5	40
Fats from bovine animals and sheep	fat	3.0	2.5	4.5	4.0	40
Fats from poultry	fat	2.0	1.75	4.0	3.0	40
Fats from pigs	fat	1.0	1.0	1.5	1.25	40
Mixed animal fats	fat	2.0	1.50	3.0	2.5	40
Vegetable oil and fats	fat	0.75	0.75	1.5	1.25	40
Marine oils	fat	2.0	1.75	10.0	6.0	200
Foods for infants and young children	w.w.	---	0.1	---	0.2	1.0

w.w. – wet weight

^a Commission Regulation (EC) No 1881/2006 (based on WHO₉₈-TEF)

^b SANCO/13329/2010 and SANCO/13331/2001 (based on WHO₀₅-TEF)

^c NDL-PCBs (sum of PCB 28, 52, 101, 138, 153, 180; ICES 6)

^d The maximum level expressed on fat is not applicable for foods containing <2% fat. For foods containing <2% fat, the maximum level applicable is the level on product basis corresponding to the level on product basis for the food containing 2% fat.

Members of the Global Organization of EPA and DHA Omega-3 (GOED; <http://www.goedomega3.com/>) have developed a voluntary monograph quality standard for EPA and DHA oils (GOED, 2006). The standards sets significant lower specifications for the PCDD/F- and DL-PCB-TEQ level in fish oils for food applications compared to present EU legislations (Table 4), i.e., maximum 2 pg WHO-PCDD/F-TEQ/g and 3 pg WHO-PCB-TEQ/g. In addition, a maximum level of 0.09 mg/kg is given for the sum of the seven indicator PCBs with IUPAC number 28, 52, 101, 118, 138, 153 and 180. The level includes PCB 118 which is a DL-PCB and not included in the proposed EU ND-PCB legislations (Table 3 and 4). The sum WHO-PCDD/F-PCB-TEQ level of GOED (5 pg/g) is in accordance with present Norwegian national law for the maximum level in marine oils (FOR 2002-09-27 nr 1028).

New EU regulations are proposed amending the current PCDD/F and PCB WHO-TEQ levels in feed and food (Table 3 and 4). The reduced levels in the proposed regulations are a combined numeric effect of the introduction of the lower WHO₂₀₀₅ TEF values (Van den Berg et al., 2006) and a real reduction of the maximum permitted level in the respective products. Especially for marine feed and food products the TEQ-levels have been considerable reduced to be more in accordance with the plant and animal products.

3.5 Impacts on Atlantic salmon feed formulation

Retention of DL-PCBs in Atlantic salmon feeding trials has been found to be higher than PCDD/Fs, 84% and 49%, respectively (Isosaari et al., 2004; Lundebye et al., 2004). Even higher retention values (95%) have been observed for PBDEs (Isosaari et al., 2005). The Science article of Hites et al. (2004a) on the content of dioxins and PCBs in farmed vs. wild salmon and follow up article on PBDEs (Hites et al., 2004b) resulted in an increased focus on the POPs level in fish. Also the health benefit of eating farmed salmon was questioned (Hites et al., 2004a; Hamilton et al., 2005). However, others have concluded that the benefits of fish intake exceed the potential risks (Mozaffarian and Rimm, 2006).

Three strategies can be used by the feed manufacturing industry to comply with the present MPLs in fish feed (Berntssen et al., 2005 and 2006): (i) Use of marine ingredients with intrinsic low levels of POPs. (ii) Use of decontaminated fish oil and fishmeal. (iii) Use on non-marine feed ingredients. The first option implies preferred use of fishmeal and fish oil from the southern hemisphere. Exchange of fishmeal and fish oil in the diet with plant protein and lipid sources have been shown to significantly lower the final flesh level (Berntssen et al., 2005; Berntssen et al., 2010a). This research area has also been given increased focus due to the continuous optimization of commercial feeds based on least cost formulation. However, challenges related to the content of anti-nutritional factors (Francis et al., 2001) and the unavoidable influence on the fatty acid composition of the final edible product have so far partly limited the industrial utilization of such feed ingredients. The salmon farming industry should aim at production of an edible product with n-3 PUFAs comparable to wild salmon to ensure that similar human health benefits will accrue from the consumption of farmed fish. If fish oil in the feed pellet is replaced with vegetable oil a significant reduction in EPA and DHA content of edible parts is observed (Torstensen et al., 2004).

Global production of fishmeal and fish oil is dependent on the sustainability of captured fisheries (FAO, 2009). Annual fish oil production has stabilized between 1 and 1.2 million tones, and global decrease of the available fish stocks require increased focus on improved utilization of by-products from the fishery and aquaculture industry (Blanco et al., 2007), and other major marine resources like Antarctic krill. The situation emphasizes the importance of utilization of all available marine resources including fishmeal and oil parcels above MPLs through use of decontamination processes. For decontaminated fish oil parcels the feed manufacturing and fish farming industry have requested documentation on possible removal of nutrients of importance for growth rate and feed utilization. Quantification of such process effects after SPD is reported in paper V. No negative effects have been documented in an 11 weeks Atlantic salmon feeding trial based on a diet containing commercial available fish oil decontaminated with the combined use of AC adsorption and thin film deodorization (Sprague et al., 2010). Berntssen et al.

(2010b) have reported an 18 month feeding trial based on fish oil from the same producer (Fiskernes Fiskeindustri, Skagen, Denmark) and found a reduction (3%) of very long chain n-3 fatty acids after the decontamination process. This was also expressed in a minor reduction (4-7%) of EPA + DHA in the salmon flesh compared to the control diet. Such effects were not observed in a 10 weeks feeding trial reported by Pratoomyot et al. (2008) based on oil from the same producer. In this study, however, a significant lower digestibility of crude protein, fat, dry matter and most fatty acids was observed for feed containing docontaminated oil. In all the above studies a highly significant reduction of PCDD/Fs and DL-PCBs was documented in the decontaminated fish oil and consequently Atlantic salmon flesh. Equivalent main conclusions have been obtained in a 16 months Atlantic salmon feeding trial based on fish oil decontaminated by SPD (Olli et al., 2010). In this study also indications of positive effects of the decontamination process on growth, feed utilization efficiency and product quality criteria was observed.

In the above referred feeding trials only the type of decontamination technology is stated without any detailed information regarding applied process conditions given under materials and methods. The reported differences in biological response based on combined AC and deodorization can therefore not be evaluated based on possible differences in the applied process conditions. Regretfully, this also makes it difficult to generalize the main process technology conclusions or relate the observations to the process optimization studies presented in paper II, IV and V. The limited information also makes it difficult to develop guidelines for good manufacturing decontamination practices within the industry based on the given studies.

3.6 Impacts on refining of fish oil for human consumption

Fish oil for human consumption in the form of cod liver oil, fish oil capsules or as fortification in formulated food products are normally refined, bleached and deodorized to remove free fatty acids, pigments, trace metals and oxidation products. Steam deodorization will to some extent remove POPs (Hilbert et al., 1998; Carbonnelle et al., 2006) and additional steps comprising use of AC in the bleaching

step (Paper II) and SPD (Paper IV) can be included to increase the efficiency. Decontamination practices were introduced by the cod liver oil refining industry in Norway end 1980ies after increased focus on high levels of dioxins in the crude oil (Breivik et al., 1990). Although details regarding applied technology are not revealed by the fish oil refining industry, the present industry practice is probably based on either the combined used of AC adsorption and deodorization (Carbonnelle et al., 2006) or SPD (Breivik and Thorstad, 2005). In Norwegian monitoring programs the mean level of WHO-PCDD/F-PCB-TEQ in cod liver oil for human consumption has been found to be 2.0 pg/g compared to 32 pg/g in cod liver (Alexander et al., 2006). Crude oil extracted from such cod liver will have a TEQ level around twice the level observed on wet weight basis and underlines the need for use of refining technology including an efficient decontamination step to comply with legislation levels (Table 4). However, recent published results from a Spanish monitoring of the POPs level in fish oil supplements has revealed products on the market with TEQ levels above present EU legislations (Marti et al., 2010). Such monitoring findings document the existence of commercial actors in the international fish oil refining industry still using inadequate decontamination technology.

4. Alternative decontamination technologies

4.1 Fishmeal

POPs are hydrophobic compounds with a $\log K_{OW} > 5$ (UNEP, 2001; Wania, 2003) and will accumulate in the fatty tissue of living organism. In the fishmeal and fish oil process they will follow the fat phase and the final fishmeal level controlled by the partitioning of fat in the process (Paper I). The fishmeal POPs level is directly proportional to the fishmeal fat level. Consequently, any process optimization addressing reduced fat content will lower the final fishmeal POPs level.

4.1.1 Improved mechanical fat separation

Fat separation steps in the fishmeal and oil process are performed based on mechanical pressing and centrifugation (Anon, 1986; Søbstad, 1992). Høstmark (1987) has studied the effect of a gentle, low temperature (60 °C) coagulation procedure on fat separation in the fishmeal process. On average, a 40% reduction of the fishmeal fat level was obtained with herring and mackerel raw material compared to a more harsh coagulation procedure (7.2% and 12.0% Soxhlet fat on a dry matter basis, respectively) aimed to simulate large scale industrial conditions. Attempts in 1995 to upscale the process failed, but part of the same improved fat separation effect has been obtained in large scale operation by use of hot press or decanter liquid in return to the cooker (Øistein Høstmark, Nofima, pers. comm.). The obtainable effect in large scale operation is, however, also dependent on raw material type and quality and the specific equipment used in the processing plant. Moll et al. (1996) used high temperature short time treatment (100-150 °C, 15-40 seconds) to split emulsions and coagulate protein before fat separation from the press liquid in the fishmeal process. The treatment gave improved separation of suspended particles and reduced fat content in the stickwater.

Generally most of the polar lipids will be membrane bound and remain in the solid phase after wet rendering. The phosphorous level in fish oil is in the range 5-100 ppm (Young, 1986) corresponding to a phospholipids level around 150-3000 ppm. The

water washing or polishing step used in the final fish oil separation will remove some of these phospholipids and can be compared to the water degumming step used in the vegetable oil industry. Improved fat separation has also been reported based on protease treatment of non-coagulated fish by-products (Dumay et al., 2009; Linder et al., 2005), partly attributed to increased release of phospholipids. However, substantial amount of phospholipids was also found emulsified in the water fraction after centrifugation. The possible use of membrane filtration has been proposed to separate the emulsified lipid phase but not experimentally demonstrated (Dumay et al., 2009).

Marmon et al. (2009) have studied the use of pH-shift processes to reduce the level of dioxins and PCBs in protein isolates from herring. Compared to herring mince the tested processing conditions gave protein isolates with 70-80% reduced fat content (based on combined *n*-hexane-acetone and *n*-hexane-diethyl ether extraction) and a WHO-PCDD/F-PCB-TEQ reduction highly correlated to the fat reduction. However, on a dry matter basis the fat content (10-11%) was on a level comparable to commercial fishmeal. Use of 5% ethanol during processing of alkali-made isolates further reduced the fat level to 5.2%.

Baron et al. (2007) has demonstrated a 20-30% transfer of the fishmeal fat content (based on analysis of the fatty acid profile) to the water phase after protease (Alcalase) treatment at pH 9 followed by readjustment to pH 6 and centrifugation. However, the used experimental protocol did not demonstrate the separation of fat from the extracted water phase and the overall effect after adding back the water soluble protein fraction.

The effects of protease (Alcalase) and heat treatment on increased fat separation in press cake and stickwater concentrate is reported in Paper I. Effect on press cake fat level was negligible. The fat level (based on Bl&D extraction) in stickwater concentrate was significantly reduced, on average by 64%, corresponding to a 13% reduction of the fat level in the final experimental fishmeal. However, the effect

could be attributed the applied laboratory scale centrifugation conditions after treatment and not the studied experimental variables.

4.1.2 Organic solvent extraction

A large range of tested alternatives for solvent extraction of oilseeds are reported in the literature (Johnsen and Lusas, 1983). Only few of these are, however, acceptable for food and feed applications, i.e., methanol, ethanol, isopropanol, ethyl acetate, acetone, methyl ethyl ketone and hexane. Hexane (i.e., commercial petroleum fractions consisting of 45-90% *n*-hexane and other branched and cyclic hexane isomers) is the main solvent alternative used for edible oil extraction (Wan and Wakelyn, 1997) and has also been utilized in Norway in the 1960 and 70ies to produce a special quality low fat fishmeal (Norsamin[®]) to be used in domestic animal feed (Opstvedt and Hansen, 1977). A new fishmeal decontamination plant based on hexane extraction was put in operation in 2005 at the fishmeal factory TripleNine Fish Protein in Esbjerg, Denmark. However, I am not aware of any published information on decontamination efficiency or residual fat content in the fishmeal after the hexane extraction.

Hexane is an apolar solvent with limited ability to extract phospholipids from a matrix. In laboratory setup based on reflux of hot hexane (i.e., Soxhlet apparatus) a large difference in total extracted lipids compared to chloroform-methanol (Bl&D) extraction (Bligh and Dyer, 1959) has been reported. The difference is mainly attributed a reduced extraction of phospholipids. Based on this assumption hot hexane is able to extract 40-60% of the phospholipids in anchovy and red eye fish meal and 54% in hake fish meal (de Koning et al., 1985). Roschke et al. (1978) reported that petroleum ether (petroleum fraction with boiling point 40-60 °C) was able to extract only 65% of the Bl&D extractable lipids in krill meal. This was explained by a low extraction rate of phospholipids.

Hexane is a highly flammable and toxic solvent and isopropanol has been studied as a more environmental friendly alternative. The main drawbacks of isopropanol,

compared to hexane have been the lower apparent solvency for oil and higher energy consumption in the recovery process (Lusas et al., 1994).

Effect on fat reduction (based on Bl&D fat) in fishmeal after hexane (79%) and isopropanol (88%) extraction was documented in Paper I. The higher effect of isopropanol was attributed to its higher polarity. Comparable results are reported by Baron et al. (2007). Opstvedt and Hansen (1977) reported the residual fat content in commercial hexane extracted fishmeal samples to be in the 3.4-6% range based on residual Bl&D fat, confirming its less efficient extraction effect on polar lipids.

4.1.3 Sub- and supercritical fluid extraction

Carbon dioxide (critical point 31 °C and 74 bars) is the most widely used alternative in supercritical fluid extraction (SFE), being the cheapest and most environmental friendly alternative (King, 1997). Examples of utilization of SFE technology in the food industry are removal of caffeine, extraction of fat, cholesterol, essential oils and antioxidants, and fractionation of lipids (Herrero et al., 2006, Sahena et al., 2009). The main advantages of SFE is relative low extraction temperature, inert conditions avoiding molecular alterations, high extraction rate (low viscosity and high diffusion rate), and simple removal of solvent by evaporation. Addition of about 5-10% of ethanol to CO₂ is reported as necessary to achieve extraction of phospholipids in egg yolk, canola, meat, soybean flakes, sunflower seed and corn germ (Boselli and Caboni, 2000). However, by utilization of high pressure (517 bars, 40 °C) these authors have obtained extraction yields comparable to conventional Bl&D extraction.

Pederssetti et al., 2011 have compared the efficiency of compressed propane and supercritical CO₂ extraction for extraction of canola seeds. Comparable yields and oil qualities were obtained, but extraction with propane was much faster making this technology an interesting alternative.

4.1.4 Oil leaching

The principle behind the oil leaching process was first presented to the fishmeal and fish feed industry in 2002 (Oral presentation I). The decontamination technology is

based on contacting the fishmeal intermediate products, press cake and decanter solids, by low-dioxin triglyceride oil (i.e., vegetable or fish oil) with subsequent separation steps to reduce the fat content back to normal level (Figure 4) (Paper I). During the extracting step the lipid phase and POPs embedded in the solid matrix will be transferred to the continuous oil phase where it subsequently can be removed by use of oil decontamination technology. The extraction principle can be utilized both on wet press cake (Paper I) and dried fishmeal (Baron et al., 2007; Oterhals, unpublished results). Oil leaching of press cake has been shown to be as effective as hexane or isopropanol extraction of fishmeal (Paper I). Compared to organic solvent extraction the process alternative offers several advantages including easy implementation in an existing fishmeal processing plant and use of a safe and nonflammable extraction medium.

4.2 Fish oil

4.2.1 Activated carbon adsorption

AC is produced from coals, peat, wood, and a wide range of organic byproducts of industry and agriculture by two standard activation methods: gas and chemical (Yang, 2003). Gas activation is performed by pyrolysis of the carbonaceous raw material at 400-500 °C followed by partial gasification at 800-1000 °C in the presence of a mild oxidizing gas such as CO₂ and steam, to develop the porosity and surface area. Chemical activation is performed by direct reaction between the raw material and an activator such as phosphoric acid and zinc chloride at 500-900 °C. The pore size distribution, available surface area and surface properties are dependent on the starting material and manufacturing procedure. The pore size distribution can be divided into micro-, meso- and macropores with effective radii of <2, 2–50 and 50 nm, respectively. AC consists of a heterogenic twisted network of defective hexagonal carbon layer planes, cross-linked by aliphatic bridging groups between which slit-like crevices are formed (Barton et al., 1999). The most widely used commercial products have surface area of about 800 to 1500 m²/g with micropore contribution of about 95% (Bansal and Goyal, 2005). The macropore surface area

normally contributes less than $0.5 \text{ m}^2/\text{g}$ and this pore structure functions mainly as transportation channels.

AC adsorption has been used in edible oil refining operations for several decades to remove PAHs from coconut oil (Biernoth and Rost, 1967) and in recent years also from olive pomace oil (Leon-Camacho et al., 2003). The use of AC treatment to remove dioxins was first reported in 1976 (Mounts et al., 1976). AC treatment has proven very efficient for removal PCDD/Fs from fish oil but is less effective for removal of DL-PCBs (Maes et al., 2005; Paper II). *Ortho*-substituted PCBs are less adsorbed compared to non-*ortho* substituted. No effect of AC on PBDEs removal was observed in study reported in Paper II. However, Ortiz et al. (2011) have obtained up to 9% reduction based on 2.5% AC at 80 °C and 38 min adsorption time. They also reported adsorption of NDL-PCBs (11%), HCB (70%) and DDT (27%) at these conditions. The discrimination between PCDD/Fs and DL-PCBs has been confirmed by many studies (Cornelissen et al. 2005; Maes et al., 2005; Paper II). The trapping mechanisms of adsorption onto AC are still only partly understood. However, planar conformation or in case of PCBs and PBDEs, steric hindrance imposed by *ortho*-substitution and energy barriers for the formation of coplanar structures with the ability to form π -electron interactions with the hexagonal carbon layer and electrostatic forces plays an important role (Paper II and III).

No negative effects of AC adsorption have been observed on tested oil quality parameters, i.e., fatty acid composition (Maes et al., 2005; Usydus et al., 2009; Ortiz et al., 2011) and oxidation level and retinol retention (Maes et al., 2005; Paper II).

4.2.2 Miscellaneous sorbent and complexation systems

Ordinary acid activated bleaching earth used to remove color compounds and polar oxidation products in edible oil refining has no effect on PCDD/F and DL-PCB removal (Maes et al., 2005; Mounts et al., 1976; Ortiz et al., 2011; Paper II). Also, no effect of silica treatment (0.5%) and filter aid (diatomaceous earth, 0.5%) has been documented (Epe et al., 2005; Ortiz et al., 2011). Phosphoric acid activated AC with an acid pH reaction is less effective compared to steam activated AC with a basic pH

(De Meulenaer et al., 2003). Yang et al. (1999) have developed a sorbent screening technique for dioxin removal based on the study of desorption activation energy. The high efficiency of AC could be explained by a much higher bond energy compared to clays, pillared clays, γ -Al₂O₃ and zeolites. Carbon nanotubes have nearly three times higher activation energy for dioxin desorption compared to AC and superior for dioxin removal (Long and Yang, 2001).

Mono-, di- and tri-chlorobiphenyls in insulation oil has been efficiently removed by inclusion complexing into channel-type γ -cyclodextrin assemblies (Kida et al., 2008). However, 3,3',5,5'-TeCB was scarcely removed and the adsorption capability of the tested channel-type γ -cyclodextrin is therefore not suited for reduction of the TEQ level in feed and edible oils. According to the authors optimization of the preparation condition for the channel-type γ -cyclodextrin is needed and further studies in progress.

4.2.3 Supercritical CO₂ extraction

High efficiency of supercritical CO₂ extraction on reduction of the TEQ level in fish oil has been obtained. The extraction efficiency is negatively correlated to the degree of chlorine substitution or molecular weight and effective removal could only be achieved of congeners with MW below 400. Obtained TEQ reduction ranged from 86% for TCDD/Fs to 14% for OCDD/Fs, and from 92% for TCBs to 69% for HpCBs (Kawashima et al., 2006). Use of counter-current compared to semi-batch type extraction reduced the consumption of CO₂, but still with a low refined oil yield of only 65% (Kawashima et al., 2009). Extracted oil increases with increasing CO₂/oil ratio (Jakobsson et al., 1994) and pressure (Kawashima et al., 2009). To reduce the PCDD/F-TEQ level below 90% the subsequent use of AC has been proposed (Kawashima et al. 2006 and 2009).

4.2.4 Steam deodorization

Deodorization is a steam stripping process where superheated steam is contacted with the oil at elevated temperature and a pressure of 3 mbar or lower. Amount of

stripping steam depends on temperature and pressure conditions and target quality parameters. Deodorization is used in the edible oil processing industry to remove undesirable volatile off-flavor compounds and free fatty acids, and thermal decomposition of hydroperoxides and pigments. Several types of commercial deodorizer designs exist based on batch, semicontinuous and continuous technology (De Greyt and Kellens, 2005). Deodorization of most vegetable oils is performed at temperature levels between 230 and 260 °C. Fish oils, however, are susceptible to thermal induced polymerization and geometrical isomerization (i.e., formation of trans isomers) at temperature levels above 180 °C and demands special attention on heat load to avoid such negative effects (Mjøseth and Solvang, 2006; Fournier et al., 2006).

The steam stripping process will also remove other volatile substances including tocopherol, sterols and POPs. Pesticides used during cultivation and storage of oil seeds, fruits and kernels are removed from the extracted oil to below detection level at commercial refining and deodorization conditions (230-240 °C for 45-70 min and a steam dosing of 8 m³/kg oil) (van Duijn, 2008). More gentle deodorization conditions (180 °C for 2 hours and 7% steam relative to oil mass) were able to reduce the level of the most volatile compounds (i.e., α -HCH, lindane, HCB) found in fish oil to below detection level (Hilbert et al., 1998). Less volatile organochlorine pesticides (i.e., dieldrin, *p,p'*-DDE and *p,p'*-DDD) and PCBs were reduced by about 50%. Low molecular weight PAHs are efficiently removed in commercial deodorization of soybean oil (Larsson et al., 1987). However, heavy PAHs were reduced to a limited extent with observed residual benzo[a]pyrene levels up to 1 µg/kg. Carbonnelle et al., 2006 have compared the use of packed column and cross-flow stripping of PCDD/Fs- and DL-PCBs in fish oil at different operation conditions. PCDD/Fs were more difficult to remove compared to DL-PCBs. Temperature levels between 210 and 220 °C were needed to reduce the WHO-PCDD/F-PCB-TEQ level below the present MPL (10 pg/g). A combination of AC adsorption and steam deodorization was used to improve the total decontamination rate.

4.2.5 Short-path distillation

SPD is characterized by the combination of very short residence time in the evaporator (1–10 s), low pressure (< 1 Pa), short distance between the evaporator and condenser (10–50 mm) and approximately collision free mass transfer of molecules in the distillation space (Lutisan and Cvengros, 1995). The short residence time is achieved by formation of a thin liquid film on a vertical cylinder (falling film evaporator, Figure 3) or on a rotating surface (centrifugal film evaporator). In the literature molecular and SPD is often used as synonyms, the first terminology referring to an apparatus with a gap between the evaporator and condenser equal or less than the mean free path of the molecules evaporated (collision free diffusion).

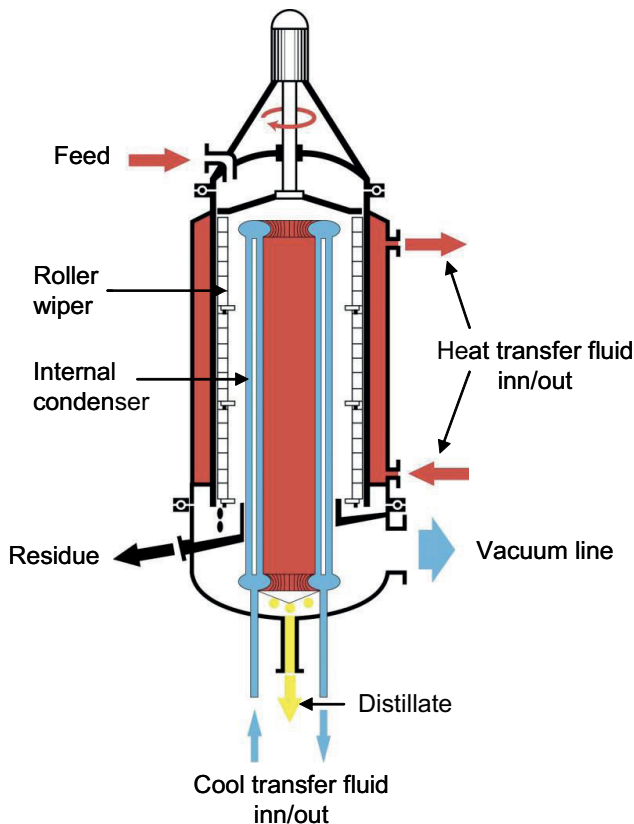


Figure 3. Cross-section of a short-path evaporator (after UIC GmbH, with permission).

SPD is established as a good manufacturing practice in the lipid processing industry to separate heat labile substances (Xu, 2005). However, few studies pertaining use of the technology to reduce POPs in edible oils are published. Bills and Sloan (1967) studied the removal of chlorinated insecticides in milk fat and achieved a reduction of 95–99% at evaporator temperature of 200 °C and 0.7×10^{-3} mbar. Julshamn et al. (1973) reported 70% removal of DDT in cod liver oil at operation conditions 200–228 °C and $21\text{--}23 \times 10^{-3}$ mbar. The use of the technology in the fish oil refining industry is also reported by Brevik et al., 1990. They obtained 95% reduction of the N-PCDD/F-TEQ (based on Nordic TEF model) level in fish oil, but with only a rough indication of the applied process conditions, i.e., 180–220 °C, <100 Pa. Breivik and Thorstad (2005) reported >90% reduction of PCDD/Fs, DL-PCBs, PBDEs and OCPs in fish oil based on SPD and the improvement of the process by addition of 3–6% of a volatile “working fluid” consisting of fatty acid ethyl esters. Details regarding preferred process conditions are given in the corresponding patent (Breivik and Thorstad, 2004), i.e., pressure between 0.1 and 0.001 mbar, temperature 180–200 °C. Similar improvement of process efficiency has been obtained by flushing the inner condenser with a “washing liquid” (Albers and Graverholt, 2006; Albers and Schardt, 2007). Response surface methodology has been used in Paper IV to study the effects of evaporator temperature, flow rate and addition of “working fluid” on the removal of PCDD/Fs, DL-PCBs and PBDEs. The WHO-PCDD/F-PCB-TEQ level could be reduced up to 98% based on the best experimental settings. Temperature levels up to 228 °C were used without loss of polyunsaturated fatty acids (PUFAs) or formation of trans-isomers (Paper IV). Retention of vitamins and cholesterol in the fish oil was substantially higher compared to the reduction of WHO-PCDD/F-PCB-TEQ. Generally, no adverse negative effects on the nutritional quality of the fish oil could be documented. Residual WHO-PCDD/F-PCB-TEQ level in accordance with the voluntary industrial monograph of GOED could be achieved on the basis of operation conditions giving <20% loss of vitamins (Paper V).

5. Experimental and analytical approaches

5.1 Presscake and fish oil production

The annual world fishmeal production is around 5 million metric tons with Peru, Chile, Thailand, U.S.A, Japan, Denmark, China, Norway, Mexico and Iceland as the main producing countries in descending 2009 order (Shepherd, 2010). Fishmeal is produced by use of heat coagulation of the raw material followed by a mechanical fat separation and thermal dewatering process (Anon, 1986; Søbstad, 1992). The processing equipment is fairly standardized worldwide and product quality is mainly dependent on raw material type and quality (Opstvedt et al., 2000) together with drying conditions (Opstvedt et al., 2003). A general outline of the fishmeal and fish oil process unit operations is included in Figure 4 (Paper I).

The crude fish oil used in Paper II, IV and V was purchased directly from a Scandinavian fishmeal and oil producer. The oil was of commercial quality with a free fatty acid (FFA) level of 46 g/kg and mainly produced from sprat (*Sprattus sprattus*) caught in the North Sea region.

The press cake used in Paper I was produced from herring (*Clupea harengus*) caught in Skagerrak. The fish was of food quality and delivered in frozen blocks. After thawing overnight it was processed in the pilot plant facilities of Nofima in Bergen. Details regarding processing conditions are given in Paper I. After heat treatment (80-90 °C) of the fish raw material it is run over a strainer (not shown in Figure 4) to remove free water and oil phase before entering the screw press. The fish material entering the press (Figure 5A) is transported by counter-rotating screws of reducing height. The compression ratio, normally 1:3.5-4 in a fish press, causes fish oil and water to be squeezed out of the coagulated material and through the sieve plates. The water and oil together with solubles and fine particles are collected in the bottom of the press and mixed with water phase removed over the strainer. The liquid process stream is heated and run over a Jesma sieve or decanter centrifuge to remove suspended solids before oil separation by a disc centrifuge (Figure 4). The press cake

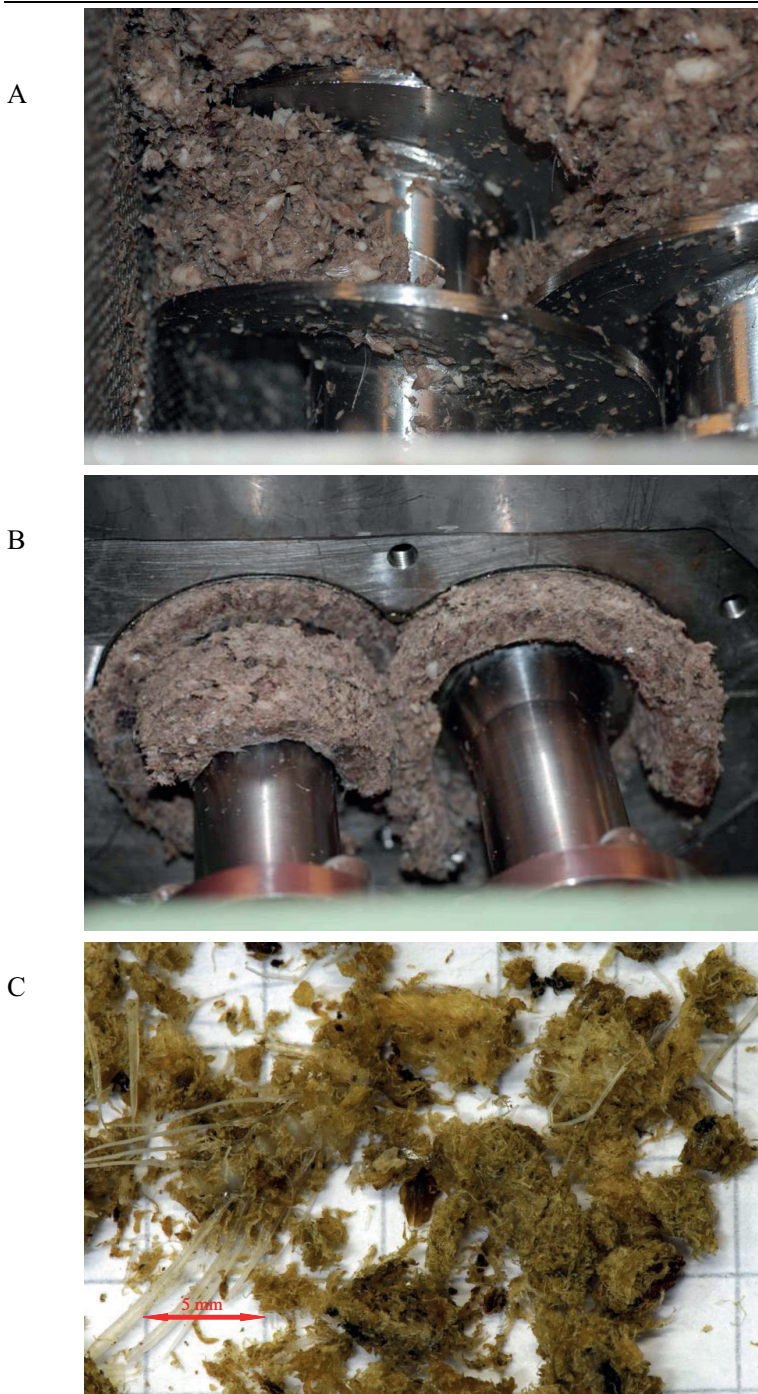


Figure 5. Cooked herring at the inlet (A) and outlet (B) of a double-screw mechanical press (screw diameter 13 cm), and (C) press cake after disintegration.

5.2 Increased fat separation in the fishmeal process

5.2.1 Mechanical separation

Improved fat separation in the fishmeal process has been studied extensively through several research projects at the Norwegian Herring Oil and Meal Industry Research Institute (SSF) and recommendations implemented in the industry. By experience, further improvements based on mechanical separation are difficult to obtain without high investments. In Paper I a two-factorial design experiment was performed to test the effect of heat (121 °C) and protease (EC 3.4.21.62) treatment on improved fat separation from press cake/jesma solids and stickwater concentrate. The design was based on the following industrial practices and principles: (i) A second pressing step (so-called double pressing) has been applied by the fishmeal industry to reduce the fat content of decanter solids. (ii) Alcalase 2.4L (Novozymes AS, Bagsvaerd, Denmark) treatment is used to lower the viscosity of stickwater concentrate and thereby improve the concentration rate. Recommended conditions are 1 g Alcalase per kg dry matter and temperature below 60 °C. (iii) High temperature treatment has been reported to improve the fat separation from decanter liquid.

Improved fat separation after the treatment protocols was measured by centrifugation of the samples. The upper oil/water layer was collected after freezing of the sample and separated oil quantified by extraction of this subsample using tetrachloromethane. The protocol reflects the maximum released fat possible to separate by use of a decanter centrifuge in case of press cake and a disc centrifuge in case of stickwater.

5.2.2 Solvent extraction

Two industrial relevant organic solvents, hexane and isopropanol, were compared in Paper I. Extraction of different seeds (soy, rape/canola, flax, sunflower, cotton etc.) to produce edible oils is performed in large scale operations worldwide primarily based on hexane extraction. It has earlier also been used in industrial scale for defatting of fishmeal (Opstvedt and Hansen, 1977) and at present in Denmark for

decontamination of fishmeal. Isopropanol has been studied extensively as a more environmental friendly alternative. Hexane is a nonpolar solvent which mainly extract neutral lipids, i.e. triglycerides. Isopropanol is more polar with a higher ability to extract phospholipids and other polar lipid compounds.

Excess solvent and temperature close to the boiling point of commercial hexane (i.e., petroleum fraction consisting of 45-90% n-hexane with a boiling point in the range 65-70 °C) used in the oilseed extraction industry was chosen as test conditions. The dry fishmeal was extracted twice with a sample to solvent ration of 1:5 at 58 °C. Residual fat, PCDD/F and DL-PCB levels were used as responses. The fat level was measured based on four laboratory extraction protocols normally used to quantify fat in food and feed products: Ethyl acetate extracting, light petroleum (boiling range 40-60 °C) Soxhlet extraction, light petroleum (boiling range 40-60 °C) Soxhlet extraction with acid hydrolysis (EC method), and monophasic chloroform-methanol-water (Bl&D) extraction. The extraction protocols represent solvent systems with different polarity and ability to extract polar lipids in the matrix.

5.3 Presscake oil leaching

The oil leaching process was tested to explore more environmental friendly alternatives to organic solvent extraction. Soybean oil was chosen as a leaching agent to enable measurement of the interchange of both POPs and fatty acids between the lipid phase embedded in the solid matrix and the continuous lipid phase. The press cake used in this study was produced from herring raw material based on the wet rendering process described under 5.1. Press cake extruded from the screw press consists of large lumps (Figure 5B) and was grinded to obtain a more homogenous material (Figure 5C). The leaching process was performed in a batch system utilizing the combination of high temperature (88 °C) and long contact time (60 min) under continuous mixing to explore the feasible use of the technology, followed by two-step water washing of the press cake to remove excess oil (Paper I). The protocol was established based on initial tests and conditions chosen to be relevant for later implementation in the fishmeal process. A schematic presentation of the used

processing steps showing the possible implementation in a fishmeal processing plant is given as shaded boxes in Figure 4.

5.4 Activated carbon adsorption

Among the adsorbents used in edible oil refining AC was the only known to be able to remove PCDD/Fs and PCBs. It is also used by the industry to remove PAHs. Several commercial producers were contacted to gather non-published knowledge and evaluate candidate products. AC adsorption was performed using a 5 L jacketed glass reactor equipped with temperature control, stirrer and vacuum pump (Paper II). The adsorption process was performed under vacuum (<20 mbar) and AC removed by use of a Büchner filter. Process conditions were chosen based on earlier experience in fish oil bleaching and general industrial practice. A 0.5% addition of a powder quality AC (Norit SA4 PAH) was used in the optimization trials. General

Table 5. General properties of Norit SA 4 PAH used in the AC adsorption trials. (Analyses provided by Norit, Amersfoort, The Netherlands)

Parameter	Level
Moisture	2%
Ash content	8%
pH	Alkaline
Particle size D10	3 μm
Particle size D50	20 μm
Particle size D90	140 μm
Total surface area (BET)*	1075 m^2/g
Total pore volume*	0.58 ml/g
- micro (<1 nm)*	0.45 ml/g
- meso (1-25 nm)*	0.13 ml/g
Average pore diameter*	2.6 nm
Iodine number*	1035 mg/g

*) Analyzed in the used lot# 2053.4.

properties of the AC quality are given in Table 5. The effect of temperature (32-88 °C) and contact time (14-56 min) on the removal of PDDD/Fs, DL-PCBs and PBDEs was studied based on a central composite design (CCD) experiment (Figure 7).

5.5 Short-path distillation

Two volatilization technologies are used in the edible oil industry: steam deodorization and SPD. Based on my earlier experience steam deodorization was known to be less effective for removal of PCDD/Fs (Opstvedt et al., 1996) and give limitations regarding maximum temperature level and overall heat load. SPD is established as a good manufacturing practice in the edible oil industry to separate heat labile compounds and was evaluated to be the most efficient and gentle technology.

SPD trials were performed on a stainless steel KD6 pilot plant at UIC GmbH (Alzenhau-Hörsteiner, Germany). A simplified process flow diagram is given in Figure 6 (Paper IV). The feedstock was heated to 123-136 °C in a heat exchanger before degassing at 0.3 mbar to remove dissolved gasses and low boiling compounds before entering the short-path evaporator. Effect of evaporator temperature (172-228 °C), feed rate (2.30-7.83 kg/h) and addition of working fluid (WF; 0-4%) was tested based on factorial design experiments. The WF consisted of a fatty acid ethyl ester fraction obtained as a distillation by-product during concentration of EPA and DHA from fish oil.

The evaporator was a falling film system (surface area = 0.06 m²) equipped with a roller wiper (400 rpm) and a vacuum system consisting of a cold trap (-25 °C), oil diffusion and rotary vane pump in series. The internal condenser temperature was 60 °C. The residue was collected in a residue cup (160 °C) and pumped through a cooler before sampling under nitrogen cover.

At ideal conditions the molar flux of volatile compounds ($j = \text{mol}/(\text{m}^2 \cdot \text{s})$) in a SPD unit can be described by the Langmuir-Knudsen equation (Lutisan and Cvengros, 1995; Salez-Cruz and Gani, 2006):

$$j_i = \gamma_i x_i P_i(T) \sqrt{\frac{1}{2\pi R M_i T}} \quad (2)$$

γ_i is the activity coefficient, x_i the mol fraction, $P_i(T)$ the saturation vapor pressure in Pa at absolute temperature T in kelvin, R the gas constant and M_i the molecular weight. As the feed runs down the evaporator it will be depleted for any volatile POP with resulting reduced congener specific molar flux. At increasing flow rate and temperature (i.e. reduced viscosity) the reduced residence time at the evaporator surface will be an additional limiting factor for the obtainable decontamination rate. Co-evaporation of other volatile compounds (i.e. vitamins, cholesterol, WF) in the feedstock and splashing (i.e., transfer of small droplets from the evaporator to the condenser) will influence the resulting concentration and thereby the re-evaporation rate in the opposite direction of the individual POPs from the condenser surface. The maximum obtainable decontamination rate will depend on the net molar flux of the individual POPs from the evaporator and condenser surfaces at the specified operation conditions and have a theoretical limit characterized by equilibrium between the two fluxes.

The vapor pressure of low volatile compounds like PCDD/Fs and PCBs are difficult to measure. Values of physical and chemical properties are published in several studies (Mackay et al., 2006) but might vary over several orders of magnitude (Åberg et al., 2008). Additionally, the majority of values have been reported at 298 K and there is a lack of data at the high temperature levels used in SPD. The PCDD/Fs, DL-PCBs and PBDEs studied in this thesis forms homologous series with linear relationship between vapor pressure and degree of chlorine or bromine substitution (Li et al., 2005; Nakajoh et al., 2005; Åberg et al., 2008; Wong et al., 2001). In addition, the number of *ortho*-substitutions will influence the vapor pressure of PCBs (Nakajoh et al., 2005) and PBDEs (Wong et al., 2001). In the model building in Paper IV these quantitative structure properties relationships (QSPRs) were combined with process variables to establish general models for each of the homologous series.

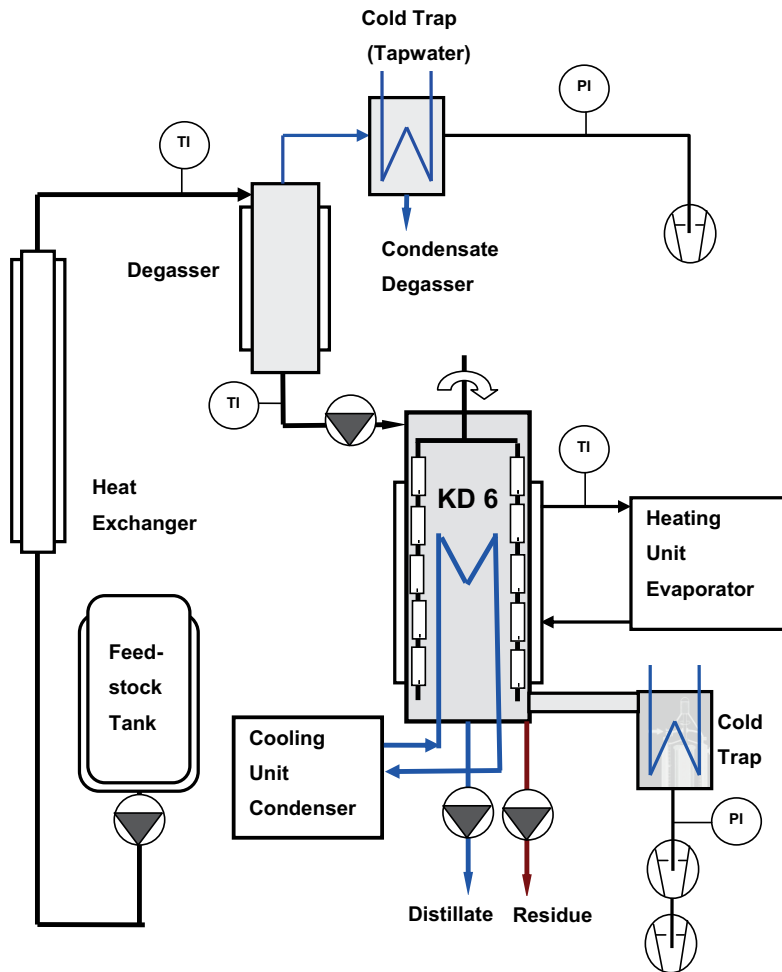


Figure 5. Simplified flow sheet of the short-path distillation (SPD) process.

5.6 Analyses of POPs

PCDD/F and DL-PCB analyses were performed by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) according to US EPA methods (US EPA: Method 1613 and 1668). The congeners analyzed included the 17 PCDD/Fs and 12 DL-PCBs for which WHO has established TEFs for human risk assessment (Table 2; Van Den Berg et al., 1998). Analyses of PBDEs (-28, -47, -99, -100, -153, -154) were performed by gas chromatography with negative chemical ionization (GC-NCI) with methane as reagent gas, as previously described (de Boer et al., 2001; Bethune et al., 2005). The analytical laboratory (NIFES, Bergen, Norway) is accredited according to ISO 17025. Expanded measurement of uncertainty ($k = 2$; i.e. level of confidence of approximately 95%) was in the low concentration range (i.e., <1 ng/kg for PCDD/Fs and DL-PCBs and < 500 ng/kg for PBDEs) approximately 40% for PCDD/Fs and DL-PCBs and 25% for PBDEs. At higher concentrations (i.e. >4 ng/kg for PCDD/Fs and DL-PCBs and >500 ng/kg for PBDEs) the maximum measurement uncertainty was approximately 25% or less.

5.7 Fishmeal and oil composition and quality assessment

The following analytical methods have been applied to assess composition and quality of materials and processed samples studied in the respective papers:

Analytical method	Reference	Paper
FFA	AOCS method Ca 5-40	II, IV
Moisture content	ISO 6496	I
Fat content – ethyl acetate	NS 9402	I
Fat content – Soxhlet	AOCS method Ba 3-38	I
Fat content - EC	Commission Directive 98/64/EC	I
Fat content – Bl&D	Bligh and Dyer, 1959	I
Fat content – tetrachloromethane	NS 4752	I
Fatty acid composition	AOCS method Ce 1b-89; Jordal et al., 2007	I, V
Fatty acid isomers	Mjøs and Solvang, 2006	V
Lipid classes – HPTLC	Bell et al. 1993; Jordal et al., 2007	V

Lipid classes- HPLC-CAD	Paper IV	IV
Unsaponifiable matter	AOCS method Ca 6b-53	V
Peroxide value	AOCS method Cd 8b-90	II, V
Anisidine value	AOCS method Cd 18-90	II, V
Conjugated double bounds	DGF method C-IV 6(a) (57)	II, V
Oxidative stability - weight gain	Olcott and Einset, 1958	V
Vitamin A	Nóll, 1996	V
Vitamin D	Horvli et al., 1994	V
Vitamin E	Lie et al., 1994	V
Vitamin K	Schurgers et al., 1999	V

5.8 Statistical experimental design and process modeling

Optimization of industrial processes often involves measuring of the effect of several independent variables on the process performance or several product quality characteristics (responses). In many cases the costs and efforts needed per experiment is high and limits the number of variable settings possible to test within given budget frames. To enable cost effective experimental work several strategies or experimental designs are described in the literature based on statistical methods (Leardi, 2009; Lundstedt et al., 1998; Myers and Montgomery, 2002). Factorial and central composite designs (CCD) have been applied in this thesis combined with model development based on ordinary multiple linear regression (MLR) and partial least squares regression (PLSR). A brief introduction to these methods is given below.

5.8.1 Factorial and central composite design

A 2^k factorial design is the simplest form of experimental design. It requires 2^k experiments where k is the number of variables studied. Each variable is studied at two levels, coded -1 and +1, and can be either quantitative (e.g., temperature, pressure, amount of ingredient) or qualitative (e.g., type of catalyst, sequence of operations). A 2^2 factorial design is embedded in Figure 7 as the cube points and the response can be described based on the following mathematical model:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \varepsilon \quad (3)$$

The parameters β_j , $j = 0, 1, \dots, k$ are the regression coefficients and ε the error or residual. This first-order response model assumes linearity in the factor effects. It is capable of representing some curvature in the response function by twisting of the plane induced by the interaction term $\beta_{12} X_1 X_2$. However, there are situations where the curvature in the response function is not adequately modeled by Equation 3 and this is solved by introducing squared effects, i.e., a complete second-order response surface model.

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon \quad (4)$$

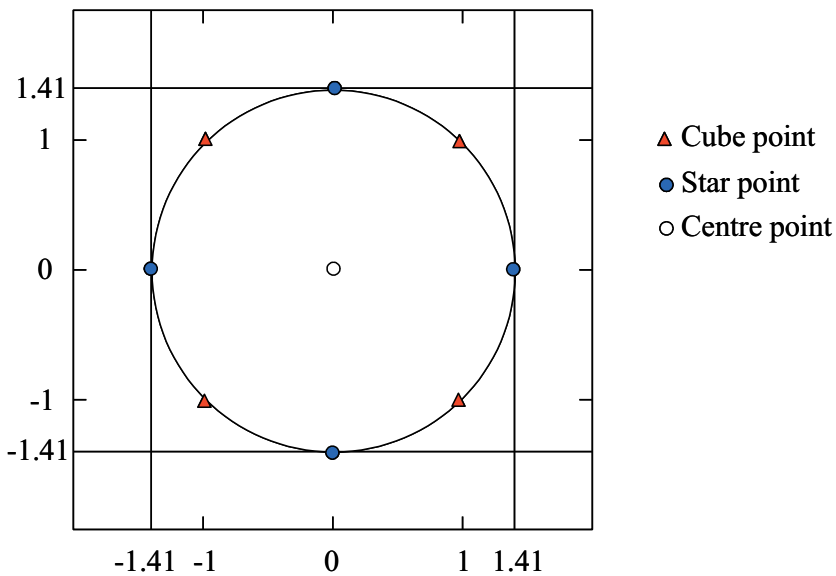


Figure 7. Graphical presentation of the two variable central composite design (CCD).

Replication of the centre point (normally 3-5 times) allows for an independent estimate of the experimental error to be obtained. A 2^2 design including centre points only has five distinct experimental settings and can not be used to estimate the unknown square effect parameters (β_{11} and β_{22}). There must be at least as many distinct design points as parameters in the model and at least three levels for each

design variable. To solve this problem the 2^2 design need to be augmented with additional experimental points. The most common way of doing this is to add axial or star points. In the rotatable CCD the star points are situated a distance from the center point equal to:

$$\alpha = \sqrt[4]{F} \quad (5)$$

where F is the number of factorial points ($F = 2^k$ if it is a full factorial design). This gives a design with $2^k + 2k$ experimental points that are equidistance from the design center (Figure 7).

5.8.2 Multivariate regression techniques

The above regression models can be written in the following matrix notation:

$$\mathbf{y} = \mathbf{X}\mathbf{b} \quad (6)$$

where \mathbf{y} is the response variables, \mathbf{X} the x-variables and \mathbf{b} the regression coefficients vector, respectively. Ordinary multiple linear regression (MLR) are normally applied to find the regression coefficients in response surface methodology (Myers and Montgomery, 2002). The regression coefficients are estimated based on the following equation:

$$\mathbf{b} = (\mathbf{X}^T\mathbf{X})^{-1} \mathbf{X}^T\mathbf{y} \quad (7)$$

Estimation of the unknown parameters (the β 's) assumes that: (i) The number of terms in the model is less than the number of independent experimental runs. (ii) The values of the predictor variables are exact so all random variation are contained in the measured response. (iii) The residuals are uncorrelated and normal distributed (Kvalheim, 1990). In cases where covariance between the studied variables exists or the used experimental settings can not be rigorously controlled at the preselected levels, MLR may lead to poor estimation of \mathbf{b} because the matrix $(\mathbf{X}^T\mathbf{X})$ is rank deficient or ill-conditioned. This can be solved by projecting the original variables to a set of orthogonal latent variables and perform the MLR on the score vectors (Martens and Martens, 2001). In principal component regression (PCR) the \mathbf{X} -matrix

is first decomposed by principal component analyses (PCA) and the MLR is then based on the PCA score vectors. In partial least squares regression (PLSR) the algorithm extracts latent variables that explain as much as possible of the common variance between the \mathbf{X} -matrix and the \mathbf{y} -vector. The structure of the data matrix can also be revealed by use of PCA and correlation loading plots used to assess the covariance among the studied experimental and response variables. PCA score plots can be used to show similarities and differences in a measured response based on the applied combinations of process conditions.

The response models obtained based on MLR or PLSR might contain insignificant or unreliable variables. Different variable reduction techniques can be used to simplify the model by selection of a subset of significant predictor variables (Andersen and Bro, 2010). The reduced model is less complex, simpler to interpretate, and normally improves the prediction ability of the model. In ordinary MLR models (Paper II, IV, V) variable selection have been based on backward elimination. The algorithm starts with a model including all candidate regressors. Then the partial F -statistic is computed for each regression coefficient as if it was the last variable to enter the model. The smallest of these partial F -statistics is compared with a pre-selected cutoff value, F_{OUT} (or F -to-remove), and if smaller the corresponding regressor is removed from the model (Myers and Montgomery, 2002). The algorithm is continued until all partial F -statistics are higher than F_{OUT} .

Prediction ability of the MLR models was validated based on the prediction error sum of squares (PRESS) (Myers and Montgomery, 2002). The PRESS statistics is calculated by leaving out one observation at a time and fit the regression model to the remaining $n-1$ observation (leave-one-out cross-validation). The new model is used to predict the withheld observation y_i and the procedure repeated for each observation $i = 1, 2, \dots, n$, producing a set of n PRESS residuals ($e_i = y_i - \hat{y}_i$). The PRESS statistics is defined as the sum of squares of the n PRESS residuals:

$$\text{PRESS} = \sum_{i=1}^n e_i^2 = \sum_{i=1}^n [y_i - \hat{y}_i]^2 \quad (8)$$

PRESS can be used to compute an approximate R^2 for prediction (Q^2):

$$Q^2 = 1 - \frac{PRESS}{SS_T} \quad (9)$$

where SS_T is the total sum of squares. In chemistry values of $Q^2 \geq 0.5$ is considered acceptable and > 0.8 excellent (Lundstedt et al., 1998).

In PLSR models (Paper IV) the variable reduction was based on cross-validation of the response models. Cross-validation of a model is performed by dividing the observations into random segments. The algorithm uses one segment as test set while the remaining observations are used for training and this is continued until all segments have been used as test set once. A new model is generated for each of the training sets and used to obtain an estimate of the standard deviation of the predicted regression coefficients ($\hat{s}(\hat{b})$). The uncertainty of the regression coefficients is tested based on a t -test of the expression $\hat{b}/\hat{s}(\hat{b})$ and used for backward elimination of unreliable regressors similar to the MLR algorithm described above (Martens and Martens, 2001).

Prediction ability of the PLSR models was validated based on the root mean square error of prediction (RMSEP(Y)) defined by

$$RMSEP(Y) = \sqrt{\frac{1}{N} \sum_{i=1}^N (y_i - \hat{y}_i)^2} \quad (10)$$

where N is the number of observations, y_i the i th response and \hat{y}_i the corresponding predicted value based on the regression model estimated without the segment including the i th response. The optimal number of principal components (PCs) used in the models were defined based on the RMSEP(Y) vs. number of PCs curve. Outlier observations were identified by use of normal probability plots of studentized y -residuals and influence (residuals vs. leverage) plots.

5.9 Molecular modeling

Molecular modeling can be a powerful tool in terms of exploring possible mechanisms for selective adsorption of POPs on different types of solid sorbents with promising properties and possible use in decontamination of fish oil. It can also provide valuable information of surfaces thermodynamics as well as transport properties related to the adsorption processes. As a first step in the direction of more extensive use of molecular modeling as a complementary tool to experimental efforts a graphite model was used in Paper III to modeling of the trapping mechanisms and selective adsorption of non- and mono-*ortho* PCB molecules to a AC surface. The molecular dynamics (MD) (Jensen, 2007) approach is based on following the trajectories of all molecules in an assemble under the influence of the intermolecular potentials. The molecular assemble can be defined by a $6N$ -dimensional space, where N is the number of particles. $3N$ of these dimensions are due to position of the particles, and $3N$ is due to the momentum. The initial arrangement of molecules or phase space holds all possible positions and momentums. PCB 77 (non-*ortho*) and PCB 118 (mono-*ortho*) were chosen as model compounds with triolein representing the solvent (i.e., fish oil). A graphite model with slit pores of 20 Å was used to represent the AC surface.

Optimized Potentials for Liquid Simulation (OPLS) force field (Jorgensen et al., 1996) was primarily used to parameterize the model. In OPLS the energy contributions are split into bonded and non-bonded contributions. Bonded contributions consist of bond stretching, angle bending and twisting of dihedral angles, while non-bonded contributions are van der Waals forces and electrostatic forces modeled by Lennard-Jones 12-6 potential and Columb's law, respectively (Paper III).

Molecular force fields employed in the simulations combined short-range parameters from the OPLS with partial atomic charges obtained via quantum chemical calculations using DFT/B3LYP/6-31**G+ and Solvation Model 6. The dihedral angle potential between the PCB aromatic rings was modified and the required force field

constants evaluated by use of Schrödinger's Jaguar package. The molecular dynamics software MDynaMix v. 5.1 (Lyubartsev et al., 2000) was used in the simulation setup. The dimensions of the system was 80 x 78 x 1100 Å after initial compression to target density of 915 kg/m³ and consisted of an AC block of 31.232 carbon atoms, 252 triolein molecules of 167 atoms, and 51 molecules of both PCB congeners 77 and 188, each comprising 22 atoms. The total number of atoms was 75.560. The time step was set to 1.0 femtoseconds and temperature 330 K (e.g., centre point used in experimental design Paper II). Total run time on the Cray XT4 supercomputer at Bergen Centre for Computational Sciences was 5 months, with the simulation extending to just over 4 nanoseconds.

6. Summary and discussion of experimental work

6.1 Reduction of fishmeal fat content (Paper I)

The PCDD/F, DL-PCB, fat, and dry matter partitioning during fishmeal production was studied in pilot scale. Fat partitioning was assessed based on four frequently applied analytical extraction protocols to cover methods used in the fishmeal and oil industry and research activity: (i) ethyl acetate extraction, (ii) light petroleum Soxhlet extraction, (iii) light petroleum Soxhlet extraction with acid hydrolysis (EC method), and (iv) chloroform-methanol extraction (Bl&D extraction). The ranking Bl&D > EC > Soxhlet was systematic for all samples and in agreement with earlier studies on fishmeal extraction. Ethyl acetate extraction gave a less systematic picture difficult to explain and render this extraction protocol less suitable to follow the fat partitioning in the fishmeal process. Most of the dry matter and lipid content in the fishmeal could be ascribed to the press cake intermediate product with contribution from stickwater around 18% and in the 15-25% range, respectively. POPs are lipophilic compounds and will preferentially partition in the lipid phase during processing of fish raw material to fishmeal and fish oil. This was reflected by the observed PCDD/F and DL-PCB partitioning data. Consequently, any actions on fat reduction directed on press cake and decanter solids will have a higher fishmeal decontamination potential compared to the stickwater concentrate fraction.

A factorial design experiment based on protease and heat treatment (121 °C) of the press cake gave negligible effects on the fat content (mean 1.2% fat reduction) with no practical interest in the fishmeal production. Stickwater concentrate treated based on the same protocol showed no main effects but the used centrifugation conditions reduced the Bl&D fat content by 64%, corresponding to a fishmeal decontamination effect of 13%. Alcalase treatment is used by the fishmeal industry to reduce the viscosity and improve the dry matter concentration obtainable in falling film evaporators. Such treatment might also improve the fat separation of the stickwater concentrate. In general, improvement of the existing fat separation unit operations used in the fishmeal process is expected to be the most cost effective way to reduce

the TEQ-level in fishmeal. However, the effect obtainable based on mechanical separation technology is limited. Although effects up to 40% have been obtained in other reported pilot scale studies, this has only partly been possible to transfer to industrial scale operation.

Optionally, organic solvent extraction can be utilized to reduce the fat and WHO-TEQ level in fishmeal. Both hexane and isopropanol extraction were able to substantially reduce the fat level with residual Bl&D fat of 31 and 17 g/kg of dry matter, respectively, corresponding to 79% and 88% fat reduction. The levels were in good agreement with the WHO-PCDD/F-PCB-TEQ reductions of 75% and 88%, respectively. The difference can be explained by the higher Bl&D fat content after hexane extraction.

Comparison of the ratio between the WHO-PCDD/F-PCB-TEQ and fat reduction after organic solvent extraction showed large differences based on the tested laboratory fat extraction protocols. Bl&D extraction gave an overall ratio closest to unity and demonstrates that this extraction protocol can be utilized to estimate the WHO-PCDD/F-PCB-TEQ reduction effect of improved fat separation in the fishmeal process or after organic solvent extraction.

6.2 The oil leaching process (Paper I)

Initial tests were performed to test a novel oil leaching process for reduction of POPs in fishmeal. The leaching process is based on contacting the wet intermediate products, presscake and decanter solids, with a feed compatible low-dioxin triglyceride oil, e.g., plant or fish oil. During the leaching operation the POPs are partitioned in the available fat phases and can be removed by a subsequent separation step aimed to remove excess fat and normalize the fat on dry matter level. A simplified process flow sheet is given in Figure 4. The process was tested by contacting wet press cake/Jesma solids with excess soybean oil for 60 min at 88 °C under continuous mixing. The oil leaching process was able to reduce the WHO-PCDD/F-PCB-TEQ level in the presscake/Jesma solids by 97%. Combined with fat

separation of the stickwater concentrate, the applied process conditions were able to give a fishmeal decontamination rate comparable to hexane and isopropanol extraction of the fishmeal.

Use of soybean oil as a leaching agent made it possible to also assess exchange of fatty acids between the solid matrix and continuous oil phase. Exchange of 56-72% of the lipids in the press cake/Jesma solids with soybean oil could be estimated based on fatty acids typical for fish oil (EPA and DHA) and soybean oil (linoleic and α -linolenic acid). The level is lower than the decontamination effect and possible caused by less mobility of the more polyunsaturated phospholipids embedded in membrane structures in the solid matrix. If desirable, the change of fatty acids composition can be minimized by use of fish oil instead of a vegetable oil in the leaching process.

6.3 Activated carbon adsorption (Paper II)

The effect of AC adsorption on the reduction of POPs in fish oil was studied based on response surface methodology at a 5 g/kg AC inclusion level. The oil was alkali refined and bleached to remove FFA and other constituents that might compete with the studied POPs for adsorption sites on the AC surface. The pre-treatment increased the POP levels proportional to the level of removed FFA, probably due to partitioning of the lipophilic compounds in the continuous lipid phase during removal of the soapstock and water washing steps during alkali refining.

The tested process variables (contact time 14-56 min and temperature 32-88 °C) affected the AC adsorption rate and significant first- and second-order response models could be established. PCDD/Fs showed very rapid adsorption behavior and the concentration and WHO-TEQ level could be reduced by 99%. Even at the least favorable processing conditions tested the PCDD concentration could be reduced by 98%. Adsorption of DL-PCBs was less effective and depended on *ortho* substitution, i.e., non-*ortho* PCBs were adsorbed more effectively than mono-*ortho* PCBs with a maximum of 87 and 21% reduction, respectively, corresponding to a WHO-PCB-

TEQ reduction of 73%. A common optimum for both PCDD/F and DL-PCB adsorption could not be identified. However, only marginal improvement of PCDD/Fs adsorption was obtained by increasing the contact time and temperature above 80 °C and 15 min, respectively. PCB adsorption could to some extent be improved by use of higher temperature and longer contact time. Mono-*ortho* PCBs contributed to only 12.7% of the WHO-TEQ level in the feedstock. Due to low adsorption it plays, however, a key role with respect to the total residual WHO-TEQ level after AC treatment of the oil. Introduction of the new WHO₂₀₀₅ TEF values (Table 2) will lower this contribution and improve the limitations of AC adsorption caused by this group of compounds.

AC treatment had no effect on the level of PBDE flame retardants. The differences in adsorption patterns may be explained based on molecular conformation. PCDD/Fs form coplanar molecular structures that can interact with the AC through π -electron interaction. PCBs have a twisted conformation with an energy barrier of rotation into coplanar conformation for non- and mono-*ortho* PCBs of 8.3-9.8 and 28.5-34.9 kJ/mol, respectively. The PCDD/F adsorption selectivity was also to some extent positively correlated to the number of Cl substitutions. In contrast, a negative correlation was observed between average congener reduction rate and number of Cl substitutions for non-*ortho* PCBs.

The feedstock contained 8.7 ng WHO-PCDD/F-TEQ/kg and 11.8 ng WHO-PCB-TEQ/kg. This could be reduced to 0.2 and 4.1 ng WHO-TEQ/kg, respectively, after 15 min AC treatment at 80 °C. Compliance with present PCDD/F and DL-PCB EC legislation levels in fish oil for food and feed applications can be achieved based on AC adsorption. However, the GOED specification of maximum 3 ng WHO-PCB-TEQ/kg could not be met based on the tested conditions.

Quality assessment of the fish oil revealed no change in the PV and a small reduction of the AV based on mean values after AC treatment compared to feedstock. Conjugated double bounds were reduced after the alkali refining and bleaching

process, but increased after AC treatment. However, the mean level was still below the crude oil.

6.4 Molecular Dynamics modeling (Paper III)

The selectivity of PCB adsorption from fish oil onto AC was investigated by means of molecular dynamics to determine the importance of molecular planarity. PCB congeners 77 (*non-ortho*) and 118 (*mono-ortho*) were selected for comparison purposes due to pronounced differences in mean adsorption efficiency and molecular geometry. Triolein, a triacylglycerol of oleic acid (C18:1), was used to represent fish oil. A graphitic carbon structure with pore diameter 20 Å was set up to serve as AC model. The complete system comprised a number of PCB molecules dissolved in triacylglycerol that overlaid and filled the pores of an AC structure.

At the start of the simulation only seven PCB molecules was situated within the pores of the AC. After the production run (4 nanoseconds) no new PCB molecules entered the pores, nor did any of the initial seven molecules leave. Due to the partial atomic charge at the pore edge, the triolein molecules tended to orient their polar heads towards the edges (Figure 2 Paper III) with the non-polar tails pointing outwards. The PCB molecules tended to accumulate in this non-polar region of the system instead of entering the pores. Inside the pores the large hydrocarbon tails of the triolein molecules tended to block the movement of the PCB molecules against the wall. This system behavior had a detrimental effect on the simulation and emphasizes the need for a more thorough investigation based on an improved assignment of the partial charges for the AC.

The majority of PCB molecules trapped in pores were attached via Cl-AC "bonding", leaving the main part of the PCB molecule free to interact with triolein. In this orientation a chlorine atom can move in-between the negatively charged AC surface and interact with the positively charged second row of carbon atoms. At the same time the molecule will have most of its surface available to interact with triolein. The Cl-AC adsorption energy was found to surpass the energy criteria conventionally

used for hydrogen bonds (-10 kJ mol^{-1}). Planar orientation to the AC surface was only observed for a PCB 77 molecule positioned on top of the graphite sheet (Figure 3 Paper 3). This position gives an energetically favorable π -cloud overlap.

Due to the lack of interchange of PCB molecules between pore and bulk phase, the simulation did not support any selectivity for adsorption of non- and mono-*ortho* PCBs to AC. Both pores in the model had a width of 20 Å, slightly exceeding the micro-pore range. A smaller pore width below 7.5 Å might increase the selectivity as this will impose rotational restrictions for PCB 118 with a resulting entropy penalty. In addition, introduction of wall defects with some negative outer carbons removed and inner positive ones exposed, may result in electrostatic interactions becoming more favorable for the planar conformation.

6.5 Short-path distillation (Paper IV)

A factorial experimental design based on temperature (172-228 °C), feed rate (2.3-7.8 kg/h) and addition of WF (0-4%; fatty acid ethyl esters) was used to model a SPD process applied for removal of PCDD/Fs, DL-PCBs and PBDEs in fish oil. The average reduction in chemical concentration of the individual congeners was linearly dependent on the number of chlorine or bromine substitutions within each homologue group. DL-PCB congeners could also be separated based on *ortho*-substitution. The pattern is consistent with reported values for saturation vapor pressures of the studied POPs, i.e., linearly correlated to the degree of chlorine or bromine substitution. In addition, the number of *ortho*-substitutions will influence the vapor pressure of PCBs and PBDEs. The analyzed PBDEs included mono-, di- and tri-*ortho*-substituted congeners that could not be separated in the correlation plot due to few observations. By including the number of Cl/Br- and *ortho*-Cl/Br substitutions of the individual congeners as new predictor variables, it was possible to develop response models representing all congeners within each homologous group based on PLSR. WF addition was highly correlated to amount of distillate and was exchanged with the latter variable to establish a more mechanistic model according to the Langmuir-

Knudsen equation. This modeling approach also makes it easier to compare the response to the optional direct addition of a “washing liquid” to the condenser.

The response models show a comparative large positive effect of the process variables temperature and amount of distillate and a negative effect of feed rate. The decrease in saturated vapor pressure and molar flux with increasing Cl/Br-substitution and molecular weight is expressed by the negative effect of the number of Cl/Br-substitutions. For DL-PCBs and PBDEs also a positive effect of the number of *ortho*-chlorine/bromine substitutions were observed. Cross validated predictive ability of the models was in the 4–9% range.

It will not be possible to define optimum operation conditions for POPs reduction in fish oil by SPD due to the large variance in vapor pressures for the multi component mixture of organic compounds. Both individual congener levels and the ratio between PCDD/Fs and DL-PCBs will influence the reduction in WHO-TEQ at specific operation conditions. Generally high temperature, low feed rate and WF addition improved the decontamination efficiency.

The feedstock WHO-TEQ-distribution showed that the dominant congeners belonged to the more volatile tetra- and penta-chlorinated groups, i.e., the relative distribution, volatility and toxicity of the dominating congeners was favorable with respect to SPD decontamination. WHO-TEQ-reduction > 90% could easily be achieved in the SPD process bringing the residual levels down to < 2.1 ng WHO-PCDD/F-PCB-TEQ/kg, i.e., considerably below present maximum permitted levels in European food and feed legislations.

6.6 Oil quality assessment after refining and short-path distillation (Paper V)

SPD is established as the most effective industrial process to remove POPs in fish oil. However, the technology involves heating of the oil to high temperature levels (>200 °C) that possibly give unwanted heat-induced side reactions and co-evaporation of minor compounds of importance for the nutritional quality of the oil. The effects on

retention of vitamins, cholesterol, and unsaponifiable compounds, geometrical isomerization, loss of PUFA, oxidation level, and oxidative stability was studied on the basis of experiments designed to optimize and model the effect of process conditions on the reduction of POPs (Paper IV). Loss of volatile nutrients was observed, but the extent will depend on the process conditions needed to obtain target decontamination level, as well as the ratio and difference in vapor pressure between free and ester forms of the studied compounds. Analysis of the ratio between free and ester forms was outside the scope of this study. However, based on reported SPD elimination curves most of vitamin A and D in cod liver oil exist in ester form resulting in a high retention relative to the TEQ-reduction at the used experimental conditions. Vitamin A was quantitatively removed by the used bleaching step but retention after SPD somewhat lower than vitamin D can be expected depending on the composition of the fatty acid moiety. Vitamin E exists in free form and retention after SPD down to 36% was observed. Vitamin K is a mixture of phylloquinone (vitamin K₁) and menaquinones (vitamins MK-n) and the degree of retention relative to the TEQ-reduction was reflected by the molecular weight: K₁ < MK-4 < MK-7 < MK-8. Cholesterol was only found in free form and to a small extent reduced (16%) after the alkali refining and bleaching step, probably as a result of incorporation into micelles transferred to the soapstock. Retention after SPD was at lowest down to 31%. Unsaponifiables were highly correlated to cholesterol ($R^2 = 0.84$), but showed a somewhat higher degree of retention (down to 54%).

Some reduction in oxidation level (measured based on PV and AV) was observed after alkali refining and bleaching, and further after SPD. The PUFA level was conserved with no detectable thermally induced trans isomerization of EPA and DHA. The oxidative stability was reduced after alkali refining and bleaching. After SPD both further reduction and improvement of the stability was observed depending on the applied process conditions. The pattern could to some extent be attributed the degree of tocopherol retention and reduction of PV. Addition of 150 ppm BHT to the fish oil after SPD improved the oxidative stability to a level above the initial crude.

Optimal process conditions were modeled that ensure removal of POPs to within legislation levels while retaining most of the vitamin levels in fish oil. A 76% reduction of the WHO-PCDD/F-PCB-TEQ level in the used feedstock was needed to be in accordance with the voluntary industrial monograph of GOED. This could be achieved on the basis of operation conditions giving <20% loss of vitamins. A 90% decontamination rate gave vitamin retentions in the 60-90% range. Any need for fortification of the oil to meet target final product specifications will depend on the specific food or feed application.

7. Conclusions

The papers included in this thesis have improved the fundamental understanding of the possibilities and limitations of alternative process technologies aimed to reduce the level of persistent organic pollutants in fishmeal (i.e., mechanical fat separation, organic solvent extraction and oil leaching) and fish oil (i.e., AC adsorption and SPD). The main conclusions are summarized in the following:

- Chloroform-methanol extraction (BI&D extraction) or equivalent is the best protocol to estimate POPs partitioning and reduction based on improved fat separation in the fish meal process.
- Optimization of the existing fat separation steps is expected to be the most cost-effective way to reduce the POPs content in fishmeal. However, the obtainable effects are limited and have to be combined with organic solvent extraction or oil leaching if a high decontamination rate (estimated to above 20-30%) is needed. Protease and high temperature (121 °C) treatment of press cake and stickwater concentrate have marginal effects. However, reduced viscosity of the stickwater after protease treatment might improve the fat separation if performed before the final concentration step. The efficiency of the proposed oil leaching process is comparable to hexane and isopropanol extraction of fishmeal.
- The level of PCDD/F, DL-PCBs and PBDEs in fish oil increase after alkali refining and bleaching. The effect is proportional to the free fatty acid level and can be attributed to the partitioning of lipophilic compounds in the continuous lipid phase during soapstock separation and water washing steps.
- Activated carbon adsorption of PCDD/Fs is highly effective with obtainable WHO-TEQ-reduction of 99%. Adsorption of DL-PCBs is less effective and dependent on *ortho*-substitution, i.e., non-*ortho*-PCBs are adsorbed more effectively than mono-*ortho*-PCBs. The maximum obtained non-*ortho* and mono-*ortho* WHO-PCB-TEQ reduction in this study was 87% and 21%,

respectively, corresponding to a total WHO-PCB-TEQ reduction of 73%. Compliance with present feed and food EC WHO-PCDD/F-PCB-TEQ legislations in fish oil can be achieved based on AC adsorption. The voluntary GOED specification of maximum 3 ng WHO-PCB-TEQ/kg could not be met based on the tested conditions.

- The level of mono-, di-, and tri-*ortho* PBDEs could not be reduced by use of AC adsorption. The selectivity between the studied POPs can be explained based on dispersive electron interaction affected by sorbate planarity and steric effects. MD simulation of the adsorption mechanisms revealed that planar adsorption of PCB molecules will be heavily favored by short range interactions and hindered by presence of electrostatic forces. This selectivity will be further promoted in case of AC with neutral rather than substantial partial charges like in our model. In addition a pore size below the gyration radius of mono-*ortho* PCBs will favor the adsorption of the more co-planar non-*ortho* PCB congeners.
- The reduction after SPD of the individual PCDD, PCDF, DL-PCB and PBDE congeners is linearly dependent on the number of chlorine or bromine substitutions within each homologue group. In addition, DL-PCB and PBDE congeners can be separated based on *ortho*-substitution. The quantitative structure property relationships (QSPR) were combined with process parameters to establish decontamination models for each homologue congener group with cross validated RMSEP in the 4-9% range.
- The efficiency of SPD is mainly dependent on the volatility of the respective compounds and, compared to AC adsorption, less influenced by the conformation and chemical nature of the POPs to be removed. High decontamination efficiency (> 90%) can be obtained by choice of favorable process conditions (i.e., high temperature, low flow rate and adequate distillate flow) giving residual levels considerable below MPLs in feed and food.

- AC adsorption and SPD did not have any negative effects on fish oil oxidation level within the tested operation ranges. The applied high temperature short time SPD conditions did not affect PUFA level nor induce geometrical isomerization. Oxidative stability of the oil after SPD was affected both negative and positive depending on processing conditions.
- Some co-evaporation and loss of vitamins, cholesterol and unsaponifiables can not be avoided during SPD. The retention level will depend on the applied process conditions needed to obtain target decontamination level, as well as the concentration ratio and difference in vapor pressure between free and ester forms of the respective compounds.
- A high retention level (>80%) of volatile nutrients after SPD can be obtained by chose of process conditions giving residual WHO-PCDD/F-PCB-TEQ levels in accordance with present EC feed and food legislations and the voluntary industrial monograph of GOED.

8. Future outlooks

The levels of PCDD/Fs and DL-PCBs in several regions of the world can be expected to be of environmental and health concern for several decades to come. Although the control of main emission sources have improved and observed levels in the biota reduced the last decades, the levels of new emerging POPs create additional challenges. Development and optimization of cost-effective decontamination technologies will be one of many necessary actions needed to reduce and minimize the impact on population health.

The proposed oil leaching process for fishmeal decontamination requires further optimization, but has several advantages compared to organic solvent extraction. These include easy implementation in an existing fishmeal processing line, use of a safe and non-flammable extraction medium, and possible lower investment and operation costs. Needed studies include effects of presscake pretreatment and optimization of the oil leaching conditions. Also subsequent reduction of the fat level should be studied to obtain a final fishmeal with acceptable physical and chemical properties.

The selective sorption of PCDD/Fs vs. non- and mono-*ortho* PCBs to AC have been elucidated in this thesis. Improved understanding of the trapping mechanisms involved should be further pursued based on molecular dynamics simulation. Such knowledge might also unveil new possibilities in tailor making of novel sorbents with improved adsorption properties for different groups of POPs. Simulation studies should be followed up experimentally to validate the findings and quantify the adsorption capacity and kinetics of alternative adsorbents.

Further studies are needed to optimize and model the effect of alternative volatilization and partition based technologies on the removal of different POPs. Especially the needed process conditions for removal of new substances included in the amended Aarhus Protocol and Stockholm Convention should be studied. Both experimental based empirical modeling approaches and process simulation models

based on physical and chemical properties and computational engineering should be explored. Currently, also the large variation in reported physical data limits the possible simultaneous modeling of existing and new POPs candidates.

The reduction of persistent organic pollutants in fishmeal and fish oil will introduce additional processing costs. At present, this is mainly covered by the fishmeal and oil producers processing fish raw material caught in polluted ocean areas. An economical comparison of alternative decontamination strategies is needed to elucidate the investment and production costs involved, and to identify the most cost-effective technology. Recent reports on the possible combined negative effects of low levels of POPs in feed on fish growth and health might, together with the general population concern on POPs content in seafood, initiate the development of a new voluntary feed monograph specifying maximum levels below the official MPLs, corresponding to the existing GOED fish oil monograph. This might in the future also even out the economical burden on all the industrial actors in the value chain from feed ingredient manufacturing to fish farming.

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Errata

Paper I: Ranking of the fat levels reported in Table 1 relative to the applied extraction protocols is wrongly stated in the last paragraph on page 2015. The correct ranking is BI&D > EC > Soxhlet.