Effects of alkylphenols (C_4-C_7) on the reproductive system of Atlantic cod (*Gadus morhua*).

I. *Long-term effects of para-substituted alkylphenols (C_4-C_7) on the reproduction in cod .*

II. *Development of analytical methods for detection of low levels of alkylphenols in oil produced water and biological tissues*

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The degree of Doctor Scientiarum (dr.scient)

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Abstract

The work in this thesis is a part of a strategic work at the Institute of Marine Research, which aims to develop a variety of methods to be used in investigating potential endocrine disruption in Atlantic cod (*Gadus morhua*).

Produced water, a by-product of offshore oil production, contains significant amounts of alkylphenols (APs). Many studies have shown that long-chain para-substituated APs cause endocrine disruption in freshwater fish, but relatively little is currently known about their long-term effects on the biology of marine fish. Here we describe the results from two experiments studing in detail the effects of some APs present in produced water on the reproductive potential of first-time spawning Atlantic cod. Groups of cod were fed pastes containing four APs (4-*tert*-butylphenol, 4-*n*-pentylphenol, 4-*n*-hexylphenol and 4-*n*-heptylphenol), at different concentrations for either 4 months (experiment 1) or 5 weeks (experiment 2). AP-exposed fish were compared to unexposed fish and to fish fed paste containing natural estrogen (17 β-estradiol). The results of the present study suggest that multiple mechanisms underlie the responses in the AP treated cod. The exposure to APs influences the plasma concentration of several male and female sex hormones and the egg yolk precursor protein, vitellogenin, in Atlantic cod. This study also shows that AP-exposure down to 20 μg/kg body burden interferes with the maturation of the sex organs, and that this effect is likely caused by disruption of the sex hormone system. There were also found effects of the AP treatment on the hepatic P450 systems (CYP1A and CYP3A) as well as glutathione, glutathione-related enzymes and changes in the lipid composition in liver and brain membranes.

Even though the concentrations used in our experiments are higher than may be reasonably expected as the result of oil production alone, measurements of actual AP levels in the sea indicate that APs may still be a significant risk factor in the marine environment.

List of Abbreviations

11KT	11-ketotestosterone
11β-HSD	11β-hydroxysteroid dehydrogenase
17α,20-βP	17α, 20β-dihydroxy-4-pregnen-3-one
17β-HSD	17β-hydroxysteroid dehydrogenase /oxidoreductase
20β-HSD	20β-hydroxysteroid dehydrogenase
3β-HSD	3β-hydroxysteroid dehydrogenase
AhR	Aryl hydrocarbon receptor
AP	Alkylphenol
APE	Alkylphenol ethoxylates
APEC	Alkylphenol carboxylates
AR	Androgen receptor
BCF	Bioconcentration Factor
BP	Butylphenol
BPG	brain-pituitary-gonadal axis
BHT	2,6-di-*tert*-butyl-4-methylphenol
cAMP	Adenylate cylase
CAR	Constitutive androstane receptor
CAT	Catalase
CNS	Central-nervous-system
Da	Dopamin
DCM	Dichloromethane
E2	17β-estradiol
EDC	Endocrine disrupting chemicals
ER	Estrogen receptor
EROD	Ethoxyresorufin-O-deethylase
FSH	Follicle-stimulating hormone
GABA	γ-aminobutyric acid
GC-MS	Gas Chromatography-Mass Spectrometry
GC-ECD	Gas Chromatography-Electron capture detector
GC-FID	Gas Chromatography-Flamme ionisation detector
GnRH	Gonadotropins releasing hormones
GR	Glutathione reductase
GSH	Glutathione
GSI	Gonadosomatic index
GST	Glutathione S-transferase
GTH	Gonadotropine
HepP	Heptylphenol
HexP	Hexylphenol
HSI	Hepatosomatic index
ICE 182,780	7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]-17β-estradiol
LC	Lethal concentration
LH	Luteinising hormone
LOEL	Lowest observed effect level
MAPK	Mitogen activated protein kinase
NSC	Neural stem cells
NOAEL	No observable adverse effect level
NP	Nonylphenol
NPE	Nonylphenol ethoxylates
OP	Octylphenol
P45011β	11β-hydroxylase

P450c17	17α-hydroxylase/17,20 lyase
P450C21	21-hydroxylase
P450scc	P450 cholesterol side chain clevage
PGC	Primordial germ cell
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PNEC	Predicted no effect concentrations values
PP	Pentylphenol
PUFA	Poly unsatureted fatty acids
PXR	Pregnane X receptor
QSAR	Quantitative structure – activity relationship
ROS	Reactive oxygen species
SFA	Saturated fatty acids
SOD	Superoxide dismutase
SPE	Solid phase extraction
StAR	Steroidogenic acute regulatory protein
SULT	Sulfotransferases
T	Testosterone
UDPGT	Uridine diphospho-glucuronosyltransferases
VTG	Vitellogenin
Zrp	*Zona radiata* protein

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1. Introduction

1.1 Background

There is need for more knowledge of the effects on the marine environment due to discharges to the sea from the offshore oil and gas industry. Norway is currently in a period where discharges of produced water from the petroleum sector are increasing rapidly as the oil fields ages. Produced water is defined as the water that comes up with oil and gas from sea bed reservoirs, separated on the platform from the oil and discharged into the sea. In 2004 it was estimated that 143 million m^3 of produced water was released (OLF, 2005). The prognoses show that the increasing trend will continue until 2011, and the discharges may reach 180 million m^3 a year before it starts to decline. More knowledge of the long-term effects on the marine environment are essential for the authorities to ensure a healthy development of this sector and to coordinate the exploitation of Norwegian oil and gas reserves with other uses of the marine environment. A central aspect of this is that the total impact on the marine environment must not lead to changes in biological diversity or in the marine ecosystem.

In 1997, the Institute of Marine Research started the project "The hormonal effects of alkylphenols on cod (*Gadus morhua*)" that aimed to clarify potential harmful effects of alkylphenols (APs) on cod. Significant quantities of APs are released into the sea by petroleum installations as a result of discharges of produced water. It has been shown that APs may have estrogenic (feminising) effects on fish and animals, resulting in reproductive disturbances. The question was whether cod, Norway's most important commercially fished species, might be similarly affected. Experiments with long-term exposure of cod by environmental relevant doses of selected APs were therefore carried out.

1.2 Brief overview of the endocrine regulation of fish reproduction

The endocrine system plays a essential role for a successful reproduction, and is involved in multiple reproductive functions in vertebrates, like: sex differentiation and development of sexual organs, initiation of puberty, development of secondary sexual characteristics, sexually behavior and controlling the reproductive cycles.

There are more than 24000 different fish species and between these there are large variety both in mechanism of sex determination and reproductive physiology (Nelson, 1994). In thise thesis, the focus will be on gonochoristic teleosts, like cod (gonochoristic: species with separate sexes, the male and female reproductive organs being in different individuals, as opposed to hermaphroditic, gynogenetic, and hybridogenetic).

Sex determination and sex differentiation.

Sex determination and sex differentiation are defined as two different, but closely connected processes (reviewed in Devlin and Nagahama, 2002). The sex determination is the primary control (often predetermined genetically at fertilization) that leads to sex differentiation, the development and expression of the male or female phenotypes (development of testis or ovary). In fish embryos, the germ cells are only present as undifferentiated primordial germ cells (PGCs) and are similar for both sexes, and will later differentiate into oogonia (females) or spermatogoina (Males). Sex differentiation takes first place after hacthing, but there are large differences between species where in the larvae development this takes place. However, it appears to be common that there are a relative short “critical period” where the fish larvae is especially sensitive for the hormonal signals that initiate cell differentiation of PGC and somatic gonadal cells. The endocrine regulation of sex differentiation is not completely understood, but it involves a complex interplay between the brain and gonad and it is clear that sex steroid plays a very important role. Steroid producing enzymes (chapter 1.3) can be detected prior to sex differentiation and especially aromatase, the enzyme responsible

of the last steep in the estrogen synthesis, is found present in the brain (of both sexes) and the gonad (for females) in the time of sex differentiation (Devlin and Nagahama, 2002). Since the appearance of steroid synthesizing cells requires cells differentiation, it is unlikely that the steroids themselves are the primary factors involved in determination of sex ("who came first the hen or the egg"?). Nevertheless, steroid production is very closely correlated with early steps of gonadal differentiation. This is clearly showed by the adverse affect on sex differentiation that can be caused by interfering with the steroid balances. For example, inhibition of estrogen synthesis in early development using aromatase inhibitors can cause masculinization while treatment with exogenous estrogen can cause feminization in many fish species.

The essential role of steroid hormone makes the sex differentiation event vulnerable for endocrine disruption as will bediscussed later in the thesis.

Puberty.

Puberty is the development that brings an immature juvenile to a mature adult reproductive system (Schulz and Goos, 1999). The timing of puberty is in addition to genetic factors also controlled by a variety of external stimuli like photoperiod, water temperature and availability of food. The pubertal maturation is synchronized via the brain-pituitary-gonadal (BPG) axis, and the onset of puberty starts with stimulation of the synthesis of the neuroendocrine decapeptide gonadotropins-releasing hormones (GnRH) in the brain (Welzien et al., 2004; Whitlock et al., 2006). These do in turn control the secretion of gonadotropins (GTH) from the pituitary (follicle-stimulating hormone (FSH) and luteinising hormone (LH)). The GTHs are heterdimeric glycoproteins, consisting of a common glycoprotein α-subunit and a hormone-specific β-subunit. The GTHs are transported by the blood to the gonads and binds to specific membranes reseptors on the gonadal somatic celles, Leydig and Sertoli cells in testis and thecal and granulosa cells in the ovary. The GTHs stimulate the maturation of the gonads and cause these to produce sex steroid hormones, 17 β-estradiol (E2) and

testosterone (T) in female fish; T and 11-ketotestosterone (11KT) in males. Sex steroids have important feedback effects on secretions of hormones from the pituitary and the brain, but are also required in the gonads for germ cell maturation (for details, see (Baroiller et al., 1999; Nagahama, 2000)). Puberty is the first step into oogenesis and spermatogenesis.

Oogenesis

After sex differentiation at larvae stages, the oogonia increase in numbers in the gonads through mitotic proliferation. Oogenesis begins at puberty, when a portion of the oogonia entry into meiosis and becomes primary oocytes. The meiosis is arrested in diploytene stages of prophases I, and the oocyte stays like that through out the growth phases, and until final oocyte maturation where the first meiotic division is completed. The second meiotic division of the oocyte is first completed after fertilization. Together with the onset of previtellogenic growth the folliculogenesis is started and the ovarian follicle is formed (figure 1). In the follicle the oocyte is covered with granulosa cells, which in turn is surrounded by thecal cells. A part of the formation of the follicle is the zonagenesis. Eggshell proteins, *zona radiator* proteins (Zrp) are synthesized in the liver under the influence of E2 and transported to the ovary and incorporated in the corian around the ooctye. Vitellogenisis is the major growth phases of the oocyte and account for as much as 90 % of the final egg weight. Vitellogening (VTG) is a glycophospholipoprotein and the main source of yolk proteins and lipids in the growing oocyte. VTG is, like Zrp, synthesized in the liver in response to E2 and transported by the blood and taken up by the oocyte through receptor-mediated endocytosis (Tyler and Sumter, 1996; Tyler et al., 1999).

The BPG axis plays a central role in regulating the oogenesis by controlling the synthesis of sex steroids (T and E2) that in turn are stimulating the oocyte growth (illustrated in figure 3). In the end of vitellogenisis the BPG axis stimulates a shift in the steroidogenesis of the ovary from synthesizing E2 to produce maturation-inducing

steroids (MIS), which leads to the breakdown of germinative vesicles, maturation of the oocyte and ovulation. At least two different steroid hormones have been identified to induce final maturation in teleost: 17α,20β,-dihydroxy-4-pregnen-3-one (17α,20β-P) and 17,20β,21-trihydroxy-4-pregnen-3-one (17,20β,21-P) (Nagahama, 1997).

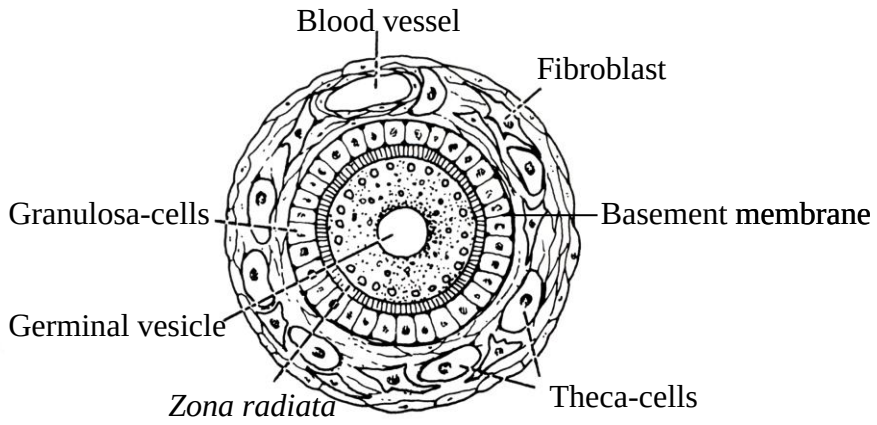

Fig 1. *Diagram of a primær ovarian follicle in fish. The oocyte is covered by zone radiata (also known as chorion and wich lather becomes the egg shell). The oocyte is closely connected with granulosa cells by microvilli located in the chorionic pores. The oocyte and granulosa cells are separated from the surrounding theca cells, blood vessel and fibroblast by a basement membrane. (Illustration: Stein H. Mortensen, IMR).*

Spermatogenesis

During spermatogenesis the male germ cells go through four major phases:
1). Mitotic proliferation, where the Spermatogonial stem cells undergo a specific number of mitotic cycles, leading to both new stem cells and differentiated spermatogonia. 2). Meiosis, where the differentiated spermatogonia undergo meiosis and becomes primary spermatocytes, secondary spermatocytes and finally haploid spermatides. 3). Spermiogenesis, where spermatides are transformed into flagellated spermatozoa. 4). Sperm maturation, where nonfunctional spermatozoa develop into mature spermatozoa (fully capable of motility and fertilization) (Schulz and Miura,

2002; Miura and Miura, 2003; Welzien et al., 2004). The germ cells development is depending on close association with Sertoli cells. Sertoli cells enclosed the germ cells into so called spermatocyst. Each spermatocyst contains clone of germ cells that all are in the same stages of development (figure 2). Cell-Cell communication through gap junctions between Sertoli-Sertoli, Sertoli-germ and germ-germ cells in the spermatocyst is essential for the spermatogenesis. This junctional complexe does together with the basement membrane result in a blood-testis barrier, isolating the germ cells to a Sertoli cell determinated enviroment. The testis lobules are separated by connective tissue containing fibroblast, blood vessels and Leydig cells.

Sex steroids play an important role several places in the spermatogenesis. E2 is part of the regulation of spermatogonia renewal, spermatogonial proliferation toward meiosis is promoted by 11-KT and sperm maturation is regulated by 17α,20β-P (MIS) (Miura and Miura, 2003). Figure 3 gives a simplified schematic diagram of hormone regulation through the BPG axis.

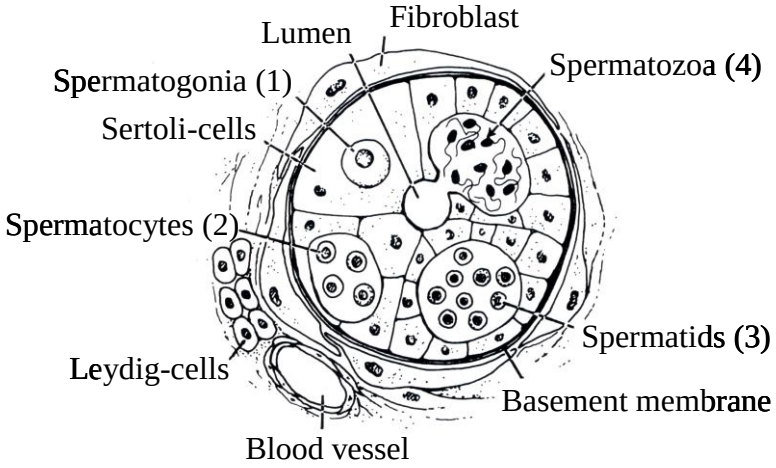

Fig. 2. *Cross-section of testicular lobule with spermatocysts (germ cells surrounded by Sertoli-cells) containg the different stades of sperm development. The number shows the chronological order in development. (Illustration: Stein H. Mortensen, IMR).*

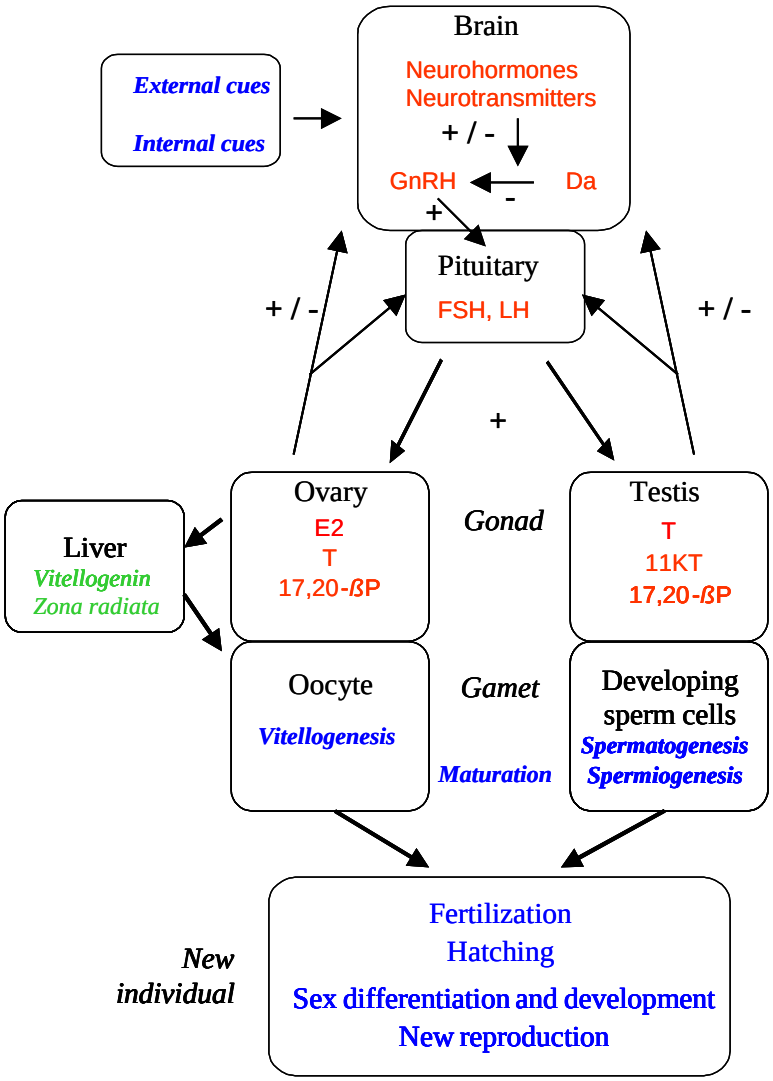

Fig. 3*. The reproductive system of fish and possible sites of action of contaminants. The brain-pituitary-gonadal axis is aktivated by external stimuli (like temperature, photoperiod, pheromones, social behavere, etc) and internal stimuli (biological cloks, nutritional status, etc). The hormonal system is regulated by a series of complex feedback mechanisms between the organs involved. (Da = dopamine; GnRH = gonadotropin-releasing hormone; FSH = follicle-stimulating hormone; LH = luteinising hormone; E2 = 17β-Estradiol; T = testosterone; KT = 11-ketotestosterone; 17,20-βP = 17α, 20β-dihydroxy-4-pregnen-3-one). Black – structures, red – hormones/neurotransmitter, green – protein, blue – processes.*

1.3 Steroid biosynthesis

The steroidogenesis is a complex process converting cholesterol into biological active steroids. The biosynthesis of steroid hormones is mainly happening in the gonads (ovaries and testes), the adrenals and the brain (Kime, 1987; Nagahama, 2000; Schumacher et al., 2003).

Figure 4 shows the biosynthesis pathways, even though it looks very complex it is only involving a small numbers of enzymes:

- Desmolase/lyase that cleave the side chain between carbon 20 – 22 (P450scc = $P450_{c11A}$) and carbon 17 – 20 ($P450_{c17}$).
- Hydroxylases that incorporate hydroxyl groups at different places ($P450_{11\beta}$, $P450_{c17}$, $P450_{C21}$).
- Hydroxysteroid dehydrogenase/oxidoredutase that oxidize hydroxyl-groups into keto-groups or reducing keto-groups to hydroxyl-groups (3β-HSD, 11β-HSD, 17β-HSD, 20β-HSD).
- Aromatase that converts androgens into aromatic estrogens ($P450_{arom} = P450_{C19}$).

The steroidogenic enzymes are located both in the mitochondria and in the endoplasmic reticulum and the synthesis involves transport between the different organelles. The rate-limiting step in the steroidogenesis is the transport of cholesterol between the outer and inner mitochondria membrane where the P450scc is located and the first conversion of cholesterol to pregnenolone takes place. Cholesterol cannot move over the intermembranal space by itself, but is actively transported by the steroidogenic acute regulatory (StAR) protein (Stocco and Clark, 1996).

In the teleost gonads both the StAR and the steroidogenic enzymes are regulated by the GTHs (FSH and LH). The seasonal pattern of FSH and LH differ between different species (Hellqvist et al., 2006), but there are some common mechanisms. In female fish increasing secretion of GTH from the pituitary glands stimulates increased synthesis of sex steroids (E2, T) in the gonads. Plasma levels of E2 and T are rising during vitellogenesis and peaks just before the start of spawning. Changes in the GTH signal then create a shift in the steroidogenesis by down-regulation of $P450_{arom}$ and up-regulating 20β-HSD, leading to a drop in E2 production and a rise in 17α,20β-P (inducing final maturation of the oocyte) (Senthilkumaran et al., 2004). Similar regulation of the steroid synthesis is also seen throughout the spermatogenesis (Schulz and Miura, 2002). The synthesis of E2 in the ovary is mediated by a two-cell system, where thecal cells in the outer follicular layer are converting cholesterol into T. T is secreted from the thecal cells and taken up by the granulosa cells in the inner follicular layer, where T is aromatized into E2 (Nagahama, 1994). In the testis, all steroid synthesis occurs in the Leydig cells.

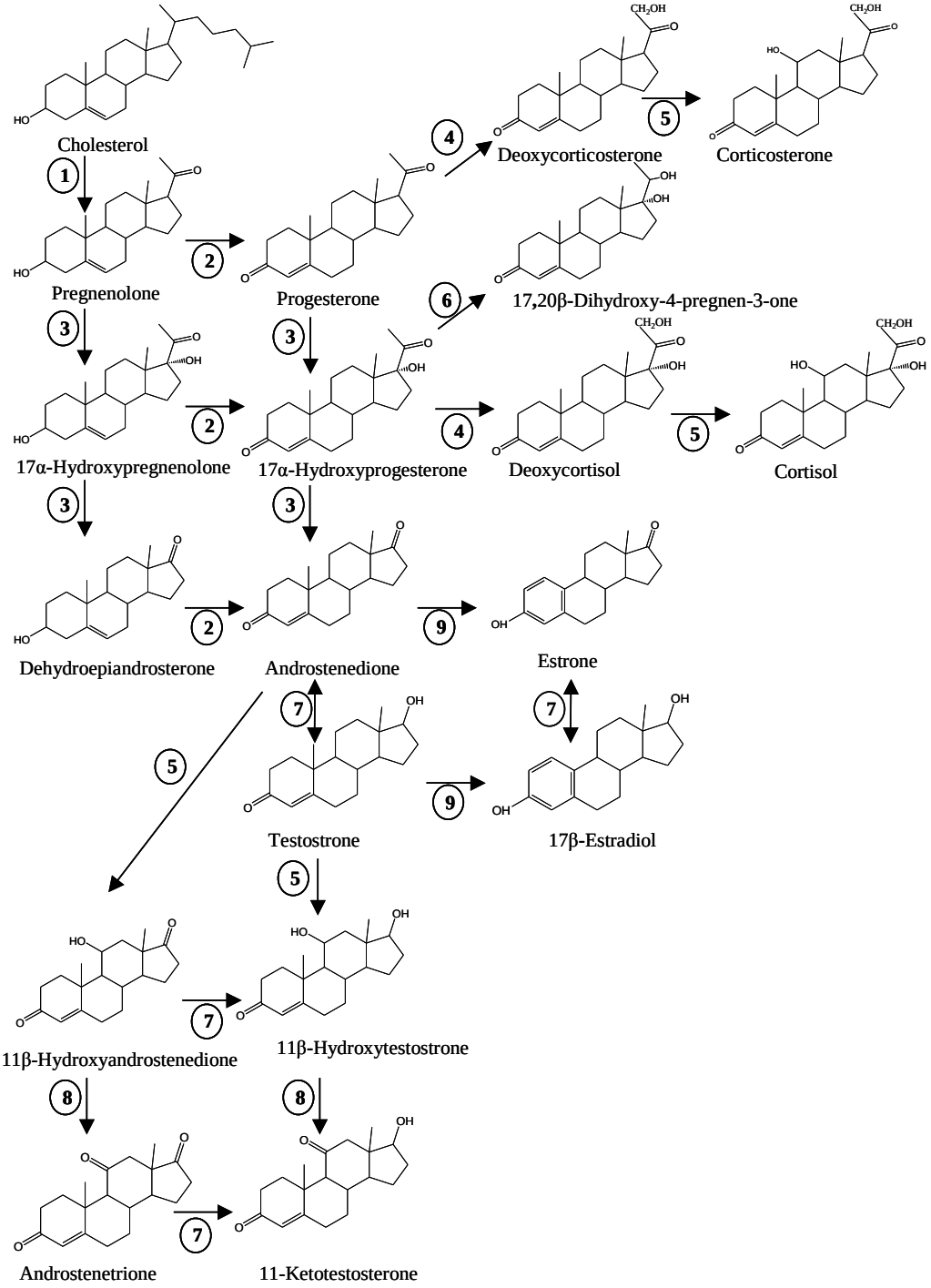

Fig. 4. *Biosynthesis pahtways of steroids in teleosts. Cholesterol is converted to pregnenolone by the enzyme (1) cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc). The other enzymes involved in the steroid synthesis are: (2) 3β-hydroxysteroid dehydrogenase (3β-HSD); (3) 17α-hydroxylase/17,20 lyase ($P450_{c17}$); (4) 21-hydroxylase ($P450_{C21}$); (5) 11β-hydroxylase ($P450_{11\beta}$); (6) 20β-hydroxysteroid dehydrogenase (20β-HSD); (7) 17β-hydroxysteroid dehydrogenase-oxidoredutase (17β-HSD); (8) 11β-hydroxysteroid dehydrogenase (11β-HSD); (9) aromatase ($P450_{arom}$).*

1.4 Distribution and reproduction physiology of Atlantic cod

There are a number of separated stocks of Atlantic cod spread all over the north Atlantic from the coast of Newfoundland Canada/USA in west to the Barents Sea in east. Atlantic cod is a cold-water species and the southern distribution limit is in the English Channel (ICES, 2005). The Arcto-Norwegian cod in the Barents Sea is the largest cod stock in the world and is one of the few stocks that are in reasonably good conditions. Many of the other cod stocks have experienced a dramatic decline since the late 1980s, and several stocks have collapsed and have problems recovering (Myers et al., 1996; Cook et al., 1997; Fu et al., 2001). In the Norwegian part of the Atlantic, the North Sea cod stock is now at a historically low size (Cook et al., 1997; Rice, 2006). The main factor of the collapse in the cod stocks is overfishing. However, there are speculation on whether ecosystem regime shifts, probably driven by climate changes, can be the reason for the lack of recovery that are observed (Gao, 2002; Beaugrand et al., 2003; Alheit et al., 2005). This study has been initiated by the question if pollution and especially endocrine disrupting chemicals (EDC) from the oil industry discharges play a role in the poor recruitment of the North Sea cod.

The Atlantic cod is an asynchronous batch-spawner. The ovary of the cod contains therefore ooctyes at many different stages of development through out the oogenesis and the process of vitellogenesis, final maturation and ovulation are ongoing paralleled in the spawning period. Large cod can spawn 20 batches of eggs over a period of 6-8 weeks from February to April (Kjesbu et al., 1996). The cod have small eggs and a very high fecundity. It is normal that large cod spawn more than 2 million eggs. The cod, as a species (or in local terms, as a stock) has a long spawning season of more than two months and sometimes as long as three months (Brander, 1993). However, in UK waters as many as two thirds of the eggs are spawned during a period of four to six weeks. The spawning season appears to be centred on the period of plankton blooms, with *Calanus finmarchicus* as an important species (Brander, 1994). It is important to ensure that as many eggs as possible will hatch at a time when the availability of food

and the level of predation are optimal, thus ensuring good larval survival (Ellertsen et al., 1986; Gotceitas et al., 1996).

The photoperiod is considered the most important factor for the timing of the sexual maturation of the cod (Norberg et al., 2004). Vitellogenesis starts in October (Kjesbu and Holm, 1994) but the main oocyte growth phase is in the month just prior to spawning. As for other teleost species, the oocyte grow by taking up VTG, which is synthesized in the liver and regulated by E2 (Silversand et al., 1993). The steroid hormone levels in the plasma reflect well the timing of maturation and spawning of cod. In female cod, the E2 levels rise from < 1 ng/ml early in the vitellogenesis up to 40 ng/ml prior to spawning. Testosterone follows a similar seasonal fluctuation as E2, but with lower concentrations (maximum 3-4 ng/ml) (Norberg et al., 2004). Male fish mature earlier that the females and the males often have testis with running sperm many weeks before the spawning. The plasma levels of T and 11-KT are strongly correlated with testis growth (Dahle et al., 2003).

Aquacultured cod mature much earlier than wild fish, and it is normal that farmed cod are first-time spawners at the age of 2 years due to optimal food conditions (Karlsen et al., 1995). Wild cod on the other hand mature between 4 and 8 years old (Norwegian coastal cod and Arcto-Norwegian cod, respectively) (Godo and Moksness, 1987).

1.5 Effects of pollution on reproduction

Aquatic pollution may have severe effects at several different levels in the reproductive cycle of fish (Kime, 1995). Since the beginning of the 90s there has been a sharp focus on hormone-disrupting substances. A large number of chemical compounds have been shown to “resemble” hormones or in other ways to affect the hormonal balance, thus disturbing natural reproductive processes. Chemicals with “estrogen mimicking” effects have caused most concern (Reviewed by (Arukwe and Goksoyr, 1998)). Growing attention is now also paid to other classes of hormones, such as the androgen system (Kelce and Wilson, 1997; Fang et al., 2003) and the thyroid hormones

(Oberdorster and Cheek, 2001; Brown et al., 2004). Among the xenobiotics that have been shown to have estrogen-disrupting effects (whether agonistic or antagonistic) we find APs, phthalates, bisphenol A, chlorinated hydrocarbons such as polychlorinated biphenyls (PCBs), dioxins and pesticides such as chlordane, dieldrin, DDT and its metabolite DDE (Arukwe and Goksoyr, 1998).

The endocrine apparatus is a complex system with many factors and is therefore liable to suffer disturbances at many levels as described by the general definition of hormone-disrupting substances, i.e. that they are "exogenous agents that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones" (Kavlock et al., 1996).

The high degree of "plasticity" in the sexual development of fish results in the existence of "critical windows" in early life stages. During these periods fish are particularly sensitive to effects from EDCs. Even brief exposures or exposures to low concentrations may have important and irreversible consequences. This phenomenon is actively exploited in aquaculture in order to produce monosex fish cultures. Hormonal treatment of fish in aquaculture is forbidden in Norway, although it is widely used in many other countries. Hormonally controlled feminisation of a number of different species of fish is widely used. This is primarily carried out by treating eggs and/or larvae with estrogens (Piferrer, 2001).

The sensitivity of early life stages to the effects of estrogen is also reflected in results from field works. The clearest evidence of hormonal disturbance in wild fish comes from reports of the feminisation of male fish, with findings of intersex/ovo-testis gonads (testis that contain morphological characteristics of female fish; i.e. hermaphroditism) in a number of freshwater fish species (Jobling and Tyler, 2003) and saltwater fish (Matthiessen, 2003).

The yolk protein VTG is a sensitive biomarker, widely used in studies of the effects of estrogen mimics in fish. Even though VTG is a protein specific to female fish, males also possess all of the genetic system needed for VTG protein synthesis. Estrogen

induces VTG synthesis in the liver of both males and females, and a rise in the level of VTG can therefore be used as an indication of estrogen influence. Several studies have found increased VTG levels in wild male fish and in fish kept in cages in polluted areas. Most of these studies have been done on freshwater fish (Jobling and Tyler, 2003). Abnormally high levels of VTG have also been found in saltwater fish: flounder (*Platichthys flesus*) caught off the British coast (Allen et al., 1999a; Allen et al., 1999b; Lye et al., 1997; Kirby et al., 2004; Kleinkauf et al., 2004) and near offshore installations in the UK sector of the North Sea (Matthiessen et al., 1998), flounder (*Pleuronectes yokohamae*) and goby (*Acanthogobius flavimanus*) caught in coastal areas around Japan (Hashimoto et al., 2000, Ohkubo et al., 2003), swordfish (*Xiphias gladius*) and red mullet (*Mullus barbatus*) in the Mediterranean (De Metrio et al., 2003; Fossi et al., 2004; Martin-Skilton et al., 2006b) and cod from the North Sea (Scott et al., 2006).

Unlike the great deal of interest that has been shown in estrogenic effects and feminisation of male fish, there are only a few reports of masculinizing effects on females. It is known that eels (*Anguilla anguilla*) are particularly sensitive to early exposure to environmental hormones, and it has been suggested that the high proportion of male eels that are found in European rivers is due to environmental factors (Beullens et al., 1997). It has also been shown that discharges of wastewater from papermills can contain substances with androgenic or anti-estrogenic effects (Bortone et al., 1989; Bortone and Cody, 1999; Bortone and Davis, 1994; Karels et al., 1999; Hegrenes, 1999; Larsson et al., 2000).

A few laboratory studies have shown that certain environmental toxins may interact with receptors for maturation-stimulating hormones, but there are no data from field studies that confirm this (Thomas et al., 1998; Das and Thomas, 1999; Thomas, 2000; Tokumoto et al., 2005). Similarly, there is little information in the literature regarding disruptions of the thyroid hormones in fish (Oberdorster and Cheek, 2001; Zhou et al., 2000; Brown et al., 2004).

In spite of the relative comprehensive list of field studies given above a, most of the evidence for hormonal disturbances caused by hormone mimics is the result of laboratory studies. There has been some criticism of the fact that many of these studies have been carried out using unrealistically high concentrations in comparison with the concentrations that are actually found in nature (Cooper and Kavlock, 1997; Oberdorster and Cheek, 2001; Tyler et al., 1998). More field studies, and lower more realistic concentrations in the laboratory studies, as well as a sharper focus on long-term effects have been called for.

1.6 Alkylphenols in the aquatic environment.

Most of the research in this field has dealt with the two long-chain APs nonylphenol (NP) and octylphenol (OP). These are derivatives of degradation products of the non-ionic surfactants known as alkylphenol ethoxylates (APE). APE consist of an alkylphenol group, principally NP (82%) but also OP or dodecylphenol, coupled to long ethylene oxide chains (see (Nimrod and Benson, 1996b)). APE is and has been utilised in a large number of products, including herbicides, paint and industrial cleaning and degreasing agents (Naylor et al., 1992). APE is one of the most widely used surfactants in the world, with an annual production of around 500,000 tons (Renner, 1997). In Norway, the use of APE has been very limited, and has fallen significantly during the 90s, from 615 tons in 1995 to 113 tons in 2000 (www.SFT.no, 2001). The use of NP, OP and their APEs has been forbidden in Norway since January 2002 (www.miljoverndepartementet.no, 2001). The EuropeanUnion is also planning to forbid the use of these substances (Directive 2003-53-EC, 2003). The APE and APs are on the Oslo-Paris Commission's (OSPAR) list of toxic chemicals, which ought to be phased out.

The long-chain APEs have low toxicity and have no hormone-mimicking effects. However, they are broken down gradually and relatively rapidly in waste-treatment plants into the more resistant alkylphenol mono- and di-ethoxylates AP1E and AP2E

and the short-chain carboxylic acid derivatives (the alkylphenol carboxylates AP1EC and AP2EC). These are in turn partly broken down into pure APs (Nimrod and Benson, 1996b). A large proportion of these degradation products finally end up in the aquatic environment. There have been concern about AP in environment for more than two decades (McLeese et al., 1981; Giger et al., 1984; Waldock and Thain, 1986), but the research and monitoring of AP was intensified in middle of the 1990es. NP and OP have now been found in a large number of freshwater systems all over the world, in water concentrations of up to 644 µg/l in particularly highly polluted areas, but with typical values from ng/l to the low µg/l range. In sediment, concentrations are found up to 60 mg/kg (Ying et al., 2002).

The APs are transported by the rivers and eventually ends up in the marine environment. Measurements of seawater from coastal areas near cities and river estuaries have shown concentrations of up to 9 µg/l (table 1), while values from sediment samples can be as high as 15 mg/kg at exposed sites (table 2). The effuents from the great rivers are the main sources of AP into the oceans (Heemken et al., 2001; Stachel et al., 2003; Jonkers et al., 2005a). However, NP, OP and their APE have been found in atmospheric samples (Dachs et al., 1999; VanRy et al., 2000; Cincinelli et al., 2003; Berkner et al., 2004; Xie et al., 2006). It is therefore also possible that air-sea exchanges contribute to distribution of AP into the sea (Xie et al., 2006). The concentrations of APs in the open sea are, as one should expect, much lower than in coastal areas. Kannan *et al*. (1998) found very low levels of NP in the Sea of Japan (0.002 - 0.093 ng/l), while measurement from the North Sea showed significantly higher values. In samples from the German Bight, NP and OP concentrations were found between 0.09 - 4.4 ng/l and 0.013-0.3 ng/l respectively (Bester et al., 2001; Heemken et al., 2001; Xie et al., 2006). In sediment, sampled more than 100 km offshore, concentrations up to 13 µg/kg NP were found (Bester et al., 2001). The concentration of NP in water from the Dutch coastal zone was found to be as high as 1700 ng/l (median concentration 77 ng/l) (Jonkers et al., 2005b).

In addition to the long-chain OP and NP, there are a number of other APs that are used in industrial chemicals and also found in the environment (Remberger et al., 2003). 2,6-Di-*tert*-butyl-4-methylphenol (butylated hydroxytoluene = BHT) is a commonly used antioxidant and stabiliser in large groups of products. BHT is found in river water in concentrations up to 365 ng/l (Kolpin et al., 2002; Fries and Puttmann, 2004) and in marine sediments around the coast of UK in concentrations up to 90 μg/kg (CEFAS, 2006). 4-*tert*-butylphenol (4-*tert*-BP) are widely used in paint, plastics, rubber and glue industry and is found in rivers and coastal areas in both water (up to 2300 ng/l) and sediments (up to 3.2 mg/kg) (Heemken et al., 2001; Kannan et al., 2001; Inoue et al., 2002; Remberger et al., 2003; Uguz et al., 2003; Basheer et al., 2004; Brossa et al., 2004; Kawaguchi et al., 2004; Koh et al., 2006). Other long-chain APs, 4-*n*-pentylphenol (4-*n*-PP); 4-*n*-hexylphenol (4-*n*-HexP) and 4-*n*-heptylphenol (4-*n*-HepP) are reported found in Japanese rivers (30-80 ng/l) (Inoue et al., 2002), coastal waters from Singapore (10-2920 ng/l) (Basheer et al., 2004) and 4-*n*-PP are found in the North Sea in concentration up to 8 ng/l (Heemken et al., 2001).

APs are fully biologically degradable in water, but the degradation rate falls rapidly with increasing chain length. Brendehaug *et al*. (1992) measured the biological degradation of phenols in produced water diluted in seawater, and found that phenol and cresol (methylphenol) degraded very rapidly (only 0.1% remaining after one week), on the other hand, did 33% of the initial concentration of HexP and 60% of HepP still remained after one month (Brendehaug et al., 1992). NP also shows relatively high resistance to biodegradation. In lake water only 9 % was lost by microbial activity in a 57 days experiment (Lalah et al., 2003). Another study of the degradation of NP in seawater indicated a very slow rate at the beginning of the study (0.06% per day), but that the degradation rate increased rapidly after 28 days to 1% per day. This suggests that the microorganisms in the seawater adapt to NP as a substrate after a while. After 58 days, 50% of the original quantity of NP was still in the water (Ekelund et al., 1993).

APs have high capability of sorption to colloidal particles (Johnson et al., 1998) and the primary sink for NP is the sediments. Ahel et al., (1994) found that sediment concentrations were up to 5100 times higher than the concentrations in river water. In addition to high sedimentation rates it is also important that the degradation rate can be very low in surface sediments (Lalah et al., 2003). In anaerobic conditions deeper in the sediment core, the degradation is extremely slow and analysis of sediments cores can give information of the historical discharges of AP (Shang et al., 1999b; Isobe et al., 2001; Hashimoto et al., 2005; Heim et al., 2006; Koh et al., 2006). Profiling the NP distributions in sediment cores from Tokyo Bay showed a maximum of NP in the layer deposited around the mid-1970s (Isobe et al., 2001, Hashimoto et al., 2005). Similarly maximum NP concentrations were found in 1972 sediment cores from Venice Lagoon, Italy (Marcomini et al., 2000). Also in Yeongil Bay, Korea, was the highest sendiment concentrations found in sediment cores from 1971-1980 (Koh et al., 2006). This kind of investigation can track sedimentation of NP all the way back to 1920s in Venice Lagoon, Italy (Marcomini et al., 2000) and 1950s in Tokyo Bay (Isobe et al., 2001).

It is clear that APs (especially NP and OP) are widely distributed in the aquatic enviroment. Analyses of sediment cores from different time periodss show a declining trend in NP concentrations, indicating that the discharges to the enviroment are decreasing. Because APs are biodegradable, the potential environmental problems caused by these substances can disappear in a relatively short time when (if) the production and use of APEs are phased out. There are big differences in the view on AP legislation around the world, the European Union wants to reduce and ban the use of these substances (Directive 2003-53-EC, 2003; OSPAR Commission, 2004), while APEs are still widely used in the USA (Renner, 1997). Increasing use in Asia is also causing concern (Zhou et al., 2003).

Table 1. *Concentration of NP and OP (ng/l) in marine and estuarine waters around the World.*

Location	NP	OP	Reference
Venice lagoon, Italy	200		(Marcomini et al., 1989)
Krka River estuary, Croatia	<20-1200		(Kvestak and Ahel, 1994)
Tyne and Tees estuaries, UK	<80-5200	<100-1300	(Blackburn and Waldock, 1995)
Sea of Japan	0.002 - 0.093		(Kannan et al., 1998)
Tyne and Tees estuaries, UK	30-9050	2-340	(Lye et al., 1999)
Shipyards in Virginia, USA	1.0–6300		(Hale et al., 2000)
North Sea, Germany	1-33		(Bester et al., 2001)
Jamaica Bay, USA	77-416	1.6-8.3	(Ferguson et al., 2001)
North Sea, Germany	0.3-84	0.1-16	(Heemken et al., 2001)
The coast of Spain	150-4100		(Petrovic et al., 2002b)
Tokyo Bay, Japan	10-100		(Hando et al., 2003)
The coast of China	1-10		(Hando et al., 2003)
San Francisco estuary, USA	<0.25-4		(Oros et al., 2003)
Costal water from Singapore	200-2760	10-540	(Basheer et al., 2004)
Coastal area, Okinawa and Ishigaki Islands, Japan	<50-150		(Kawahata et al., 2004)
Tokyo Bay, Japan	0.5-104		(Hashimoto et al., 2005)
Baltic Sea, Germany	2.5-13.8	0.4-0.95	(Beck et al., 2005)
Scheldt and Rhine estuaries, Holland	12-962		(Jonkers et al., 2005a)
North Sea, Holland	31-1700		(Jonkers et al., 2005b)
Ariake sea, Japan	11-49		(Kim et al., 2005)
Saemangeum Bay, Korea	7-298		(Li et al., 2005)
North Sea, Germany	0.09-1.4	0.013-0.3	(Xie et al., 2006)

Table 2. *Concentration of NP and OP (μg/kg dry weight) in marine surface sediments around the world.*

Location	NP	OP	Reference
Barcelona, Spain	6-70		(Chalaux et al., 1994)
Nile estuary, Egypt	19-44		(Chalaux et al., 1994)
10 estruaries, UK	<100-15000		(Blackburn et al., 1999)
Masam Bay, Korea	113-3890		(Khim et al., 1999)
Tyne and Tees estuaries, UK	30-9050	2-340	(Lye et al., 1999)
Strait of Georgia, British Columbia, Canada	280-320		(Shang et al., 1999a)
Jamaica Bay, USA	7-13700	<2-45	(Ferguson et al., 2001)
Shipyards in Virginia, USA	0.5–14100		(Hale et al., 2000)
Tokyo Bay, Japan	30-13000	3-670	(Isobe et al., 2001)
North Sea, Germany	<10-153		(Bester et al., 2001)
Elbe estuary, Germany	370-480		(Heemken et al., 2001)
The coast of Spain	8-1050		(Petrovic et al., 2002b)
Delaware river estuary, USA	0.14-13		(Ashley et al., 2003)
Coastal area, Okinawa and Ishigaki Islands, Japan	<5-44		(Kawahata et al., 2004)
Urdaibai estuary, Spain	140-1100		(Bartolome et al., 2005)
Pearl River estuary and South China Sea, China	59-571	1-18	(Chen et al., 2005)
Scheldt and Rhine estuaries, Holland	3-1026		(Jonkers et al., 2005a)
North Sea, Holland	0.3-86		(Jonkers et al., 2005b)
Tokyo Bay, Japan	2-4560		(Hashimoto et al., 2005)
Bohai Bay, Japan	203		(Hu et al., 2005)
Southern California bight, USA	122-3200	<2-8	(Schlenk et al., 2005)
The coast of UK	<10-5888	<10-530	(CEFAS, 2006)
Yeongil Bay, Korea	2-1430	<1-24	(Koh et al., 2006)
Odense fjord, Denmark	800-3300		(Madsen et al., 2006)

1.7 Alkylphenols and offshore oil and gas production

Historically, large quantities of APE have been used in offshore petroleum production, both as detergents for platform washing purposes and as additives in the production process. Blackburn et al., (1999) suggest that discharges on the British continental shelf may have been as much as 100 tons a year per platform. NP and NPE are found in high concentrations (up to 68 mg/kg) in the sediments around North Sea platforms (CEFAS, 2005, Jonkers et al., 2005b). The use of APE is now forbidden in the Norwegian sector of the North Sea (letter from SFT to all operators on the Norwegian shelf, dated 31.08.98). The Danish and UK authorities are also working on phasing out APE in their sectors of the North Sea (Lye, 2000).

In addition to being degradation products of the APEs, APs are natural components of crude oil (Ioppolo-Armanios et al., 1992, Ioppolo-Armanios et al., 1995, Taylor et al., 1997, Rolfes and Andersson, 2001, Bastow et al., 2005). As a result of their solubility in water, a high proportion of APs will be found in the aqueous phase after water/oil separation and discharged into the sea with the produced water. The APs are typically found in concentrations of 0.6 - 10.0 mg/l in produced water. About 80 % of the total amount consists of the most water-soluble APs (phenol and cresol). Of the remaining components, the higher APs from BP - to HepP occur in low concentrations of 0.07 - 237 µg/l (Grahl-Nielsen, 1987; Brendehaug et al., 1992; Røe and Johnsen, 1996; Boitsov et al., 2004).

It is showed that produced water contains estrogen receptor agonists and APs have been identified to be the major contributor to this effect (Thomas et al., 2004a; Thomas et al., 2004b; Tollefsen et al., 2006). *In vitro* screening have found estrogen equivalents form <0.03 – 91 ng E2 /l in produced water from different installations in the UK sector of the North Sea (Thomas et al., 2004a).

Very little is known about the fate of these substances in the marine offshore environment. There are no empirical data on concentrations of long-chain APs in the

sea around North Sea offshore installations. One study showed that phenol and lighter APs (C1–C4) occur at the concentrations of 486 and 140 ng/l, respectively (Riksheim and Johnsen, 1994). The discharges of produced water from the Norwegian petroleum sector are continuously increasing with the age of the oil fields, and were in 2004 143 million m^3. In 2004, approximately 13 tons of long-chain ($\geq C_4$) APs were released from installations on the Norwegian continental shelf in connection with discharge of produced water (OLF, 2005).

1.8 Bioconcentration of alkylphenols

NP and OP are both bioconcentrated and have been identified in aquatic organisms in nature. Ahel *et al.* (1993) found concentrations of NP of up to 1600 µg/kg (dry weight) in various freshwater fish in Swiss rivers. NP has been found in carp (*Cyprinus carpio*) caught in Lake Mead, Nevada (up to 184 µg/kg) (Snyder et al., 2001a) and in Cuyahoga River, Ohio (32-920 µg/kg) (Rice et al., 2003). Fish from various lakes in Michigan, USA had tissue concentrations of <3.3 to 29.1 µg/kg NP (Keith et al., 2001). Fish from Japanese rivers have been shown to contain from 1 - 110 µg/kg NP (Tsuda et al., 2000b) and similar concentrations are also found in periphytons, 8-130 µg/kg NP and benthos, 8-140 µg/kg NP (Takahashi et al., 2003). Lower levels NP were found in fish from Chinese rivers (up to 2 µg/kg) (Shao et al., 2005). Breams (*Abramis Brama*) caught in German rivers contain up to 130 µg/kg NP (Klein et al., 2005). Retrospective monitoring of APs in aquatic biota (from the German Environmental Specimen Bank) from 1985 to 2001 shows a decrease of NP concentration in biota from all sampling sites after 1997, the NP content in mussels from the German Bight dropped from 4 µg/kg in 1985 to 1.1 µg/kg in 1995 (Gunther et al., 2001; Wenzel et al., 2004). Wahlberg et al., (1990) found between 200 and 400 µg/kg NP in mussels gathered from the sea near the wastewater outlet of a Swedish plant that produced APE. Molluscs, crustaceans and fish from the Adriatic Sea, Italy, contained 9.5-1431 µg/kg NP and 0.3-4.3 µg/kg OP (Ferrara et al., 2001; Ferrara et al., 2005). NP and OP were found in the bile of red mullet from the Franch coast of the

Mediterranean Sea (Martin-Skilton et al., 2006b). Flounders caught in brackish water outside the rivers Tyne and Tees in England have been shown to contain 5 - 118 µg/kg NP (Lye et al., 1999). NP was not found (above a detection threshold of 100 µg/kg) in fish caught in the British offshore sector of the North Sea (Blackburn et al., 1999). Apart from one special case in the Detroit River in the USA, where large amounts of 2,4 di-*tert*-pentylphenol were found (Shiraishi et al., 1989), all the studies of APs of which we are aware of concerned OP and NP. We have found no field studies that have analysed petroleum-related APs.

APs are a highly diverse group of substances in terms of their physico-chemical properties. The water solubility of phenol and the short-chain APs are high, but falls drastically with increasing chain length and therefore increasing hydrophobicity. Table 3 presents an overview of three important physico-chemical properties of importance for the behavior of these substances in the environment, aqueous solubility, the logarithm of the water/octanol partitition coefficient (K_{ow}) and the bioconcentration factor (BCF). A number of studies have shown that OP and NP are readily taken up by fish, both via exposure in the water (Lewis and Lech, 1996; Arukwe et al., 2000b; Ferreira-Leach and Hill, 2001; Pedersen and Hill, 2002; Pickford et al., 2003) and by the food (Thibaut et al., 1998b; Arukwe et al., 2000b; Madsen et al., 2002; Pickford et al., 2003; Madsen et al., 2006). The APs are rapidly metabolised, mainly by phase II enzymes that conjugate intact APs to their corresponding glucuronides. The APs are excreted primarily in the bile and faeces (Ferreira-Leach and Hill, 2001; Thibaut et al., 2002; Smith and Hill, 2004). The APs accumulate particularly in the bile, digestive system and liver, but it has also been shown that AP is taken up by the brain in Atlantic salmon (*salmo salar*) (Arukwe et al., 2000b), rainbow Trout (*Oncorhynchus Mykiss*) (Ferreira-Leach and Hill, 2001; Thibaut et al., 2002), roach (*Rutilus rutilus*) (Smith and Hill, 2004) and cod (Tollefsen et al., 1998). This is of particular interest with respect to hormone-disrupting effects in the central-nervous-system. Studies with PCB have shown that cod (lean fish) are more likely than trout (fat fish) to accumulate lipophilic compounds in the brain (Ingebrigtsen et al., 1990).

Arukwe *et al.* (2000) have compared tissue distributions of NP in salmon following two different exposure regimes, via the water and via food. They found that dosing in the water results in higher uptake and a more regular distribution throughout the body than oral dosing, where NPs are more concentrated around the digestive system. Similarly, Pickford *et al.* (2003) found a 10 fold higher sensitivity for NP in fish exposed via the water compared to oral exposure of corresponding doses.

The bioaccumulation factor (BCF) for long-chain APs (>C4) is in the range of 75 - 1250 (Table 3). In fish, the biological uptake of chemicals with log K_{ow} < 4 (logarithm of the octanol/water partitition coefficient) mainly takes place via the water (theoretically 20 times as fast as uptake via food). For more hydrophobic substances with log K_{ow} > 6, the situation is reversed, with uptake via food being more important (Mackay and Fraser, 2000). As far as the APs are concerned, this means that bioaccumulation in nature properly takes place primarily via uptake through the gills and skin and not by being biomagnified through the food chain. However, little is known about the metabolism of these compounds in organisms that belong to the lower end of the food chain. High BCFs have been reported for NP in estuarine amphipods and this indicates that biomagnification can be an important source of NP in higher trophic levels, such as juvenile fish (Hecht et al., 2004).

***Table 3**. Selection of physicochemical properties of APs that may have relevance for their environmental fate. Aqueous solubility, the logarithm of the water/octanol partitition coefficient (K_{ow}) and the bioconcentration factor (BCF[1]). The overview is from (Shiu et al., 1994, Servos, 1999)*

	Water solubility (mg/l)	Log K_{ow}	BCF in fish	Species	Reference
Phenol	67000-93325	1,46-1,6	17-158	Div. fish	(Servos, 1999)
p-Cresol	1800-53000	1,62-2,06	-		(Servos, 1999)
4-Ethylphenol	5000	2,39-2,58	-		(Servos, 1999)
4-*n*-Propylphenol	1278	3,18-3,20	-		(Servos, 1999)
4-*sec*-BP	-	2,1	37	Salmon	(McLeese et al., 1981)
4-*tert*-BP	580-1848	3,04-3,31	118	Golden Ide	(Freitag et al., 1985)
			125	Cod	(Sundt and Baussant, 2003)
4-*n*-PP	-	-	90	Cod	(Sundt and Baussant, 2003)
4-HexP	-	3,60	346	Salmon	(McLeese et al., 1981)
			592	Cod	(Sundt and Baussant, 2003)
4-*n*-HepP	-	4,00	578	Cod	(Tollefsen et al., 1998)
			520	Cod	(Sundt and Baussant, 2003)
4-*tert*-OP	12,6	4,12	261	Killifish	(Tsuda et al., 2001)
			1134	Roach	(Ferreira-Leach and Hill, 2000)
4-NP	5,4-7	4,20-6,36	75-1250	Div. fish	(Servos, 1999)

[1)] The **Bioconcentration Factor (BCF)** is the relationship between the concentration in the fish and the concentration in the water; and describes only uptake via gills and skin (Mackay and Fraser, 2000).

1.9 Estrogen receptor (ER) and the binding affinities of alkylphenols to ER.

The steroid hormone, E2 is a key regulator of growth, differentiation and physiological functions in a wide number of target tissues, including the male and female reproductive system, neuronal, skeletal and cardiovascular systems. The predominating mechanisms of estrogen action are mediated through binding to the nuclear estrogen receptor (ER), which induces transcription of target genes containing estrogen response element (ERE) (Zhang and Trudeau, 2006). The ER is part of a large nuclear receptor superfamily that shares common structure and function/domains. This receptor family acts as the signal transmitter for most of the known fat-soluble hormones, including steroids (androgen receptor, (AR); progesterone receptor, glucocorticoid receptor, mineralocorticoid receptor), retinoids, thyroid hormones and vitamin D (Mangelsdorf et al., 1995). Other groups of nuclear receptors, so called "Orphan" receptors (the ligands are unknown), pregnane X-receptor (PXR) and

constitutive androstane receptor (CAR) are regulating some of the cytochrome P450 genes and other detoxification genes, (Kretschmer and Baldwin, 2005).

In addition to the classical mechanism of genomic effects there are also increasing evidence for non genomic effects of E2 and other steroids, possible mediated through membrane receptors and secondary messenger cascades (such as release of intracellular Ca^{2+}, mitogen activated protein kinase (MAPK), protein kinase A (PKA) and C (PKC), phospholipase C (PLC), phosphoinositide turnover and adenylate cylase (cAMP)) (Sak and Everaus, 2004, Zhang and Trudeau, 2006). The genomic effect has time delays from hours to days, while the non-genomic mechanism are characterised by very fast signal transmission, from seconds to minutes. Two novel seven-transmembrane spanning steroid membrane receptors, membrane progestin receptor α and membrane estrogen receptor (mER), GRP30, have resently been identified in several vertebrates (Thomas et al., 2006).

In mammals, there have been found two distint forms of nuclear estrogen receptors, ERα and ERβ (Enmark and Gustafsson, 1999). Teleosts have in addition to ERα, two different forms of ERβ (ERβa and Erβb) (Hawkins et al., 2000; Menuet et al., 2002; Hawkins and Thomas, 2004; Sabo-Attwood et al., 2004). The tissue distribution in teleost of the different ERs largely overlaps, and ERs are mainly found in the brain, pituitary gland, liver and gonads (Menuet et al., 2002). There are reported differences in the relative affinity of AP between the ERα and Erβ. In channel catfish, NP showed 100-fold lower affinity for ERα and 10000-fold lower affinity for ERβ than for E2 (Gale et al., 2004). On the other hand, difference in NP affinity to the two ERs in an assay using recombinant human ERα and ERβ, was not observed (Kuiper et al., 1998).

The capability of synthetic non-steroid compounds to bind and activate the ER have been known for more than 70 years (Cook et al., 1933). Dodds and Lawson, (1938) found that among many others compounds, also 4-*tert*-PP and 4-*n*-propylphenol had weak estrogenic effects measured by changes in vaginal cytology in ovariectomized rats. The estrogenicity of APs were further studied by Mueller and Kim, (1978) and many AP isomers were showed to be able to bind and displace E2 from the ER. But it

was first in the beginning of the 1990es, after Soto et al., (1991) had rediscovered the estrogenic effects of NP that the scientific community started the massive focus that have made this group of compounds some of the most intensively studied endocrine disruptors.

Amino acid sequence of the ER and the crystal structures of the E2–ER complex (see figure 5) have together with quantitative structure – activity relationship (QSAR) study of antagonists identified several criteria for high binding affinity to the ER: (1) Phenolic ring with hydrogen-bonding ability; (2) H-bond donor mimicking the 17β-OH and right O-O distance between 3- and 17β-OH; (3) Hydrophobic moiety mimicking the ring structure of E2 (Brzozowski et al., 1997; Sadler et al., 1998; Schmieder et al., 2000; Tanenbaum et al., 1998; Fang et al., 2001; Klopman and Chakravarti, 2003; Tong et al., 2003).

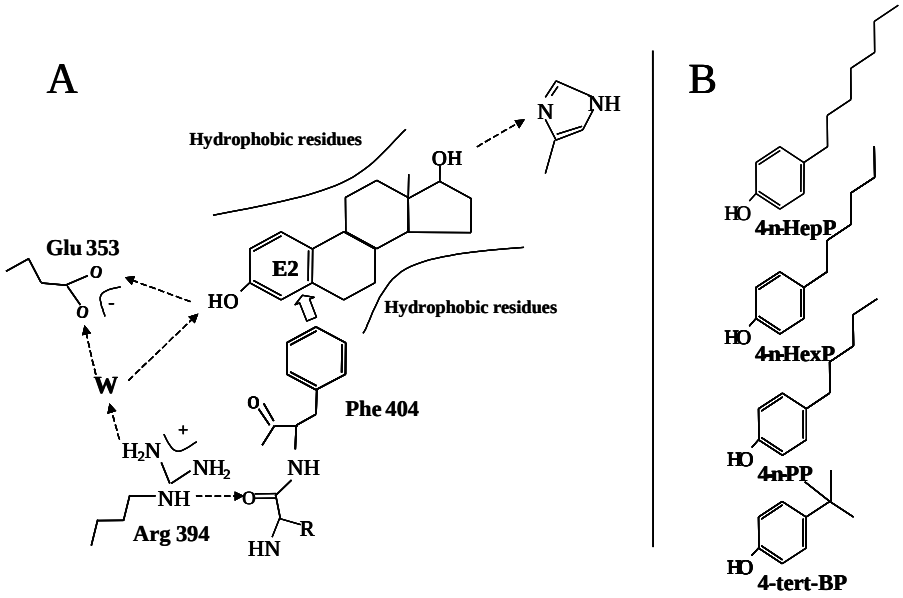

Fig 5. A)*. Model of the ERα-ligand binding domain holding E2. The specific binding domain of the ER is holding the 3-hydroxy-group of E2 in a water (**W**) mediated hydrogen-bonding network involving glutamic acid (**Glu**), arginine (**Arg**) and phenylalanine (**Phe**). The 17-hydroxy-group of E2 is bound by hydrogen bonds to histidine (**His**). In addition, the ligand-binding pocket is covered with hydrophobic amino acids making van der Waals contacts with the carbon skeleton of E2. The figure are taken from the crystal structure of the E2 complex with the human ERα (Tanenbaum et al., 1998).* ***B)****. Chemical structure of the four alkylphenols used in this study: 4-tert-butylphenol (4-tert-BP), 4-n-pentylphenol (4-n-PP), 4-n-hexylphenol (4-n-HexP) and 4-n-heptylphenol (4-n-HepP).*

Beacause of the phenol ring, APs fulfil the must important criteria for binding to the ER. APs do also have the capability of hydrophobic interaction by the alkyl-chain, but as seen on figure 5B the APs are lacking other important structures, like hydrogen-bond donor capability mimicking the 17β-OH of E2. The APs have therefore relatively low affinity to the ER.

In vitro studies (table 4) have found that the size and degree of the branching of the alkyl chain, as well as its position relative to the phenolic hydroxy-group are important for binding affinity to the ER. The most vital factor for high estrogenic activity of APs are that the alkyl chain is in the *para*-position (*para*>*meta*>*ortho*) and that the chain-length is $\geq C_6$. Maximum activity (400 - 6000 times less potent than E2) has been found for $C_6 - C_9$ *para*-substituted tertiary APs, but *para*-substituted C_5, C_4 and C_3 APs are also have weak estrogenic effects (10^5 - 10^7 times less potent than E2) (table 5). Routledge and Sumpter, (1997) found that the tertiary isomers have the highest estrogenic effects (tertiary>secondary=normal), and that 4-*tert*-OP is 60 times more potent than 4-*sec*-OP. Similarly 4-*tert*-HepP is 25 times more potent than 4-*n*-HepP, while 4-*n*-PP on the other hand is three times more potent than 4-*tert*-PP. Other investigations confirm that tertiary isomers are more potent than the normal isomers, but with less difference than found by Routledge and Sumptor (1997). 4-*tert*-OP was 2-10 times more potent than 4-*n*-OP (Tabira et al., 1999; Blair et al., 2000; Schultz et al., 2000). However, the structure of the carbon chain is important. A recent study has examined the estrogenic effects of the alkyl chain of 22 isomers of 4-NP in detail. They found that high "bulkiness" on the β-carbon was the most important factor for the high estrogenic activity and that the activity could differ as much as 3000 times between the most and the least potent 4-NP (Shioji et al., 2006).

In vivo studies also suggest that the estrogenicity of branched APs is higher than that of linear isomers (Pedersen et al., 1999; Chikae et al., 2003).

Table 4. *Estrogen receptor (ER) binding affinities of APs. The most potent isomers are given in bold. IC_{50} value: the concentration of competitor needed to displace half of the bound ligand.*

	1) (Routledge and Sumpter, 1997)	**2)** (Tabira et al., 1999)	**3)** (Blair et al., 2000)	**2)** (Schmieder et al., 2000)	**1)** (Schultz et al., 2000)	**2)** (Hu and Aizawa, 2003)
Compound	Relativ to E2	IC_{50} (M)	IC_{50} (M)	IC_{50} (M)	IC_{50} (M)	IC_{50} (M)
E2	*1*	*2.1 x 10 $^{-9}$*	*9.0 x 10 $^{-10}$*	-	*3.9 x 10 $^{-11}$*	*2.3 x 10 $^{-8}$*
4-dodecylphenol	-	2.0 x 10 $^{-4}$	4.6 x 10 $^{-6}$	9.3 x 10 $^{-4}$	-	-
4-*sec*-decylphenol	1/100000	-	-	7.6 x 10 $^{-6}$	-	-
2-*sec*-decylphenol	Nonactive	-	-	Nonactive	-	-
4-NP	1/30000	**3.7 x 10 $^{-6}$**	**2.4 x 10 $^{-6}$** – 4.7 x 10 $^{-6}$	3.4 x 10 $^{-6}$	-	-
4-*n*-NP	-	4.2 x 10 $^{-6}$	2.8 x 10 $^{-5}$	-	-	**9.5 x 10 $^{-6}$**
4-*tert*-OP	**1/1000**	6.3 x 10 $^{-6}$	6.0 x 10 $^{-6}$	**1.1 x 10 $^{-7}$**	**1.8 x 10 $^{-7}$**	1.4 x 10 $^{-5}$
4-*sec*-OP	1/60000	-	-	-	-	-
4-*n*-OP	-	1.0 x 10 $^{-5}$	1.9 x 10 $^{-5}$	-	1.9 x 10 $^{-6}$	-
2.6-di-butylphenol	Nonactive	-	-	Nonactive	-	-
2.4-di-butylphenol	Nonactive	-	-	-	-	-
4-*tert*-HepP	1/3000	-	-	2.6 x 10 $^{-7}$	-	-
4-*n*-HepP	1/75000	-	-	9.8 x 10 $^{-6}$	-	-
4-*tert*-HexP	1/6000	-	-	5.8 x 10 $^{-7}$	-	-
4-*n*-HexP	-	1.7 x 10 $^{-5}$	-	-	-	-
4-*tert*-PP	1/100000	-	1.7 x 10 $^{-4}$	3.1 x 10 $^{-6}$	4.8 x 10 $^{-6}$	-
4-*n*-PP	1/30000	-	-	1.3 x 10 $^{-5}$	9.5 x 10 $^{-6}$	-
4-*tert*-BP	1/1500000	-	3.7 x 10 $^{-4}$	1.6 x 10 $^{-4}$	-	1.0 x 10 $^{-3}$
3-*tert*-BP	Nonactive	-	-	Nonactive	-	-
2-*tert*-BP	Nonactive	-	-	Nonactive	-	-
4-*sec*-BP	1/3900000	-	2.1 x 10 $^{-4}$	3.9 x 10 $^{-4}$	-	5.8 x 10 $^{-4}$
2-*sec*-BP	-	-	3.2 x 10 $^{-4}$	Nonactive	-	1.4 x 10 $^{-3}$
4-*n*-BP	-	8.5 x 10 $^{-5}$	-	-	-	-
4-*n*-Propylphenol	1/20000000	-	-	2.2 x 10 $^{-3}$	1.5 x 10 $^{-4}$	-
4-Ethylphenol	Nonactive	6.0 x 10 $^{-3}$	1.3 x 10 $^{-3}$	-	Nonactive	1.7 x 10 $^{-3}$
3-Ethylphenol	-	-	6.6 x 10 $^{-4}$	-	-	-
2-Ethylphenol	-	-	> 1.0 x 10 $^{-3}$	-	-	-
Phenol	Nonactive	2.9 x 10 $^{-3}$	-	-	-	9.5 x 10 $^{-2}$

[1] Recombinat Yeast assay (YES screen)
[2] Estrogen receptor competitive-binding assay (Recombinant human oestrogen receptor. hERa)
[3] Estrogen receptor competitive-binding assay (ER from uterine cytosol from Sprague-Dawley rats)

In addition to be an agonist for the ER, APs have also been shown to interfere with several other classes of nuclear receptors. Even though this is not well investigated and the results are not as consistent as for effects on the ER, it tells us that AP can act as a

endocrine disruptor in a much broader way than only being an estrogen mimic (Goksoyr and Male, 2006).

4-*tert*-OP (IC_{50}=5 x 10^{-6} M) and NP (IC_{50}=2.6 x 10^{-6} M) are potent antagonists for AR and can induce anti-androgen effects (Paris et al., 2002; Lee et al., 2003a). On the contrary, Sohoni and Sumpter, (1998) found NP to be a weak agonist to AR. It is also reported that NP and 4-*tert*-OP can be both agonists to PR (Scippo et al., 2004) or PR antagonist (Tran et al., 1996). The thyroid hormone function can be disrupted by APs (Ghisari and Bonefeld-Jorgensen, 2005; Schmutzler et al., 2004). The two orphan nuclear receptors that are involved in regulation of several detoxifications enzymes are also target for AP endocrine disruption, NP is agonist for PXR and CAR (Masuyama et al., 2000, Mikamo et al., 2003, Kretschmer and Baldwin, 2005).

There are also evidence for NP to induce similar effects as E2 via membrane initiated signalling pathways (Loomis and Thomas, 2000; Bulayeva and Watson, 2004; Watson et al., 2005; Wozniak et al., 2005; Thomas and Dong, 2006). The binding affinity of AP to the nuclear ER is about 1000 times weaker than E2, but the effect-concentration of the membrane initiated effect seems to the more equal for E2 and NP. Intracellular Ca^{2+} changes are induced in pituitary tumor cell lines by 10^{-12} M of both E2 and NP within 30 sec of administration, resulting in prolactin (PRL) secretion (Wozniak et al., 2005). Thomas and Dong, (2006) found that NP binds 47 times weaker than E2 to plasma membranes prepared from HEK293 cells transfected with the seven-transmembrane estrogen receptor, GPR30.

1.10 Estrogen-receptor mediated and receptor-independent mechanisms for the biological effects of alkylphenols

Numerous *in vitro* screening systems have been developed to characterise the binding affinity of chemicals to ER (reviewed in (Zacharewski, 1997; Soto et al., 2006)). APs are shown to bind and induce effects through ER, similar to E2. It has also been shown that the effects can be blocked by ER antagonists like tamoxifen or ICI 182,780. The

variety of assays used in studying the estrogenic effects of APs includes: competitive ER binding assay (White et al., 1994); cell proliferation assay, (e.g. E-screen (MCF7-cells) (Soto et al., 1995)); protein expression assay, (e.g. VTG expression in fish hepatocyte culture (Jobling and Sumpter, 1993)); recombinant assays, (e.g. yeast-based screen (YES-screen) (Routledge and Sumpter, 1996) and cell lines (Shelby et al., 1996)).

Recent developments in screening for xenoestrogenic effects are by use of toxicogenomics (Moggs, 2005) where cDNA microarrays containing multiple estrogen-responsive genes can be used both *in vitro* and *in vivo* (Terasaka et al., 2004; Naciff et al., 2005; Terasaka et al., 2006). Such approache clearly show the complexity in estrogen signalling and the disturbers therein. Moggs (2005) reported as many as 3538 genes to be E2-responsive in the mouse uterus, and through gene ontology, the genes are categorised into 35 different biological partways. Toxicogenomics have an enormous potential in providing detailed information regarding the molecular response to xenoestrogens and revealing new biomarkers. Microarray analysis of gene expression profiles in mouse exposed for NP or E2 reveal tissue differences in response to E2 and xenobiotics. The gene expression in the gonade was very similar after E2 and NP exposure, indicating that these effects mainly are induced through the ER or other estrogen receptors. Gene expression in liver, on the other hand was more affected by NP than by E2 and activation of many genes involved in lipid and fatty acids metabolism were only found in the NP groups (Watanabe et al., 2004). Undoubtedly, the "omics" technologies (Genomics, proteomics, metabolomics, lipomics) will also play a very important role in the future for studying endocrine disruption in fish and other aquatic organisms. Today there is still some limitation in that the genome is only sequenced in very few teleosts, but smaller scale DNA microarrays are now available for several model species (Miracle and Ankley, 2005; Moens et al., 2006; Watanabe and Iguchi, 2006). However, the challenge for this approach is the same as for all other use of biomarkers; to create bridges between information of gene expression to physiological and toxicological endpoints that can

be used to extrapolate the effect to fitness of individuals and populations. ("Fitness = the relative contribution of an allele, genotype or phenotype to future generations" Wikipedia, the free encyclopedia).

The following chapters give an overview of different physiological events affected by AP exposure.

1.10.1 Effect of AP on sex differentiation and gonad development.

In non-mammalian vertebrates the genotypic sex can be overridden by exposure to steroid hormones and the sex differentiation are therefore vulnerable to EDC. Exposure to AP in the early life stages of fishes (Gimeno et al., 1996; Gray and Metcalfe, 1997) and amphibians (Kloas et al., 1999; Mosconi et al., 2002) can induce feminization of males and result in intersexuality or higher number of female phenotypes.

AP are stimulating estrogen-dependent uterine growth in rodents (Bicknell et al., 1995). The rat uterotrophic bioassay is validated as "standard *in vivo* method" for screening of xenobiotics by the Organisation for Economic Co-operation and Development (OECD). The lowest observed effect level (LOEL) in the uterotrophic assay is found to be 75 mg/kg/day for NP (Kanno et al., 2003; Owens and Koeter, 2003). Testis development in rats can also be affected by AP, and reduction in testis growth and induction of apoptosis have been reported (Han et al., 2004b; Kim et al., 2004). NP induced apoptosis in rat testis in a similar way as E2 does through the FAS/FASL Pathway (Wang et al., 2003; Han et al., 2004a). Apoptosis is also induced by NP and OP in human embryonic stem cells, and these effects are also related to the FAS/FASL Pathway (Kim et al., 2006b). The FAS-signalling pathway is important in the paracrine-signalling system between Sertoli cells and germ cells (Richburg et al., 2002). In addition to the FAS/FASL pathway, apoptosis can also be induced by a variety of other signal transitions leading to stimulation of calcium flux, cAMP production, PLC activation, inositol phosphate generation and mitochondrial

membrane transition pore permeability. OP and NP also induce apoptosis in Sertoli cell lines by inhibiting endoplasmic reticulum Ca^{+} pumps (Hughes et al., 2000).

Even though the evidences that AP give endocrine disruption in mammalian reproductive tracts are clear, it is important to note that the *in vivo* effects are only seen at relative high doses. Multigenerational studies with rats show a "no observable adverse effect level" (NOAEL) of NP > 100 mg/kg/day for effect on the reproduction development, (Chapin et al., 1999; Nagao et al., 2001; Tyl et al., 2006).

The reproductive system of fish seems more sensitive for AP exposure than that of the mammalians. Concentrations down to 5 μg/l levels of NP or OP are inhibiting the spermatogenesis of male fish, resulting in reduced testis growth, trigging of necrosis and apoptosis and altertion of testis morphology (Jobling et al., 1996; Gimeno et al., 1998; MilesRichardson et al., 1999; Weber et al., 2002). The oogenesis in the female fish is also affected by APs, but at higher dose than what is seen for the male fish. As example, 100 μg/l NP reduces the ovary weight and increases follicle atresia in zebrafish (Weber et al., 2003). End point like fertilization success from life cycle tests with zebrafish comfirm the high teleost sensibility for AP, EC_{50} values = 28 μg/l for 4-*tert*-OP (Segner et al., 2003b).

1.10.2 Effect of AP on brain and the central-nervous-system (CNS).

Estrogens are one of many neuroactive steroids and play a vital role in many neurophysiologic events such as the sexual differentiation and early development of the brain; feedback effect on brain-pituitary-gonad axis; higher cognitive functions like behavior, memory, etc.; and have neuroprotective effects. (McEwen, 2002; Melcangi and Panzica, 2006). The multiple effects are found mediated both by nuclear ER receptors and membrane signalling pathways. However, much are still not known and there are increasing discoveries of new targets and mechanisms of estrogen effects to the CNS (Toran-Allerand, 2004; Ronnekleiv and Kelly, 2005).

A particular attention has been put on APs and neuroendocrine disturbances on the brain-pituitary-gonadal axis in fish (Jones et al., 1998; Piva and Martini, 1998; Harris et al., 2001; van Baal et al., 2000; Zilberstein et al., 2000; Yadetie and Male, 2002; Maeng et al., 2005; Vetillard and Bailhache, 2006). The underlying mechanisms of the effects of AP on the GnRH and GTH are still unknown and the literature demonstrates contradictory effects in different fish species; Harris *et al.* (2001) found that NP reduces the expression of FSH gene in the pituitary gland and FSH secretion to the plasma in water exposed female rainbow trout, even at very low concentrations (they found a significant effect at the lowest exposure dose; 0.7 µg/l). Similarly, the quantity of LH-mRNA is reduced in the pituitary gland on exposure to NP (8.3 µg/l) (Harris et al., 2001). As opposed to this, Yadetie and Male (2002) stated that intraperitoneal injection of NP (50 mg/kg) strongly induces gene expression of LH in female juvenile Atlantic salmon pituitary gland. No effects were seen in male fish. The gene expression of FSH was unaffected in both sexes (Yadetie and Male, 2002). Injection of low dose (10 mg/kg) of NP induced the GTHα and LHβ mRNA levels in the pituitary gland of juvenile masu salmon (*Oncorhynchus masou*). A high dose (50 mg/kg) did, however, not induce this effect on GTHα and LHβ mRNA, but did instead slightly reduce FSHβ mRNA levels (Maeng et al., 2005). Tilapia (*Oreochromis niloticus*) showed a suppressed expression of FSH mRNA, but not LH mRNA in the pituitary gland after 5 weeks water exposure to NP (10 µg/l) (Zilberstein et al., 2000). In African catfish (*Clarias garipinus*) the amount of LH (protein) was increased in the pituitary gland of both sexes, but not in plasma after 7-14 days water exposure of NP (10 µg/l) (van Baal et al., 2000). *In vitro* studies showed an inhibitory effect of NP on the secretion of LH from African catfish cultured pituitary cells (van Baal et al., 2000). Water esposure of NP (2.2 µg/l to 2.2 mg/l) reduced GnRH in the brain of juvenile rainbow trout in a dose dependent manner (Vetillard and Bailhache, 2006).

The effects from AP on the CNS may be mediated through mimicking estrogenic feedback effects. In general, E2 (and other sex steroids) is known to exert positive feedback effects on LH levels, but there is species related variation. The feedback

control of E2 on FSH levels is much less clear, and both positive, negative or no effects are reported from juvenile teleost (Dufour et al., 2000: Kah et al. 2000). It is important to recognize that estrogen often shows reproductive stage-dependent effects on the gonadotropin secretion (Thomas, 2000).

Bevan et al., (2003) found a high increase of apoptotic cells in the nervous systems of developing tadpoles (*Xenopus laevis*) after low NP exposure (100 nM). This observation is also correlated with increased morphological deformations and high mortality. Stimulation of apoptosis by NP exposure are also found in embryonic murine neural stem cells (NSC) (Kudo et al., 2004). NP disturbs the cell cycle of NSC by accumulation of cells in the G_2/M phase by down-regulation the expression of cyclin A and B1, which are the major regulatory proteins for the G_2 to M transition of the cell cycle. The NP exposure can also lead to apoptosis of NSCs by activating the caspase cascade (Kudo et al., 2004). Apoptosis is also suggested as the mechanism for reduction in tyrosine hydroxylase active cells in the brain of neonatal rats exposed for 4-*n*-OP. The effects are thought to explain the hyperactivity behavior in exposed rats (Ishido et al., 2004).

On the contrary, NP can also disturb the neuronal functions by stimulating the synthesis of catecholamine (dopamine, epinephrine and norepinephrine) in bovine adrenal medullary cells after increased tyrosine hydroxylase activity (Yanagihara et al., 2005). The effects were not inhibited by ER antagonist (ICI182,780) or protein synthesis inhibitors (actinomycin D and cycloheximide), suggesting that NP stimulates tyrosine hydroxylase and catecholamine synthesis in a nongenomic manner. This was confirmed by the finding of effects of short-term treatment (10 min), and the authors suggest that activation of MAP kinase system induces the effects. Behavioral studies show that NP exposure has effects on fear response in rats. This is probably induced through alterations of the catecholamine systems (Negishi et al., 2004).

The neurotransmitter acetylcholine can also be affected by AP exposure, both by inhibition of acetylcholinesterase activity (Talorete et al., 2001) and modulation of the

nicotinic acetylcholine receptor (Nakazawa and Ohno, 2001) or the muscarinic acetylcholine receptor (Jones et al., 1998).

The focus on AP as a environmental problem have mainly been for the *para*-substituated APs, but the APs found in crude oil and in produced water contains a large number of isomers (Ioppolo-Armanios et al., 1995). It may therefore be interesting to draw attention to the *ortho*-substituated APs. Propofol® is the name of the widely used intravenous general anaesthetic, 2,6-diisopropylphenol. Propofol® has an inhibitory effect on the neurotransmiter γ-aminobutyric acid (GABA) by binding to the $GABA_A$ receptor, a property in common with many other general anaesthetics (Trapani et al., 2000). Similar effects are found with other *ortho*-substituted AP analogues to 2,6-diisopropylphenol, like 2,6-dimethylphenol, 2,6-diethylphenol etc. Also mono *ortho*-substituted isomers like 2-isopropylphenol show such effects (Krasowski et al., 2001a; Krasowski et al., 2001b). No studies on the effects of *ortho*-substituted APs in wild life were found in the literature. However, when working with complex mixtures of AP, one should keep in mind that other isomers than *para*-substituted can also have specific biological effects at low concentrations.

1.10.3 Effects of AP on biosynthesis and metabolism of steroids.

In addition to affecting the steroid biosynthesis indirectly through the GTH and the brain-pituitary-gonadal axis, APs can also act directly on steroidogenesis enzymes.

In vitro studies with Leydig cells from rats show that 4-*tert*-OP has a biphasic effect on T biosynthesis, with induction of T synthesis at low concentrations (1 and 10 nM) and a reduction of T synthesis at high concentrations (100 - 2000 nM). By using different steroid precursors like 22(R)-hydroxycholesterol, pregnenolone, progesterone and androstenedione as substrate for the T synthesis, inhibitory effects of OP were shown early in the biosynthesis of $P450_{scc}$, 3β-HSD and $P450_{c17,}$ but not 17β-HSD, (the enzyme that converts androstenedione to T). No similar effect was seen with E2 and the effects of OP were not inhibited by ER antagonist (ICE 182,780), demonstrating

that these effects are not modulated through the ER (Murono et al., 1999; Murono et al., 2001). NP are also found to decrease T synthesis by inhibiting $P450_{C17}$ *in vitro* in cells from rat testis, but only minimal effects were seen on T-dependent endpoints *in vivo* (Laurenzana et al., 2002a). 4-*tert*-PP, 4-*tert*-OP and 4-NP are inhibiting $P450_{scc}$ and the hydroxylases ($P450_{11\beta}$, $P450_{c17}$, $P450_{C21}$) in human andrenocortical H295R cells, resulting in a decrease in cortisol secretion (Nakajin et al., 2001). In microsomes from carp testis, NP had no effects on 17β-HSD, but increased 20β-HSD activity dramatically and induced production of MIS (17α,20β-P) (Thibaut and Porte, 2004). Expression of $P450_{11\beta}$ mRNA was completely inhibited in the testis of medaka (*Oryzias latipes*) exposed to $\geq$ 413 µg/l 4-*tert*-PP (Yokota et al., 2005). Similar inhibition of $P450_{11\beta}$ mRNA is found in the brain of salmon exposed to NP (Arukwe, 2005). The same study also found induction of StAR protein mRNA and $P450_{scc}$ in the brain of NP exposed salmon, showing a possible stimulation of the early steps of the steroid synthesis (**Chapter 1.3**). Several studies have found that NP are inducing aromatase ($P450_{arom}$) mRNA expression in the brain (Kazeto et al., 2003; Kazeto et al., 2004; Meucci and Arukwe, 2006a) and the liver (Min et al., 2003), but not in the gonad (Kazeto et al., 2004) of fish.

Beside effects on the biosynthesis, an increase or a reduction of the metabolic elimination rate can also alter the steroid levels. NP is an agonist of the PXR and the CAR, and may therefore alter several phase I, II and III enzymes that are impotant for the metabolism of natural steroids (Masuyama et al., 2000; Mikamo et al., 2003; Kretschmer and Baldwin, 2005; Meucci and Arukwe, 2006b). PXR and CAR are regulating many important phase I cytochrome P450 enzymes (CYP2A, CYP2B, CYP2C and CYP3A), phase II enzymes, like uridine diphospho-glucuronosyltransferases (UDPGT), glutathione-S-transferases (GST) and sulfotransferases (SULT), and phase III transporters (multidrug resistance proteins that are active in transporting polar metabolites across the membranes for excretion). NP exposure increases the hepatic microsomal progesterone hydroxylase activity and CYP3A proteins in rat liver (Lee et al., 1996). Gender-specific induction of

cytochrome P450s is seen in NP treated mice. NP exposure increase expression of CYP1B subfamily members in both males and females, but CYP3A is exclusive down regulated in the females and CYP2A is induced only in the males (Hernandez et al., 2006). Arukwe et al., (1997a) found that low levels of NP induce steroid hydroxylase activity, but high doses inhibit the activity *in vivo* in juvenile Atlantic salmon. They also found a reduction in CYP1A, CYK2K-like and CYP3A-like proteins in the highest exposed group (125 mg NP/kg) together with reduction in UDPGT activity. Jurgella et al., (2006) demonstrated that NP (100 μM) did not effects E2 metabolism in neither liver nor kidny tissue from lake trout (*Salvelinus namaycuch*). OP (100 μM) inhibit E2 metabolism in the liver tissue but not in kidney tissue. As seen from this discussion is it not clear if AP increase or decrease steroid metabolism, some investigations have found increased metabolism (Baldwin et al., 2005), other have found reduction or no effects (Laurenzana et al., 2002b; Vaccaro et al., 2005; Jurgella et al., 2006).

The effects of APs on the steroidogenic or metabolic enzymes, either by direct inhibition or by altering the gene-expression and protein synthesis (up or down) may affect the seasonal pattern of steroids that is so important for synchronising all the reproductive events. Induction of $P450_{arom}$ can increase the production of E2, and unnatural high levels of E2 have been reported in juvenile male flounders (Mills *et al.* 2001), male and female fathead minnow (Giesy *et al.* 2000) exposed to OP and NP. Offspring of NP exposed rainbow trout had increased levels of E2 in males and T in females, even though the offspring were grown in clean water for 3 years (Schwaiger et al., 2002). However, reduction in E2 and other steroids in plasma have also been reported as results of AP exposure. Arukwe *et al.* (1997) found a reduction in the plasma levels of E2 in juvenile Atlantic salmon at relatively low AP concentrations (1 and 5 mg/kg, injected into the abdomen) but found no effect at higher concentrations (25 and 125 mg/kg). Female rainbow trout exposed to NP has reduced E2 concentration in plasma, but only at the high doses (85.6 μg/l) (Harris *et al.* 2001). NP exposure reduced androgen and estrogen levels in plasma and testis of juvenile male

turbot, while no effect was found in female turbot (Labadie and Budzinski, 2006). In the same study, the amount of glucoronidated steroids in the bile was reduced in male fish, indicating that the drop in steroid concentration was not a result of increased metabolism.

The effects of AP on the steroidogenesis are rather contradictory and several studies find biphasic responses with different doses. However, a lack of a linear dose-response relationship is typical for the steroid system where the nature of the response often is different with low and high doses of steroids. For example, low doses of E2 and testosterone stimulate the secretion of gonadotropin in fish, while is inhibited by high doses (Jalabert *et al.* 2000).

1.10.4 Effects of AP on the immune system

Estrogen plays an important role in the immune system and are involved in differentiation and maturation of T-cells in the thymus and B cells in the bone marrow, and has other immunoregulatory properties as secretion of cytokines and production of antibodies (Sakazaki et al., 2002). NP have been found to mimic estrogenic effects on the immune system like inhibition of lymphocyte mitogenesis (Sakazaki et al., 2002) and to induce thymocyte apoptosis (probably by the FAS/FASL pathway) (Yao and Hou, 2004, Yao et al., 2005, Yao et al., 2006). NP inhibit lipopolysaccharide induced nitric oxide (NO) and tumor necrosis factor-α (TNF-α) production in mouse macrophages (You et al., 2002, Hong et al., 2004). 4-*tert*-OP increases the production of the pro-inflammatory cytokine, interleukin-4 in T-cells. The effect was blocked by a calcineurin inhibitor, FK506, but not by the ER antagonist ICI 182.780, showing that the effect was activated by the Ca^{2+}-calcineurin partway independent of ER (Lee et al., 2003b, Lee et al., 2004). NP is found *in vitro* to have inhibiting effects on one of the key enzymes, cyclooxygenase-1 (COX-1), that converts arachidonic acid (20:4 n-6) to prostaglandines. This can also affect the immune system since prostaglandins play a

central role in regulation of inflammation, together with many other physiological processes (Fujimoto et al., 2005). Multi generation exposure experiments on rats show that NP alters the activity of splenic natural killer cell and increases the numbers of splenocyte subpopulations in second generation (F_1), while no effect was seen in first generation, (F_0) (Karrow et al., 2004). NP has also myelotoxic potency in F_1 male rats (Guo et al., 2005). The expermints found that the effects on the immune system were gender-specific. Although we not are aware of any studies on effects of AP on the immune responses in teleost, AP may probly also interact with the immune systems of fish.

1.10.5 AP induction of oxidative stress and DNA damage.

Ortho-substituted APs have good antioxidant properties because of the ability to stabilise free radicals and thereby reduce autooxidation. BHT is a well known antioxidant. A side effect of the anaesthetic, 2,6-diisopropylphenol (propofol®) is also that it protect cells against oxidative stress (De la Cruz et al., 1999). Many other phenolic compounds also have antioxidant properties, including estrogen. It has been suggested that the neuroprotective effects that are found for estrogens are mediated by antioxidant activity, even though it is not likely to be the most important mechanism (Amantea et al., 2005). As for most antioxidants, phenols can have the opposite pro-oxidant effect leading to production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-), and induction of lipid autooxidation. There is several defence systems that can be activated for protection against oxidative stress: glutathione, glutathione peroxidases, superoxide dismutase (SOD) and catalase (CAT). NP exposure of male rats is found to increase H_2O_2 generation and lipid peroxidation in the sperm. This increase in oxidative stress is over-activating the antioxidant defense systems, resulting in reduced activity of CAT, SOD, glutathione peroxidase and glutathione reductase (Chitra et al., 2002). Formation of hydroxy radicals is also found in rat striatum (Obata and Kubota, 2000) and rat Sertoli cells (Gong and Han, 2006) after NP exposure . The NP induced inhibition of

cell growth in bacteria and yeast cultures can be suppressed by adding antioxidants showing that the NP effects possibly are associated with ROS generation (Okai et al., 2000a, Okai et al., 2000b). Similar does DJ-1 (a protein having anti-oxidative function) protect against NP induced cell death in cultured medaka cells (Li et al., 2006).

Both estrogens and APs can be metabolized to catechols, phenoxyl radicals, o-quinones, and semiquinone radicals, all of which could cause damage to cells through alkylation or oxidation of cellular macromolecules including DNA (Krol and Bolton, 1997; Schweigert et al., 2001; Bolton, 2002). DNA damages can in the ultimate consequences lead to cancer. Studies of biotransformation show that the majority of NP are rapidly conjugated at the phenol group by glucuronidase followed by excreted through the bile (Lewis and Lech, 1996). But small amounts of AP are also oxidated to catechols, and covalently bound residues are found in trout (1.7 % of the total labelled NP) and in rudd (*Scardinius erythrophtalmus*) (12-62 % of total 4-tert-OP) (Coldham et al., 1998, Pedersen and Hill, 2000).

The metabolism of AP into reactive metabolites shows a potential for DNA damage. However, NP is not carcinogenic by itself (Sakai, 2001). NP are, on the other hand, shown to promote rat lung carcinogenesis, possibly via mechanisms involving DNA damage caused by ROS (Seike et al., 2003). Absence of promoting effects by NP have been seen in other carcinogenesis models, like thyroid carcinogenesis (Son et al., 2000b, Son et al., 2000a), and prostate carcinogenesis (Inaguma et al., 2004). Even inhibitory effects of NP are reported for rat ovarian carcinogenesis (Tanaka et al., 2002) and 4-*n*-OP and NP are reducing mammary tumor development (Han et al., 2002). DNA damages in human sperm and lymphocytes after NP exposure have been found by the Comet assay (Anderson et al., 2003). DNA damage after 4-*n*-NP exposure is found in larvae of barnacle, an aquatic invertebrate (Atienzar et al., 2002). Exposure of turbot to 30 µg/l NP for 3 weeks did not give chromosomal damage, determined as micronuclei frequency in the fish erythrocytes. On the other hand, a mixture of North Sea oil + APs (oil related isomers) , induced a very high micronuclei

frequency showing genotoxicity (Bolognesi et al., 2006). 24 hours exposure to high doses of NP (890 μg/l) to juvenile sea bass induced erythrocytic nuclear abnormalities (Teles et al., 2004).

1.10.6 Effects of AP on the cell membrane

Evidence of APs as membrane active compounds including membrane swelling, increase in fluidity, lowering of the phase transition temperature and increased ion permeability have been established from two intensively studied APs: the antioxidant BHT (Lanigan and Yamarik, 2002) and the intravenous anaesthetic Propofol® (Singer, 1977; James and Glen, 1980; Tsuchiya, 2001). There are also good support of *para*-substituted long-chain APs can be related to membrane effects that are independent of the estrogenic pathways. NP provokes vesiculation of the Golgi apparatus of epidermis cells from fish at concentration of 20 μM (Lamche and BurkhardtHolm, 2000). Similarly, 4-*tert*-BP and 4-*tert*-OP cause formation of lipid droplets and other changes in Leydig cell membrane structures of rats (Haavisto et al., 2003). Schwaiger et al., (2000) suggest that anaemia found in NP exposed fish is a consequence of an interaction between NP and the erythrocyte membrane. NP increases membrane permeability of mitochondria membranes to protons and act therefore as an uncoupler of the oxidative phosphorylation (Bragadin et al., 1999). Mitochondrial depolarization by NP has also been suggested as one of the mechanisms behind NP induced thymocyte apoptosis (Yao et al., 2006). Several investigations have shown that APs disrupt Ca^{2+} homeostasis by affecting Ca^{2+} membrane channels (Michelangeli et al., 1990; Beeler and Gable, 1993; Ruehlmann et al., 1998; Hughes et al., 2000; Logan-Smith et al., 2002; Kirk et al., 2003; Khan et al., 2003; Lee et al., 2003b; Walsh et al., 2005; Wang et al., 2005). Gap junctional intercellular communication is reduced in murine Sertoli cell line by NP, the effect is partly explained by reduction in phosphorylation of connexin 43 (Aravindakshan and Cyr, 2005), but the gap junction may also be affected by changes in the membrane lipid bilayer (Cascio, 2005).

1.10.7 Alkylphenols and their potential effects on fish reproduction and recruritment.

As discussed in the previous chapters is it quite clear that APs can interfere with a large number of biological pathways. There are particularly good evidence from laboratory studies that AP can induce endocrine disruption and alter the reproduction in fish, and there are also many indications that NP in combinations with other EDCs are involved in reproductive disruption in wild freshwater fish (Jobling and Tyler, 2003) and marine fish (Matthiessen, 2003). Linking the impacts of EDCs with effects on population levels is however still lacking and is one of the largest challenges within this scientific field (Mills and Chichester, 2005).

Table 8 (Appendix 1) gives an overview of 176 *in vivo* laboratory studies that have been investigating effects of APs on teleost fish. The majority of the literature is related to freshwater fish and the toxicology model species dominate, with Cyprinids (zebrafish, fathead minnow, sheephead minnow and goldfish), Salmonides (trout and salmons) and Beloniformes (medaka) constituting for more than 60 % of the total reports. It is therefore clear that our knowledge of the effects of ECDs on teleost only cover a limited number of the more than 24000 different teleost. Especially, there is lacking information on endocrine disruption on marine fish, even though the numbers of reports are increasing rapidly.

Table 8 lists the species, exposure regime and lowest effect concentrations for the different effect parameters, including: toxicity data (lethal dose), growth inhibition, inappropriate production of VTG in male and juvenile fish, inhibited ovarian or testicular development (lower GSI), abnormal blood steroid concentrations, up regulation of ER, alteration in pituitary hormones, alteration in sterodogenisis enzymes, intersexuality and/or feminisation of the gonads, skewed sex ratio, changes in male and/or female maturation, increased ovarian atresia, decreased sexual behaviour in males, reduced spawning success, reduced hatching success and/or larval survival, altered growth and malformations in early development. Some of these measurements can directly be correlated to adverse endpoints like survival, growth,

morphological development and reproduction. These endpoints tell about the fitness of the individual and the effect concentrations can be transferred into "predicted no effect concentrations values" (PNECs) necessary for modelling the risk for damages on population levels. On the other hand, many of the biomarkers are not easily linked to adverse effects.

Many of the studies on the effects of APs on fish are short-time experiments presenting results with different biomarkers. These experiments are very useful in identifying which compounds that are having endocrine disruption effects and it can give mechanistic information, but it is difficult to transfer results from such studies into fitness parameters. One example; APs induce VTG in fish at doses down to 0.1-5 μg/l (Jobling et al., 1996; Fent et al., 2000; Hemmer et al., 2001; Kashiwada et al., 2002). Induction of VTG is the most used estrogen specific biomarker, because of its very high sensitivity and clear link to estrogenic effects. However, the relationship between VTG induction and adverse effects on fish reproduction is unclear. Pathological effects in liver and kidney have been seen in connection with very high VTG induction after exposure of high potent estrogens like E2 or ethynylestradiol (Herman and Kincaid, 1988; Folmar et al., 2001; Palace et al., 2002), but these effects are found after million-fold increase of VTG, resulting in plasma concentrations at the high mg/ml levels. Exposure to weak xenoestrogens like APs is mostly resulting in lower-level induction of VTG and the impact of this is not well defined (Mills and Chichester, 2005).

The optimal experimental design for EDC testing is full life-cycle tests, where multiendpoints are used for investigation of both developmental and reproductive effects. In these tests, the fish are exposed from embryos till the stages of sexual maturation and through the spawning periods. In some cases also multigenerational studies are preformed and the exposure is continued on the second generation. Of practical reasons, full life-cycle tests have only been done on small fish with short generation time (≤ 4 month) like the zebrafish, fathead minnow and medaka. However, even with small laboratory fish that mature rapidly, full-life toxicity tests require very long experiment time and are very costly and work intensive. Therefore most studies

are done by partial life-cycle test, where the experiments focus on special sensitive periods in the fish life. Embryonic and larval development, especially during the critical stages of sexual differentiation and gonadal development, has received much attention. Similarly, many experiments with adult fish are done in the time of vitellogenisis and gonadal maturation.

The lethal concentrations (LC) of NP are found to be between 18-940 µg/l for different species and developmental stages. New hatched fish larvae are most sensitive for the acute toxicity of AP, while the LC for juvenile and adult fish are over 100 µg/l for NP (table 7).

Full-life-cycle test shows that NP and OP exposure reduces the reproduction potential in zebrafish at 28-100 µg/l (Hill and Janz, 2003; Segner et al., 2003b) and in medaka at 2-50 µg/l (Gray and Metcalfe, 1997; Gray et al., 1999b; Yokota et al., 2001; Knorr and Braunbeck, 2002; Seki et al., 2003b), while the AP with shorter chain length, like 4-*tert*-PP are less potent, inducing reproduction disturbance around 200 µg/l both in medaka (Seki et al., 2003b) and fathead minnow (Panter et al., 2006). These effect concentrations are in good agreement with the results from partial life-cycle tests, but there are some differences in sensitivity between different species. The lowest adverse effect concentrations reported in the literature are in rainbow trout. Lahnsteiner *et al*. (2005) found that 60 days of exposure to 750 ng/l NP completely inhibits male semen production and doses down to 130 ng/l NP significant reduced semen production.

As discussed in **Chapter 1.6** NP is found in freshwater systems, mostly in the concentration range ng/l to the low µg/l, but up to 644 µg/l in highly polluted areas. In seawater the concentrations are lower, from low ng/l to 9 µg/l. The environmental water concentrations correspond well with reported levels of NP found in wild freshwater fish, being of the order 1-1600 µg/kg, in view of the fact that the BCF are reported to occur from 75-1250 (table 3). As seen here NP is found at exposed sites in the natural environment at concentrations high enough for adverse effects on fish to occur, but the majority of the measurements are below the known PNEC values. It has

been suggested that APs in the marine environment are partly responsible for testicular abnormalities and VTG induction in male flounder (*Platichthys flesus*) from the UK estuary (Lye et al., 1999), but no clear evidence is available. It is important to note that real environmental exposure will always be a complex mixture of many different compounds, never APs alone. Nevertheless, there have been several attempts to use the available toxicological data to risk assessment. Brown *et al* (2003, 2005) have estimated that long-time exposure (20 years) to 30 μg/l NP could lead to severe decline in population levels of freshwater fish. The U.S. Environmental Protection Agency estimated that NPs PNEC values for freshwater organisms is 28 μg/l (acute toxicity) and 6.6 μg/l (chronic toxicity) and NPs PNEC values for saltwater organisms are 7 μg/l (acute toxicity) and 1.7 μg/l (chronic toxicity) (EPA, 2005). These PNEC values agree with those of Staples *et al.* (2004) who estimated the chronic effect value of NP to be 5.7 μg/l.

1.11 Analytical methods for determination of alkylphenol in biological tissue and produced water.

APs can be analyzed with different chromatographic and electrophoresis methods (reviewed in (Lee, 1999, Petrovic et al., 2002a)). For analysis of the whole profile of individual isomers of Aps, gas chromatography (GC) is preferred because of the high-resolution power. Many are analysing phenols directly without derivatisation both with GC-FID (Ioppolo-Armanios et al., 1992; Chee et al., 1996; Lye et al., 1999) or GC-MS (Giger et al., 1981; Bhatt et al., 1992; Wheeler et al., 1997; Gunther et al., 2001; Espejo et al., 2002).

The APs contain an “active” hydrogen atom and are therefore often converted to thermally stable and less polar compounds to improve their chromatographic performance before GC. Most of the derivatization methods are used together with GC-MS or for the halogenated derivatives with GC-ECD as the detectors. Many different derivatization techniques are used for GC these involve: Akylation to methyl

ethers (Bolz et al., 2000, Fiamegos et al., 2003), 3,5-bis(triflurомethyl)benzyl ethers (Cheung and Wells, 1997), pentaflurobenzyl ethers (Chalaux et al., 1994; Nakamura et al., 2000; Doerge et al., 2002) and 4-tetrafluoropyridyl derivatives (Kojima et al., 2003); silylation to trimethylsilyl ethers (Heberer and Stan, 1997; Mol et al., 2000; Li et al., 2001; Guenther et al., 2002). There are also several arylation based derivatization methods of phenols. Among the most used methods are acetylation (Llompart et al., 1997; Louter et al., 1997; Croley and Lynn, 1998) and pentafluorobenzoyl derivatization (McCallum and Armstrong, 1973; Renberg, 1981; Granmo et al., 1986; Wahlberg et al., 1990; Bao et al., 1996; Kuch and Ballschmiter, 2001; Xiao et al., 2001; Bianchi et al., 2002). Other not so common arylation methods are determination of phenols in crude oil as ferrocenecarboxylic acid esters using GC withatomic emission detection (Rolfes and Andersson, 2001) and extractive derivatization of phenols in oil produced water with methylchloroformat (Grahl-Nielsen and Landgren-Skjellerudsveen, 1982).

Table 9 (Appendix 2) gives an overview of different methods developed for analysing AP in biota samples, and table 10 (Appendix 2) shows methods for AP analysis in produced water.

2. Aims of the Thesis

The work in this thesis is a part of a strategic work at the Institut of Marine Research, which aim to develop a variety of methods to be used in investigating potential endocrine disruption in Atlantic cod. The main concern that initiated the project were whether discharges of APs from the oil industry give endocrine disruption in Atlantic cod and thereby effect the reproduction and recruitment of cod and other species in the North Sea.

The major aims of this thesis are:

- Development of analytical methods for determination of low levels of APs in produced water and fish tissue (**Paper I, Paper II)**
- To study long-term effects of selected *para*-substituted APs (4-*tert*-BP, 4-*n*-PP, 4-*n*-HexP; 4-*n*-HepP) on the reproduction of male and female cod. The objective of this investigation has been to study a wide spectra of biological end points (growth and morphological development), biomarkers (GSI, plasma steroids, VTG and gonad histology) (**Paper III, Paper IV**), effects on the redox status (glutathione and glutathione-related enzymes) (**Paper V),** effects on the hepatic CYP1A and CYP3A protein expressions and enzyme activities **(Paper VI)** and effects on the phospholipids in the liver and brain (**Paper VII**) of AP exposed cod.

3. List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

Paper I

S. Boitsov, S. Meier, J. Klungsøyr, and A. Svardal. Gas chromatography-mass spectrometry analysis of alkylphenols in produced water from offshore oil installations as pentafluorobenzoate derivatives. *Journal of Chromatography A* 1059 (1-2):131-141, 2004.

Paper II

S. Meier, J. Klungsøyr, S. Boitsov, T. Eide, and A. Svardal. Gas chromatography-mass spectrometry analysis of alkylphenols in cod (*Gadus morhua*) tissues as pentafluorobenzoate derivatives. *Journal of Chromatography A* 1062 (2):255-268, 2005.

Paper III

Sonnich Meier, Tom Einar Andersen, Birgitta Norberg, Geir Lasse Taranger, Anders Thorsen Olav Sigurd Kjesbu, Jarle Klungsøyr and Asbjørn Svardal. Effects of long-term exposure of alkylphenols on the reproductive system of Atlantic cod (*Gadus morhua*). Manuscrip.

Paper IV

Sonnich Meier, Tom Einar Andersen, Birgitta Norberg, Anders Thorsen, Geir Lasse Taranger, Olav Sigurd Kjesbu, Roy Dale, H. Craig Morton, Jarle Klungsøyr and Asbjørn Svardal . Effects of alkylphenols on the reproductive system of Atlantic cod (*Gadus morhua*). *Aquatic toxicology* 81 (2): 207-218 (2007).

Paper V

L. Hasselberg, S. Meier, and A. Svardal. Effects of alkylphenols on redox status in first spawning Atlantic cod (*Gadus morhua*). *Aquatic toxicology* 69 (1):95-105, 2004.

Paper VI

L. Hasselberg, S. Meier, A. Svardal, T. Hegelund, and M. C. Celander. Effects of alkylphenols on CYP1A and CYP3A expression in first spawning Atlantic cod (*Gadus morhua*). *Aquatic toxicology* 67 (4):303-313, 2004.

Paper VII

Sonnich Meier, Thorny Cesilie Andersen, Kristin Lind-Larsen, Asbjørn Svardal and Holm Holmsen. Effects of alkylphenols on glycerophospholipids and cholesterol in liver and brain from cod (*Gadus morhua*). *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 145 (3): 420-430, 2007.

4. General discussion

4.1 Analytical methods for determination of alkylphenols in produced water and biological tissues

The aim of the analytical chemistry study was to develop selective and sensitive methods for determination of APs, from phenol to NP, in produced water (**Paper I**) and biota samples (**Paper II**).

Establishment of analytical methods has three main challenges.

1. Extraction of target compounds.

2. Sample Clean-up to remove matrix effects.

3. Sensitive and selective analysis.

Different techniques for all of the above parts of the method development have been tested.

Extraction of target compounds.

For the biota analysis, cyclic steam-distillation was the first extraction technique tested. This method was originally developed by Veith and Kiwus (1977) for pesticide analysis in water, sediments and biota. The method uses a water distillation to concentrate the analytes and the condensated steam is extracted with a small amount of organic solvent in a special apparatus, before it is transferred back to the distillation chamber. This method have also been used to investigate AP levels in biota (Ahel et al., 1993; Lye et al., 1999; Gunther et al., 2001; Keith et al., 2001; Snyder et al., 2001a). However, even extensive attempts to optimize the steam distillation with out-salting, pH adjustment and extraction time did not make this method work satisfactory for AP spiked cod liver samples. At the best, only an AP recovery of 30% was obtained. It was therefore concluded that steam distillation extraction not is a suitable

method for extraction of APs in extremly lipid-rich samples like cod liver. The focus was thereafter subjected to liquid-liquid extraction and dichloromethane (DCM) was found to be a good solvent for the biota samples with recovery 67-90 % (**Paper II**).

The extraction of AP in produced water was done with solid-phases extraction (SPE). Three different SPE cartridges were tested (500 mg superclean envi-chromp columns (supelco), 200 mg Oasis® HBL and 150 mg Oasis® MAX columns (Waters)). All three cartridges contants polymers of styrene-divinyl, this type of sorbents has been shown to possess better extraction abilities than other typically used ons (e.g. C_{18}) (Liu et al., 2004). The MAX column contains quaternary amino groups bound in the styrene-divinyl polymers, this give these cartridges the ability to extract both acidic and hydrophopic compounds. Oasis Max column were used further because they gave the highest recovery and the purest extracts (**Paper I**).

Sample clean-up.

Biological samples have a very complex matrix containing a high amount of lipids, proteins, etc. Therefore, purification of the extracts is usually necessary. The lipids from the biota extracts were effectively removed by gel permeation chromatography (GPC). It was found that a columns switch method using two GPC columns removed more than 98 % of the lipids in the extracts (**Paper II**) and that thise were satisfactory for GC-MS analysis. The produced water extracts from the Oasis® MAX columns was so clean that it did not need any additionally clean-up before derivatisation (**Paper I**).

There were problems with background contamination of APs, especially from 4-NP in the procedure blanks. It seems that some APs are widely spread in most indoor environments (Rudel et al., 2003) and phenol and *para*-substituted APs (*p*-cresol, 4-*tert*-BP and 4-NP) are intensively used in plastics industry (Cascaval et al., 1996). In our work, 4-NP was found in most of the plastic and rubber products used in the laboratory, including vinyl gloves, rubber stoppers for glass funnels and plastic tubes used for the nitrogen evaporator. However, despite a significant effort to avoid these problems, we still detect small amounts of phenol, cresols, 4-*tert*-BP, 4-*tert*-OP and 4-NP in the blank samples. It is therefore important to have a good and intensive control

of procedural blanks. The trace amounts of APs in blank samples increase the risk of false positive results and the levels of contaminants may limit the use of the analytical method (**Paper II**).

Analysis.

Pentafluorobenzoyl derivatisation was selected for the studies, being a sensitive and selective method for the derivatisation of APs for GC-ECD and GC-MS (McCallum and Armstrong, 1973, Renberg, 1981). The derivatisation methods were optimised by the use of factorial experimental designs. Our results show that pentafluorobenzoyl derivatisation is a good and robust method for analysing *meta*- and *para*-substituted APs. The variations of the 7 parameters tested had no significant influence on the recovery of the long chain *para*-substituted APs, but the *ortho*-substituted and most water-soluble APs were significantly affected. The recoveries of the *ortho*-substituted APs were low. For the most sterically hindered APs (like 2,6-dimethylphenol and 2,3,6-trimethylphenol) the recoveries were less than 10 % even at the most optimal conditions. From this result it was concluded that the pentafluorobenzoyl derivatisation is not suitable for analysis of sterically hindered *ortho*-substituted APs (**Paper I**). Capillary GC with ECD detection was found to be a highly sensitive method for analysis of standard solutions. However, GC-MS-NCI (negative ion chemical ionisation) methods were preferred when analysing real complex samples (produced water and biota) due to matrix effects. The complexity of APs in samples of produced water (**Paper I**) makes it difficult to find good internal standards for GC-ECD. In GC-MS on the other hand, it is possible to use an isotope-dilution method where deuterium-labelled APs are used as internal standards.

Both methods are now in use at the laboratory of the Institute of Marine Research.

4.2 Long-term effect study on alkylphenol effects on the reproduction in cod .

4.2.1 Experimental design

Paper III-VII present the results from a project carried out during 1997-2001 where the goal was to study long-term biological effects of very low concentrations of selected C_4-C_7 APs on sex development in Atlantic cod. The study was carried out under controlled laboratory conditions. The compounds tested were 4-*tert*-BP, 4-*n*-PP, 4-*n*-HexP and 4-*n*-HepP.

Very little is known about the fate of these substances in the marine environment. There are no empirical data on concentrations of long-chain APs in the sea around the North Sea offshore installations (**Chapter 1.8**). We were therefore forced to use models when estimating the levels to which fish may be exposed. Rye et al., (1996) simulates the spread of AP discharges from produced water from the Halten Bank, and calculates the likely uptake by pelagic fish using a model. The model simulates the distribution of total AP discharges from two platforms, and includes biological response (Bioconcentration Factor (BCF) and constants for uptake and elimination). The calculations of a "worst case scenario" show that the body burden of AP in the fish modelled will be up to 10 µg/kg (Rye et al., 1996).

Given the lack of field data, we used the model values indicated in Rye *et al.*(1996) as a basis for choosing the exposure regimes in our experiments. Using an equal mixture of the four components with differing chain lengths (C4 to C7), an attempt has been made to take into account the wide range of different APs found in produced water. The intention of the tests was to dose the fish to a body burden within the range of Rye *et al.*'s estimates. Using the available information, it was concluded that 5 µg/kg of each of the four AP correspond to a fairly realistic dose.

Two independent experiments were carried out (table 5 and table 6): In Experiment I (**Paper III**), two groups of cod were exposed through regular food *per os* with a

mixture of the four APs, from October to the end of January (14 weeks): 0.02 mg/kg in the low dose group and 2 mg/kg in the high dose group. In Experiment II (**Paper IV-VII**) five groups of cod were exposed to the same mixture of APs, ranging between 0.02 and 80 mg/kg APs and a positive control of 5 mg/kg E2, for 1 or 5 weeks. In experiment II the APs were administered to the fish by a plastic tube directly to the stomach. The way of exposere assured that each individual got the same defined dose per unit weight.

Table 5. *Exposure and sampling scheme.*

	Experiment I	Experiment II
Start of exposure	1997-09-30	1999-11-16
Sample 1	1997-10-30	1999-11-23
Sample 2	1997-11-27	1999-12-21
Sample 3	1997-12-16	-
Sample 4	1998-01-26	-

Table 6*. Treatment and doses (sum of 4-tert-BP, 4-n-PP, 4-n-HexP and 4-n-HepP) for the two experiments.*

Groups	Experiment I	Experiment II
Control	Untreated	Untreated
Positive control	-	5 mg E2/kg
AP 1	0.020 mg AP/kg	0.020 mg AP/kg
AP 2	2 mg AP/kg	2 mg AP/kg
AP 3	-	20 mg AP/kg
AP 4	-	40 mg AP/kg
AP 5	-	80 mg AP/kg

The results of the present study suggest a multiple mechanism response in the AP treated cod. The exposure to APs can influence the plasma concentration of several male and female sex hormones and the egg yolk precursor protein, vitellogenin, in Atlantic cod. This study also shows that AP-exposure down to 20 µg/kg body burden interferes with the maturation of the sex organs, and that this effect is likely caused by disruption of the sex hormone system. There were also found effects of the AP treatment on the hepatic P450 systems (CYP1A and CYP3A) as well as glutathione, glutathione-related enzymes and changes in the lipid composition in liver and brain membranes.

4.2.2 Effects of APs on the glutathione-dependent antioxidant system in cod.

The effects of AP on the glutathione-dependent antioxidant system were studied in experiment II (**Paper V**). Total glutathione (reduced + oxidized forms)) increased in the livers of female fish after one week of exposure to APs. Males were not sampled after one week, so we do not know the early response of this group to AP exposure. The second sampling (after 5 weeks exposure) showed a smaller difference in GSH levels between the control and exposed groups. The level of reduced glutathione was also measured and the ratio of reduced to total glutathione was calculated. This relationship was relatively constant, and was similar in controls, positive controls and the exposed groups. Overall, the results show that there may be a temporary effect on glutathione level, but that the redox ratio remains unchanged. High, relatively stable redox ratios also indicate that the system that keeps glutathione in its reduced form, i.e. glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH), (which generates NADPH) function adequately under the experimental conditions reported.

Neither the activity of glutathione S-transferase (GST) nor of G6PDH appeared to be affected by AP exposure. This may indicate that neither of these two enzymes is particularly important in AP metabolism. It is known that glucuronidation is the major phase 2 metabolism of APs (Lewis and Lech, 1996; Meldahl et al., 1996; Thibaut et al., 1998a; Arukwe et al., 2000b; Ferreira-Leach and Hill, 2001), and these results may indicate that this metabolic pathway is sufficient to metabolise such quantities of APs as the fish were exposed to in our experiments.

4.2.3 Effects of APs on CYP1A and CYP3A in cod.

In vivo and *in vitro* effects of APs exposure in cod have been studied in **Paper VI**. Fish from Experiment II showed a dose-related increase in hepatic CYP1A and CYP3A protein in male cod, but no effect was observed in the females. However, this increase of CYP1A protein levels in the male fish was not linked to an increase in CYP1A-

mediated ethoxyresorufin-*O*-deethylase (EROD) activity, implying that APs inhibit the CYP1A activity *in vivo*. In addition, *in vitro* studies on the cod hepatocytes showed a strong AP dose-dependent reduction in both the CYP1A and CYP3A activity. Similar effects were also see in NP treated juvenile cod (Hasselberg et al., 2005; Sturve et al., 2006). Kinetic study of recombinant medaka CYP3A activity also found that NP are binding CYP3A and blocking the activity (Kullman et al., 2004). AP effects on the P450 systems are further discussed in (**Chapter 1.10.3, Paper VI**).

4.2.4 Effects of APs on membrane lipids in cod liver and brain.

Paper VII demonstrated that APs and E2 alter the fatty acid profile in the polar lipids of the liver to contain more saturated fatty acids (SFA) and less n-3 polyunsaturated fatty acids (n-3 PUFA) compared with the control. In the brain of the exposed groups, a similar effect was found, although with higher saturation of the fatty acids found in the neutral lipids (mainly cholesterol ester). No effects were found in the polar lipids. The AP and E2 exposure also gave a decline in the cholesterol levels in the brain. The *in vitro* studies showed that APs increased the mean molecular areas of the phospholipids in the monolayers at concentrations down to 5 μM, most likely due to intercalation of the APs between phospholipids molecules. The increase in molecular area increased with the length of the alkyl side chain. There are several other investigations that support that APs can affect the lipid environment in the cell membrane (see **Chapter 1.10.6**). Cakmak et al., (2006) found support for that NP exposure induce a decrease the membrane fluidity by increasing the lipid order in the liver of rainbow trout. This agrees with our finding of increased amount of SFA in the polar lipids. The biological consequences of changes in the lipid compositions of the membrane are unknown and need future studies.

4.2.5 Effects of APs on sex steroids in plasma of cod.

A striking observation from both experiments is that AP exposure brings about a considerable drop in the plasma E2 level in the females even at very AP low doses (0.02 mg/kg). The effects depended on the maturation status of the cod. In experiment I the reduction in E2 was first visible after 2 months of exposure in November. In the low-dose group and the high-dose group the E2 level were 68 % and 44 % of control, respectively. This effect became stronger in December (low-dose 71 % and high-dose 35 % of the control), but the difference was not significant before January. This was probably because the number of fish was to low to give statistically significant effects in November and December (see **table 6, Paper III**). In experiment II the exposure first started in November. The down-regulation of E2 levels by AP treatment was confirmed in this experiment and significant effects were found both after 1 and 5 weeks of exposure (**Paper IV**). The plasma level of T in female fish was also affected, but the results were more ambiguous than for E2.

In male cod, AP exposure also affected the plasma levels of 11-KT and T, basically by lowering the levels. However, like T in the female fish, there were large seasonal variations and no dose-related trend.

One hypothesis explaining reductions in the steroid concentrations may be that AP exposure increases the steroid catabolism. NP is shown to be an agonist of the orphan nuclear receptors, PXR and CAR that are involved in regulation of several detoxification enzymes, such as the CYP2B and CYP3A family members, which are responsible for the metabolism of steroids and this may alter their physiological levels (**Chapter 1.10.3**). The fact that we found a dose-related increase in hepatic CYP1A and CYP3A protein in male cod could indicate an induced metabolism, however, such effect was not observed in the females (**Paper VI**). The increase of CYP1A protein levels in the male fish was not linked to an increase in EROD activity, implying that APs inhibit the CYP1A activity *in vivo*. In addition, *in vitro* studies of cod hepatocytes showed a strong AP dose-dependent reduction in both the CYP1A and CYP3A

activities. The increase in the amount of CYP3A protein may indicate an increase in the potential steroid catabolism in the male cod, but not in the females. A general increase in steroid catabolism does not explain the observed decrease in E2 (female) and 11-KT levels (male), because the level of testosterone was maintained or even increased. There is also support for that AP inhibit phase II enzymes and therby decrease steroid catabolism. Kirk et al., (2003) finds that *para*-substituated APs (C1-C9) reduces E2 sulfations in liver cytosol of chub (*Leuciscus cephalus*). Additionally, NP exposure has an inhibiting effect on glucuronidation of T and E2 in juvenile turbot (*Scophthalmus maximus*) and juvenile cod (Martin-Skilton et al., 2006c) and NP is also reducing E2 sulfation and glucuronidation in carp testicular microsomes (Thibaut and Porte, 2004) and E2 sulfation in liver cytosols from two marine fish, *mullus barbatus* and *Lepidorhombus boscii* (Martin-Skilton et al., 2006a).

Therefore, another explanation to the drop seen in E2 and 11-KT levels could be that AP exposure affects the steroidogenic enzymes (**Chapter 1.10.3, Paper IV**). One interesting finding in the present study has been that there is no clear dose-response relationship for E2 to AP exposure. The group that received the lowest dose (0.02 mg/kg) displayed the same decrease in E2 level as those that received higher doses. This may indicate that E2 down-regulation is a result of exceeding a threshold level. Currently we are doing further work to reveal the mechanism of action of APs on the steroid levels in cod. Several studies indicate that APs disrupt the natural endocrinal feedback system of the fish somewhere in the central nervous system and this affects the secretion of GTH from the pituitary and thereby indicretly affect the steroid synthesis (see **Chapter 1.10.2**).

4.2.6 Effects of APs on VTG in plasma of cod.

A massive induction of VTG in female and male cod following exposure to E2 (5mg/kg) confirms the VTG's sensitivity as a biomarker for estrogen (**Paper IV**). It

confirms previous reports on the cod's suitability as a model organism in these studies (Hylland and Haux, 1997; Hylland, 2000).

We demonstrated that the AP exposure gave a weak induction of VTG in the male cod. Experiment I gave statistically inconclusive VTG values. Nevertheless, more male fish were producing VTG in the exposed groups than in the control (**Paper III**). In Experiment II, there was a weak dose-related induction of VTG (**Paper IV**). It should be noted that even though there was an induction of VTG following AP exposure, the induction was several thousand times lower than in the fish exposed to E2.

Some surprising seasonal differences were observed in the control groups. November samples from both experiments showed higher plasma VTG levels in the males than fish from the other samples. After 1 week in Experiment II (November), measurable quantities of VTG were found in as much as 80% of the fish in the control group, while at the end of December (5 weeks) only 30% of the control fish had detectable levels of VTG in their plasma. This may be the effect of normal seasonal fluctuations of endogenous E2.

E2 plays an important role in the early part of spermatogenesis, regulating the renewal of spermatogonia (Miura and Miura, 2003). In the few studies reporting E2 in plasma from male teleost fish, concentrations are generally below 1 ng/ml (eel (*Anguilla japonica*): 0.5 ng/ml (Miura et al., 1999); huchen (*Hucho perryi*): 0.35 ng/ml (Amer et al., 2001); flounder (*Platichthys flesus*): 1.2 ng/ml (Scott, 2000); carp (*Cyprinus carpio*): 0.25 ng/ml (Villeneuve et al., 2002): Atlantic cod: 0.04-0.37 ng/ml (Scott et al., 2006). It is an unanswered question if these naturally occurring levels of E2 can be enough to induce VTG in male fish. Scott et al., (2006) measure induction of VTG in blood plasma of male cod caught in the North Sea and other areas around UK and Norway. A positive relationship between VTG and fish size was reported, but there were not found any correlation between plasma concentrations of E2 and VTG. It is suggested that large cod are exposed for estrogenic compounds through the food chain. Difference in the feeding ecology between large cod (feeding close to the bottom after

large prey) and small cod (feeding on free-swimming organisms in the water column) may explain the observed differents in VTG levels.

In aquaculture, there may be other sources of estrogenic substances. In mature male aquaculture cod VTG concentrations of 6.7 ± 4.5 µg/ml have been found and this is higher than generally found in wild fish (CEFAS, 2005). This points to the presence of elevated estrogen levels in farmed fish. At present, it can only be speculated regarding the reason for this. It is possible that phytoestrogens found in commercial fish diets play a role. In experimental settings, it is also possible that natural estrogen or its metabolites, secreted through urine from female fish, influence the male hormone levels. The recent findings of low, natural E2 levels in male fish, as well as the potency of E2 as a VTG inductor, may indicate that VTG is unsuitable as a biomarker for xenoestrogens at very low concentrations. Our data suggest that the hormone system may be affected by environmental contaminants at very low concentrations, while VTG-induction is only moderately susceptible to such influence. As research moves towards effect-studies of lower and lower concentrations of contaminants, we believe it will be necessary to use additional biomarkers for estrogenic substances than VTG induction.

4.2.7 Do AP exposure have adverse effects on the reproduction of cod

The results from the present thesis (**Paper III and IV**) show a reduction in steroid levels, ovary growth and testis maturation status at the lowest concentration tested, 20 µg/kg nominal body burden (sum of four APs), but it is not clear if these effects are causing adverse effects on the cod reproduction. Table 7 gives corresponding water concentrations and body burden (assumes a BCF factor of 500), useful when comparing the doses of different exposure regimes. Back calculations of the 20 µg/kg are equivalent to a theoretical total concentration of 40 ng/l, which are very low effect concentrations and below levels reported from other studies. Our finding need to be

confirmed in water exposure experiments, but it brings to attention to that there can be large differences in the response to AP between different species and that effects concentrations may be down in the ng/l levels.

Table 7. *Corresponding water concentration and body burdens, using BCF = 500*

Water concentration		Body burden
1 ng/l	↔	0.5 µg/kg
40 ng/l	↔	20 µg/kg
100 ng/l	↔	50 µg/kg
1 µg/l	↔	500 µg/kg
100 µg/l	↔	50 mg/kg

Experiment II showed considerable effects on the pattern of maturation of the testis in males (**Paper IV**). There was an increase in the amount of spermatogonia. There also appeared to be an increase in the amount of spermatocytes and a reduction in spermatozoa. Similar effects after AP exposure have been seen in a number of other fish species, but it still remains to find out if the changes in maturation status are leading to reduced sperm quality and thereby affecting the cability of the male cod to fertilize the eggs.

As discused above, the most significant effects found from these experiments are an anti-estrogenic effect of the APs, possibly by APs causing a down-regulation of natural E_2 synthesis (**Paper III and IV**). Normally, there is a direct relationship between E2, vitellogenin and gonadal growth. It is therefore not surprising that low E2 levels were accompanied by a drop in gonadal weight. The fish from the positive control group aborted the oocyte maturation, resorbing the oocytes through atresia. The groups exposed to APs did not show an increased occurrence of atresia. However, the oocytes had a significant reduction in oocyte diameter. Histologically, the oocytes seem to develop slowly but otherwise normally. The oocytes of the exposed groups were in the beginning of vitellogenesis at a time when the controls were in late vitellogenesis. The significantly smaller oocytes in the exposed groups predicted delayed spawning.

The natural spawning time for the North Sea cod stock are in spring, around the time of the initiation of the seasonal plankton development. The copepod *Calanus*

finmarchicus is a key zooplankton species in Norwegian waters, and early developmental stages (nauplius larvae) of this species are the main prey for fish larvae (Sundby, 2000). Variation in the timing of the plankton development *versus* the spawning and larval development of fish has been considered in the match-mismatch hypothesis to be a major cause for variable recruitment of fish (Cushing, 1990; Beaugrand et al., 2003; Platt et al., 2003). A delay in start of spawning, as estimated for our AP-exposed fish increases the chances of the eggs being spawned too late relative to the optimum. Data from the Baltic Sea show that over the last decade, there has been a shift towards spawning several weeks later in the season than has been the case in the past (Wieland et al., 2000). The temporal overlap between the developing Baltic cod larvae and their prey has decreased since the mid-1980s and this coincides with a massive reduction in the recruitment of this cod stock. (Hinrichsen et al., 2005). The main theories trying to explain the shift in spawning times are changes in water temperature during the period of gonadal maturation, density-dependent processes related to the size of the spawning stock, and food availability. But as shown in the present work, endocrine disrupting chemicals also effect the maturation of the gonads. This indicates that EDCs may be at least partially responsible for the changes seen in fish populations in the highly polluted Baltic Sea.

As support for such a theory, long-term monitoring (1988-2000) in the Baltic Sea of the gonadal size of female perch (*Perca fluviatilis*) revealed a strong trend towards decreased GSI. Pollution are suggested to play an important role as causative for this phenomenon (Hansson et al., 2006). Similarly, Noaksson *et al.* found a delay in the gonadal maturation of wild female perch, roach (*Rutilus rutilus*) and brook trout (*Salvelinus fontinalis*) (Noaksson et al., 2001; Noaksson et al., 2003; Noaksson et al., 2005) living in lakes receiving leakage water from old refuse dumps. The compounds causing endocrine disruption are not identified. The observed reduction in plasma steroid levels (T and E2) in combination with decreased GSI is, however, similar to the findings in cod in the present publication.

In medaka, it has been found that exposure to NP results in reduced realised fecundity (Gronen et al., 1999; Shioda and Wakabayashi, 2000a). These authors also noted a tendency for fewer eggs to hatch when an unexposed female spawned with an exposed male. This aspect has not been examined in our study, but there is every reason to look further into it. If exposure reduces both realised fecundity and the proportion of eggs that actually hatch, this would increase the effects of delayed spawning.

It is not possible from our results to conclude that the AP exposure is resulting in advanced effects on the cod reproduction, but the findings presented clearly calls for more studies.

4.2.8 Bioaccumulation and oral uptake of APs in cod

Our decision to expose fish through their food in our experiments was due to the practical and environmental consequences of exposure through the water. Large fish were used, and they were kept in large tanks. The fish require a continuous supply of large quantities of water (20000 l/h). A large-scale exposure experiment would require large amounts of APs and the building of costly infrastructure to properly handle the discharged water.

The body burden of 5µg AP/kg was expected to be equivalent to the quantity that the fish might absorb if exposed to seawater with 10 ng/l of the individual APs. This simplified calculation assumes a BCF factor of 500 for all four APs. 5 µg/kg is equivalent to a theoretical total concentration (sum of all four APs) of 40 ng/l. This concentration is lower than the levels that have previously been reported to affect the endocrine system in fish.

It may be argued that oral exposure results in APs being distributed in the body to a lesser extent than with exposure through the water. This should, however, result in an underestimation of the effects of the exposure. There seems to be no reason to suggest

that oral exposure leads to increased bioaccumulation. Consequently, our results more likely underestimate than overestimate the effects of AP exposure. Furthermore, the concentrations of APs actually found in the tissues are more likely overestimated than underestimated. On this background, it is clear that the present findings represent a minimum of expected detrimental effects of exposure to the tested concentrations of AP. Sundt and Baussant (Sundt and Baussant, 2003) compared the uptake and tissue distribution of the four APs used in our study in cod, using oral and waterborne exposure. They found that the bioconcentation from seawater was much higher than via absorption through the gut wall. A similar situation is also found in fathead minnows (*Pimephales promelas*) (Pickford et al., 2003). These studies suggest that the actual AP body burden in the current experiment can be only 10% of the nominal body burden and, furthermore, that the exposure level giving significant disruptions in the reproductive system of female cod may be as low as 2 µg/kg body burden, which is equivalent to 4 ng/l in the seawater.

4.3 Alkylphenols from offshore oil production

Limited data have been available on the contents of long-chain APs (C4 - C7) in produced water (Brendehaug et al., 1992; Røe and Johnsen, 1996). The analytical methods generally used (GC-MS of underivatised phenols with cluster analysis of all isomers with specific masses) have low selectivity and overestimations are likely to have occurred. Methods with higher sensitivity and more selective detection, have now been developed (**Paper 1**). A large number of APs are found in an average produced water sample. Theoretically, there can be hundreds of isomers of C_4-C_9 APs. Most of them are not commercially available. Only the long-chain, *para*-substituted APs have significant estrogenic effects. *Ortho*-substituted, *meta*-substituted and short-chain APs have very little or no estrogen effect (Routledge and Sumpter, 1997). At the IMR, an effort is currently made to synthesise as many as possible of the long-chain *para*-substituted AP isomers. We hope to be able to identify and quantify more of the *para*-

substituted AP from C_4 to C_9 and then estimate the total estrogenic potential from APs in produced water. Until such data are available, it will only be possible to obtain long-chain AP concentrations of a few standard compounds or as the sum of all isomers. The method described in **Paper 1**, have been used for determination of APs in produced water from 9 different oil fields on the Norwegian sector and the total concentrations of APs > C_4 are found to be in the range of 5-81 µg/l (in preparation). Thomas *et al* used *in vitro* methods (yeast estrogen screen, YES) to detect estrogen receptor (ER) agonists in produced water from the North Sea oil installation (Thomas et al., 2004b; Thomas et al., 2004a). They found that produced water contains ER agonists in amounts corresponding to E_2 equivalents from the low ng/l and up to 91 ng/l. This corresponds well with the levels of APs, considering that APs are in the order of 1000 times weaker ER agonist than E2 (Routledge and Sumpter, 1997).

The produced water is rapidly diluted after being discharged from the platform. Computer simulations show 30 and 100 times dilution 10 m and 100 m from the outlet, respectively. Further dilution is, however, slower, and the model showed that 1:1000 dilution occurred as far as 1000 m from the outlet (Neff, 2002). The results from the computer model and field data indicate that dispersed oil may be found in concentrations from 1-3 µg/l in an area with a radius of 50 to 100 km around the largest oil fields in the North Sea (Rye et al., 1998). This corresponds to a dilution factor of approx. 1:10.000.

There are no empirical data available on concentrations of long chain APs in the sea around North Sea offshore installations, but as discussed above, the concentrations in the marine environment should be low. On the other hand, the discharges are continuous and prognoses indicate increased discharges as the oil field age. Little is known about the fate of long-chain APs in produced water after it enters the sea. The degradation rate of APs falls rapidly with increasing chain length and APs have high sedimentation rates (**Chapter 1.6**). Measurement of long chain APs in seawater and sediment around oil installations should therefore be a priority in future risks assessment studies.

The results from the presented experiments have been used in a theoretical study titled: “Risk Assessment of reproductive effects of alkyl phenols in produced water on fish stocks in the North Sea” (Myhre et al., 2005). The modeling is done using the DREAM software (developed by Sintef, RF-Akvamiljø and TNO, Delft, the Netherlands). The model includes the combined discharges from three major Norwegian oil fields (Tampen, Ekofisk and Sleipner). The fish stock distributions (cod, saithe and haddock, from the international bottom trawl surveys (IBTS) database) and a Predicted No Effect Concentration (PNEC) for APs of 4 ng/l were used as basis data for the calculations. The total amount of APs>C_4 discharged from all the oil installation was estimated to be 25.6 kg/day, dissolved in 364.300 m^3/day produced water. The conclusion of the risk assessment was: “The overall results of the simulations with DREAM show that there is no significant risk potential. In other words there were no fish particles, which accumulated APs above the critical body burden of 2 µg/kg in any of the simulations. The highest accumulated body burden in any of the fish particles was 0.09 µg/kg” (Myhre et al., 2005). This new modelling work indicates that the article of Rye *et al.* (1996) overestimated the body burden and that the doses used in these experiments may not be expected to arise from produced water discharges alone. However, both models are encumbered with uncertainty, primarily because the fate of the long chain APs in the sea is not known. It is reasonable to believe that these relatively hydrophobic substances will bind to biological particles in the sea. This may affect distribution, degradation and also uptake of APs in the food chain. All these unknown factors urge for proper field studies in order to be able to forecast the impact of these biologically active chemicals on the marine environment.

4.4 Conclusions.

The results presented in this thesis have shown that cod is sensitive to AP contamination of the environment. Even though the concentrations used in our experiments are higher than may reasonably be expected as the result of oil production alone, measurements of real AP levels in the sea indicate that APs may still be a significant factor in the marine environment. Only when the environmental fate of the long-chain APs has been more thoroughly understood, the APs may possibly be ruled out as a significant detrimental factor of proper growth and development of the relevant fish populations. Collecting information about the concentrations of long-chain APs in the open water and in marine sediments is mandatory for sound evaluations of the environmental effects of long-chain APs.

4.5 Future perspectives.

New experiments have been conducted in 2005 to supplement and clarify the results from the present study. These include exposure to lower doses of APs to provide knowledge about the true "no effect" concentration. Furthermore, the fish have also been exposed to real produced water with its natural high complexity of components. Cod have also been reared through spawning after long-time exposure (20 weeks) to APs and produced water, aiming to confirm the estimated delay in spawning shown in this thesis, as well as searching for any effects on realised fecundity and fertilisation.

To begin elucidating the complex mechanisms involved in the response to APs, pitutitary gene expression related to FSH and LH will be analysed, as well as aromatase activity in the gonads and the brain. Steroid profiles will be analysed in blood plasma, gonads and brain, together with the amount of conjugated steroids in the bile.

Appendix 1. *In vivo* studies of the effects of alkylphenols on teleost fish

Table 8. *In vivo studies of the effects of alkylphenols on teleost fish. The table provides an overview of various measurement variables and the lowest effect dose that has response. (where concentrations are quoted per kg, the substance was administered by injection or oral; when quoted per litre, the substance was provided in the water*

Species, dose and exposure time	**Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis**	**Other effects**	**Reference**
Order: Cypriniformes			
Family: Cyprinidae			
Juvenile Carp (*Cyprinus carpio*). 100, 320 and 1000 µg/l 4-*tert*-PP at different times in the early lift stages.	Oviduct formed in testis by exposure during sexual differentiation. Reduction in number of primary sex cells.		(Gimeno et al., 1996; Gimeno et al., 1997; Gimeno et al., 1997)
Mature carp. 32 µg/l 4-*tert*-PP for 3 month.	Reduced number of spermatogenetic cysts. Reduced GSI after three months of exposure.		(Gimeno et al., 1998)
Zebrafish (*Danio rerio*). Fed NP containing food corresponding 40 µg/fish/day for 21 days.	Induction of VTG.		(Allner et al., 1999)
Fathead minnow (*Pimephales promelas*). 0.05–3.4 µg/l NP for 42 days.	1.1-3.4 µg/l NP increase Sertoli cell hypertrophy and necrotic aggregates of germ cells in testes.		(MilesRichardson et al., 1999)
Fathead minnow. 0.05–3.4 µg/l NP for 42 days	0.1 µg/L NP induced a 10-fold raise of E2 in female and induced VTG in the female fish.	BCF for NP from 203-268.	(Giesy et al., 2000)
Fathead minnow. 0.6–63 µg/l NP for 3 weeks.	Induction of VTG at 8 µg/l NP in males and at 58 µg/l NP in females. Doses of NP above 48 µg/l NP inhibited reproduction completely. (measured conc.)	Reduction in the prominence of secondary sexual characteristics in males at 8 µg/l NP.	(Harries et al., 2000)
Juvenile sheepshead minnow (*Cyprinodon variegatus*). Acute toxicity test for NP.		96-hr. LC50 = 142	(Lussier et al., 2000)
Juvenile carp.1–15 µg/l NP for 70 days.		Effects on blood parameters. Enlarged erythrocytes, and possible anaemia from 10 µg/l NP.	(Schwaiger et al., 2000)
Sheepshead minnow. Exp1 0.6 – 43 µg/l NP. Exp 2: 6 and 60 µg/l NP for 16 days. Samples were taken up to 96 days post-exposure.	Induction of VTG above 5 µg/l NP. VTG mRNA rapidly diminishes, but plasma VTG clearance is concentration and time dependent and can be detected months after exposure.		(Hemmer et al., 2001; Hemmer et al., 2002)
Pre-larval zebrafish embryos. 24 hr. exposure for 22 µg/l NP.	Minor but significant change in anterior-posterior distribution of primordial germ cells.		(Willey and Krone, 2001)
Juvenil carp. Injected with 1, 10 and 100 mg/kg NP, repeated after 7 days. Sampling after 7 days.	Induction of VTG at 100 mg/kg NP.		(Casini et al., 2002)
Transgene zebrafish. 220 µg/l NP for 96 hr.	No induction of estrogen receptor (ER)-mediated luciferase gene expression in the transgene fish.	Uptake of NP after 96 hr. 8940 µg/kg NP.	(Legler et al., 2002)
Male carp. 0.05–5.4 µg/l NP for 28-31 days.	No changes in steroid (E2 and T) or VTG levels in plasma. No differents in GSI.	Detection of 0.5-3.5 mg/kg NP in pooled plasma or tissue samples.	(Villeneuve et al., 2002)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Zebrafish. 10-100 µg/l NP. 60 days post hatch (dph), full life cycle tests.	Induction of VTG mRNA above 30 µg/l NP. Shift in sex ratio towards females at 100 µg/l NP (10 % males and 45 % in control). Two fish with ovatestis were found at 100 µg/l NP. 60 days after end of exposure (120 dph) there were no differens in sex ratio from any groups.		(Hill and Janz, 2003)
Embryos of zebrafish. Exposure (1, 10, 1000 µg/l NP) started one hr. after fertilization and til 48 hr.		10 % of the genes on the 230 gene chip were altered even at 1 µg/l NP. 9 gene were selected as potential biomarkers for NP exposure.	(Hoyt et al., 2003)
Male sheepshead minnow. 11.5–61.1 µg/l 4-*tert*-OP for 24 days. Spawning experiment with exposed male and non exposed females.	Induction of VTG at 11.5 µg/L OP. Testis anomalies in male at 33.6 µg/L OP.	Reduction in viable eggs when the male have been exposed for 33.6 µg/L OP. No effects on second generation: embryonic development, egg hatching or fry survival.	(Karels et al., 2003)
Juvenile zebrafish. 2.2, 22 and 220 µg/l NP for 3 days.		Up-regulation of whole fish transcript of aromatase CYP19A2 gene (mainly found in the brain and pituitary) at 2.2 µg/l NP. CYP19A1 (mainly found in the gonads) were unaffected.	(Kazeto et al., 2003; Kazeto et al., 2004)
Fathead minnow. Study of the route of exposure: 2 weeks exposure for 1-50 µg/L NP or 100–1000 µg/kg/day NP oral exposure.	VTG mRNA induced at 50 µg/l NP and 500 µg/kg/day NP. VTG found in plasma also at 10 µg/l NP.	It was concluded that exposure via the water gives a 10–fold higher sensitivity that via exposure by the diet.	(Pickford et al., 2003)
Zebrafish. 1-38 µg/l 4-*tert*-OP for 78 days, full life cycle tests	Fertilization success EC50=28 µg/l OP.		(Segner et al., 2003b; Segner et al., 2003a)
Embryonic zebrafish. 100 and 1000 µg/l NP for 48 or 72 hr.		30 % of the proteins from embryo homogenates were specific to the NP groups (two-dimensional electrophoresis, 2DE).	(Shrader et al., 2003)
Zebrafish and rainbow trout. 12.5-100 µg/l 4-*tert*-OP and 20-500 µg/l NP for 3 weeks.	Rainbow trout were 3 times more sensitive than zebrafish to OP and NP. Induction of VTG at 30 µl/L OP and 100 µl/l NP.		(Van den Belt et al., 2003)
Zebrafish. 2 days post-hatch (dph) – 60 dph. 10, 30 and 100 µg/l NP.	Delayed gametogenesis in both male and female from 100 µg/l NP. Increased ovarian follicle atresia.	Kidney pathology at 10 µg/l NP.	(Weber et al., 2003)
Male goldfish (*Carassius auratus)*. 0.1–100 µg/l NP in 28 days.	Induction of VTG at 100 µg/l NP.	No effect on CYP1A or GST.	(Ishibashi et al., 2004)
Female zebrafish. 30–500 µg/l NP for 3 weeks.	Induction of VTG at 250 µg/l NP. Reduced GSI at 500 µg/l NP.		(Van den Belt et al., 2004)
Fathead minnow. 0.4-7 µg/l NP and 1.5-35 µg/l OP. Mixtures of different xenobiotic. Exposure for 14 days.	EC_{50} for VTG induction 7 µg/l NP and 48 µg/l for OP. Additive effect of mixtures.		(Brian et al., 2005)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Male zebrafish. 500 µg/l 4-*tert*-OP for 7 and 15 days.	Induction of VTG. No difference in testis histology	Increased peroxisomal surface and numerical densities and activities of the peroxisomal β-oxidation enzyme acyl-CoA oxidase (AOX) in the liver. No induction of catalase activity.	(Ortiz-Zarragoitia and Cajaraville, 2005)
Juvenile goldfish. 22 and 220 µg/l NP for 3 weeks.	Induction of VTG at 22 µg/l NP. 22 µg/l NP affects the steroids levels in plasma: decrease in androgens (ELISA with cross reaction of many androgens) and increase of E2.	Induction of gene expression of ERβ-1. Accumulation of NP in liver and muscle.	(Soverchia et al., 2005)
Male carp. 690, 1380 and 2300 µg/l 4-*tert*-BP for 4 weeks. Sampling after 7, 14 , 21 and 28 days.	Reduced Male GSI ≥ 690 µg/l 4-*tert*-BP. Induction of VTG ≥ 690 µg/l 4-*tert*-BP. Totally loss of histo-morphology architecture in testis at all concentrations (disintegration of the lumen wall and necrosis of spermatozoa).	96 hr-LC = 6900 µg/l 4-tert-BP. Increased HIS and reno-somatic index ≥ 690 µg/l 4-tert-BP. Pathological changes was found in liver tissue ≥ 690 µg/l 4-tert-BP (hyperplasia of connective tissue, decrease in the number of hepatocytes, increased vacuolization in extracellular space and fibrous lesions). Decrease in alkaline phosphatase and aspartate aminotransferase activity ≥ 690 µg/l 4-tert-BP. Increrase alanine aminotransferase and acid phosphatase activity ≥ 690 µg/l 4-tert-BP.	(Barse et al., 2006)
Male fathead minnow larvae. 5 µg/l NP for 64 days.	No effects on VTG or GSI.	No effect on larvae survival. The NP exposed showed increased competitive reproductive behavior.	(Bistodeau et al., 2006)
Juvenile carp. 5, 50 and 500 ng/l NP or injection of 50 mg/kg NP. Sampling after 24 hr and 96 hr.	Gene expression analysis using a carp cDNA microarray (consisting of 960 hormone-responsive and gender-associated gene fracments) found that 111 genes were regulated in the liver of NP exposed fish. Examples of significant upregulated genes were: VTG, cytochrome c oxidase and cytochrome P450 monooxygenase (CYP2K1v2). No dose response data are giving.		(Moens et al., 2006)
Fathead minnow embryos. 56, 180 and 560 µg/l 4-*tert*-PP for up to 107days post hatch (dph), some fish were exposed for 30 or 60 dph and followed in clean water on to 107 dph.	No effects on hatching succes or survival, but a delay in time to hatch for the 560 µg/l PP. Induction of VTG at 180 µg/l PP in both female and males. Lower GSI in females at 560 µg/l PP. All female and no testis development at 560 µg/l PP. Intersex and ovarian-like cavity were found in all male fish at 180 µg/l PP.	Lower condition factor in females at all doses. No effect on liver or kidney histologi.	(Panter et al., 2006)
Mature zebrafish. 0.1, 1, 10, 50, 100 and 500 µg/L NP for 3 weeks.	Induction of VTG in male fish at 100 µg/l NP. Reduced GSI for females at 500 µg/l NP. Prenatal exposure of females to 50 µg/L of NP resulted in effect on the offspring by inhibition of CAT D activity, decrease of eggshell thickness and elevation of malformation rate.		(Yang et al., 2006)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Zebrafish. 10, 25 and 75 µg/l for 3 weeks through out the spawning period	Reduction of egg production at all concentrations.		(Zoller, 2006)
Adult rare minnow (*Gobiocypris rarus*). 3, 10 and 30 µg/l NP for 28 days.	Induction of VTG in males at 10 µg/l NP. Ovatestis was found in some males at 30 µg/l NP.	Pathological changes were found in liver and renal tissue of the 30 µg/l NP group (hypertrophy of hepatocytes, damages to cellular structure and accumulation of eosinophilic material).	(Zhai et al., 2007)
Order: Siluriformes.			
Family: Ictaluridae.			
Cannel catfish (*Ictalurus punctatus*). Injection of 79 and 237 mg/kg NP, sampling on day 7.	Induction of VTG in males at 237 mg/kg NP.		(Nimrod and Benson, 1996a)
Cannel catfish. Injected three times (day 1, 4 and 7) with 60 mg/kg NP, the fish were sampled on day 10.	Induction of VTG in males.	Up regulation of ER in the liver.	(Nimrod and Benson, 1997)
Cannel catfish. Seven different diets containing 10 –100 mg/kg NP, sampling after 7 and 21 days		Phagocyte function was enhanced by 10 mg/kg NP on day 7.	(Rice et al., 1998)
Family: Claridae			
African catfish (*Clarias garipinus*). 10 µg/l NP		Increase in LH content in the pituitary of male catfish.	(van Baal et al., 2000)
Order: Salmoniformes			
Family: Salmonids			
Juvenile Atlantic salmon (*salmo salar*). Acute toxicity test for 10 different Aps		96-h LC50 was from 0.13 mg/l for NP to 0.74 mg/l for 4-sec-BP.	(McLeese et al., 1981)
Rainbow trout males. 0.6 – 44 µg/l 4-*tert*-OP; 1.0 – 54.3 µg/l NP, 3 weeks exposure.	Induction of VTG at 4.8 µg/l OP and 20.3 µg/l NP. Decrease in GSI (4.8 µg/l OP; 54.3 µg/l NP). Spermatogonia accumulation. Decrease in spermatocyte fraction.		(Jobling et al., 1996)
Rainbow trout. 10 – 250 µg/l NP for 72 hr.	Induction of VTG gene expression at 10 µg/l NP.	LC50 was194 µg/l NP.	(Lech et al., 1996,Ren et al., 1996)
Juvenile Atlantic salmon. 1 – 125 mg/kg NP injected. Sampling after 14 days	Induction of Zrp and VTG at 1 mg/kg NP. Reduction in plasma concentrations of E2 at 1 and 5 mg/kg NP. No effects at higher doses.	Dose-related reduction in EROD. Higher activity of steroid-hydrogenase at 1 and 5 mg/kg NP, lower at higher doses.	(Arukwe et al., 1997b; Arukwe et al., 1997a)
Atlantic salmon. 130 mg/kg NP injected once a week in 30 days under smoltification	Increased calcium and protein levels in plasma =>induction of VTG.	Inhibited progress of smoltification.	(Madsen et al., 1997)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Juvenile Atlantic salmon. 25 mg/kg NP injected. Sampling after 14-30 days	Induction of Zrp and VTG.		(Arukwe et al., 1998; Arukwe et al., 2000a; Arukwe et al., 2001b; Arukwe et al., 2001a)
Juvenile female rainbow trout. 1-50 µg/l NP and 4-*tert*-OP for 22-35 days, the fish were followed in 431 days.	Increased GSI at 50 µg/l NP.	Growth inhibition at 10 µg/l of both NP and OP.	(Ashfield et al., 1998)
3 cold water fish (rainbow trout, apache trout, lahanton trout) and 4 warm water fish (fathead minnow, razorback sucker, bonytail chub, colorado squawfish). 50-220 µg/l NP for 96 hr.		220 µg/l NP decrease brain muscarinic cholinergic receptor (MChR) in three cold water species.	(Jones et al., 1998)
Juvenile rainbow trout. Injection of 5 and 50 mg/kg 4-*tert*-OP	Induction of Zrp at both concentration OP.	Up regulation of E2 binding capacity in the liver at both concentrations.	(Knudsen et al., 1998)
Male rainbow trout and adult roach (*Rutilus rutilus*). 1-100 µg/l 4-*tert*-OP	VTG induction at 100 µg/l OP for rainbow trout. Roach was 10 times less sensitive.		(Routledge et al., 1998)
Juvenile rainbow trout. 25-100 µg/l NP for 21 days.	Induction of VTG at 50 µg/l NP. NP exposure did not affect plasma levels of cholesterol, pregnenolone or T levels.		(Tremblay and Van der Kraak, 1998)
Juvenile Atlantic salmon. 125 mg/kg NP injected. Samples after 14 days.	Immunohistochemical identification of VTG in liver.	No histopathological changes.	(Arukwe et al., 1999)
Juvenile Atlantic Salmon feed with 300 mg NP /kg food for 4 weeks or injected with 80 mg/kg NP, sampling after 14 days.	No significant induction of VTG. No difference in sex ratio.		(Norrgren et al., 1999)
Rainbow trout. Comparing branched 4-*tert*-OP, 4-NP and linear 4-*n*-OP, 4-*n*-NP. Injection with 50 mg/kg (sampling after 12 days) and water exposure with 150 µg/l for 9 days.	VTG induction only for the branched Aps and not for the normal Aps.		(Pedersen et al., 1999)
Juvenile Atlantic salmon. 5-125 mg/kg NP injected. Sampling after 2, 4 or 7 days.	Induction of VTG and Zrp mRNA and protein at 25 mg/kg NP in liver.	Up-regulation of ER mRNA in liver at 25 mg/kg NP.	(Yadetie et al., 1999)
Rainbow trout. 1-10 µg/L NP during the embryonic, larval and juvenile life stage for 1 year.	Induction of VTG at 1 µg/l NP and ZRP at 10 µg/l NP. No testisova was observed and the sex-ratios were unchanged.		(Fent et al., 2000; Ackermann et al., 2002)
Juvenile rainbow trout. 0.32-100 µg/l NP for 21 days.	Induction of VTG 16 µg/l NP. No effect on GSI.	Increases in hepatosomatic index at 53 µg/l NP.	(Thorpe et al., 2000)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Female rainbow trout. 0.7, 8.3, and 85.6 µg/L NP, for 18 weeks.	Induction of VTG above 8.3 µg/l. Reduction in E2 in plasma at 85.6 µg/l NP. Decrease in GSI at 85.6 µg/l NP.	Plasma FSH levels and FSH gene expression in the pituitary were reduced at the lowest dose employed (0.7 µg/L NP). Pituitary LH content was significantly lower in fish exposed to 85.6 µg/L NP, and LH gene expression was suppressed in fish exposed to 8.3 and 85.6 µg/L NP.	(Harris et al., 2001)
All-male embryos of Rainbow trout. 10 µg/l and 100 µg/l NP, 1 h exposure per day (in 10 days) from eyed stage to hatching.	*In situ* hybridization showed high levels of estrogen receptors (ER) in embryos receiving 100 µg/l NP. No effect on testis morphology was found (8 months after exposure).		(Madigou et al., 2001)
Juvenile rainbow trout. 2.4 – 24 µg/l NP for 14 days.	Induction of VTG above 6.1 µg/l NP.		(Thorpe et al., 2001)
Chinook salmon (*Oncorhynchus tshawytscha*). 0.1-10 µg/L NP, 29 days post hatching (DPH), fish were allowed to grow until 103 and 179 DPH.	No effect on sex differentions or gonadal morfologi.		(Afonso et al., 2002)
Juvenile rainbow trout. 25-50 mg/kg 4-*tert*-OP injected at day 1, 4 and 7. Samples were taken on day 9.	Induction of VTG in both sexes at both concentrations.	Down-regulation of P450 protein levels in liver (CYP2K1, CYP2M1, CYP3A27). No effect on EROD.	(Katchamart et al., 2002)
All-male amago salmon. 100 µg/l NP for 50 days after hatching	38 % of the fish were completely feminizated. 37 % had intersex gonads.		(Nakamura et al., 2002)
Mature rainbow trout. Intermittently exposed for 1 and 10 µg/l NP (10 days a month in 4 months).	Induction of VTG in males at 1 µg/l NP. The F_1 generation had significant increased E2 in the male fish and T in the females. VTG was induced in F_1 female. One feminized male at 1 µg/l NP and 2 at 10 µg/l NP.	Increased mortality of early egg stage at 1 µg/l NP. Decreased hatching rate at 10 µg/l NP.	(Schwaiger et al., 2002)
Rainbow trout. 0.25-53 µg/l NP for 14 days.	Induction of VTG at 16 µg/l NP.		(Tyler et al., 2002)
Juvenile Atlantic Salmon. Injected with 10, 50 and 125 mg/kg NP. Sampled after 3 days.	Induction of VTG and Zrp in males at 10 mg/kg NP and in females at 50 mg/kg NP.	Induction of LHβ mRNA in the pituitary of the females (not in the males) at 50 and 125 mg/kg NP. Induction of pituitary specific transcription factor (Pit-1) mRNA at 125 mg/kg NP. No effect gene expression of SFHβ, GH or prolactin.	(Yadetie and Male, 2002)
Atlantic salmon smolt. 5-20 µg/L NP for 7 days during the migration period.	No significant induction of VTG.	No effect on gill Na^+K^+ATPase or hypoosmo regulatory.	(Moore et al., 2003)
Juvenile rainbow trout. 0.4-50 mg/kg 4-*tert*-OP administered orally for 11 days.	Induction of VTG at 30 mg/kg OP. Significant correlation between concentration of OP in liver and VTG in plasma.	Only 1-2 ‰ of the total amount of OP was found in the liver and muscle 24 h after last administration.	(Pedersen et al., 2003)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Atlantic salmon smolt. Two 24 h pulses (days 1 and 5) of 20 µg/l NP. Sampling after 1, 2 and 3 month.		Reduced weight after the exposure for 2 and 3 months. Reduction in plasma insulin-like growth factor I (IGF-I) concentrations.	(Arsenault et al., 2004)
Atlantic salmon. 6 serial injection with 120 mg/kg NP over 20 days. The fish were released in a natural stream.	Induction of VTG in both female and males.	Impaired smolt development and survival and delayed downstream migration.	(Madsen et al., 2004)
Juvenile Atlantic salmon. 5, 15 and 50 µg/l NP for 7 days.		Gene expression of brain StAR mRNA was induced at 15 µg/l NP but not at 5 or 50 µg/l NP. P450scc mRNA was induced at 5 and 15 µg/l NP, but not at 50 µg/l NP. CYP1A mRNA was induced at 5 and 15 µg/l NP, but reduced at 50 µg/l NP. CYP3A mRNA was induced at all NP concentrations.	(Arukwe, 2005)
Rainbow trout. 1-250 µg/l NP for 21 days.	Induction of VTG at 13 µg/l NP.		(Dussault et al., 2005)
4 salmonidae and 13 other fish species. Acute toxicity test of NP. 96 hr.		96 hr. LC50 from 50 µg/l – 460 µg/l NP.	(Dwyer et al., 2005)
Juvenile coho salmon (*Oncorhynchus kisutch*). Fed with NP (0.002 – 2000 mg/kg) for 4 weeks in freshwater before transferred to seawater.	No effects on plasma levels of thyroid hormones (T_4 or T_3).	No effect was observed on osmoregulatory performance or subsequent growth performance. Fast elimination via the biliary-fecal pathway.	(Keen et al., 2005)
Rainbow trout. 100 – 750 ng/l NP during the spawning period (60 days).	Semen production was completely inhibited at 750 ng/L NP, and reduced at 280 and 130 ng/l NP (sperm density, sperm motility and sperm fertility were not affected).	There was lower larval survival over 280 ng/L NP. NP did not directly affect sperm motility or fertilizability at any of the concentrations.	(Lahnsteiner et al., 2005)
Sockeye salmon smolt. Injected with 15 and 150 mg/kg NP at day 0 and day 2. The fish were sampled at day 7.		NP had different effects on gene expression of Erα at different times during smolting. In March and May NP did not have any effects, but in April NP did decrease liver and gill Erα mRNA.	(Luo et al., 2005)
Female juvenile Masu salmon (Oncorhynchus masou). Injection of 10 or 50 mg/kg NP and sampling after 3 days.	Induction of VTG mRNA in liver at 50 mg/kg NP.	Low dose of NP (10 mg/kg) induced the GTHα and LHβ mRNA levels. High dose of NP (50 mg/kg) slightly reduced FSHβ mRNA levels.	(Maeng et al., 2005)
Juvenile Atlantic salmon. Injected with 0.5, 2, 10, 40 or 150 mg/kg NP for times over 11 days during parr-smolt transformation. After 14 days the fish were exposed to saltwater.	Induction of VTG and total calcium in plasma at 150 mg/kg NP.	Lower salinity tolerance at 150 mg/kg NP. Decreased plasma insulin-like growth factor I (IGF-I) at 150 mg/kg NP. Decreased plasma thyroxine at 10-150 mg/kg NP after 7 days exposure, but only at 150 mg/kg NP after 14 days. Plasma cortisol levels were not affected by any of the treatments.	(McCormick et al., 2005)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Rainbow trout. 220 µg/l NP for 3 weeks		Fourier transform infrared spectroscopy (FT-IR) analysis showed compositinal and structural changes in the liver of NP exposed fish. Decrease in glycogen and protein levels and increase in hepatic lipids (especially triacylglycerides). The FT-IR spectra also indicated a decrease in membrane fluidity after increased lipid order in the membranes.	(Cakmak et al., 2006)
Juvenile Atlantic salmon. 5, 15 and 50 µg/L NP for 7 days.	Induction of VTG and Zrp mRNA in liver at 15 µg/l NP.	In the brain the gene expression of ERα and ERβ decreased after 3 days, but increased after 7 days exposure to 5-50 µg/l NP. Brain aromatase mRNA was also induced after 7 days exposure to 5-50 µg/l NP. In the liver the gene expression of CYP3A, CYP1A, PXR and AhR were induced after 7 days exposure to 5-50 µg/l NP	(Meucci and Arukwe, 2006c; Meucci and Arukwe, 2006b)
Juvenile female rainbow trout. 2.2 µg/l – 2200 µg/l NP	Induction of VTG gene expression in the lever at 2200 µg/l NP, No effect on ER mRNA	Dose-dependent reduction of sGnRH2 gene expression in the brain, no effect on sGnRH1 or ER mRNA levels	(Vetillard and Bailhache, 2006)
Juvenile female rainbow trout. 40 and 80 µg/l NP for 5 days.		The exposed fish showed changes in behaviour and were were more likely to be attacked by other fish, and were less successful when competing for food resources than control fish. The behavioural effects of the NP exposure do not appear to be related to its estrogenic potential, as there were no effects seen with the estrogen-positive control.	(Ward et al., 2006)
Order: Gadiformes			
Family: Gadidae			
Juvenile Atlantic cod (*Gadus morhua*). Injected with 220 mg/kg NP, sampled after 1-40 days.	Induction of VTG.		(Hylland and Haux, 1997)
Juvenile Atlantic cod. 96 - 806 µg/l 4-*n*-HepP for 168 hr.		168-hr. LC50 = 518 µg/l. Biological half-life ($t_{1/2}$)=13 hr. and BCF = 555	(Tollefsen et al., 1998)
Juvenile Atlantic cod. 29 µg/l NP for 3 weeks.		No induction of micronuclei in peripheral erythrocytes.	(Bolognesi et al., 2006)
Juvenile Atlantic cod. 29 µg/l NP for 3 weeks.	Induction of VTG and Zrp.	SELDI-TOF found 146 peaks (peptides) that were significantly altered (up or down) in the plasma of the exposed cod compared with control.	(Larsen et al., 2006)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Juvenile Atlantic cod. 29 µg/l NP for 3 weeks.	No effect on T and E2 levels in plasma.	No effect on P450 aromatase activity in ovaries. Reduced glucuronidation of E2 (UGT-E2), no effect on UGT-T. No effect on E2-sulfotransferase activity.	(Martin-Skilton et al., 2006c)
Juvenile Atlantic cod. 29 µg/l NP for 3 weeks.		Decrease of hepatic CYP1A and CYP3A levels, decrease in EROD activity. Increase in conjugation enzyme GST activity.	(Sturve et al., 2006)
Mature Atlantic cod. Fed with food containing an AP mixture (4-*tert*-BP, 4-*n*-PP, 4-*n*-HexP, 4-*n*-HepP) corresponding to 0.02 and 2 mg/kg/day for 4 months.	Reduction of E2 and T in the plasma of female at both doses. Induction of VTG in males.		This study (Paper III)
Mature Atlantic cod. Oral exposure for 5 weeks with an AP mixture (4-*tert*-BP, 4-*n*-PP, 4-*n*-HexP, 4-*n*-HepP) 0.02 mg/kg/week - 80 mg/kg/week.	Significant effects at lowest dose. Reduction of E2 and T in the plasma of female, Reduced 11-KT in male plasma. Induction of VTG in males. Delayed ovary growth, lower GSI and smaller oocytes. Increase in the amount of spermatogonia and a reduction in the amount of spermatozoa in testis.	Increased weight loss at 20 mg/kg/week AP for females. Increased hepatic total glutathione in females after 1 week exposure, an effect not seen after 4 weeks. Increased glutathione reductase catalytic activities in both males and females at 0.02 mg/kg/week AP for 4 weeks. The glutathione *S*-transferase activity was only affected in male fish at 0.02 mg/kg/week AP, and glucose-6-phosphate dehydrogenase activity increased in female fish exposed to 0.02 mg/kg/week AP for 1 week. Dose dependent increase of hepatic CYP1A and CYP3A protein levels in males, but not in females. No increase in EROD activity in any of the sexes.	This study (**Paper IV-VII**)
Order: Atheriniformes			
Family: Atherinosidae			
Inland silverside (Menidia beryllina). Acute toxicity test for NP.		96 hr. LC50 = 70 µg/l NP	(Lussier et al., 2000)
Order: Beloniformes			
Family:Adrianichthyidae			
Medaka (*Oryzias latipes*). Full life-cycle exposure, 50 and 100 µg/l NP from hacthing to 3 months.	50% of the male fish from 50-µg/L NP and 86% of the males in the 100-µg/L NP developed testis-ova. The ratios of males to females in the control treatment (2:1, M:F) and the 100 µg/L NP treatment (1:2, M:F) were different.	The medaka embryo/larval LC50 for NP was 460 µg/l NP.	(Gray and Metcalfe, 1997)
Medaka. 0.5, 0.8, 1.9 µg/l NP from hacthing to 1 month. Sampled one month after end of exposure.	No alteration in sex ratios was observed. There was no depreciation in reproductive capability measured by fecundity, viability of eggs, or hatchability of eggs.		(Nimrod and Benson, 1998)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Medaka. Full life-cycle exposure, 10 – 100 µg/l 4-*tert*-OP from hacthing to 3 month.	Ovotestis at 50 and 100 µg/l OP.	Reduced pairing activity (25 µg/l OP). Poor hatching or deformities in offspring of exposed fish (10 – 100 µg/l OP).	(Gray et al., 1999a; Gray et al., 1999b)
Embryo medaka. 0 – 1000 µg/l 4-*tert*-OP from fertilization to day 17 (swim-up)		LC50 = 450, 830 and 940 µg/l OP (3 replicate experiment.	(Gray and Metcalfe, 1999)
Medaka . 10 – 100 µg/l OP for 21 days.	Dose-dependent and reversible induction of VTG at 20 µg/l OP. Ovotestis in two fish (one at 74 µg/l OP and one at 230 µg/l OP). More spermatogonia (A and B) at concentrations above 41 µg/l OP.	Larval survival correlated with VTG serum levels. Unexposed female fish together with exposed males spawned fewer eggs (50%) than controls. Abnormal embryonic development.	(Gronen et al., 1999)
Medaka. 20 µg/l NP (two different sources of NP) for 4 days	Induction of VTG in males. No difference between the different NPs.		(Foran et al., 2000)
Medaka. 150 – 1500 µg/l BP; 6.6 – 66 µg/l NP for 2 weeks.		Reduced hatching fraction with pairs of which the male had been exposed to 66 µg/l NP.	(Shioda and Wakabayashi, 2000a)
Male Medaka. 6.6 – 66 µg/l NP for 2 weeks.		Decrease in the number of hatching from eggs fertilized by male fish dosed 66 µg/l NP	(Shioda and Wakabayashi, 2000b)
Medaka. 0.1 – 100 µg/l NP for 5 weeks.	Female specific proteins (VTG + ZR + ?) induced at 0.1 µg/l NP (immunodetection). Measurable concentration at 100 µg/l NP. Abnormal gonadal development in two (of seven) individuals at 100 µg/l NP.	Reduced survival at more than 50 µg/l NP. LC50 for larvae 130 µg/l NP, and 860 µg/l NP for mature fish.	(Tabata et al., 2001)
Medaka. 4.2-183 µg/l NP. Full life-cycle exposure for 105 days. The offspring (F_1) was followed until 60 d posthatch.	Feminization, altered sex distribution at 51.5 µg/l NP and observation of ovotestis 17.7 µg/l NP. Intersex was found in sekund generation (F_1) from the parents treated with 8.2 µg/l NP.	Embryo survival were lower at 183 µg/l NP. Larval mortality was increased at 17.7 µg/l NP.	(Yokota et al., 2001)
Medaka. 2, 20 or 50 µg/l NP for 7 days.	Induction of VTG at 20 µg/l NP.		(Islinger et al., 2002)
Medaka. 0.1, 10 or 100 µg/l NP for 5 weeks.	Induction of VTG at 0.1 µg/l NP. IC50 values for inhibition to egg hatching were 850 µg/l NP.	72 hr. LC50 = 850 µg/l NP (males); 870 µg/l NP (females); 130 µg/l (recently hatched larvae)	(Kashiwada et al., 2002)
Medaka. 2-50 µg/l OP. Full life-cycle exposure. Exposed for 2-4 hr. post fertilization until maturity (12-13 weeks of exposure)	Ovotestis induced by 50 µg/l OP Shift in sex ratio towards females > 2 µg/l OP.	Cross-mating experiment, mating exposed males with control females resulting in up to 11 % lower fertilization rate. Increased mortality in the progeny derived from exposed females (>20 µg/l OP) and unexposed males. Growth inhibition at > 50 µg/l OP. Increase in mortality both before and after hatch at > 2 µg/l OP.	(Knorr and Braunbeck, 2002)
Medaka. 5-500 µg/l NP for 6 days.	ZRP mRNA was induced at 50 µg/l NP.		(Lee et al., 2002)
Medaka. 100 µg/l NP for 6 weeks.	Increased cellular apoptosis in spermatocytes, Sertoli cells and Leydig cells, but not in spermatids		(Weber et al., 2002)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Male medaka. 0.02 - 40 mg/g diet of 4-*tert*-OP, NP and 4-*n*-NP. The fish were fed for 7 days (≈0.06 g pr 10 fish pr day).	EC_{50} for VTG induction 2600 µg/kg OP, 940µg/kg NP. 4-*n*-NP did induce VTG to a lower extent than the branched isomers.	There was high mortality in the group fed 40 mg/kg diet.	(Chikae et al., 2003)
Medaka. 24.8-184 µg/l NP. Eight breeding pairs were exposed for 3 weeks.	Induction of VTG in both sexes at 50.9 µg/l NP. Ovotestis induced by all concentrations. GSI reduced at 184 µg/L.	Egg production was decreased in the 101 µg/l group and fertility was decreased at 184 µg/L.	(Kang et al., 2003)
Medaka. 20 and 100 µg/l NP for 2 weeks.	Reduced number of motile spermatozoa after 20 µg/l NP.		(Kawana et al., 2003)
Medaka. 75 µg/l NP for 1, 2, 4 and 10 days.		Rapid induction of ER mRNA in the liver after 1 day and maximum expression after 10 days. Increase in aromatase (CYP19) mRNA expression from day 2 and maximum at day 10. p53 mRNA was increased after 1 days but decrease after day 2.	(Min et al., 2003)
Medaka. 51–931 µg/l 4-*tert*-PP. Full life-cycle test. Exposed from fertilized eggs to 101 d posthatch (F_0). Studied effects on second generation (F_1).	Induction of VTG in males at 51 µg/l PP. Feminization (altered sex distribution and observation of ovotestis) at 224 µg/l PP.	Lethal and sublethal toxicity for F_0 at 931 µg/l PP. In F_1 the lethal and sublethal toxicity were 224 µg/l PP.	(Seki et al., 2003b)
Medaka. 3.3-44.7 µg/l NP and 6.9-94 µg/l 4-*tert*-OP. Exposed fertilized eggs to 60 d post hatch.	Induction of VTG at 11.6 µg/l NP and 11.4 µg/l OP. Feminization (altered sex distribution and observation of Ovotestis) at 11.6 µg/l NP and 48.1 µg/l OP.	Growth inhibition at > 23.5 NP.	(Seki et al., 2003a)
Medaka.100 µg/l NP for 5 weeks.	Induction of VTG. The VTG concentration decreased only slowly after exposure and was not return to initial normal levels even after 5 weeks.		(Tabata et al., 2003)
Mature medaka. 24.8, 50.9, 101 and 181 µg/l for 21 days.	Egg production was decreased at ≥101 µg/l NP and fertility was decreased at 181 µg/l NP. Induction of testis-ova in male was found in all NP concentrations, whereas abnormal spermatogenesis and lower GSI was only seen in the males from the 181 µg/l NP group. VTG was induced in the liver of both sexes ≥50.9 µg/l NP		(Kang et al., 2003)
Medaka. 500 µg/l NP for 1-12 days.	Induction of VTG in the liver and in the testis (immunohistochemically).		(Kobayashi et al., 2005)
Transgenic medaka. 0.01 - 403 µg/l NP for 16 days.	No induction of the estrogen-responsive choriogenin H gene (fused to the green fluorescent protein gene). No induction of VTG mRNA at any concentration.	Increased mortality (62 %) at 80 µg/l NP and 100 % at 403 µg/l NP.	(Scholz et al., 2005)
Male medaka. 50 and 500 µg/l NP for 8 hr.	Induction of VTG II mRNA and ERα mRNA at 500 µg/l NP.		(Yamaguchi et al., 2005)
Medaka. 62-783 µg/l 4-*tert*-PP Exposed fertilized eggs for 60 d post hatch.	Feminization (altered sex distribution) at ≥ 238 µg/l PP.	Expression of Cytochrome P450 11β-hydroxylase ($P450_{11\beta}$) mRNA was completely inhibited by ≥ 413 µg/l PP.	(Yokota et al., 2005)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Medaka. 1-100 µg/l NP from day 1 after hacthing to 100 days.	80 % of the exposed males had ovotestis at 29 µg/l NP (measured conc.) and 5 % at 9 µg/l NP. Mixed secondary sex characteristics in 40 % at 29 µg/l NP and 20 % at 9 µg/l NP.		(Balch and Metcalfe, 2006)
Medaka. 10, 50 and 100 µg/l NP for 21 days in the spawning period.	Induction of VTG in males at 10 µg/l NP. Reduction of eggs at 100 µg/l NP. Reduced fertilisation of eggs at 100 µg/l NP. Reduced hatchability and delayed hatching of eggs from the 100 µg/l NP group.	Increased HSI in both gender at 100 µg/l NP. Increased mortality (40 %) of males at 100 µg/l NP. Matenal transfere of NP into the eggs (2-7 µg/g egg) for the 100 µg/l NP group. (BCF = 30-100).	(Ishibashi et al., 2006)
Medaka. 20 and 100 µg/l NP for 96 hr.	Induction of VTG mRNA and Zrp mRNA after 96 hr. (not after 24 hr.)	75 different genes were found altered (using 3.4 K gene microarray) after 96 h. Dose related respons.	(Kim et al., 2006a)
Order: Cyprinodontiformes			
Family: Poecilidae			
Guppy (*Poecilia reticulata*). 150 µg/l 4-*tert*-OP for 4 weeks.		Males displayed reduced aggressiveness to competitors.	(Bayley et al., 1999)
Mosquitofish (*Gambusia holbrooki*). 0.5–50 µg/l NP for 75 days.	100% female secondary sex characteristics at 50 µg/l NP. At 0.5 and 5 µg/l NP, occurrence of individuals with partially developed gonopodia. Only female or undeveloped gonads at 50µg/l NP, none with testis.	Effect on liver at 50 µg/l NP (reduced lipids, perivascular necrosis, and hepatocytes with pyknotic or hypertrophic cores.	(Dreze et al., 2000)
Platyfish (*Xiphophorus maculatus*). 80 – 1280 µg/l NP for 28 days.	Induction of VTG above 80 µg/l NP. Reduced number of cysts in testis; hypertrophy of Sertoli cells (dose-related response found from 80 µg/l NP).	Free sperm in enlarged ductus spermaticus (dose-related)	(Kinnberg et al., 2000a; Kinnberg et al., 2000b)
Swordtail (*Xiphophorus helleri*). 4 – 100 µg/l NP for 60 days.	VTG-mRNA expressions at lowest dose 4 µg/l NP. Increased apoptosis and necrosis. Lesions in testis at 100 µg/l NP. Minor effects also found at 4 µg/l NP.		(Kwak et al., 2001)
Male guppy. 100 – 900 µg/l 4-*tert*-OP for 30 days.	Reduced GSI at 100 µg/l OP Increased quantity of spermatozoa in ejaculate (from 100 µg/l OP). Variable results among groups.	Reduced size and intensity of sexually attractive orange marks (from 100 µg/l OP).	(Toft and Baatrup, 2001)
Platyfish, neonates, juvenile and mature. 14 µg/l NP for 8 months.	Neonates and juvenile showed a significant delay in gonadal development, and did never develop functioning gonads.	High mortality (35 %) in exposed groups (2% in control). Down regulation of GTH in the pituitary.	(Magliulo et al., 2002)
Guppy. 100, 300 and 900 µg/l 4-*tert*-OP for 60 days.	Reduced number of spermatogenetic cysts at 900 µg/l OP.	60 % mortality at 900 µg/l OP (15 % in the control group).	(Kinnberg and Toft, 2003)
Guppy. 26 µg/l 4-*tert*-OP 26-36 days.	Indications of blocked spermatogonial mitosis for the adult males. There were no effects on the weight, length, gonopodiurn index or sex distribution of the offspring.		(Kinnberg et al., 2003)
Guppy. 0-1600 µg/l 4-*tert*-OP for 96 h and 1.7 – 149 µg/l 4-tert-OP for 90 days.	Reduced GSI of female at 100 µg/l Increased quantity of spermatozoa in ejaculate (from 100 µg/l OP). Variable results among groups.	Reduced size and intensity of sexually attractive orange marks (from 100 µg/l OP). 96 h LC_{50} for OP were 495 µg/l.	(Toft and Baatrup, 2003)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
New born guppies. 100 µg/l NP for 90 days.	Induction of VTG mRNA in liver of both males and females. Gender balance towards female (sex ratio = 0.3 males per female).		(Cardinali et al., 2004)
Male guppies, 10, 60, and 150 µg/L NP for 7-21 days.	Induction of VTG at 60 µg/l NP.		(Li and Wang, 2005)
Family: Fundulidae			
Killifish (*Fundulus heteroclitus*). Embryos and larvae. 107-2140 µg/l NP and 100-2000 µg/l 4-*tert*-OP.		Larvae (96-h LC50 = 204 µg/L NP) are more sensitive than embryos (96-h LC50 = 5 mg/l NP). Sub lethal abnormalities at 40 µg/l NP. NP have higher toxicity than OP.	(Kelly and DiGiulio, 2000)
Male killifish. Injection of 10-50 mg/kg NP and 10-50 mg/kg 4-tert-OP. Sampling after 32 days.	Induction of VTG in males at 10 mg/kg NP and 100 mg/kg OP. Decrease in GSI at 50 mg/kg NP.		(Pait and Nelson, 2003)
Male mummichog (*Fundulus heteroclitus*). 65 µg/l NP for 4 days.	Induction of VTG.		(Garcia-Reyero et al., 2004)
Family: Rivulidae			
Rivulus marmoratus (Hermaphroditic fish). 150 or 300 µg/l NP for 60 days.	No fish exposed to 300 µg/l and only 2 of 9 fish exposed to 150 µg/l developed testicular tissue.		(Tanaka and Grizzle, 2002)
Rivulus marmoratus. 300 µg/l NP for 96 hr.		Induction of glutathione S-transferase gene (GSTa mRNA).	(Lee et al., 2005a)
Rivulus marmoratus. 300 µg/l NP for 96 hr.		Induction of P450 1A (CYP1A) gene.	(Lee et al., 2005b)
Rivulus marmoratus. 300 µg/l NP or 300 µg/l 4-tert-OP for 96 hr.		Brain aromatase gene (cyp19b) were up-regulated and ovarian aromatase (cyp19a) were down-regulated by NP. OP up-regulated both brain and ovarian aromatase.	(Lee et al., 2006b)
Rivulus marmoratus. 300 µg/l NP for 96 hr.		Up-regulation of c-KI-*ras* mRNA in liver and Ha-*ras* in the brain. (The *ras* genes are associated with carcinogen exposure in fish).	(Lee et al., 2006a)
Rivulus marmoratus. 300 µg/l NP for 96 hr.		Down-regulation of androgen receptor (rm-AR) and estrogen receptor (ER-α and ER-β) in the gonads and liver.	(Seo et al., 2006)
Order: Gasterosteiformes			
Family: Gasterosteidae			
Three-spined stickleback (Gasterosteus aculeatus). Acute toxicity test of NP		96-hr LC50 of 370 µg/L	(Granmo et al., 1991).
Order: Scorpaeniformes			

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Family: Agonidae			
Pogge (*Agonus cataphractus*). Acute toxicity test of NP for 96 hr and 7 days.		LC50 was 510 and 360 µg/l for 96 hr. and 7 days exposure, respectively.	(Waldock and Thain, 1986)
Family: Sebastidae			
Rockfish (*sebastes schlegeli*). Injection of 10 and 25 mg/kg NP. Sampling after 12 and 48 hr.	Induction of VTG mRNA in the liver of both females and males at 10 mg/kg NP.		(Jung et al., 2003)
Rockfish. Injection of 10 and 25 mg/kg NP. Sampling after 2-14 days.	Induction of VTG mRNA in the liver of both females and males at 10 mg/kg NP. Maximum VTG mRNA levels 48 hr. in females and 72 hr. in males, after injection. Induction of plasma VTG at 10 mg/kg, maximum after 72 hr. in females and 168 hr. in males.		(Jung et al., 2006)
Order: Perciformes			
Family: Centrarchidae			
Bluegill sunfish (*Lepomis macrochirus*). NP was applied to enclosures every 48 h over a 20-d period (11 applications) at rates of 3, 30, 100, and 300 µg/L.		Significant mortality at 300 µg/l NP. Tissue concentrations of NP from enclosures treated with 3 and 30 µg/L NP ranged from 0.01 to 2.94 µg/g wet weight. BCF = 87 ± 124.	(Liber et al., 1999)
Largemouth bass (*Micropterus salmoides).* 50 mg/kg NP injected. Sampled after 48 hr.	Gene array showed 9 genes increased by a factor 2: four VTG genes, Choriogenin 2 and 3, aspartic protease, signal peptidase, and an unidentified gene. 2 genes were down-regulated: transferrin and one unknown.		(Larkin et al., 2002)
Largemouth bass. Injection of 5 mg/kg and 50 mg/kg NP. Sampled after 2, 7 or 14 days.	Induction of VTG at 5 mg/kg NP.	NP decreased glutathione S-transferases (GST) mRNA levels in the liver, but not GST catalytic activity. No effect on glutathione (GSH) levels. 5 mg/kg NP increased hepatic quinone reductase (QR)	(Hughes and Gallagher, 2004)
Family: Cichlidae			
Tilapia (*Oreochromis niloticus*). 2.2-2200 µg/l NP for 3-5 weeks.		FSHβ mRNA is suppressed and LH release from the pituitary is increased at all concentrations, dose response.	(Zilberstein et al., 2000)
Family: Gobiidae			
Japanese common goby (*Acanthogobius flavimanus*). 5, 25 and 50 µg/L NP for 3 weeks.	Induction of VTG in males 13 µg/L NP (measured conc.).		(Ohkubo et al., 2003)
Black goby (*Gobius niger*). Injected with 50 or 500 mg/kg NP. Sampled after 72 hr.	Induction of VTG at both doses. No effects were seen on testis histologi.	Both doses of NP caused reduction of CYP1A mRNA expression and EROD activity in the liver. Increased expression of AhR in liver.	(Maradonna et al., 2004)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Japanese common goby. 0.2, 1, 5, 25 µg/l NP for 3 weeks	Induction of VTG at 25 µg/l NP.	5 and 25 µg/l NP down regulated the expression of ubiquitin C-terminal hydrolase mRNA in testis, but not in the brain.	(Mochida et al., 2004).
Sand goby (*Pomatoschistus minutus*). 3 –100 µg/l 4-*tert*-OP for 101 days.	Induction of VTG at 31 µg/l OP after 28 days exposure. No effect on GSI, but inhibition of the sperm duct glands at 28 µg/l OP.	195 µg/l OP were acutely toxic, 119 µg/l OP gave 100 % mortality over 40 days. LC_{50} was 29 µg/l OP after 8 weeks exposure.	(Robinson et al., 2004)
Family: Moronidae			
Juvenile sea bass (*Dicentrarchus labrax*). 891 µg/l NP for 2-24 hr.	No effect on cortisol.	Induction of erythrocytic nuclear abnormalities frequency. No effect on EROD or GST.	(Teles et al., 2004)
Male sea bass. Injection with 5 or 50 mg/kg NP. Sampling after 3-28 days.	Induction of VTG only for 50 mg/kg NP. Maximum VTG levels 14 days after injection.	Inhibition of CYP1A at 50 mg/kg NP. No effects on 6β-T hydroxylase (CYP3A) or GST.	(Vaccaro et al., 2005)
Family: Mugilidae			
Juvenile and adults grey mullet (*Liza aurata*). Juveiles were exposed for 25, 100 or 1000 µg/l NP for 7 days. Adults were injected with 0.25 or 250 mg/kg. Sampling after 48 hr or 72 hr.	No induction of VTG was seen after exposure of juveniles. In adults were VTG induced at highst dose.NP	NP decreased CYP1A1 mRNA expression and EROD activity in both juveniles and adults at all concentrations	(Cionna et al., 2006)
Family: Sparidae			
Juvenile gilthead seabream (*Sparus Auratus*). Injected with 100 and 200 mg/kg NP. Sampling after 10 days.	Increased plasma levels of E2 at 200 mg/kg NP.	Plasma glucose and protein levels were not affected. Increased levels of triacylglycerol in plasma at both doses.Reduction in kedney Na^+,K^+-ATPase activity and increases in plasma osmolality at 200 mg/kg NP. Decrease in EROD and increase in GST activity at 200 mg/kg NP.	(Carrera et al., 2007)
Family: Zoarcidae			
Male eelpout (*Zoarces viviparus*). Injected with 10 mg/kg/week and 100 mg/kg /week of NP. Sampled after 25 days.	Dose-dependent induction of VTG. Decrease in GSI. Effects on testicular structure and cytology and germ cells and sertoli cells.		(Christiansen et al., 1998)
Male eelpout. 10-1000 µg/l NP for 3 weeks.	Induction of VTG at 100 µg/l NP.		(Korsgaard and Pedersen, 1998)
Eelpout. 10 mg/kg 4-*tert*-OP injected 4 times.	Induction of VTG.	Upregulation of E2 binding capacity in the liver => induction of receptor levels.	(Andreassen and Korsgaard, 2000)
Eelpout. 25 and 100 µg/l 4-*tert*-OP for 3 weeks.	Induction of VTG at 14 µg/l OP (measured conc.).		(Rasmussen et al., 2002)
Eelpout. 100 mg/kg 4-*tert*-OP three times over 10 days.	Massiv induction of VTG. Reduction in GSI and milt volume. Increased spermatocrit.		(Rasmussen and Korsgaard, 2004)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Eelpout . 0.5 and 25 µg/l 4-*tert*-OP for up to 168 hr.	Induction of VTG at 25 µg/l OP after 168 hr.	Induction of gene expression of ERα mRNA in the liver after 48 hr.	(Andreassen et al., 2005)
Male Eelpout. 10, 50 and 100 µg/l 4-*tert*-OP for 3 weeks.	Induction of VTG at 35 µg/l OP (measured conc.).	100 µg/l OP gave effect on the offspring (viviparous fish).	(Rasmussen et al., 2005)
Order: Pleuronectiformes			
Family: Pleuronectidae			
Flounder (*Platichthys flesus*). 10, 30 and 100 µg/l NP for 3 weeks.	No induction of VTG.	Higher HSI at 30 µg/l NP. Lerthal concentration at 100 µg/l NP.	(Matthiessen et al., 1998)
Flounder. 10-200 mg/kg NP injected once a weeks for two weeks. Sampled after 2 weeks after last injection.	Induction of VTG at 10 mg/kg NP after one week. No effect on GSI	High mortality at 200 mg/kg NP (71 %).	(Christensen et al., 1999)
Larvae of winter flounder (*Pleuronectes americanus*). Acute toxicity test.		96 hr. LC50 = 17 µg/l	(Lussier et al., 2000)
Male summer flounder (*Paralichthys dentatus)*. Injected twice with 2, 20 and 200 mg/kg 4-*tert*-OP. Sampled after 4, 6 and 8 weeks.	Increased plasma E2 and decreased T at all concentrations. Reduced GSI at 200 mg/kg OP.		(Mills et al., 2001)
Male summer flounder. 100 mgkg/week 4-*tert*-OP injected 2 times, sampled after 4-6 weeks.	Retadation of testicular development: Reduced GSI, thickened tubule walls and no developing sperm cysts at 100 mg/kg.	No histopathologically effect in liver or kidney	(Zaroogian et al., 2001)
Flounder. 10, 50 and 100 mg/kg OP administered orally every second day during a period of 11 days	Induction of VTG at 10 mg/kg OP	OP accumulated in liver and muscle, the tissue concentration were positive correlated with VTG induction	(Madsen et al., 2002)
Flounder. Exp.1: 10, 50 and 100 mg/kg 4-*tert*-OP and Exp.2: 1, 2,5, 5, 7,5, 10, 25 mg/kg 4-*tert*-OP administered orally every 2 days for 10 days (5 exposure). Samples taken after day 6 and day 11.	Induction of VTG at 5 mg/kg OP. No effect on GSI	OP was found in both liver, muscle and testis tissue. Only 8% of the OP administered to the group receiving 50 mg/kg OP was retained in the liver and the muscles.	(Madsen et al., 2003)
Male winter flounder. Injected with 100 mg/kg/day NP for 2 days. Sampled after 48 hr.		NP induced testosterone metabolism and induction of CYP3A protein.	(Baldwin et al., 2005)
Eggs and larvae of marbled sole (*Pleuronectes yokohamae*). Acute toxicity test, 0-2430 µg/l NP.		Increased mortality at 68 µg/l NP of eggs. Increased mortality at 13 µg/l NP for larvae (96 hr.). No survival of neither eggs nor larvae over 200 µg/l NP.	(Kume et al., 2006)

Species, dose and exposure time	Vitellogenin (VTG), zona radiata protein (Zrp), steroid, sex differentiation and gametogenesis	Other effects	Reference
Flounder. Oral administration of 50 mg/kg 4-*tert*-OP. Sampled after 2-216 hr.	Induction of VTG after 48 hr. The VTG levels increase until the end of the experiment 9 days after the administration of OP.	OP was found in liver, testis, muscle and plasma 3-18 hr. after administration. The maximum concentrations of OP in liver, muscle and testis were 67, 3.2 and 6.8 µg/g, respectively.	(Madsen et al., 2006)
Family: Scophthalmidae			
Juvenile turbot (*Scophthalmus maximus*). 29 µg/l NP for 3 weeks.	Reduction of plasma and testis levels of androgens (T, 11-KT and androstenedione) and estrogens (E1 and E2). Also biliary levels of steroids were highly depressed in male turbot. No effects of NP were found on the steroid profile of the females.		(Labadie and Budzinski, 2006)
Juvenile turbot (*Scophthalmus maximus*). 29 µg/l NP for 3 weeks.	Induction of VTG and Zrp	Surfaced enhanced laser desoption ionisation-time of flight (SELDI-TOF) found 121 peaks (peptides) that were significantly altered (up or down) in the plasma of the exposed turbot compared with control.	(Larsen et al., 2006)
Juvenile turbot (*Scophthalmus maximus*). 29 µg/l NP for 3 weeks.	Lower levels of T and E2 in plasma	Reduced P450 aromatase activity in ovaries. Reduced glucuronidation of T and E2. No effect on E2-sulfotransferase activity	(Martin-Skilton et al., 2006c)

Appendix 2. Analytical methods for determination of alkylphenols.

Table 9. *Analytical methods for determination of alkylphenol in biota.*

Compounds	Matrix	Extraction	Clean-up	Analysis	LOQ	Reference
NP	mussels	Liq/Liq (DCM)	Partitioning (NaOH:AN)	GC-MS (EI and NCI), pentafluorobenzoyl derivatives	1 µg/kg	(Wahlberg et al., 1990)
NP	Algae, fish and duck	Steam distillation	non	HPLC (fluorimetric)	30 µg/kg dry weight	(Ahel et al., 1993)
4-tert-OP	Rats	Liq/Liq(MTBE)	non	GC-MS (EI)	5-10 µg/kg	(Certa et al., 1996)
NP	Fish	Soxhlet (DCM)	Silica and alumina column	GC-MS (EI)	not given	(Blackburn et al., 1999)
NP	Fish	Liq/Liq (Hex)	Florisil column	GC-MS (EI)	10 µg/kg	(Liber et al., 1999)
NP and OP	Fish	Steam distillation	Alumina column	GC-FID and GC-MS (EI)	not given	(Lye et al., 1999)
4-tert-OP	Fish	MASE	SPE (aminopropyl)	LC-MS (APCI)	10 µg/kg – 50 µg/kg	(Pedersen and Lindholst, 1999)
NP and OP	Fish	Liq/Liq (AN)	Partitioning (Hex:AN)	GC-MS (EI)	2 µg/kg (OP), 20 µg/kg (NP)	(Tsuda et al., 1999)
NP	Fish and mussels	MSPD	Direct in the extraction	HPLC (fluorimetric)	10-30 µg/kg	(Zhao et al., 1999)
NP and OP	Mussels	Soxhlet (Hex)	GPC	GC-MS (EI)	1 µg/kg (OP), 13 µg/kg (NP)	(Bennett and Metcalfe, 2000)
NP and OP	Mussels	MSPD	Alumina column	GC-FID and GC-MS (EI)		(De Voogt et al., 2000)
NP and OP	Fish	Liq/Liq (AN)	Partitioning (Hex:AN), Florisil PR	HPLC (fluorimetric)	1 µg/kg (OP), 2 µg/kg (NP)	(Tsuda et al., 2000a)
NP and OP	Mussels	Liq/Liq (DCM)	Silica column	GC-MS (EI)	3 µg/kg	(Cathum and Sabik, 2001)
NP	Food	Steam distillation	HPLC (silica column)	GC-MS (EI), silylated derivatives	27 µg/kg	(Gunther et al., 2001)
NP	Fish	Steam distillation	HPLC (silica column)	GC-MS (EI)	3,3 µg/kg	(Keith et al., 2001)
NP	Fish	Steam distillation	HPLC (silica column)	GC-MS (EI)	4,8 µg/kg	(Snyder et al., 2001a)
NP	Fish	Soxhlet (DCM)	Silica column	HPLC (fluorimetric)	67 µg/kg	(Snyder et al., 2001b)
NP	Fish	Soxhlet (Hex)	SPE (aminopropyl)	HPLC (UV)	10 µg/kg	(Corsi and Focardi, 2002)
NP	Fish	ASE (DCM)	GPC and SPE (aminopropyl)	HPLC (fluorimetric)	5 µg/kg	(Datta et al., 2002)
NP	Rats	Liq/Liq(AN)	SPE (silica column)	LC-MS (APCI) pentafluorobenzyl derivatives	220 µg/kg	(Doerge et al., 2002)
NP and OP	Fish	ASE	Florisil column	LC-MS (ESI)	5 µg/kg (OP), 20 µg/kg (NP)	(Tavazzi et al., 2002)

Table 7. continue on the next page

Table 7 continue

Compounds	Matrix	Extraction	Clean-up	Analysis	LOQ	Reference
NP	Fish	Steam distillation	HPLC (silica column)	GC-MS (EI)	3,3 µg/kg	(Kannan et al., 2003)
NP	Mussels	MASE (Ac:Hex)	Silica column	GC-MS (EI), pentafluorobenzyl derivatives	3 µg/kg	(Sabik et al., 2003)
NP and OP	Fish	ASE (cyclohex/EA 95:5)	GPC and SPE (Aminopropyl)	GC-MS-MS (EI) silylated derivatives	0.2 µg/kg (OP), 2 µg/kg (NP)	(Wenzel et al., 2004)
NP and OP	Oyster	Steam distillation	None	GC-MS (EI)	20 µg/kg	(Cheng et al., 2005)
NP and OP	Fish	Liq./Liq. (AC-Hex 3.5:1)	Alkali extraction (AN/NaOH)	GC-MS (EI)	0.5 µg/kg (OP), 8 µg/kg (NP)	(Ferrara et al., 2005)
HP, OP, NP and DP	Fish	Steam distillation	SPE (aminopropyl)	LC-MS/MS (ESI)	3-4 µg/kg	(Keen et al., 2005)
NP and OP	Fish	Liq./Liq. (AN)	Florisil column	HPLC (fluorimetric)	5 µg/kg (OP), 9 µg/kg (NP)	(Mao et al., 2006)
32 APs from phenol to NP	Fish	Liq./Liq. (DCM)	GPC	GC-MS (NCI) pentafluorobenzoyl derivatives	1-23 µg/kg	**Paper II**

Techniques: ASE = Accelerated solvent extraction; GPC = Gel permeation chromatography; Liq: Liq = Liquid: liquid extraction; MASE = Microwave-assisted solvent extraction, MSPD = Matrix solid-phase dispersion; SPE = Solid phase extraction; **Solvent**: AC = Acetone; AN = Acetonitrile; DCM = Dichloremethane; EA = Ethylacetate; Hex = Hexane; MTBE = Methyl-tert-butylether;

Tabel 10. *Analytical methods for determination of alkylphenol in produced water.*

Compounds	Extraction	Clean-up	Analysis	LOQ	Reference
C_0-C_2 AP	Liq./Liq.(DCM)	None	GC-MS (EI)	Not given	(Grahl-Nielsen, 1987)
Clusters of C_0-C_7	SPE (C_{18})	None	GC-MS (EI)	Not given	(Brendehaug et al., 1992)
C_0-C_3 AP	SPE (C_{18})	None	GC-MS (EI)	Not given	(Bennett et al., 1996)
Clusters of C_0-C_7	SPE (C_{18})	None	GC-MS (EI)	Not given	(Røe and Johnsen, 1996)
32 APs (C_0-C_9)	Liq./Liq.(DCM)	None	GC-MS (EI)	1-1010 ng/l	(Durell et al., 2002)
31 APs (C_0-C_9)	SPE (MAX)	None	GC-MS (NCI) pentafluorobenzoyl derivatives	3-1051 ng/l	**Paper I**
4-APs (C_0-C_9)	Liq./Liq.(DCM)	None	GC-MS (EI)	11-980 ng/l	(Thomas et al., 2004a)

Reference list

Ackermann G E, Schwaiger J, Negele R D, Fent K. 2002. Effects of long-term nonylphenol exposure on gonadal development and biomarkers of estrogenicity in juvenile rainbow trout (*Oncorhynchus mykiss*). Aquatic toxicology; (60): 203-221.

Afonso L O B, Smith J L, Ikonomou M G, Devlin R H. 2002. Y-chromosomal DNA markers for discrimination of chemical substance and effluent effects on sexual differentiation in salmon. Environmantal Health Perspectives; (110): 881-887.

Ahel M, Giger W, Schaffner C. 1994. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment-II. Occurrence and transformation in rivers. Water Research; (28): 1143-1152.

Ahel M, McEvoy J, Giger W. 1993. Bioaccumulation of the lipophilic metabolites of nonionic surfactactants in freshwater organisms. Enviromental pollution; (79): 243-248.

Alheit J, Mollmann C, Dutz J, Kornilovs G, Loewe P, Mohrholz V, Wasmund N. 2005. Synchronous ecological regime shifts in the central Baltic and the North Sea in the late 1980s. ICES Journal of Marine Science; (62): 1205-1215.

Allen Y, Matthiessen P, Scott A P, Haworth S, Feist S, Thain J E. 1999a. The extent of oestrogenic contamination in the UK estuarine and marine environments - further surveys of flounder. Science of the Total Enviroment; (233): 5-20.

Allen Y, Scott A P, Matthiessen P, Haworth S, Thain J E, Feist S. 1999b. Survey of estrogenic activity in United Kingdom estuarine and coastal waters and its effects on gonadal development of the flounder *Platichthys flesus*. Environmental Toxicology and Chemistry; (18): 1791-1800.

Allner B, Wegener G, Knacker T, StahlschmidtAllner P. 1999. Electrophoretic determination of estrogen-induced protein in fish exposed to synthetic and naturally occurring chemicals. Science of the Total Enviroment; (233): 21-31.

Amantea D, Russo R, Bagetta G, Corasaniti M T. 2005. From clinical evidence to molecular mechanisms underlying neuroprotection afforded by estrogens. Pharmacological Research; (52): 119-132.

Amer M A, Miura T, Miura C, Yamauchi K. 2001. Involvement of sex steroid hormones in the early stages of spermatogenesis in Japanese huchen (*Hucho perryi*). Biology of Reproduction; (65): 1057-1066.

Anderson D, Schmid T E, Baumgartner A. 2003. Cemeli-Carratala E, Brinkworth M H, Wood J M. Oestrogenic compounds and oxidative stress (in human sperm and lymphocytes in the Comet assay). Mutation Research-Reviews in Mutation Research; (544): 173-178.

Andreassen T K, Korsgaard B. 2000. Characterization of a cytosolic estrogen receptor and its up-regulation by 17 beta-estradiol and the xenoestrogen 4-tert-octylphenol in the liver of eelpout (*Zoarces viviparus*). Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology; (125): 299-313.

Andreassen T K, Skjoedt K, Korsgaard B. 2005. Upregulation of estrogen receptor alpha and vitellogenin in eelpout (*Zoarces viviparys*) by Waterborne exposure to 4-tert-octylphenol and 17 beta-estradiol. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology; (140): 340-346.

Aravindakshan J, Cyr D G. 2005. Nonylphenol alters connexin 43 levels and connexin 43 phosphorylation via an inhibition of the p38-mitogen-activated protein kinase pathway. Biology of Reproduction; (72): 1232-1240.

Arsenault J T M, Fairchild W L, MacLatchy D L, Burtidge L, Haya K, Brown S B. 2004. Effects of water-borne 4-nonylphenol and 17 beta-estradiol exposures during parr-smolt transformation on growth and plasma IGF-I of Atlantic salmon (*Salmo salar L.*). Aquatic toxicology; (66): 255-265.

Arukwe A. 2005. Modulation of brain steroidogenesis by affecting transcriptional changes of steroidogenic acute regulatory (StAR) protein and cholesterol side chain cleavage (P450scc) in juvenile Atlantic salmon (*Salmo salar*) is a novel aspect of nonylphenol toxicity. Environmental Science & Technology; (39): 9791-9798.

Arukwe A, Celius T, Walther B T, Goksoyr A. 2000a. Effects of xenoestrogen treatment on zona radiata protein and vitellogenin expression in Atlantic salmon (*Salmo salar*). Aquatic toxicology; (49): 159-170.

Arukwe A, Celius T, Walther B T, Goksoyr A. 1998. Plasma levels of vitellogenin and eggshell *zona radiata* proteins in 4-nonylphenol and o,p-DDT treated juvenile atlantic salmon (*Salmo salar*). Marine Environmental Research; (46): 133-136.

Arukwe A, Forlin L, Goksoyr A. 1997a. Xenobiotic and steroid biotransformation enzymes in atlantic salmon (*Salmo salar*) liver treated with an estrogenic compound, 4-Nonylphenol. Environmental Toxicology and Chemistry; (16): 2576-2583.

Arukwe A, Goksoyr A. 1998. Xenobiotics, xenoestrogens and reproduction disturbances in fish. SARSIA; (83): 225-241.

Arukwe A, Knudsen F R, Goksoyr A. 1997b. Fish zona radiata (eggshell) protein: A sensitive biomarker for environmental estrogens. Environmantal Health Perspectives; (105): 418-422.

Arukwe A, Kullman S W, Hinton D E. 2001a. Differential biomarker gene and protein expressions in nonylphenol and estradiol-17 beta treated juvenile rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology; (129): 1-10.

Arukwe A, Nilsen B M, Berg K, Goksoyr A. 1999. Immunohistochemical analysis of the vitellogenin response in the liver of Atlantic salmon exposed to environmental oestrogens. Biomarkers; (4): 373-380.

Arukwe A, Thibaut R, Ingebrigtsen K, Celius T, Goksoyr A, Cravedi J P. 2000b. In vivo and in vitro metabolism and organ distribution of nonylphenol in Atlantic salmon (*Salmo salar*). Aquatic toxicology; (49): 289-304.

Arukwe A, Yadetie F, Male R, Goksoyr A. 2001b. In vivo modulation of nonylphenol-induced zonagenesis and vitellogenesis by the antiestrogen, 3,3 ' 4,4 '-tetrachlorobiphenyl (PCB-77) in juvenile fish. Environmental Toxicology and Pharmacology; (10): 5-15.

Ashfield L A, Pottinger T G, Sumpter J P. 1998. Exposure of female juvenile rainbow trout to alkylphenolic compounds results in modifications to growth and ovosomatic index. Environmental Toxicology and Chemistry; (17): 679-686.

Ashley J T, Moore A, Stapleton H M, Velinsky D J, Wilhelm M P. 2003. Sedimentary nonylphenol contamination in an urbanized, industrialized segment of the Delaware River estuary, USA. Bulletin of Environmental Contamination and Toxicology; (70): 978-984.

Atienzar F A, Billinghurst Z, Depledge M H. 2002. 4-n-nonylphenol and 17-beta estradiol may induce common DNA effects in developing barnacle larvae. Environmental pollution; (120): 735-738.

Balch G, Metcalfe C. 2006. Developmental effects in Japanese medaka (*Oryzias latipes*) exposed to nonylphenol ethoxylates and their degradation products. Chemosphere; (62): 1214-1223.

Baldwin W S, Roling J A, Peterson S, Chapman L M. 2005. Effects of nonylphenol on hepatic testosterone metabolism and the expression of acute phase' proteins in winter flounder (*Pleuronectes americanus*): Comparison to the effects of Saint John's Wort. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology; (140): 87-96.

Bao M L, Barbieri K, Burrini D, Griffini O, Pantani F. 1996. Traces determination of phenols in water by solid phase extraction followed by pentafluorobenzoylation. Annali di Chimica; (86): 343-356.

Baroiller J F, Guigen Y, Fostier A. 1999. Endocrine and environmental aspects of sex differentiation in fish. Cellular and Molecular Life Sciences; (55): 910-931.

Barse A, Chakrabarti T, Ghosh T K, Pal A K, Jadhao S B. 2006. One-tenth dose of LC50 of 4-tert-butylphenol causes endocrine disruption and metabolic changes in Cyprinus carpio. Pesticide Biochemistry and Physiology; (86): 172-179.

Bartolome L, Cortazar E, Raposo J C, Usobiaga A, Zuloaga O, Etxebarria N, Fernandez L A. 2005. Simultaneous microwave-assisted extraction of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phthalate esters and nonylphenols in sediments. Journal of Chromatography A; (1068): 229-236.

Basheer C, Lee H K, Tan K S. 2004. Endocrine disrupting alkylphenols and bisphenol-A in coastal waters and supermarket seafood from Singapore. Marine pollution bulletin; (48): 1161-1167.

Bastow T P, van Aarssen B G K, Alexander R, Kagi R I. 2005. Origins of alkylphenols in crude oils: Hydroxylation of alkylbenzenes. Organic Geochemistry; (36): 991-1001.

Bayley M, Nielsen J R, Baatrup E. 1999. Guppy sexual behavior as an effect biomarker of estrogen mimics. Ecotoxicology and environmental safety; (43): 68-73.

Beaugrand G, Brander K M, Lindley J A, Souissi S, Reid P C. 2003. Plankton effect on cod recruitment in the North Sea. Nature; (426): 661-664.

Beck I C, Bruhn R, Gandrass J, Ruck W. 2005. Liquid chromatography-tandem mass spectrometry analysis of estrogenic compounds in coastal surface water of the Baltic Sea. Journal of Chromatography A; (1090): 98-106.

Beeler T J, Gable K S. 1993. Activation of Ca2+ Release from Sarcoplasmic-Reticulum Vesicles by 4-Alkylphenols. Archives of Biochemistry and Biophysics; (301): 216-220.

Bennett B, Bowler B F J, Larter S R. 1996. Determination of C-0-C-3 alkylphenols in crude oils and waters. Analytical chemistry; (68): 3697-3702.

Bennett E R, Metcalfe C D. 2000. Distribution of degradation products of alkylphenol ethoxylates near sewage treatment plants in the lower Great Lakes, North America. Environmental Toxicology and Chemistry; (19): 784-792.

Berkner S, Streck G, Herrmann R. 2004. Development and validation of a method for determination of trace levels of alkylphenols and bisphenol A in atmospheric samples. Chemosphere; (54): 575-584.

Bester K, Theobald N, Schroder H FR. 2001. Nonylphenols, nonylphenol ethoxylates, linear alkylbenzenesulfonates (LAS) and bis (4-chlorophenyl)-sulfone in the German Bight of the North Sea. Chemosphere; (45): 817-826.

Beullens K, Eding E H, Ollevier F, Komen J, Richter C J. 1997. Sex differentiation, changes in length, weight and eye size before and after metamorphosis of European eel (*Anguilla anguilla L*) maintained in captivity. Aquaculture; (153): 151-162.

Bevan C L, Porter D M, Prasad A, Howard M J, Henderson L P. 2003. Environmental Estrogens alter early development in *Xenopus laevis*. Environmental Health Perspectives; (111): 488-496.

Bhatt B D, Prasad J V, Kalpana G, Ali S. 1992. Separation and characterization of isomers of p-nonylphenols by capillary GC/GC-MS/GC-FTIR techniques. Journal of Chromatographic Science; (30): 203-210.

Bianchi F, Careri M, Mucchino C, Musci M. 2002. Improved determination of chlorophenols in water by solid-phase microextraction followed by benzoylation and gas chromatography with electron capture detection. Chromatographia; (55): 595-600.

Bicknell R J, Herbison A E, Sumpter J P. 1995. Oestrogenic activity of an environmentally persistent alkylphenol in the reproductive tract but not the brain of rodents. J Steroid Biochem Molec Biol; (54): 7-9.

Bistodeau T J, Barber L B, Bartell S E, Cediel R A, Grove K J, Klaustermeier J, Woodard J C, Lee K E, Schoenfuss H L. 2006. Larval exposure to environmentally relevant mixtures of alkylphenolethoxylates reduces reproductive competence in male fathead minnows. Aquatic toxicology; (79): 268-277.

Blackburn M A, Kirby S J, Waldock M J. 1999. Concentrations of alkyphenol polyethoxylates entering UK estuaries. Marine pollution bulletin; (38): 109-118.

Blackburn M A, Waldock M J. 1995. Concentrations of alkylphenols in rivers and estuaries in England and Wales. Water Research; (29): 1623-1629.

Blair R M, Fang H, Branham W S, Hass B S, Dial S L, Moland C L, Tong W D, Shi L M, Perkins R, Sheehan D M. 2000. The estrogen receptor relative binding affinities of 188 natural and xenochemicals: Structural diversity of ligands. Toxicological Sciences; (54): 138-153.

Blazquez M, Bosma P T, Fraser E J, VanLook K J, Trudeau V L. 1998. Fish as models for the neuroendocrine regulation of reproduction and growth. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology; (119) :345-364,.

Boitsov S, Meier S, Klungsoyr J, Svardal A. 2004. Gas chromatography-mass spectrometry analysis of alkylphenols in produced water from offshore oil installations as pentafluorobenzoate derivatives. Journal of Chromatography A; (1059): 131-141.

Bolognesi C, Perrone E, Roggieri P, Pampanin D M, Sciutto A. 2006. Assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions. Aquatic toxicology; (78): S93-S98.

Bolton J L. 2002. Quinoids, quinoid radicals, and phenoxyl radicals formed from estrogens and antiestrogens. Toxicology; (177): 55-65.

Bolz U, Korner W, Hagenmaier H. 2000. Development and validation of a GC/MS method for determination of phenolic xenoestrogens in aquatic samples. Chemosphere; (40): 929-935.

Bortone S A, Cody R P. 1999. Morphological masculinization in poeciliid females from a paper mill effluent receiving tributary of the St. Johns River, Florida, USA. Bulletin of Enviromental Contamination and Toxicology; (63): 150-156.

Bortone S A, Davis W P. 1994. Fish intersexuality as indicator of environmental stress. Bioscience; (44): 165-172.

Bortone S A, Davis W P, BundrickC M. 1989. Morphological and behavioral characters in mosquitofish as potential bioindication of exposure to kraft mill effluent. Bulletin of Enviromental Contamination and Toxicology; (43): 370-377.

Bragadin M, Perin G, Iero A, Manente S, Rizzoli V, Scutari G. 1999. An in vitro study on the toxic effects of nonylphenols (NP) in mitochondria. Chemosphere; (38): 1997-2001.

Brander K. 1993. Comparison of spawning characteristics of cod (*Gadus morhua*) stocks in the North Atlantic. ICES NAFO Sci Coun Studies; (18): 13-20.

Brander K M. 1994. The location and timing of cod spawning around the British isles. ICES Journal of Marine Science; (51): 71-89.

Brendehaug J, Johnsen S, Bryne K H, Gjøse A L, Eide T H, Aamot E. 1992. Toxicity testing and chemical characterization of produced water - A preliminary study. In: Produced water. (Eds.Ray JP, Engelhart FR). New York: Plenum Press,; 245-256.

Brian J V, Harris C A, Scholze M, Backhaus T, Booy P, Lamoree M, Pojana G, Jonkers N, Runnalls T, Bonfa A, Marcomini A, Sumpter J A. 2005. Accurate prediction of the response of freshwater fish to a mixture of estrogenic chemicals. Environmental Health Perspectives; (113): 721-728.

Brossa L, Pocurull E, Borrull F, Marce R M. 2004. Solid-phase extraction/high-performance liquid chromatography-electrospray mass spectrometry to determine endocrine disruptors in water samples. Chromatographia; (59): 419-423.

Brown A R, Riddle A M, Cunningham N L, Kedwards T J, Shillabeer N, Hutchinson T H. 2003. Predicting the effects of endocrine disrupting chemicals on fish populations. Human and Ecological Risk Assessment; (9): 761-788.

Brown A R, Riddle A M, Winfield I J, Fletcher J M, James J B. 2005. Predicting the effects of endocrine disrupting chemicals on healthy and disease impacted populations of perch (perca fluviatilis). Ecological Modelling; (189): 377-395.

Brown S B, Adams B A, Cyr D G, Eales J G. 2004. Contaminant effects on the teleost fish thyroid. Environmental Toxicology and Chemistry; (23): 1680-1701.

Brzozowski A M, Pike A C W, Dauter Z, Hubbard R E, Bonn T, Engstrøm O, Ohman L, Greene G L, Gustafsson J Å, Carlquist M. 1997. Molecular basis of agonism and antagonism in the oestrogen receptor. Nature; (389): 753-758.

Bulayeva N N, Watson C S. 2004. Xenoestrogen-induced ERK-1 and ERK-2 activation via multiple membrane-initiated signaling pathways. Environmental Health Perspectives; (112): 1481-1487.

Cakmak G, Togan I, Severcan F. 2006. 17 beta-estradiol induced compositional, structural and functional changes in rainbow trout liver, revealed by FT-IR spectroscopy: A comparative study with nonylphenol. Aquatic toxicology; (77): 53-63.

Cardinali M, Maradonna F, Olivotto I, Bortoluzzi G, Mosconi G, Polzonetti-Magni A M, Carnevali O. 2004. Temporary impairment of reproduction in freshwater teleost exposed to nonylphenol. Reproductive Toxicology; (18): 597-604.

Carrera E P, Garcia-Lopez A, delRio M d P M, Martinez-Rodriguez G, Sole M, Mancera J M. 2007. Effects of 17β-estradiol and 4-nonylphenol on osmoregulation and hepatic enzymes in gilthead seabream (*Sparus auratus*). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology; (in press).

Cascaval C N, Rosu D, Agherghinei I. 1996. The thermal behaviour of some epoxy-acrylic polymers based on phenol and para-alkyl substituted phenols. *Polymer Degradation and Stability* (52) :253-257

Cascio M. 2005. Connexins and their environment: effects of lipids composition on ion channels. Biochimica et Biophysica Acta-Biomembranes; (1711): 142-153.

Casini S, Fossi M C, Mori G, Bjornstad A. 2002. Vitellogenin induction in *Cyprinus carpio* treated with 17 beta-estradiol and 4-nonylphenol. Environmental Monitoring and Assessment; (75): 235-239.

Cathum S, Sabik H. 2001. Simultaneous determination of alkylphenol polyethoxylate surfactants and their degradation products in water, effluent and mussel using gas chromatography-mass spectrometry. Chromatographia; (53): S400-S405.

CEFAS. The significance of offshore chemicals as endocrine disruptors in fish. 1-19. 2005. London, Department for Environment, Food and Rural Affairs.
Ref Type: Report

CEFAS. Monitoring of the quality of the marine environment, 2003-2004. Lowestoft: Cefas. Sci.Ser.Aquat.Environ.Monit. no 58:1-168, 2006.
Ref Type: Report

Certa H, Fedtke N, Wiegand H J, Muller A M F, Bolt H M. 1996. Toxicokinetics of *p*-tert-octylphenol in male Wistar rats. Archives of Toxicology; (71): 112-122.

Chalaux N, Bayona M, Albaiges J. 1994. Determination of nonylphenols as pentafluorobenzyl derivateres by capillary gas chromatography whit eletroncapture and mass spectrometric detektor in environmental matrices. Journal of Chromatography A; (686): 275-281.

Chapin R E, Delaney J, Wang Y F, Lanning L, Davis b, Collins B, Mintz N, Wolfe G. 1999. The effects of 4-nonylphenol in rats: A multigeneration reproduction study. Toxicological Sciences; (52): 80-91.

Chee K K, Wong M K, Lee H K. 1996. Determination of 4-nonylphenol .1. Optimization of the gas chromatographic determination of 4-nonylphenol by orthogonal array design and electronic pressure programming. Journal of Microcolumn Separations; (8): 29-36.

Chen B, Mai B X, Duan J C, Luo X J, Yang Q S, Sheng G Y, Fu J M. 2005. Concentrations of alkylphenols in sediments from the Pearl River estuary and South China Sea, South China. Marine pollution bulletin; (50): 993-997.

Cheng C Y, Liu L L, Ding W H. 2005. Determination of xenoestrogens alkylphenols in oyster and snail tissues by extractive steam distillation and gas chromatography-mass spectrometry. Journal of the Chinese Chemical Society; (52): 1257-1262.

Cheung J, Wells R J. 1997. Analysis of phenolic compounds in effluent by solid-phase extraction and gas chromatography mass spectrometry with direct on-column benzylation: Sensitive negative ion chemical ionisation gas chromatography mass spectrometry detection of phenyl benzyl ethers . Journal of Chromatography A; (771): 203-211.

Chikae M, Ikeda R, Hasan Q, Morita Y, Tamiya E. 2003. Effect of alkylphenols on adult male medaka: plasma vitellogenin goes up to the level of estrous female. Environmental Toxicology and Pharmacology; (15): 33-36.

Chitra K C, Latchoumycandane C, Mathur P P. 2002. Effect of nonylphenol on the antioxidant system in epididymal sperm of rats. Archives of Toxicology; (76): 545-551.

Christensen L J, Korsgaard B, Bjerregaard P. 1999. The effect of 4-nonylphenol on the synthesis of vitellogenin in the flounder *Platichthys flesus*. Aquatic toxicology; (46): 211-219.

Christiansen T, Korsgaard B, Jespersen Å. 1998. Effects of nonylphenol and 17β-ostradiol on vitellogenin synthesis, testicular structure and cytology in male eelpout *Zoarces viviparus*. Journal of Experimental Biology; (201): 179-192.

Cincinelli A, Mandorlo S, Dickhut R M, Lepri L. 2003. Particulate organic compounds in the atmosphere surrounding an industrialised area of Prato (Italy). Atmospheric Environment; (37): 3125-3133.

Cionna C, Maradonna F, Olivotto I, Pizzonia G, Carnevali O. 2006. Effects of nonylphenol on juveniles and adults in the grey mullet, *Liza aurata*. Reproductive Toxicology; (22): 449-454.

Coldham N G, Sivapathasundaram S, Dave M, Ashfield L A, Pottinger T G, Goodall C, Sauer M J. 1998. Biotransformation, tissue distribution, and persistence of 4-nonylphenol residues in juvenile rainbow trout (*Oncorhynchus mykiss*). Drug Metabolism and Disposition; (26): 347-354.

Cook J W, Dodds E C, Hewett C L. 1933. A synthetic oestrus-exciting compound. Nature; (131): 56-57.

Cook R M, Sinclair A, Stefansson G. 1997. Potential collapse of North Sea cod stocks. Nature; (385): 521-522.

Cooper R L, Kavlock R J. 1997. Endocrine disruptors and reproductive development: A weight-of-evidence overview. Journal of Endocrinology; (152): 159-166.Corsi I, Focardi S. 2002. Nonylphenols in a lagoon environment: p-nonylphenol and nonylphenol ethoxylates in fish tissue. Bulletin of Enviromental Contamination and Toxicology; (68): 908-914.

Croley T R, Lynn B C. 1998. Molecular ion stabilization for enhanced tandem mass spectrometry through derivatization for alkylphenols. Rapid Communications in mass spectrometry; (12): 171-175.

Cushing D H. 1990. Plankton Production and Year-Class Strength in Fish Populations - An Update of the Match Mismatch Hypothesis. Advances in Marine Biology; (26): 249-293.

Dachs J, VanRy D A, Eisenreich S J. 1999. Occurrence of estrogenic nonylphenols in the urban and coastal atmosphere of the lower Hudson River estuary. Environmental Science & Technology; (33): 2676-2679.

Dahle R, Taranger G L, Karlsen O, Kjesbu O S, Norberg B. 2003. Gonadal development and associated changes in liver size and sexual steroids during the reproductive cycle of captive male and female Atlantic cod (*Gadus morhua L.*). Comparative Biochemistry and Physiology A Mol Integr Physiol; (136): 641-653.

Das S, Thomas P. 1999. Pesticides interfere with the nongenomic action of a progestogen on meiotic maturation by binding to its plasma membrane receptor on fish oocytes. Endocrinology; (140): 1953-1956.

Datta S, Loyo-Rosales J E, Rice C P. 2002. A simple method for the determination of trace levels of alkylphenolic compounds in fish tissue using pressurized fluid extraction, solid phase cleanup, and high-performance liquid chromatography fluorescence detection. Journal of Agricultural and Food Chemistry; (50): 1350-1354.

De la Cruz J P, Zanca A, Carmona J A, de la Cuesta F S. 1999. The effect of propofol on oxidative stress in platelets from surgical patients. Anesthesia and Analgesia; (89): 1050-1055.

De Metrio G, Corriero A, Desantis S, Zubani D, Cirillo F, Deflorio M, Bridges C R, Eicker J, de la Serna J M, 2003. Megalofonou P, Kime D E. Evidence of a high percentage of intersex in the Mediterranean swordfish (*Xiphias gladius L.*). Marine pollution bulletin; (46): 358-361.

De Voogt P, Kwast O, Hendriks R, Jonkers N. 2000. Alkylphenol ethoxylates and their degradation products in abiotic and biological samples from the environment. Analusis; (28): 776-782.

Devlin R H and Nagahama Y. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture; (208) :191-364,.

Directive 2003-53-EC. Amending for the 26th time council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate and cement). Official Journal of the European Union 178, 24-26. 2003. European Parliament and the Council.
Ref Type: Report

Dodds E C, Lawson W. 1938. Molecular structure in relation to ostrogenic activity. Compounds without a phenanthrene nucleus. Proceedings of the royal society of London Series B, Biological Sciences; (125): 222-232.

Doerge D R, Twaddle N C, Churchwell M I, Chang H C, Newbold R R, Delclos K B. 2002. Mass spectrometric determination of p-nonylphenol metabolism and disposition following oral administration to Sprague-Dawley rats. Reproductive Toxicology; (16): 45-56.

Dreze V, Monod G, Cravedi J P, BiagiantiRisbourg S, Legac F. 2000. Effects of 4-nonylphenol on sex differentiation and puberty in mosquitofish (Gambusia holbrooki). Ecotoxicology; (9): 93-103.

Dufour S, Huang Y S, Rousseau K, Sbaihi M, Le Belle N., Vidal B, Marchelidon J, Querat, B, Burzawa-Gerard E, Chang C F, Schmitz, M. 2000. Puberty in teleost: New insights into the role of peripheral signals in the stimulation of pituitary gonadotropins. Proceedings of the 6th international symposium on the Reproduction physiology of fish:455-461,.

Durell GS, Liu B, Faksness L. 2002. Produced water phenol analysis. Summary report from the Round Robin Study. 1-63. SINTEF Applied Chemistry, N-7465 Trondheim, Norway.
Ref Type: Report

Dussault E B, Sherry J P, Lee H B, Burnison B K, Bennie D T, Servos M R. 2005. In vivo estrogenicity of nonylphenol and its ethoxylates in the Canadian environment. Human and Ecological Risk Assessment; (11): 353-364.

Dwyer F J, Mayer F L, Sappington L C, Buckler D R, Bridges C M, Greer I E, Hardesty D K, Henke C E, Ingersoll C G, Kunz J L, Whites D W, Augspurger T, Mount D R, Hattala K, Neuderfer G N. 2005. Assessing contaminant sensitivity of endangered and threatened aquatic species: Part I. Acute toxicity of five chemicals. Archives of Environmental Contamination and Toxicology; (48): 143-154.

Ekelund R, Granmo A, Magnusson K, Berggren M. 1993. Biodegradation of 4-nonylphenol in seawater and sediment. Environmental pollution; (79): 59-61.

Ellertsen B, Fossum P, Solemdal P, Sundby S, Tilseth S. 1986. The effect of biological and physical factors on the survival of arcto-Norwegian cod and the influence on recruitment variability. Proceedings of the third Soviet-Norwegian symposium, Murmansk;101-125.

Enmark E, Gustafsson J A. 1999. Oestrogen receptors - an overview. Journal of Internal Medicine; (246): 133-138.

EPA. 2005. Aquatic Life Ambient Water Quality Criteria - Nonylphenol Final. EPA-822-R-05-005, 1-89. Washington, DC, U.S. Environmental Protection Agency.
Ref Type: Report

Espejo R, Valter K, Simona M, Janin Y, Arrizabalaga P. 2002. Determination of nineteen 4-alkylphenol endocrine disrupters in Geneva municipal sewage wastewater. Journal of Chromatography A; (976): 335-343.

Fang H, Tong W, Branham W S, Moland C L, Dial S L, Hong H, Xie Q, Perkins R, Owens W, Sheehan D M. 2003. Study of 202 natural, synthetic, and environmental chemicals for binding to the androgen receptor. Chemical Research in Toxicology; (16): 1338-1358.

Fang H, Tong W D, Shi L M, Blair R, Perkins R, Branham W, Hass B S, Xie Q, Dial S L, Moland C L, Sheehan D M. 2001. Structure-activity relationships for a large diverse set of natural, synthetic, and environmental estrogens. Chemical Research in Toxicology; (14): 280-294.

Fent K, Ackermann G, Schwaiger J. 2000. Longterm effects of nonylphenol on vitellogenin and zona radiata protein expression in juvenile rainbow trout. Proceedings of the 6th international symposium on the Reproduction physiology of fish;356-358.

Ferguson P L, Iden C R, Brownawell B J. 2001. Distribution and fate of neutral alkylphenol ethoxylate metabolites in a sewage-impacted urban estuary. Environmental Science & Technology; (35): 2428-2435.

Ferrara F, Fabietti F, Delise M, Bocca A P, Funari E. 2001. Alkylphenolic compounds in edible molluscs of the Adriatic Sea (Italy). Environmental Science & Technology; (35): 3109-3112.

Ferrara F, Fabietti F, Delise M, Funari E. 2005. Alkylphenols and alkylphenol ethoxylates contamination of crustaceans and fishes from the Adriatic Sea (Italy). Chemosphere; (59): 1145-1150.

Ferreira-Leach A M R, Hill E M. 2001. Bioconcentration and distribution of 4-tert-octylphenol residues in tissues of the rainbow trout (*Oncorhynchus mykiss*). Marine Environmental Research; (51): 75-89.

Ferreira-Leach A M R, Hill E M. 2000. Bioconcentration and metabolism of 4-tert-octylphenol in roach (*Rutilus rutilus*) fry. Analusis; (28): 789-792.

Fiamegos Y C, Nanos C G, Pilidis G A, Stalikas C D. 2003. Phase-transfer catalytic determination of phenols as methylated derivatives by gas chromatography with flame ionization and mass-selective detection. Journal of Chromatography A; (983): 215-223.

Folmar L C, Gardner G R, Schreibman M P, Magliulo-Cepriano L, Mills L J, Zaroogian G, Gutjahr-Gobell R, Haebler R, Horowitz D B, Denslow N D. 2001. Vitellogenin-induced pathology in male summer flounder (*Paralichthys dentatus*). Aquatic toxicology; (51): 431-441.

Foran C M, Bennett E R, Benson W H. 2000. Exposure to environmentally relevant concentrations of different nonylphenol formulations in Japanese medaka. Marine Environmental Research; (50): 135-139.

Fossi M C, Casini S, Marsili L, Ancora S, Mori G, Neri G, Romeo T, Ausili A. 2004. Evaluation of ecotoxicological effects of endocrine disrupters during a four-year survey of the Mediterranean population of swordfish (*Xiphias gladius*). Marine Environmental Research; (58): 425-429.

Freitag D, Ballhorn L, Geyer H, Korte F. 1985. Environmental hazard profile of organic chemicals. An experimental methodfor the assessment of the behaviour of organic chemicals in the ecosphere by means of simple laboratory tests with ^{14}C labelled chemicals. Chemosphere; (14): 1589-1616.

Fries E, Puttmann W. 2004. Monitoring of the antioxidant BHT and its metabolite BHT-CHO in German river water and ground water. Science of the Total Environment; (319): 269-282.

Fu C H, Mohn R, Fanning L P. 2001. Why the Atlantic cod (*Gadus morhua*) stock off eastern Nova Scotia has not recovered. Canadian Journal of Fisheries and Aquatic Sciences; (58): 1613-1623.

Fujimoto Y, Usa K, Sakuma S. 2005. Effects of endocrine disruptors on the formation of prostaglandin and arachidonoyl-CoA formed from arachidonic acid in rabbit kidney medulla microsomes. Prostandins, Leukotrienes and essential fatty acids; (73): 447-452.

Gale W L, Patino R, Maule A G. 2004. Interaction of xenobiotics with estrogen receptors alpha and beta and a putative plasma sex hormone-binding globulin from channel catfish (*Ictalurus punctatus*). General and Comparative Endocrinology; (136): 338-345.

Gao Y W. 2002. Regime shift signatures from stable oxygen isotopic records of otoliths of Atlantic cod (Gadus morhua). Isotopes Environ Health Stud; (38): 251-263.

Garcia-Reyero N, Raldua D, Quiros L, Llaveria G, Cerda J, Barcelo D, Grimalt J O, Pina B. 2004. Use of vitellogenin mRNA as a biomarker for endocrine disruption in feral and cultured fish. Analytical and Bioanalytical Chemistry; (378): 670-675.

Ghisari M, Bonefeld-Jorgensen E C. 2005. Impact of environmental chemicals on the thyroid hormone function in pituitary rat GH3 cells. Molecular and Cellular Endocrinology; (244): 31-41.

Giesy J P, Pierens S L, Snyder E M, MilesRichardson S, Kramer V J, Snyder S A, Nichols K M, Villeneuve D A. 2000. Effects of 4-nonylphenol on fecundity and biomarkers of estrogenicity in fathead minnows (*Pimephales promelas*). Environmental Toxicology and Chemistry; (19): 1368-1377.

Giger W, Brunner P H, Schaffner C. 1984. 4-Nonylphenol in sewage sludge: Accumulation of toxic Metabolites from nonionic surfactants. Science; (225): 623-625.

Giger W, Stephanou E, Schaffner C. 1981. Persistent organic chemicals in sewage effuents: I. Identifications of nonylphenols and nonylphenolethoxylates by glass capillary gas chromatography / mass spectrometry. Chemosphere; (10): 1253.

Gimeno S, Gerritsen A, Bowmer T, Komen H. 1996. Feminization of male carp. Nature; (384): 221-222.

Gimeno S, Komen H, Gerritsen A G, Bowmer T. 1998. Feminisation of young males of the common carp, Cyprinus carpio, exposed to 4-tert-pentylphenol during sexual differentiation. Aquatic toxicology; (43): 77-92.

Gimeno S, Komen H, Venderbosch P W M, Bowmer T. 1997. Disruption of sexual differentiation in genetic male common carp (*Cyprinus carpio*) exposed to an alkylphenol during different life stages. Environmental Science & Technology; (31): 2884-2890.

Godo O R, Moksness E. 1987. Growth and Maturation of Norwegian Coastal Cod and Northeast Arctic Cod Under Different Conditions. Fisheries Research; (5): 235-242.

Goksoyr A, Male R. 2006. Endocrine disrupters and the aquatic environment: Studies on mechanisms of toxicity and influence on nuclear receptor signalling pathways in fish. Endocrine Disruptors.

Gong Y, Han X D. 2006. Nonylphenol-induced oxidative stress and cytotoxicity in testicular Sertoli cells. Reproductive Toxicology; (22): 623-630.

Gotceitas V, Puvanendran V, Leader L L, Brown j A. 1996. An experimental investigation of the 'match mismatch' hypothesis using larval Atlantic cod. Marine Ecology Progress Series; (130): 29-37.

Grahl-Nielsen O. 1987. Hydrocarbons and phenols in discharge water from offshore operations. Fate of the hydrocarbons in the recipient. SARSIA; (72): 375-382.

Grahl-Nielsen O, Landgren-Skjellerudsveen B. 1982. A new method for analysis of phenols in water. 8, 64-65.. Dubendorf, Switzerland, Commission of the european communities. Report of the workshop of the working party 8.
Ref Type: Report

Granmo A, Ekelund E, Berggren M, Magnusson K. 1991. Toxicity of 4-nonylphenol to aquatic organisms and potential for bioaccumulation. 53-75.. Sweden, Proccedings, Swedish Environmental Protection Agency Seminar on Nonylphenol Ethoxylates/nonylphenol, Saltsjobaden, Sweden, February 6-8.
Ref Type: Conference Proceeding

Granmo Å, Kvist E, Mannheimer J, Renberg L, Rosengarden A, Solyom P. 1986. Miljegenskaper hos några utvalda typer av tensides. Naturvårdsverket, informationsenheten. 3024, 1-20.. Solna, Sverige, Naturvårdsverket.
Ref Type: Report

Gray M A, Metcalfe C D. 1999. Toxicity of 4-tert-octylphenol to early life stages of Japanese medaka (*Oryzias latipes*). Aquatic toxicology; (46): 149-154.

Gray M A, Metcalfe C D. 1997. Induction of testis-ova in japanese medaka (*Oryzias latipes*) exposed to p-nonylphenol. Environmental Toxicology and Chemistry; (16): 1082-1086.

Gray M A, Niimi A J, Metcalfe C D. 1999a. Factors affecting the development of testis-ova in medaka, Oryzias latipes, exposed to octylphenol. Environmental Toxicology and Chemistry; (18): 1835-1842.

Gray M A, Teather K L, Metcalfe C D. 1999b. Reproductive success and behavior of Japanese medaka (Oryzias latipes) exposed to 4-tert-octylphenol. Environmental Toxicology and Chemistry; (18): 2587-2594.

Gronen S, Denslow N, Manning S, Barnes S, Barnes D, Brouwer M. 1999. Serum vitellogenin levels and reproductive impairment of male Japanese medaka (*Oryzias latipes*) exposed to 4-tert-octylphenol. Environmantal Health Perspectives; (107): 385-390.

Guenther K, Heinke V, Thiele B, Kleist E, Prast H, Raecker T. 2002. Endocrine disrupting nonylphenols are ubiquitous in food. Environmental Science & Technology; (36): 1676-1680.

Gunther K, Durbeck H W, Kleist E, Thiele B, Prast H, Schwuger M. 2001. Endocrine-disrupting nonylphenols - ultra-trace analysis and time-dependent trend in mussels from the German bight. Fresenius Journal of Analytical Chemistry; (371): 782-786.

Guo T L, Germolec D R, Musgrove D L, Delclos K B, Weis C, White K L. 2005. Myelotoxicity in genistein-, nonylphenol-, methoxychlor-, vinclozolin- or ethinyl estradiol-exposed F-1 generations of Sprague-Dawley rats following developmental and adult exposures. Toxicology; (211): 207-219.

Haavisto T E, Adamsson N A, Myllymaki S A, Toppari J, Paranko J. 2003. Effects of 4-tert-octylphenol, 4-tert-butylphenol, and diethylstilbestrol on prenatal testosterone surge in the rat. Reproductive Toxicology; (17): 593-605.

Hale R C, Smith C L, deFur P O, Harvey E, Bush E O. 2000. Nonylphenols in sediments and effluents associated with diverse wastewater outfalls. Environmental Toxicology and Chemistry; (19): 946-952.

Han B S, Fukamachi K, Takasuka N, Ohnishi T, Maeda M, Yamasaki T, Tsuda H. 2002. Inhibitory effects of 17 beta-estradiol and 4-n-octylphenol on 7,12-dimethylbenz[a]anthracene-induced mammary tumor development in human c-Ha-ras proto-oncogene transgenic rats. Carcinogenesis; (23): 1209-1215.

Han X, Tu Z, Wang X, Shen S, Hou Y. 2004a. Nonylphenol induced apoptosis in rat testis through the Fas/FasL pathway. Bulletin of Environmental Contamination and Toxicology; (73): 620-627.

Han X D, Tu Z G, Gong Y, Shen S N, Wang X Y, Kang L N, Hou Y Y, Chen J X. 2004b. The toxic effects of nonylphenol on the reproductive system of male rats. Reproductive Toxicology; (19): 215-221.

Hando R, Abo M, Okubo A. 2003. Determination of alkylphenols and bisphenol A in sea environment by GC/MS. Bunseki Kagaku; (52): 695-699.

Hansson T, Lindesjoo E, Forlin L, Balk L, Bignert A, Larsson A. 2006. Long-term monitoring of the health status of female perch (*Perca fluviatilis*) in the Baltic Sea shows decreased gonad weight and increased hepatic EROD activity. Aquatic toxicology; (79): 341-355.

Harries J E, Runnalls T, Hill E, Harris C A, Maddix S, Sumpter J P, Tyler C R. 2000. Development of a reproductive performance test for endocrine disrupting chemicals using pair-breeding fathead minnows (*Pimephales promelas*). Environmental Science & Technology; (34): 3003-3011.

Harris C A, Santos E M, Janbakhsh A, Pottinger T G, Tyler C R, Sumpter J P. 2001. Nonylphenol affects gonadotropin levels in the pituitary gland and plasma of female rainbow trout. Environmental Science & Technology; (35): 2909-2916.

Hashimoto S, Bessho H, Hara A, Nakamura M, Iguchi T, Fujita K. 2000. Elevated serum vitellogenin levels and gonadal abnormalities in wild male flounder (*Pleuronectes yokohamae*) from Tokyo Bay, Japan. Marine Environmental Research; (49): 37-53.

Hashimoto S, Horiuchi A, Yoshimoto T, Nakao M, Omura H, Kato Y, Tanaka H, Kannan K, Giesy J P. 2005. Horizontal and vertical distribution of estrogenic activities in sediments and waters from Tokyo Bay, Japan. Archives of Environmental Contamination and Toxicology; (48): 209-216.

Hasselberg L, Grosvik B E, Goksoyr A, Celander M. 2005. Interactions between xenoestrogens and ketoconazole on hepatic CYP1A and CYP3A, in juvenile Atlantic cod (*Gadus morhua*). Comparative Hepatology; (4:2).

Hawkins M B, Thomas P. 2004. The unusual binding properties of the third distinct teleost estrogen receptor subtype ER beta a are accompanied by highly conserved amino acid changes in the ligand binding domain. Endocrinology; (145): 2968-2977.

Hawkins M B, Thornton J W, Crews D, Skipper J K, Dotte A, Thomas P. 2000. Identification of a third distinct estrogen receptor and reclassification of estrogen receptors in teleosts. Proceedings of the National Academy of Sciences of the United States of America; (97): 10751-10756.

Heberer T, Stan H J. 1997. Detection of more than 50 substituted phenols as their t-butyldimethylsilyl derivatives using gas chromatography mass spectrometry. Analytica chimica acta; (341): 21-34.

Hecht S A, Gunnarsson J S, Boese B L, Lamberson J O, Schaffner C, Giger W, Jepson P C. 2004. Influences of sedimentary organic matter quality on the bioaccumulation of 4-nonylphenol by estuarine amphipods. Environmental Toxicology and Chemistry; (23): 865-873.

Heemken O P, Reincke H, Stachel B, Theobald N. 2001. The occurrence of xenoestrogens in the Elbe river and the North Sea. Chemosphere; (45): 245-259.

Hegrenes S G. 1999. Masculinization of spawning channel catfish in the Red River of the North. COPEIA;491-494.

Heim S, Hucke A, Schwarzbauer J, Littke R, Mangini A. 2006. Geochronology of anthropogenic contaminants in a dated sediment core of the Rhine River (Germany): emission sources and risk assessment. Acta Hydrochimica et Hydrobiologica; (34): 34-52.

Hellqvist A, Schmitz M, Mayer I, Borg B. 2006. Seasonal changes in expression of LH-beta and FSH-beta in male and female three-spined stickleback, Gasterosteus aculeatus. General and Comparative Endocrinology; (145): 263-269.

Hemmer M J, Bowman C J, Hemmer B L, Friedman S D, Marcovich D, Kroll K J, Denslow N D. 2002. Vitellogenin mRNA regulation and plasma clearance in male sheepshead minnows, (*Cyprinodon variegatus*) after cessation of exposure to 17 beta-estradiol and p-nonylphenol. Aquatic toxicology; (58): 99-112.

Hemmer M J, Hemmer B L, Bowman C J, Kroll K J, Folmar L C, Marcovich D, Hoglund M D, Denslow N D. 2001. Effects of p-nonylphenol, methoxychlor, and endosulfan on vitellogenin induction and expression in sheepshead minnow (*Cyprinodon variegatus*). Environmental Toxicology and Chemistry; (20): 336-343.

Herman R L, Kincaid H L. 1988. Pathological effects of orally administered estradiol to rainbow trout. Aquaculture; (72): 165-172.

Hernandez J P, Chapman L M, Kretschmer X C, Baldwin W S. 2006. Gender-specific induction of cytochrome P450s in nonylphenol-treated FVB/NJ mice. Toxicology and applied pharmacology; (216): 186-196.

Hill R L, Janz D M. 2003. Developmental estrogenic exposure in zebrafish (*Danio rerio*): I. Effects on sex ratio and breeding success. Aquatic toxicology; (63): 417-429.

Hinrichsen H H, Schmidt J O, Petereit C, Mollmann C. 2005. Survival probability of Baltic larval cod in relation to spatial overlap patterns with their prey obtained from drift model studies. ICES Journal of Marine Science; (62): 878-885.

Hong C C, Shimomura-Shimizu M, Muroi M, Tanamoto K I. 2004. Effect of endocrine disrupting chemicals on lipopolysaccharide-induced tumor necrosis factor-alpha and nitric oxide production by mouse macrophages. Biological & Pharmaceutical Bulletin; (27): 1136-1139.

Hoyt P R, Doktycz M J, Beattie K L, Greeley M S. 2003. DNA Microarrays detect 4-nonylphenol-induced alterations in gene expression during zebrafish early development. Ecotoxicology; (12): 469-474.

Hu J Y, Aizawa T. 2003. Quantitative structure-activity relationships for estrogen receptor binding affinity of phenolic chemicals. Water Research; (37): 1213-1222.

Hu J Y, Wan Y, Shao B, Jin X H, An W, Jin F, Yang M, Wang X J, Sugisaki M. 2005. Occurrence of trace organic contaminants in Bohai Bay and its adjacent Nanpaiwu River, North China. Marine chemistry; (95): 1-13.

Hughes E M, Gallagher E P. 2004. Effects of 17-beta estradiol and 4-nonylphenol on phase II electrophilic detoxification pathways in largemouth bass (*Micropterus salmoides*) liver. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology; (137): 237-247.

Hughes P J, McLellan H, Lowes D A, Khan S Z, Bilmen J G, Tovey S C, Godfrey R E, Michell R H, Kirk C J, Michelangeli F. 2000. Estrogenic alkylphenols induce cell death by inhibiting testis endoplasmic reticulum Ca2+ pumps. BioChemical and Biophysical Research Communications; (277): 568-574.

Hylland K. The use of juvenile atlantic cod to monitor environmental estrogens. 2000. Proceedings of the 6th international symposium on the Reproduction physiology of fish;366.

Hylland K, Haux C. 1997. Effects of environmental oestrogens on marine fish species. TRAC-Trends in Analytical Chemistry; (16): 606-612.

ICES. 2005. Spawning and life history information for North Atlantic Cod stockes. Brander, K. No. 274. Copenhagen, Denmark, ICES Cooperative Research Report.
Ref Type: Report

Inaguma S, Takahashi S, Imaida K, Suzuki S, Shirai T. 2004. p-nonylphenol pretreatment during the late neonatal period has no effect on 3,2 '-dimethyl-4-aminobiphenyl-induced prostate carcinogenesis in male F344 rats. Cancer Letters; (212): 159-166.

Ingebrigtsen K, Hektoen H, Andersson T, Bergman A, Brandt I. 1990. Species-specific accumulation of the polychlorinated biphenyl (PCB) 2,3,3',4,4'-pentachlorobiphenyl in fish brain - A comparison between cod (*Gadus morhua*) and rainbow-trout (*Oncorhynchus-mykiss*). Pharmacology & Toxicology; (67): 344-345.

Inoue K, Yoshie Y, Kondo S, Yoshimura Y, Nakazawa H. 2002. Determination of phenolic xenoestrogens in water by liquid chromatography with coulometric-array detection. Journal of Chromatography A; (946): 291-294.

Ioppolo-Armanios M I, Alexander R, Kagi R I. 1992. Identification and analysis of C_0-C_3 phenol in Australian crude oils. Organic Geochemistry; (18): 603-609.

Ioppolo-Armanios M I, Alexander R, Kagi R I. 1995. Geosynthesis of organic compounds. 1. Alkylphenols. Geochimica Et Cosmochimica ACTA; (59): 3017-3027.

Ishibashi H, Hirano M, Matsumura N, Watanabe N, Takao Y, Arizono K. 2006. Reproductive effects and bioconcentration of 4-nonylphenol in medaka fish (*Oryzias latipes*). Chemosphere; (65): 1019-1026.

Ishibashi H, Tachibana K, Tsuchimoto M, Soyano K, Tatarazako N, Matsumura N, Tomiyasu Y, Tominaga N, Arizono K. 2004. Effects of nonylphenol and phytoestrogen-enriched diet on plasma vitellogenin, steroid hormone, hepatic cytochrome P450 1A, and glutathione-S-transferase values in goldfish (*Carassius auratus*). Comparative Medicine; (54): 54-62.

Ishido M, Masuo Y, Oka S, Niki E, Morita M. 2004. Intracisternal administration of p-n-octylphenol into neonatal rats causes hyperactivity concomitantly with the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL)-positive cells in the mesencephalon where immunoreactivity for tyrosine hydroxylase is reduced by the chemical. Journal of Health Science; (50): 407-412.

Islinger M, Yuan H, Voelkl A, Braunbeck T. 2002. Measurement of vitellogenin gene expression by RT-PCR as a tool to identify endocrine disruption in Japanese medaka (*Oryzias latipes*). Biomarkers; (7): 80-93.

Isobe T, Nishiyama H, Nakashima A, Takada H. 2001. Distribution and behavior of nonylphenol, octylphenol and nonylphenol monoethoxylate in okyo metropolitan area: Their association with aquatic particles and sedimentary distributions. Environmental Science & Technology; (35): 1041-1049.

James R, Glen J B. 1980. Synthesis, biological evaluation, and preliminary structure-activity considerations of a series of alkylphenols as intravenous anesthetic agents. Journal of Medicinal Chemistry; (23): 1350-1357.

Jobling S, Sheahan D, Osborne J A, Matthiessen P, Sumpter J P. 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environmental Toxicology and Chemistry; (15): 194-202.

Jobling S, Sumpter J P. 1993. Detergent components in sewage effluent are weakly oestrogenic to fish: An in vitro study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Aquatic toxicology; (27): 361-372.

Jobling S, Tyler C R. 2003. Endocrine disruption in wild freshwater fish. Pure and Applied Chemistry; (75): 2219-2234.

Johnson A C, White C, Besien T J, Jurgens M D. 1998. The sorption potential of octylphenol, a xenobiotic oestrogen, to suspended and bed-sediments collected from industrial and rural reaches of three English rivers. Science of the Total Enviroment; (210): 271-282.

Jones S B, King L B, Sappington L C, Dwyer F J, Ellersieck M, Buckler D R. 1998. Effects of carbaryl, permethrin, 4-nonylphenol, and copper on muscarinic cholinergic receptors in brain of surrogate and listed fish species. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology; (120): 405-414.

Jonkers N, Laane R W P M, de Graaf C, De Voogt P. 2005a. Fate modeling of nonylphenol ethoxylates and their metabolites in the Dutch Scheldt and Rhine estuaries: validation with new field data. Estuarine Coastal and Shelf Science; (62): 141-160.

Jonkers N, Laane R W P M, De Voogt P. 2005b. Sources and fate of nonylphenol ethoxylates and their metabolites in the Dutch coastal zone of the North Sea. Marine chemistry; (96): 115-135.

Jung J H, Shim W J, Addison R F, Baek J M, Han C H. 2006. Protein and gene expression of VTG in response to 4-nonylphenol in rockfish (*Sebastes schlegeli*). Comparative Biochemistry and Physiology C-Toxicology & Pharmacology; (143): 162-170.

Jung J H, Sohn Y C, Lee J Y, Kim D J, Lee J S, Jeon J K, Han C H. 2003. Molecular cloning of vitellogenin cDNA in rockfish (*Sebastes schlegeli*) and effects of estrogenic substances on its gene expression. Fish Physiology and Biochemistry; (28): 419-420.

Jurgella G F, Marwah A, Malison J A, Peterson R, Barry T P. 2006. Effects of xenobiotics and steroids on renal and hepatic estrogen metabolism in lake trout. General and Comparative Endocrinology; (148): 273-281.

Kah O, Madigou T, Mazurais D, Le Drean G. 2000. Aspects of the central regulation of reproduction in teleost fish. *Proceedings of the 6th international symposium on the Reproduction physiology of fish*:27-34.

Kang I J, Yokota H, Oshima Y, Tsuruda Y, Hano T, Maeda M, Imada N, Tadokoro H, Honjo T. 2003. Effects of 4-nonylphenol on reproduction of Japanese medaka, *Oryzias latipes*. Environmental Toxicology and Chemistry; (22): 2438-2445.

Kannan K, Keith T L, Naylor C G, Staples C A, Snyder S A, Giesy J P. 2003. Nonylphenol and nonylphenol ethoxylates in fish, sediment, and water from the Kalamazoo River, Michigan. Archives of Environmental Contamination and Toxicology; (44): 77-82.

Kannan K, Kober J L, Kang Y S, Masunaga S, Nakanishi J, Ostaszewski A, Giesy J P. 2001. Polychlorinated naphthalenes, biphenyls, dibenzo-p-dioxins, and dibenzofurans as well as polycyclic aromatic hydrocarbons and alkylphenols in sediment from the Detroit and Rouge Rivers, Michigan, USA. Environmental Toxicology and Chemistry; (20): 1878-1889.

Kannan N, Yamashita N, Petrick G, Duinker J C. 1998. Polychlorinated biphenyls and nonylphenols in the Sea of japan. Environmental Science & Technology; (32): 1747-1753.

Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens W. 2003. The OECD program to validate the rat uterotrophic bioassay. Phase 2: Dose-response studies. Environmental Health Perspectives; (111): 1530-1549.

Karels A, Soimasuo M, Oikari A. 1999. Effects of pulp and paper mill effluents on reproduction, bile conjugates and liver MFO (mixed function oxygenase) activity in fish at Southern Lake Saimaa, Finland. Water Science and Technology; (40): 109-114.

Karels A A, Manning S, Brouwer T H, Brouwer M. 2003. Reproductive effects of estrogenic and antiestrogenic chemicals on sheepshead minnows (*Cyprinodon variegatus*). Environmental Toxicology and Chemistry; (22): 855-865.

Karlsen O, Holm J C, Kjesbu O S. 1995. Effects of periodic starvation on reproductive investment in 1st-time spawning Atlantic cod (*Gadus Morhua L.*). Aquaculture; (133): 159-170.

Karrow N A, Guo T L, Delclos K B, Newbold R R, Weis C, Germolec D R, White K L, Mccay J A. 2004. Nonylphenol alters the activity of splenic NK cells and the numbers of leukocyte subpopulations in Sprague-Dawley rats: a two-generation feeding study. Toxicology; (196): 237-245.

Kashiwada S, Ishikawa H, Miyamoto N, Ohnishi Y, Magara Y. 2002. Fish test for endocrine-disruption and estimation of water quality of Japanese rivers. Water Reasarch; (36): 2161-2166.

Katchamart S, Miranda C L, Henderson M C, Pereira C B, Buhler D R. 2002. Effect of xenoestrogen exposure on the expression of cytochrome P450 isoforms in rainbow trout liver. Environmental Toxicology and Chemistry; (21): 2445-2451.

Kavlock R J, Daston G P, DeRosa C, Fenner-Crisp P, Gray L E, Kaattari S, Lucier G, Luster M, Mac M J, Maczka C, Miller R, Moore J, Rolland R, Scott G, Sheehan D M, Sinks T, Tilson H A. 1996. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: A report of the U.S. EPA-sponsored workshop. Environmantal Health Perspectives; (104): 715-740.

Kawaguchi M, Inoue K, Yoshimura M, Sakui N, Okanouchi N, Ito R, Yoshimura Y, Nakazawa H. 2004. Trace analysis of phenolic xenoestrogens in water samples by stir bar sorptive extraction with in situ derivatization and thermal desorption-gas chromatography-mass spectrometry. Journal of Chromatography A; (1041): 19-26.

Kawahata H, Ohta H, Inoue M, Suzuki A. 2004. Endocrine disrupter nonylphenol and bisphenol A contamination in Okinawa and Ishigaki Islands, Japan - within coral reefs and adjacent river mouths. Chemosphere; (55): 1519-1527.

Kawana R, Strussmann C A, Hashimoto S. 2003. Effect of p-Nonylphenol on sperm motility in Japanese medaka (Oryzias latipes). Fish Physiology and Biochemistry; (28): 213-214.

Kazeto Y, Goto-Kazeto R, Place A R, Trant J M. 2003. Aromatase expression in zebrafish and channel catfish brains: changes in transcript abundance associated with the reproductive cycle and exposure to endocrine disrupting chemicals. Fish Physiology and Biochemistry; (28): 29-32.

Kazeto Y, Place A R, Trant J M. Effects of endocrine disrupting chemicals on the expression of CYP19 genes in zebrafish (*Danio rerio*) juveniles. Aquatic toxicology 2004; (69): 25-34.

Keen P L, Higgs D A, Hall K J, Ikonomou M. 2005. Effects of dietary exposure of 4-nonylphenol on growth and smoltification of juvenile coho salmon (*Oncorhynchus kisutch*). Science of the Total Environment; (349): 81-94.

Keith T L, Snyder S A, Naylor C G, Staples C A, Summer C, Kannan K, Giesy J P. 2001. Identification and quantitation of nonylphenol ethoxylates and nonylphenol in fish tissues from Michigan. Environmental Science & Technology; (35): 10-13.

Kelce W R, Wilson E M. 1997. Environmental antiandrogens: Developmental effects, molecular mechanisms, and clinical implications. Journal of Molecular Medicine-JJM; (75): 198-207.

Kelly S A, DiGiulio R T. 2000. Developmental toxicity of estrogenic alkylphenols in killifish (*Fundulus heteroclitus*). Environmental Toxicology and Chemistry; (19): 2564-2570.

Khan S Z, Kirk C J, Michelangeli F. 2003. Alkylphenol endocrine disrupters inhibit IP(3)-sensitive Ca(2+) channels. Biochem Biophys Res Commun; (310): 261-266.

Khim J S, Kannan K, Villeneuve D L, Koh C H, Giesy J P. 1999. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 1. Instrumental analysis. Environmental Science & Technology; (33): 4199-4205.

Kim I C, Lee Y M, Lee C, Kim H M, Oda S, Lee Y S, Mitani H, Lee J S. 2006a. Expression profiles of 4-nonylphenol-exposed medaka (*Oryzias latipes*) analyzed with a 3.4 K microarray. Marine Environmental Research; (62): S141-S146.

Kim S K, Kim B K, Shim J H, Gil J E, Yoon Y D, Kim J H. 2006b. Nonylphenol and octylphenol-induced apoptosis in human embryonic stem cells is related to fas-fas ligand pathway. Toxicological Sciences; (94): 310-321.

Kim S K, Lee H J, Yang H W, Kim H S, Yoon Y D. 2004. Prepubertal exposure to 4-tert-octylphenol induces apoptosis of testicular germ cells in adult rat. Archives of Andrology; (50): 427-441.

Kim Y S, Katase T, Horii Y, Yamashita N, Makino M, Uchiyama T, Fujimoto Y, Inoue T. 2005. Estrogen equivalent concentration of individual isomer-specific 4-nonylphenol in Ariake sea water, Japan. Marine pollution bulletin; (51): 850-856.

Kime D E. 1987. The steroids. In: Fundamentals on comparative vertebrate endocrinology. (Ed.Chester-Jones IP). New York: Plenum press,; Chester-Jones, Ingelton, Phillips: 3-56.

Kinnberg K, Korsgaard B, Bjerregaard P. 2003. Effects of octylphenol and 17 beta-estradiol on the gonads of guppies (*Poecilia reticulata*) exposed as adults via the water or as embryos via the mother. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology; (134): 45-55.

Kinnberg K, Korsgaard B, Bjerregaard P. 2000a. Concentration-dependent effects of nonylphenol on testis structure in adult platyfish *Xiphophorus maculatus*. Marine Environmental Research; (50): 169-173.

Kinnberg K, Korsgaard B, Bjerregaard P, Jespersen A. 2000b. Effects of nonylphenol and 17 beta-estradiol on vitellogenin synthesis and testis morphology in male platyfish *Xiphophorus maculatus*. Journal of Experimental Biology; (203): 171-181.

Kinnberg K, Toft G. 2003. Effects of estrogenic and antiandrogenic compounds on the testis structure of the adult guppy (*Poecilia reticulata*). Ecotoxicology and environmental safety; (54): 16-24.

Kirby M F, Allen Y T, Dyer R A, Feist S W, Katsiadaki I, Matthiessen P, Scott A P, Smith A, Stentiford G D, Thain J E, Thomas K V, Tolhurst L, Waldock M J. 2004. Surveys of plasma vitellogenin and intersex in male flounder (*Platichthys flesus*) as measures of endocrine disruption by estrogenic contamination in United Kingdom estuaries: Temporal trends, 1996 to 2001. Environmental Toxicology and Chemistry; (23): 748-758.

Kirk C J, Bottomley L, Minican N, Carpenter H, Shaw S, Kohli N, Winter M, Taylor E W, Waring R H, Michelangeli F, Harris R A. 2003. Environmental endocrine disrupters dysregulate estrogen metabolism and Ca2+ homeostasis in fish and mammals via receptor-independent mechanisms. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology; (135): 1-8.

Kjesbu O S, Holm J C. 1994. Oocyte recruitment in first-time spawning Atlantic cod (*Gadus-morhua*) in relation to feeding regime. Canadian Journal of Fisheries and Aquatic Sciences; (51): 1893-1898.

Kjesbu O S, Solemdal P, Bratland P, Fonn M. 1996. Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences; (53): 610-620.

Klein R, Bartel M, He X H, Muller J, Quack M. 2005. Is there a linkage between bioaccumulation and the effects of alkylphenols on male breams (Abramis brama)? Environmental Research; (98): 55-63.

Kleinkauf A, Scott A P, Stewart C, Simpson M G, Leah R T. 2004. Abnormally elevated VTG concentrations in flounder (*Platichthys flesus*) from the Mersey Estuary (UK)--a continuing problem. Ecotoxicology and environmental safety; (58): 356-364.

Kloas W, Lutz I, Einspanier R. 1999. Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals in vitro and in vivo. Science of the Total Enviroment; (225): 59-68.

Klopman G, Chakravarti S K. 2003. Structure-activity relationship study of a diverse set of estrogen receptor ligands (I) using MultiCASE expert system. Chemosphere; (51): 445-459.

Knorr S, Braunbeck T. 2002. Decline in reproductive success, sex reversal, and developmental alterations in Japanese medaka (*Oryzias latipes*) after continuous exposure to octylphenol. Ecotoxicology and environmental safety; (51): 187-196.

Knudsen F R, Arukwe A, Pottinger T G. 1998. The in vivo effect of combinations of octylphenol, butylbenzylphthalate and estradiol on liver estradiol receptor modulation and induction of zona radiata proteins in rainbow trout: no evidence of synergy. Environmental pollution; (103): 75-80.

Kobayashi K, Tamotsu S, Yasuda K, Oishi T. 2005. Vitellogenin-immunohistochemistry in the liver and the testis of the Medaka, *Oryzias latipes*, exposed to 17 beta-estradiol and p-nonylphenol. Zoological Science; (22): 453-461.

Koh C H, Khim J S, Villeneuve D L, Kannan K, Giesy J P. 2006. Characterization of trace organic contaminants in marine sediment from Yeongil Bay, Korea: 1. Instrumental analyses. Environmental pollution; (142): 39-47.

Kojima M, Tsunoi S, Tanaka M. 2003. Determination of 4-alkylphenols by novel derivatization and gas chromatography-mass spectrometry. Journal of Chromatography A; (984): 237-243.

Kolpin D W, Furlong E T, Meyer M T, Thurman E M, Zaugg S D, Barber L B, Buxton H T. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. Environmental Science & Technology; (36): 1202-1211.

Korsgaard B, Pedersen K L. 1998. Vitellogenin in *Zoarces viviparus*: Purification, quantification by ELISA and induction by estradiol-17 beta and 4-nonylphenol. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology; (120): 159-166.

Krasowski M D, Jenkins A, Flood P, Kung A Y, Hopfinger A J, Harrison N L. 2001a. General anesthetic potencies of a series of propofol analogs correlate with potency for potentiation of gamma-aminobutyric acid (GABA) current at the GABA(A) receptor but not with lipid solubility. Journal of Pharmacology and Experimental Therapeutics; (297): 338-351.

Krasowski M D, Nishikawa K, Nikolaeva N, Lin A, Harrison N L. 2001b. Methionine 286 in transmembrane domain 3 of the GABA(A) receptor beta subunit controls a binding cavity for propofol and other alkylphenol general anesthetics. Neuropharmacology; (41): 952-964.

Kretschmer X C, Baldwin W S. 2005. CAR and PXR: Xenosensors of endocrine disrupters? Chemico-Biological Interactions; (155): 111-128.

Krol E S, Bolton J L. 1997. Oxidation of 4-alkylphenols and catechols by tyrosinase: Ortho-substituents alter the mechanism of quinoid formation. Chemico-Biological Interactions; (104): 11-27.

Kuch H M, Ballschmiter K. 2001. Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range. Environmental Science & Technology; (35): 3201-3206.

Kudo C, Wada K, Masuda T, Yonemura T, Shibuya A, Fujimoto Y, Nakajima A, Niwa H, Kamisaki Y. 2004. Nonylphenol induces the death of neural stem cells due to activation of the caspase cascade and regulation of the cell cycle. Journal of Neurochemistry; (88): 1416-1423.

Kuiper G G J M, Lemmen J G, Carlsson B, Corton J C, Safe S H, Van der Saag P T, van der Burg P, Gustafsson J A. 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology; (139): 4252-4263.

Kullman S W, Kashiwada S, Hinton D E. 2004. Analysis of medaka cytochrome P450 3A homotropic and heterotropic cooperativity. Marine Environmental Research; (58): 469-473.

Kume G, Horiguchi T, Goto A, Isobe T, Serizawa S, Shiraishi H, Morita M. 2006. Lethal effects of nonylphenol on fertilized eggs and larvae of marbled sole Pleuronectes yokohamae. Fisheries Science; (72): 217-219.

Kvestak R, Ahel M. 1994. Occurrence of toxic metabolites from nonionic surfactants in the Krka river Estuary. Ecotoxicology and environmental safety; (25): 25-34.

Kwak H I, Bae M O, Lee M H, Lee Y S, Lee B J, Kang K S, Chae C H, Sung H J, Shin J S, Kim J H, Mar W C, Sheen Y Y, Cho M H. 2001. Effects of nonylphenol, bisphenol A, and their mixture on the viviparous swordtail fish (Xiphophorus helleri). Environmental Toxicology and Chemistry; (20): 787-795.

Labadie P, Budzinski H. 2006. Alteration of steroid hormone balance in juvenile turbot (*Psetta maxima*) exposed to nonylphenol, bisphenol A, tetrabromodiphenyl ether 47, diallylphthalate, oil, and oil spiked with alkylphenols. Archives of Environmental Contamination and Toxicology; (50): 552-561.

Lahnsteiner F, Berger B, Grubinger F, Weismann T. 2005. The effect of 4-nonylphenol on semen quality, viability of gametes, fertilization success, and embryo and larvae survival in rainbow trout (Oncorhynchus mykiss). Aquatic toxicology; (71): 297-306.

Lalah J O, Schramm K W, Henkelmann B, Lenoir D, Behechti A, Gunther K, Kettrup A. 2003. The dissipation, distribution and fate of a branched C-14- nonylphenol isomer in lake water/sediment systems. Environmental pollution; (122): 195-203.

Lamche G, BurkhardtHolm P. 2000. Nonylphenol provokes a vesiculation of the Golgi apparatus in three fish epidermis cultures. Ecotoxicology and environmental safety; (47): 137-148.

Lanigan R S, Yamarik T A. 2002. Final report on the safety assessment of BHT. International Journal of Toxicology; (21): 19-94.

Larkin P, Sabo-Attwood T, Kelso J, Denslow N D. 2002. Gene expression analysis of largemouth bass exposed to estradiol, nonylphenol, and p,p '-DDE. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology; (133): 543-557.

Larsen B K, Bjornstad A, Sundt R C, Taban I C, Pampanin D M, Andersen O K. 2006. Comparison of protein expression in plasma from nonylphenol and bisphenol A-exposed Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) by use of SELDI-TOF. Aquatic toxicology; (78): S25-S33.

Larsson D G J, Hallman H, Forlin L. 2000. More male fish embryos near a pulp mill. Environmental Toxicology and Chemistry; (19): 2911-2917.

Laurenzana E M, Balasubramanian G, Weis C, Blaydes B, Newbold R R, Delclos K B. 2002a. Effect of nonylphenol on serum testosterone levels and testicular steroidogenic enzyme activity in neonatal, pubertal, and adult rats. Chemico-Biological Interactions; (139): 23-41.

Laurenzana E M, Weis C C, Bryant C W, Newbold R, Delclos K B. 2002b. Effect of dietary administration of genistein, nonylphenol or ethinyl estradiol on hepatic testosterone metabolism, cytochrome P-450 enzymes, and estrogen receptor alpha expression. Food and Chemical Toxicology; (40): 53-63.

Lech J J, Lewis S K, Ren L. 1996. *In Vivo* estrogenic activity of nonylphenol in rainbow trout. Fundam Appl Toxicol; (30): 229-232.

Lee C, Na J G, Lee K C, Park K. 2002. Choriogenin mRNA induction in male medaka, Oryzias latipes as a biomarker of endocrine disruption. Aquatic toxicology; (61): 233-241.

Lee H B. 1999. Review of analytical methods for the determination of nonylphenol and related compounds in environmental samples. Water Quality Research Jouenal of Canada; (34): 3-35.

Lee H J, Chattopadhyay S, Gong E Y, Ahn R S, Lee K. 2003a. Anti-Androgenic Effects of Bisphenol A and Nonylphenol on the Function of Androgen Receptor. Toxicol Sci.

Lee M H, Chung S W, Kang B Y, Park J, Lee C H, Hwang S Y, Kim T S. 2003b. Enhanced interleukin-4 production in CD4(+) T cells and elevated immunoglobulin E levels in antigen-primed mice by bisphenol A and nonylphenol, endocrine disruptors: involvement of nuclear factor-AT and Ca2+. Immunology; (109): 76-86.

Lee M H, Kim E, Kim T S. 2004. Exposure to 4-tert-octylphenol, an environmentally persistent alkylphenol, enhances interleukin-4 production in T cells via NF-AT activation. Toxicology and applied pharmacology; (197): 19-28.

Lee P C, Patra S C, Struve M. 1996. Modulation of rat hepatic CYP3A by nonylphenol. Xenobiotica; (26): 831-838.

Lee Y M, Chang S Y, Jung S O, Kweon H S, Lee J S. 2005a. Cloning and expression of alpha class glutathione S-transferase gene from the small hermaphroditic fish *Rivulus marmoratus* (Cyprinodontiformes, Rivulidae). Marine pollution bulletin; (51): 776-783.

Lee Y M, Jung S O, Seo J S, Yoon Y D, Lee J S. 2006a. Cloning of Ki-ras and Ha-ras cDNAs from the hermaphroditic fish *Rivulus marmoratus* (Cyprinodontiformes, Rivulidae) and its expression after exposure to 4-nonylphenol. Marine Environmental Research; (62): S136-S140.

Lee Y M, Seo J S, Kim I C, Yoon Y D, Lee J S. 2006b. Endocrine disrupting chemicals (bisphenol A, 4-nonylphenol, 4-tert-octylphenol) modulate expression of two distinct cytochrome P450 aromatase genes differently in gender types of the hermaphroditic fish *Rivulus marmoratus*. BioChemical and Biophysical Research Communications; (345): 894-903.

Lee Y M, Williams T D, Jung S O, Lee J S. 2005b. CDNA cloning and expression of a cytochrome P450 1A (CYP1A) gene from the hermaphroditic fish *Rivulus marmoratus*. Marine pollution bulletin; (51): 769-775.

Legler J, Zeinstra L M, Schuitemaker F, Lanser P H, Bogerd J, Brouwer A, Vethaak A D, De Voogt P, Murk A J, van der Burg B. 2002. Comparison of in vivo and in vitro reporter gene assays for short-term screening of estrogenic activity. Environmental Science & Technology; (36): 4410-4415.

Lewis S K, Lech J J. 1996. Uptake, disposition and persistence of nonylphenol from water in rainbow trout (*Oncorhynchus mykiss*). Xenobiotica; (26): 813-819.

Li D, Park J, Oh J R. 2001. Silyl derivatization of alkylphenols, chlorophenols, and bisphenol A for simultaneous GC/MS determination. Analytical chemistry; (73): 3089-3095.

Li D H, Dong M H, Shim W J, Hong S H, Oh J R, Yim U H, Jeung J H, Kanan N, Kim E S, Cho S R. 2005. Seasonal and spatial distribution of nonylphenol and IBP in Saemangeum Bay, Korea. Marine pollution bulletin; (51): 966-974.

Li H M, Taira T, Maita C, Ariga H, Iguchi-Ariga S M M. 2006. Protection against nonylphenol-induced cell death by DJ-1 in cultured Japanese medaka (*Oryzias latipes*) cells. Toxicology; (228): 229-238.

Li M H, Wang Z R. 2005. Effect of nonylphenol on plasma vitellogenin of male adult guppies (*Poecilia reticulata*). Environmental Toxicology; (20): 53-59.

Liber K, Gangl J A, Corry T D, Heinis L J, Stay F S. 1999. Lethality and bioaccumulation of 4-nonylphenol in bluegill sunfish in littoral enclosures. Environmental Toxicology and Chemistry; (18): 394-400.

Liu R, Zhou J L, Wilding A. 2004. Simultaneous determination of endocrine disrupting phenolic compounds and steroids in water by solid-phase extraction-gas chromatography-mass spectrometry. Journal of Chromatography A (1022):179-189.

Llompart M P, Lorenzo R A, Cela R, Pare J R J, Belanger J M R, Li K. 1997. Phenol and methylphenol isomers determination in soils by in- situ microwave-assisted extraction and derivatisation. Journal of Chromatography A; (757): 153-164.

Logan-Smith M J, East J M, Lee A G. 2002. Evidence for a global inhibitor-induced conformation change on the Ca2+-ATPase of sarcoplasmic reticulum from paired inhibitor studies. Biochemistry; (41): 2869-2875.

Loomis A K, Thomas P. 2000. Effects of estrogens and xenoestrogens on androgen production by Atlantic croaker testes in vitro: Evidence for a nongenomic action mediated by an estrogen membrane receptor. Biology of Reproduction; (62): 995-1004.

Louter A J H, Jones P A, Jorritsma J D, Vreuls J J, Brinkman U A T. 1997. Automated derivatization for on-line solid-phase extraction gas chromatography; Phenolic compounds. HRC-Journal of High Resolution Chromatography; (20): 363-368.

Luo Q, Ban M, Ando H, Kitahashi T, Bhandari R K, McCormick S D, Urano C. 2005. Distinct effects of 4-nonylphenol and estrogen-17 beta on expression of estrogen receptor alpha gene in smolting sockeye salmon. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology; (140): 123-130.

Lussier S M, Champlin D, LiVolsi J, Poucher S, Pruell R J. 2000. Acute toxicity of para-nonylphenol to saltwater animals. Environmental Toxicology and Chemistry; (19): 617-621.

Lye C M. 2000. Impact of oestrogenic substances from oil production at sea. Toxicology Letters; (112): 265-272.

Lye C M, Frid C L J, Gill M E, Cooper D W, Jones D M. 1999. Estrogenic alkylphenols in fish tissues, sediments, and waters from the UK Tyne and Tees estuaries. Environmental Science & Technology; (33): 1009-1014.

Lye C M, Frid C L J, Gill M E, McCormick D. 1997. Abnormalities in the reproductive health of flounder Platichthys flesus exposed to effluent from a sewage treatment works. Marine pollution bulletin; (34): 34-41.

Mackay D, Fraser A. 2000. Bioaccumulation of persistent organic chemicals: mechanisms and models. Environmental pollution; (110): 375-391.

Madigou T, LeGoff P, Salbert G, Cravedi J P, Segner H, Pakdel F, Valotaire Y. 2001. Effects of nonylphenol on estrogen receptor conformation, transcriptional activity and sexual reversion in rainbow trout (*Oncorhynchus mykiss*). Aquatic toxicology; (53): 173-186.

Madsen L L, Korsgaard B, Bjerregaard P. 2003. Estrogenic effects in flounder Platichthys flesus orally exposed to 4-tert-octylphenol. Aquatic toxicology; (64): 393-405.

Madsen L L, Korsgaard B, Bjerregaard P. 2006. Oral single pulse exposure of flounder *Platichthys flesus* to 4-tert-octylphenol: Relations between tissue levels and estrogenic effects. Marine Environmental Research; (61): 352-362.

Madsen L L, Korsgaard B, Bjerregaard P. 2002. 4-tert-octylphenol and 17 beta-estradiol applied by feeding to flounder *Platichthys flesus*: induction of vitellogenin and accumulation in tissues. Marine Environmental Research; (54): 729-733.

Madsen S S, Mathiesen A B, Korsgaard B. 1997. Effects of 17 β-estradiol and 4-nonylphenol on smoltification and vitellogenesis in atlantic salmon (*Salmo salar*). Fish Physiology and Biochemistry; (17): 303-312.

Madsen S S, Skovbolling S, Nielsen C, Korsgaard B. 2004. 17-[beta] Estradiol and 4-nonylphenol delay smolt development and downstream migration in Atlantic salmon, *Salmo salar*. Aquatic toxicology; (68): 109-120.

Maeng S, Jung Y, Choi E, Jeon J K, Kim S, Gen K, Sohn Y C. 2005. Expression of gonadotropin subunit genes following 4-nonylphenol exposure in masu salmon: Effects on transcript levels and promoter activities via estrogen receptor alpha. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology; (142): 383-390.

Magliulo L, Schreibman M P, Cepriano J, Ling J. 2002. Endocrine disruption caused by two common pollutants at "acceptable" concentrations. Neurotoxicology and Teratology; (24): 71-79.

Mangelsdorf D J, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans R M. 1995. The Nuclear Receptor Superfamily - the 2Nd Decade. Cell; (83): 835-839.

Mao I F, Lu Y Y, Chen M L. 2006. A simplified method for simultaneous quantitation of alkylphenols and alkylphenol ethoxylates in meat and fish using high-performance liquid chromatography with fluorescence detection. International Journal of Environmental Analytical Chemistry; (86): 713-722.

Maradonna F, Polzonetti V, Bandiera S M, Migliarini B, Carnevali O. 2004. Modulation of the hepatic CYP1A1 system in the marine fish *Gobius niger*, exposed to xenobiotic compounds. Environmental Science & Technology; (38): 6277-6282.

Marcomini A, Pojana G, Sfriso A, Alonso J M. 2000. Behavior of anionic and nonionic surfactants and their persistent metabolites in the Venice lagoon, Italy. Environmental Toxicology and Chemistry; (19): 2000-2007.

Marcomini A, Stelluto S, Pavoni B. 1989. Determination of Linear Alkylbenzenesulfonates and Alkylphenol Polyethoxylates in Commercial Products and Marine Waters by Reversed-Phase and Normal-Phase Hplc. International Journal of Environmental Analytical Chemistry; (35): 207-218.

Martin-Skilton R, Coughtrie M W H, Porte C. 2006a. Sulfotransferase activities towards xenobiotics and estradiol in two marine fish species (*Mullus barbatus* and *Lepidorhombus boscii*): Characterization and inhibition by endocrine disrupters. Aquatic toxicology; (79): 24-30.

Martin-Skilton R, Lavado R, Thibaut R, Minier C, Porte C. 2006b. Evidence of endocrine alteration in the red mullet, Mullus barbatus from the NW Mediterranean. Environmental pollution; (141): 60-68.

Martin-Skilton R, Thibaut R, Porte C. 2006c. Endocrine alteration in juvenile cod and turbot exposed to dispersed crude oil and alkylphenols. Aquatic toxicology; (78): S57-S64.

Masuyama H, Hiramatsu Y, Kunitomi M, Kudo T, MacDonald P N. 2000. Endocrine disrupting chemicals, phthalic acid and nonylphenol, activate Pregnane X receptor-mediated transcription. Mol Endocrinol; (14): 421-428.

Matthiessen P. 2003. Endocrine disruption in marine fish. Pure and Applied Chemistry; (75): 2249-2261.

Matthiessen P, Allen Y, Allchin CR, Feist SW, Kirby MF, Law RJ, Scott AP, Thain JE, Thomas KV. 1998. Oestrogenic endocrine disruption in flounder *(Platichthys flesus L.)* from United kingdom estruarine and marine waters. CEFAS. 107, 1-49. Lowestoft, CEFAS. Science series technical report.
Ref Type: Report

McCallum n K, Armstrong R J. 1973. The derivatisation of phenols for gas chromatography using electron capture detection. Journal of Chromatography A; (78): 303-307.

McCormick S D, O'Dea M F, Moeckel A M, Lerner D T, Bjornsson B T. 2005. Endocrine disruption of parr-smolt transformation and seawater tolerance of Atlantic salmon by 4-nonylphenol and 17 beta-estradiol. General and Comparative Endocrinology; (142): 280-288.

McEwen B. 2002. Estrogen actions throughout the brain. Recent Progress in Hormone Research, Vol 57; (57): 357-384.

McLeese D W, Zitko V, Sergeant D B, Burridge L, Metcalfe C D. 1981. Lethality and accumulation of alkylphenols in aquatic fauna. Chemosphere; (10): 723-730.

Melcangi R C, Panzica G C. 2006. Neuroactive steroids: Old players in a new game. Neuroscience; (138): 733-739.

Meldahl A C, Nithipatikom K, Lech J J. 1996. Metabolism of several ^{14}C-nonylphenol isomers by rainbow trout (*Oncorhynchus mykiss*) : *In vivo* and *in vitro* microsomal metabolites. Xenobiotica; (26): 1167-1180.

Menuet A, Pellegrini E, Anglade I, Blaise O, Laudet V, Kah O, Pakdel F. 2002. Molecular characterization of three estrogen receptor forms in zebrafish: Binding characteristics, transactivation properties, and tissue distributions. Biology of Reproduction; (66): 1881-1892.

Meucci V, Arukwe A. 2006b. The xenoestrogen 4-nonylphenol modulates hepatic gene expression of pregnane X receptor, aryl hydrocarbon receptor, CYP3A and CYP1A1 in juvenile Atlantic salmon (*Salmo salar*). Comparative Biochemistry and Physiology C-Toxicology & Pharmacology; (142): 142-150.

Meucci V, Arukwe A. 2006c. Transcriptional modulation of brain and hepatic estrogen receptor and P450arom isotypes in juvenile Atlantic salmon (*Salmo salar*) after waterbome exposure to the xenoestrogen, 4-nonylphenol. Aquatic toxicology; (77): 167-177.

Meucci V, Arukwe A. 2006a. The environmental estrogen, 4-nonylphenol modulates brain estrogen-receptor- and aromatase (CYP19) isoforms gene expression patterns in Atlantic salmon (*Salmo salar*). Marine Environmental Research; (62): S195-S199.

Michelangeli F, Orlowski S, Champeil P, East J M, Lee A G. 1990. Mechanism of inhibition of the (Ca^{2+} - Mg^{2+}) -ATPase by nonylphenol. Biochemistry; (29): 3091-3101.

Mikamo E, Harada S, Nishikawa J I, Nishihara T. 2003. Endocrine disruptors induce cytochrome P450 by affecting transcriptional regulation via pregnane X receptor. Toxicology and applied pharmacology; (193): 66-72.

MilesRichardson S R, Pierens S L, Nichols K M, Kramer V J, Snyder E M, Snyder S A, Render J A, Fitzgerald S D, Giesy J P. 1999. Effects of waterborne exposure to 4-nonylphenol and nonylphenol ethoxylate on secondary sex characteristics and gonads of fathead minnows (*Pimephales promelas*). Environmental Research; (80): S122-S137.

Mills L J, Chichester C. 2005. Review of evidence: Are endocrine-disrupting chemicals in the aquatic environment impacting fish populations? Science of the Total Environment; (343): 1-34.

Mills L J, Gutjahr-Gobell R E, Haebler R A, Horowitz D J, Jayaraman S, Pruell R J, McKinney R A, Gardner G R, 2001. Zaroogian G E. Effects of estrogenic (o,p '-DDT; octylphenol) and anti-androgenic (p,p '-DDE) chemicals on indicators of endocrine status in juvenile male summer flounder (*Paralichthys dentatus*). Aquatic toxicology; (52): 157-176.

Min J H, Lee S K, Gu M B. 2003. Effects of endocrine disrupting chemicals on distinct expression patterns of estrogen receptor, cytochrome P450 aromatase and p53 genes in Oryzias latipes liver. Journal of Biochemical and Molecular Toxicology; (17): 272-277.

Miracle A L, Ankley G T. 2005. Ecotoxicogenomics: linkages between exposure and effects in assessing risks of aquatic contaminants to fish. Reproductive Toxicology; (19): 321-326.

Miura T, Miura C, Ohta T, Nader M R, Todo T, Yamauchi K. 1999. Estradiol-17 beta stimulates the renewal of spermatogonial stem cells in males. BioChemical and Biophysical Research Communications; (264): 230-234.

Miura T, Miura C I. 2003. Molecular control mechanisms of fish spermatogenesis. Fish Physiology and Biochemistry; (28): 181-186.

Mochida K, Ohkubo N, Matsubara T, Ito K, Kakuno A, Fujii K. 2004. Effects of endocrine-disrupting chemicals on expression of ubiquitin C-terminal hydrolase mRNA in testis and brain of the Japanese common goby. Aquatic toxicology; (70): 123-136.

Moens L N, Van der Ven K, Van Remortel P, Del-Favero J, De Coen W M. 2006. Expression profiling of endocrine-disrupting compounds using a customized Cyprinus carpio cDNA microarray. Toxicological Sciences; (93): 298-310.

Moggs J G. 2005. Molecular responses to xenoestrogens: Mechanistic insights from toxicogenomics. Toxicology; (213): 177-193.

Mol H G, Sunarto S, Steijger O M. 2000. Determination of endocrine disruptors in water after derivatization with N-methyl-N-(tert.-butyldimethyltrifluoroacetamide) using gas chromatography with mass spectrometric detection. Journal of Chromatography A; (879): 97-112.

Moore A, Scott A P, Lower N, Katsiadaki I, Greenwood L. 2003. The effects of 4-nonylphenol and atrazine on Atlantic salmon (Salmo salar L) smolts. Aquaculture; (222): 253-263.

Mosconi G, Carnevali O, Franzoni M F, Cottone E, Lutz I, Kloas W, Yamamoto K, Kikuyama S, Polzonetti-Magni A M. 2002. Environmental Estrogens and reproductive biology in amphibians. General and Comparative Endocrinology; (126): 125-129.

Mueller G C, Kim U. 1978. Displacement of estradiol from estrogen receptors by simple alkylphenols. Endocrinology; (102): 1429-1435.

Murono E P, Derk R C, deLeon J H. 2001. Differential effects of octylphenol, 17 beta-estradiol, endosulfan, or bisphenol A on the steroidogenic competence of cultured adult rat Leydig cells. Reproductive Toxicology; (15): 551-560.

Murono E P, Derk R C, deLeon J H. 1999. Biphasic effects of octylphenol on testosterone biosynthesis by cultured Leydig cells from neonatal rats. Reproductive Toxicology; (13): 451-462.

Myers R A, Hutchings J A, Barrowman N J. 1996. Hypotheses for the decline of cod in the North Atlantic. Marine Ecology Progress Series; (138): 293-308.

Myhre JP, Baussant T, Sundt RC, Sanni S, Vabo R, Skjoldal HR, Klungsoyr J. Risk Assessment of reproductive effects of alkyl phenols in produced water on fish stocks in the North Sea. Report AM-2004/018. 2005. Stavanger, Norway, RF-Akvamiljø; Ref Type: Report

Naciff J M, Hess K A, Overmann G J, Torontali S M, Carr G J, Tiesman J P, Foertsch L M, Richardson B D, Martinez J E, Daston G P. 2005. Gene expression changes induced in the testis by transplacental exposure to high and low doses of 17 alpha-ethynyl estradiol, genistein, or bisphenol A. Toxicological Sciences; (86): 396-416.

Nagahama Y. 2000. Gonadal steroid hormones: major regulators of gonadal sex differentiation and gamatogenesis in fish. Proceedings of the 6th international symposium on the Reproduction physiology of fish;211-222.

Nagahama Y. 1994. Endocrine regulation of gametogenesis in fish. International Journal of Developmental Biology; (38): 217-229.

Nagahama Y. 1997. 17α,20β-Dihydroxy-4-pregnen-3-one, a maturation-inducing hormone in fish oocytes: Mechanisms of synthesis and action. Steroids; (62): 190-196.

Nagao T, Wada K, Marumo H, Yoshimura S, Ono H. 2001. Reproductive effects of nonylphenol in rats after gavage administration: a two-generation study. Reproductive Toxicology; (15): 293-315.

Nakajin S, Shinoda S, Ohno S, Nakazawa H, Makino T. 2001. Effect of phthalate esters and alkylphenols on steroidogenesis in human adrenocortical H295R cells. Environmental Toxicology and Pharmacology; (10): 103-110.

Nakamura M, Nagoya H, Hirai T. 2002. Nonylphenol induces complete feminization of the gonad in genetically controlled all-male amago salmon. Fisheries Science; (68): 1387-1389.

Nakamura S, Takino M, Daishima S. 2000. Determination of alkylphenols by GC/negative-ion chemical-ionization MS. Bunseki Kagaku; (49): 329-336.

Nakazawa K, Ohno Y. 2001. Modulation by estrogens and xenoestrogens of recombinant human neuronal nicotinic receptors. European Journal of Pharmacology; (430): 175-183.

Naylor C G, Mieure J P, Adams W J, Weeks J A, Castaldi F J, Ogle l D, Romano R R. 1992. Alkylphenol ethoxylates in the environment. JAOCS; (69): 695-703.

Neff J M. 2002. Bioaccumulation in marine organisms. Effect of contaminants from oil well produced water. ELSEVIER Science Ltd, Oxford, UK.

Negishi T, Kawasaki K, Suzaki S, Maeda H, Ishii Y, Kyuwa S, Kuroda Y, Yoshikawa Y. 2004. Behavioral alterations in response to fear-provoking stimuli and tranylcypromine induced by perinatal exposure to bisphenol a and nonylphenol in male rats. Environmental Health Perspectives; (112): 1159-1164.

Nelson,J.S., 1994. Fishes of the the world. Wiley, New York, US, 600 pp.

Nimrod A C, Benson W H. 1996b. Environmental estrogenic effcts of alkylphenol ethoxylates. Critical reviews in toxicology; (26): 335-364.

Nimrod A C, Benson W H. 1996a. Estrogenic responses to xenobiotics in channel catfish (*Ictalurus punctatus)*. Marine Environmental Research; (42): 155-160.

Nimrod A C, Benson W H. 1998. Reproduction and development of Japanese medaka following an early life stage exposure to xenoestrogens. Aquatic toxicology; (44): 141-156.

Nimrod A C, Benson W H. 1997. Xenobiotic interaction with and alteration of channel catfish estrogen receptor. Toxicology and applied pharmacology; (147): 381-390.

Noaksson E, Linderoth M, Bosveld A T C, Norrgren L, Zebuhr Y, Balk L. 2003. Endocrine disruption in brook trout (*Salvelinus fontinalis*) exposed to leachate from a public refuse dump. Science of the Total Environment; (305): 87-103.

Noaksson E, Linderoth M, Tjarnlund U, Balk L. 2005. Toxicological effects and reproductive impairments in female perch (*Perca fluviatilis*) exposed to leachate from Swedish refuse dumps. Aquatic toxicology; (75): 162-177.

Noaksson E, Tjarnlund U, Bosveld A T, Balk L. 2001. Evidence for endocrine disruption in perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) in a remote Swedish lake in the vicinity of a public refuse dump. Toxicology and applied pharmacology; (174): 160-176.

Norberg B, Brown C L, Halldorsson A, Stensland K, Bjornsson B T. 2004. Photoperiod regulates the timing of sexual maturation, spawning, sex steroid and thyroid hormone profiles in the Atlantic cod (*Gadus morhua*). Aquaculture; (229): 451-467.

Norrgren L, Blom A, Andersson P L, Borjeson H, Larsson D G J, Olsson P E. 1999. Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (*Salmo salar*). Aquatic Ecosystem Health and Management; (2): 311-317.

Obata T, Kubota S. 2000. Formation of hydroxy radicals by environmental estrogen-like chemicals in rat striatum. Neuroscience Letters; (296): 41-44.

Oberdorster E, Cheek A O. 2001. Gender benders at the beach: Endocrine disruption in marine and estuarine organisms. Environmental Toxicology and Chemistry; (20): 23-36.

Ohkubo N, Mochida K, Adachi S, Hara A, Hotta K, Nakamura Y, Matsubara T. 2003. Estrogenic activity in coastal areas around Japan evaluated by measuring male serum vitellogenins in Japanese common goby *Acanthogobius flavimanus*. Fisheries Science; (69): 1135-1145.

Okai Y, HigashiOkai K, Machida K, Nakamura H, Nakayama K, Fijita K, Tanaka T, Taniguchi M. 2000a. Protective effects of alpha-tocopherol and beta-carotene on para-nonylphenol-induced inhibition of cell growth, cellular respiration and glucose-induced proton extrusion of bacteria. FEMS Microbiology Letters; (187): 161-165.

Okai Y, HigashiOkai K, Machida K, Nakamura H, Nakayama K, Fujita K, Tanaka T, Otani S, Taniguchi M. 2000b. Protective effect of antioxidants against para-nonylphenol-induced inhibition of cell growth in Saccharomyces cerevisiae. FEMS Microbiology Letters; (185): 65-70.

OLF. Miljørapport 2004. Oljeindustriens Landsforening (OLF). 1-32. 2005. OLF, Postpoks 8065,4068 Stavanger, OLF.
Ref Type: Report

Oros D R, Jarman W M, Lowe T, David N, Lowe S, Davis J A. 2003. Surveillance for previously unmonitored organic contaminants in the San Francisco Estuary. Marine pollution bulletin; (46): 1102-1110.

Ortiz-Zarragoitia M, Cajaraville M P. 2005. Effects of selected xenoestrogens on liver peroxisomes, vitellogenin levels and spermatogenic cell proliferation in male zebrafish. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology; (141): 133-144.

OSPAR Commission. Nonylphenol/Nonylphenolethoxylates OSPAR Commission 2001 (2004 Update). 2004.
Ref Type: Report

Owens W, Koeter H B W M. 2003. The OECD program to validate the rat uterotrophic bioassay: An overview. Environmental Health Perspectives; (111): 1527-1529.

Pait A S, Nelson J O. 2003. Vitellogenesis in male *Fundulus heteroclitus* (killifish) induced by selected estrogenic compounds. Aquatic toxicology; (64): 331-342.

Palace V P, Evans R E, Wautier K, Baron C, Vandenbyllardt L, Vandersteen W, Kidd K. 2002. Induction of vitellogenin and histological effects in wild fathead minnows from a lake experimentally treated with the synthetic estrogen, ethynylestradiol. Water Quality Research Journal of Canada; (37): 637-650.

Panter G H, Hutchinson T H, Hurd K S, Bamforth J, Stanley R D, Duffell S, Hargreaves A, Gimeno S, Tyler C R. 2006. Development of chronic tests for endocrine active chemicals - Part 1. An extended fish early-life stage test for oestrogenic active chemicals in the fathead minnow (*Pimephales promelas*). Aquatic toxicology; (77): 279-290.

Paris F, Balaguer P, Terouanne B, Servant N, Lacoste C, Cravedi J P, Nicolas J C, Sultan C. 2002. Phenylphenols, biphenols, bisphenol-A and 4-tert-octylphenol exhibit alpha and beta estrogen activities and antiandrogen activity in reporter cell lines. Molecular and Cellular Endocrinology; (193): 43-49.

Pedersen K H, Pedersen S N, Pedersen K L, Korsgaard B, Bjerregaard P. 2003. Estrogenic effect of dietary 4-tert-octylphenol in rainbow trout (*Oncorhynchus mykiss*). Aquatic toxicology; (62): 295-303.

Pedersen R T, Hill E M. 2002. Tissue distribution and depuration of 4-tert-octylphenol residues in the cyprinid fish, *Scardinius erythrophthalmus*. Environmental Science & Technology; (36): 3275-3283.

Pedersen R T, Hill E M. 2000. Identification of novel metabolites of the xenoestrogen 4-tert-octylphenol in primary rat hepatocytes. Chemico-Biological Interactions; (128): 189-209.

Pedersen S N, Christiansen L B, Pedersen K L, Korsgaard B, Bjerregaard P. 1999. In vivo estrogenic activity of branched and linear alkylphenols in rainbow trout (*Oncorhynchus mykiss*). Science of the Total Enviroment; (233): 89-96.

Pedersen S N, Lindholst C. 1999. Quantification of the xenoestrogens 4-tert.-octylphenol and bisphenol A in water and in fish tissue based on microwave assisted extraction, solid-phase extraction and liquid chromatography-mass spectrometry. Journal of Chromatography A; (864): 17-24.

Petrovic M, Eljarrat E, de Alda M J L, Barcelo D. 2002a. Recent advances in the mass spectrometric analysis related to endocrine disrupting compounds in aquatic environmental samples. Journal of Chromatography A; (974): 23-51.

Petrovic M, Fernandez-Alba A R, Borrull F, Marce R M, Mazo E G, Barcelo D. 2002b. Occurrence and distribution of nonionic surfactants, their degradation products, and linear alkylbenzene sulfonates in coastal waters and sediments in Spain. Environmental Toxicology and Chemistry; (21): 37-46.

Pickford K A, Thomas-Jones R E, Wheals B, Tyler C R, Sumpter J P. 2003. Route of exposure affects the oestrogenic response of fish to 4-tert-nonylphenol. Aquatic toxicology; (65): 267-279.

Piferrer F. 2001. Endocrine sex control strategies for the feminization of teleost fish. Aquaculture; (197): 229-281.

Piva F, Martini L. 1998. Neurotransmitters and the control of hypophyseal gonadal functions: possible implications of endocrine disrupters. Pure and Applied Chemistry; (70): 1647-1656.

Platt T, Fuentes-Yaco C, Frank K T. 2003. Spring algal bloom and larval fish survival. Nature; (423): 398-399.

Rasmussen T H, Andreassen T K, Pedersen S N, Van der Ven L T M, Bjerregaard P, Korsgaard B. 2002. Effects of waterborne exposure of octylphenol and oestrogen on pregnant viviparous eelpout (*Zoarces viviparus*) and her embryos in ovario. Journal of Experimental Biology; (205): 3857-3876.

Rasmussen T H, Korsgaard B. 2004. Estrogenic octylphenol affects seminal fluid production and its biochemical composition of eelpout (*Zoarces viviparus*). Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology; (139): 1-10.

Rasmussen T H, TEH S J, Bjerregaard P, Korsgaard B. 2005. Anti-estrogen prevents xenoestrogen-induced testicular pathology of eelpout (Zoarces viviparus). Aquatic toxicology; (72): 177-194.

Remberger M, kaj L, Palm A, Sternbeck J, Kvernes E, Brorstrom-Lunden E. 2003. Screening tertiary butylphenols, methylphenols, and long-chain alkylphenols in the Swedish environment. IVL Rapport/report B1594, 1-96.. IVL Swedish Environmental Research Institute.
Ref Type: Report

Ren L, Lewis S K, Lech J J. 1996. Effects of estrogen and nonylphenol on the post-transcriptional regulation of vitellogenin gene expression. Chemico-Biological Interactions; (100): 67-76.

Renberg L. 1981. Gas chromatographic determination of phenolic compounds in water, as their pentafluorobenzoyl derivatives. Chemosphere; (10): 767-773.

Renner R. 1997. European bans on surfactant trigger transatlantic debate. Environmental Science & Technology; (31): A316-A320.

Rice C D, Roszell L E, Banes M M, Arnold R E. 1998. Effects of dietary PCBs and Nonylphenol on immune function and CYP1A activity in channel catfish, *Ictalurus punctatus*. Marine Environmental Research; (46): 351-354.

Rice C P, Schmitz-Afonso I, Loyo-Rosales J E, Link E, Thoma R, Fay L, Altfater D, Camp M J. 2003. Alkylphenol and alkylphenol-ethoxylates in carp, water, and sediment from the Cuyahoga River, Ohio. Environmental Science & Technology; (37): 3747-3754.

Rice J C. 2006. Every which way but up: The sad story of Atlantic groundfish, featuring Northern cod and North Sea cod. Bulletin of Marine Science; (78): 429-465.

Richburg J H, Johnson K J, Schoenfeld H A, Meistrich M L, Dix D J. 2002. Defining the cellular and molecular mechanisms of toxicant action in the testis. Toxicology Letters; (135): 167-183.

Riksheim H, Johnsen S. 1994. Determination of produced water contaminants in the marine environment. Society of Petroleum Engineers; (SPE-27151): 479-484.

Robinson C D, Brown E, Craft J A, Davies I M, Moffat C F. 2004. Effects of prolonged exposure to 4-tert-octylphenol on toxicity and indices of oestrogenic exposure in the sand goby (*Pomatoschistus minutus, Pallas*). Marine Environmental Research; (58): 19-38.

Røe T I, Johnsen S. 1996. Discharges of produced water to the North Sea. In: *Produced water 2: Environmental Issues and Mitigation Technologies*. (Eds.Reed M, Johnsen S). New York: Plenum Press,; 13-25.

Rolfes J, Andersson J T. 2001. Determination of alkylphenols after derivatization to ferrocenecarboxylic acid esters with gas chromatography-atomic emission detection. Analytical chemistry; (73): 3073-3082.

Ronnekleiv O K, Kelly M J. 2005. Diversity of ovarian steroid signaling in the hypothalamus. Frontiers in Neuroendocrinology; (26): 65-84.

Routledge E J, Sheahan D, Desbrow C, Brighty G C, Waldock M, Sumpter J P. 1998. Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach. Environmental Science & Technology; (32): 1559-1565.

Routledge E J, Sumpter J P. 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environmental Toxicology and Chemistry; (15): 241-248.

Routledge E J, Sumpter J P. 1997. Structural features of alkylphenolic chemicals associated with estrogenic activity. Journal of Biological Chemistry; (272): 3280-3288.

Rudel R A, Camann D E, Spengler J D, Korn L R, Brody J G. 2003. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Environmental Science & Technology (37):4543-4553,.

Ruehlmann D O, Steinert J R, Valverde M A, Jacob R, Mann G E. 1998. Environmental estrogenic pollutants induce acute vascular relaxation by inhibiting L-type Ca^{2+} cannels in smooth muscle cells. FASEB J; (12): 613-619.

Rye H, Reed M, Ekrol N. 1998. Sensitivity analysis and simulation of dispersed oil concentrations in the North Sea with the PROVANN model. Environmental Modeling & Software; (13): 421-429.

Rye H, Reed M, Slagstad D, Melbye A, Johnsen S. 1996. Modeling transport and dilution of produced water and the resulting uptake and biomagnification in marine biota. Society of Petroleum Engineers; (SPE 35911): 231-246.

Sabik H, Gagne F, Blaise C, Marcogliese D J, Jeannot R. 2003. Occurrence of alkylphenol polyethoxylates in the St. Lawrence River and their bioconcentration by mussels (Elliptio complanata). Chemosphere; (51): 349-356.

Sabo-Attwood T, Kroll K J, Denslow N D. 2004. Differential expression of largemouth bass (*Micropterus salmoides*) estrogen receptor isotypes alpha, beta, and gamma by estradiol. Molecular and Cellular Endocrinology; (218): 107-118.

Sadler B R, Cho S J, Ishaq K S, Chae K, Korach K S. 1998. Three-dimensional quantitative structure-activity relationship study of nonsteroidal estrogen receptor ligands using the comparative molecular field analysis cross-validated r(2)-guided region selection approach. Journal of Medicinal Chemistry; (41): 2261-2267.

Sak K, Everaus H. 2004. Nongenomic effects of 17 beta-estradiol-diversity of membrane binding sites. Journal of Steroid Biochemistry and Molecular Biology; (88): 323-335.

Sakai A. 2001. p-nonylphenol acts as a promoter in the BALB/3T3 cell transformation. Mutation Research-Genetic Toxicology and Environmental Mutagenesis; (493): 161-166.

Sakazaki H, Ueno H, Nakamuro K. 2002. Estrogen receptor alpha in mouse splenic lymphocytes: possible involvement in immunity. Toxicology Letters; (133): 221-229.

Schlenk D, Sapozhnikova Y, Irwin M A, Xie L T, Hwang W, Reddy S, Brownawell B J, Armstrong J, Kelly M, Montagne D E, Kolodziej E P, Sedlak D, Snyder S. 2005. In vivo bioassay-guided fractionation of marine sediment extracts from the Southern California Bight, USA, for estrogenic activity. Environmental Toxicology and Chemistry; (24): 2820-2826.

Schmieder P K, Aptula A O, Routledge E J, Sumpter J P, Mekenyan O G. 2000. Estrogenicity of alkylphenolic compounds: A 3-D structure- activity evaluation of gene activation. Environmental Toxicology and Chemistry; (19): 1727-1740.

Schmutzler C, Hamann I, Hofmann P J, Kovacs G, Stemmler L, Mentrup B, Schomburg L, Ambrugger P, Gruters A, Seidlova-Wuttke D, Jarry H, Wuttke W, Kohrle J. 2004. Endocrine active compounds affect thyrotropin and thyroid hormone levels in serum as well as endpoints of thyroid hormone action in liver, heart and kidney. Toxicology; (205): 95-102.

Scholz S, Kurauchi K, Kinoshita M, Oshima Y, Ozato K, Schirmer K, Wakamatsu Y. 2005. Analysis of estrogenic effects by quantification of green fluorescent protein in juvenile fish of a transgenic medaka. Environmental Toxicology and Chemistry; (24): 2553-2561.

Schultz T W, Sinks G D, Cronin M T. 2000. Effect of substituent size and dimensionality on potency of phenolic xenoestrogens evaluated with a recombinant yeast assay. Environmental Toxicology and Chemistry; (19): 2637-2642.

Schulz R W, Goos H J. 1999. Puberty in male fish: concepts and recent developments with special reference to the African catfish (Clarias gariepinus). Aquaculture; (177):5-12,.

Schulz R W, Miura T. 2002. Spermatogenesis and its endocrine regulation. Fish Physiology and Biochemistry; (26): 43-56.

Schumacher M, Weill-Engerer S, Liere P, Robert F, Franklin R J M, Garcia-Segura L M, Lambert J J, Mayo W, Melcangi R C, Parducz A, Suter U, Carelli C, Baulieu E E, Akwa Y. 2003. Steroid hormones and neurosteroids in normal and pathological aging of the nervous system. Progress in Neurobiology; (71): 3-29.

Schwaiger J, Mallow U, Ferling H, Knoerr S, Braunbeck T, Kalbfus W, Negele R D. 2002. How estrogenic is nonylphenol? A transgenerational study using rainbow trout (*Oncorhynchus mykiss*) as a test organism. Aquatic toxicology; (59): 177-189.

Schwaiger J, Spieser O H, Bauer C, Ferling H, Mallow U, Kalbfus W, Negele R D. 2000. Chronic toxicity of nonylphenol and ethinylestradiol: haematological and histopathological effects in juvenile Common carp (*Cyprinus carpio*). Aquatic toxicology; (51): 69-78.

Schweigert N, Zehnder A J B, Eggen R I L. 2001. Chemical properties of catechols and their molecular modes of toxic action in cells, from microorganisms to mammals. Environmental Microbiology; (3): 81-91.

Scippo M L, Argiris C, Van de Weerdt C, Muller M, Willemsen P, Martial J, Maghuin-Rogister G. 2004. Recombinant human estrogen, androgen and progesterone receptors for detection of potential endocrine disruptors. Analytical and Bioanalytical Chemistry; (378): 664-669.

Scott AP. 2000.17β-Oestradiol in male flatfish. Proceedings of the 6th international symposium on the Reproduction physiology of fish, 382.

Scott A P, Katsiadaki I, Witthames P R, Hylland K, Davies I M, McIntosh A D, Thain J. 2006. Vitellogenin in the blood plasma of male cod (*Gadus morhua*): A sign of oestrogenic endocrine disruption in the open sea? Marine Environmental Research; (61): 149-170.

Segner H, Caroll K, Fenske M, Janssen C R, Maack G, Pascoe D, Schafers C, Vandenbergh G F, Watts M, Wenzel A. 2003a. Identification of endocrine-disrupting effects in aquatic vertebrates and invertebrates: report from the European IDEA project. Ecotoxicology and environmental safety; (54): 302-314.

Segner H, Navas J M, Schafers C, Wenzel A. 2003b. Potencies of estrogenic compounds in in vitro screening assays and in life cycle tests with zebrafish in vivo. Ecotoxicology and environmental safety; (54): 315-322.

Seike N, Wanibuchi H, Morimura K, Wei M, Nishikawa T, Hirata K, Yoshikawa J, 2003. Fukushima S. Enhancement of lung carcinogenesis by nonylphenol and genistein in a F344 rat multiorgan carcinogenesis model. Cancer Letters; (192): 25-36.

Seki M, Yokota H, Maeda M, Tadokoro H, Kobayashi K. 2003a. Effects of 4-nonylphenol and 4-tert-octylphenol on sex differentiation and vitellogenin induction in medaka (*Oryzias latipes*). Environmental Toxicology and Chemistry; (22): 1507-1516.

Seki M, Yokota H, Matsubara H, Maeda M, Tadokoro H, Kobayashi K. 2003b. Fish full life-cycle testing for the weak estrogen 4-tert-pentylphenol on medaka (*Oryzias latipes*). (vol 22, pg 1487, 2003). Environmental Toxicology and Chemistry; (22): 2214.

Senthilkumaran B, Yoshikuni M, Nagahama Y. 2004. A shift in steroidogenesis occurring in ovarian follicles prior to oocyte maturation. Molecular and Cellular Endocrinology; (215): 11-18.

Seo J S, Lee Y M, Jung S O, Kim I C, Yoon Y D, Lee J S. 2006. Nonylphenol modulates expression of androgen receptor and estrogen receptor genes differently in gender types of the hermaphroditic fish *Rivulus marmoratus*. BioChemical and Biophysical Research Communications; (346): 213-223.

Servos M R. 1999. Review of the aquatic toxicity, estrogenic responses and bioaccumulation of alkylphenols and alkylphenol polyethoxylates. Water Quality Research Jouenal of Canada; (34): 123-177.

Shang D Y, Ikonomou M G, Macdonald R W. 1999a. Quantitative determination of nonylphenol polyethoxylate surfactants in marine sediment using normal-phase liquid chromatography-electrospray mass spectrometry. Journal of Chromatography A; (849): 467-482.

Shang D Y, Macdonald R W, Ikonomou M G. 1999b. Persistence of nonylphenol ethoxylate surfactants and their primary degradation products in sediments from near a municipal outfall in the strait of Georgia, British Columbia, Canada. Environmental Science & Technology; (33): 1366-1372.

Shao B, Hu J Y, Yang M, An W, Tao S. 2005. Nonylphenol and nonylphenol ethoxylates in river water, drinking water, and fish tissues in the area of Chongqing, China. Archives of Environmental Contamination and Toxicology; (48): 467-473.

Shelby M D, Newbold R R, Tully D B, Chae K, Davis V L. 1996. Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. Environmantal Health Perspectives; (104): 1296-1300.

Shioda T, Wakabayashi M. 2000a. Evaluation of reproductivity of medaka (*Oryzias latipes*) exposed to chemicals using a 2-week reproduction test. Water Science and Technology; (42): 53-60.

Shioda T, Wakabayashi M. 2000b. Effect of certain chemicals on the reproduction of medaka (*Oryzias latipes*). Chemosphere; (40): 239-243.

Shioji H, Tsunoi S, Kobayashi Y, Shigemori T, Ike M, Fujita M, Miyaji Y, Tanaka M. 2006. Estrogenic activity of branched 4-nonylphenol isomers examined by yeast two-hybrid assay. Journal of Health Science; (52): 132-141.

Shiraishi H, Carter D S, Hites R A. 1989. Identification and determination of *tert*-alkylphenols in carp from the Trenton channel of the Detroit river, Michigan, USA. Biomedical and environmental mass spectrometry; (18): 478-483.

Shiu W Y, Ma K C, Varhanickova D, Mackay D. 1994. Chlorophenols and alkylphenols: A rewiew and correlation of environmentally relevant properties and fate in an evaluative environment. Chemosphere; (29): 1155-1224.

Shrader E A, Henry T R, Greeley M S, Bradley B P. 2003. Proteomics in zebrafish exposed to endocrine disrupting chemicals. Ecotoxicology; (12): 485-488.

Silversand C, Hyllner S J, Haux C. 1993. Isolation, immunochemical detection, and observations of the instability of vitellogenin from 4 teleosts. Journal of Experimental Biology; (267): 587-597.

Singer M A. 1977. Interaction of Dibucaine and Propranolol with Phospholipid Bilayer Membranes - Effect of Alterations in Fatty Acyl Composition. Biochemical Pharmacology; (26): 51-57.

Smith M D, Hill E M. 2004. Uptake and metabolism of technical nonylphenol and its brominated analogues in the roach (*Rutilus rutilus*). Aquatic toxicology; (69): 359-370.

Snyder S A, Keith T L, Naylor C G, Staples C A, Giesy J P. 2001a. Identification and quantitation method for nonylphenol and lower oligomer nonylphenol ethoxylates in fish tissues. Environmental Toxicology and Chemistry; (20): 1870-1873.

Snyder S A, Keith T L, Pierens S L, Snyder E M, Giesy J P. 2001b. Bioconcentration of nonylphenol in fathead minnows (*Pimephales promelas*). Chemosphere; (44): 1697-1702.

Sohoni P, Sumpter J P. 1998. Several environmental oestrogens are also anti-androgens. Journal of Endocrinology; (158): 327-339.

Son H Y, Nishikawa A, Ikeda T, Furukawa F, Hirose M. 2000a. Lack of modification by environmental estrogenic compounds of thyroid carcinogenesis in ovariectomized rats pretreated with N-bis(2-hydroxypropyl)nitrosamine (DHPN). Japanese Journal of Cancer Research; (91): 966-972.

Son H Y, Nishikawa A, Ikeda T, Nakamura H, Miyauchi M, Imazawa T, Furukawa F, Hirose M. 2000b. Lack of modifying effects of environmental estrogenic compounds on the development of thyroid proliferative lesions in male rats pretreated with N-bis(2-hydroxypropyl)nitrosamine (DHPN). Japanese Journal of Cancer Research; (91): 899-905.

Soto A M, Justicia H, Wray J W, Sonnenschein C. 1991. p-Nonyl-phenol: An estrogenic xenobiotic released from "modified" polystyrene. Environmantal Health Perspectives; (92): 167-173.

Soto A M, Maffini M V, Schaeberle C M, Sonnenschein C. 2006. Strengths and weaknesses of in vitro assays for estrogenic and androgenic activity. Best Practice & Research Clinical Endocrinology & Metabolism; (20): 15-33.

Soto A M, Sonnenschein C, Chung K L, Fernandez M F, Olea N, Serrano F O. 1995. The E-SCREEN assay as a tool to identify estrogens: An update on estrogenic environmental pollutants. Environmantal Health Perspectives; (103): 113-122.

Soverchia L, Ruggeri B, Palermo F, Mosconi G, Cardinaletti G, Scortichini G, Gatti G, Polzonetti-Magni A M. 2005. Modulation of vitellogenin synthesis through estrogen receptor beta-1 in goldfish (*Carassius auratus*) juveniles exposed to 17-beta estradiol and nonylphenol. Toxicology and applied pharmacology; (209): 236-243.

Stachel B, Ehrhorn U, Heemken O P, Lepom P, Reincke H, Sawal G, Theobald N. 2003. Xenoestrogens in the River Elbe and its tributaries. Environmental pollution; (124): 497-507.

Staples C, Mihaich E, Carbone J, Woodburn K, Klecka G. 2004. A weight of evidence analysis of the chronic ecotoxicity of nonylphenol ethoxylates, nonylphenol ether carboxylates, and nonylphenol. Human and Ecological Risk Assessment; (10): 999-1017.

Stocco D M, Clark B J. 1996. Role of the steroidogenic acute regulatory protein (StAR) in steroidogenesis. Biochemical Pharmacology; (51): 197-205.

Sturve J, Hasselberg L, Falth H, Celander M, Forlin L. 2006. Effects of North Sea oil and alkylphenols on biomarker responses in juvenile Atlantic cod (*Gadus morhua*). Aquatic toxicology; (78): S73-S78.

Sundby S. 2000. Recruitment of Atlantic cod stocks in relation to temperature and advection of copepod populations. SARSIA; (85): 277-298.

Sundt RC, Baussant T. Uptake, tissue distribution and elimination C4-C7 Alkylated phenols in cod. Dietary vs. waterborne exposure. Report AM-2003/001, 1-27. 2003. Stavanger, Norway, RF-Akvamiljø.
Ref Type: Report

Tabata A, Kashiwada S, Ohnishi Y, Ishikawa H, Miyamoto N, Itoh M, Magara Y. 2001. Estrogenic influences of estradiol-17 beta, p-nonylphenol and bis-phenol-A on Japanese Medaka (*Oryzias latipes*) at detected environmental concentrations. Water Science and Technology; (43): 109-116.

Tabata A, Miyamoto N, Ohnishi Y, Itoh M, Yamada T, Kamei T, Magara Y. 2003. The effect of chlorination of estrogenic chemicals on the level of serum vitellogenin of Japanese medaka (*Oryzias latipes*). Water Sci Technol; (47): 51-57.

Tabira Y, Nakai M, Asai D, Yakabe Y, Tahara Y, Shinmyozu T, Noguchi M, Takatsuki M, Shimohigashi Y. 1999. Structural requirements of *para*-alkylphenols to bind to estrogen receptor. European Journal of Biochemistry; (262): 240-245.

Takahashi A, Higashitani T, Yakou Y, Saitou M, Tamamoto H, Tanaka H. 2003. Evaluating bioaccumulation of suspected endocrine disruptors into periphytons and benthos in the Tama River. Water Sci Technol; (47): 71-76.

Talorete T P N, Isoda H, Maekawa T. 2001. Alkylphenolic compounds and their effect on the injury rate, survival and acetylcholinesterase activity of the rat neuronal cell line PC12. Cytotechnology; (36): 163-169.

Tanaka J N, Grizzle J M. 2002. Effects of nonylphenol on the gonadal differentiation of the hermaphroditic fish, *Rivulus marmoratus*. Aquatic toxicology; (57): 117-125.

Tanaka T, Kohno H, Tanino M, Yanaida Y. 2002. Inhibitory effects of estrogenic compounds, 4-nonylphenol and genistein, on 7,12-dimethylbenz[a]anthracene-induced ovarian carcinogenesis in rats. Ecotoxicology and environmental safety; (52): 38-45.

Tanenbaum D M, Wang Y, Williams S P, Sigler P B. 1998. Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains. Proceedings of the National Academy of Sciences of the United States of America; (95): 5998-6003.

Tavazzi S, Benfenati E, Barcelo D. 2002. Accelerated solvent extraction then liquid chromatography coupled with mass Spectrometry for determination of 4-t-octyl phenol, 4-nonylphenols, and bisphenol ain fish liver. Chromatographia; (56): 463-467.

Taylor P, Larter S, Jones M, Dale J, Horstad I. 1997. The effect of oil-water-rock partitioning on the occurrence of alkylphenols in petroleum systems. Geochimica Et Cosmochimica ACTA; (61): 1899-1910.

Teles M, Gravato C, Pacheco M, Santos M A. 2004. Juvenile sea bass biotransformation, genotoxic and endocrine responses to [beta]-naphthoflavone, 4-nonylphenol and 17[beta]-estradiol individual and combined exposures. Chemosphere; (57): 147-158.

Terasaka S, Aita Y, Inoue A, Hayashi S, Nishigaki M, Aoyagi K, Sasaki H, Wada-Kiyama Y, Sakuma Y, Akaba S, Tanaka J, Sone H, Yonemoto J, Tanji M, Kiyama R. 2004. Using a customized DNA microarray for expression profiling of the estrogen-responsive genes to evaluate estrogen activity among natural estrogens and industrial chemicals. Environmental Health Perspectives; (112): 773-781.

Terasaka S, Inoue A, Tanji M, Kiyama R. 2006. Expression profiling of estrogen-responsive genes in breast cancer cells treated with alkylphenols, chlorinated phenols, parabens, or bis- and benzoylphenols for evaluation of estrogenic activity. Toxicology Letters; (163): 130-141.

Thibaut R, Debrauwer L, Cravedi J P. 2002. Tissue residues in rainbow trout dietary exposed to (3)h-4n- nonylphenol. Revue de Medecine Veterinaire; (153): 433-436.

Thibaut R, Debrauwer L, Rao D, Cravedi J P. 1998a. Characterization of biliary metabolites of 4-n-nonylphenol in rainbow trout (*Oncorhynchus mykiss*). Xenobiotica; (28): 745-757.

Thibaut R, Debrauwer L, Rao D, Cravedi J P. 1998b. Disposition and metabolism of [^{3}H]-4-n-Nonylphenol in rainbow trout. Marine Environmental Research; (46): 521-524.

Thibaut R, Porte C. 2004. Effects of endocrine disrupters on sex steroid synthesis and metabolism pathways in fish. Journal of Steroid Biochemistry and Molecular Biology; (92): 485-494.

Thomas K V, Balaam J, Hurst M R, Thain J E. 2004a. Bio-analytical and chemical characterisation of offshore produced water effluents for estrogen receptor (ER) agonists. Journal of Environmental Monitoring; (6): 593-598.

Thomas K V, Balaam J, Hurst M R, Thain J E. 2004b. Identification of in vitro estrogen and androgen receptor agonists in North Sea offshore produced water discharges. Environmental Toxicology and Chemistry; (23): 1156-1163.

Thomas P. 2000. Chemical interference with genomic and nongenomic actions of steroids in fishes: role of receptor binding. Marine Environmental Research; (50): 127-134.

Thomas P, Breckenridge-Miller D, Detweiler C. 1998. The teleost sperm membrane progestogen receptor: Interaction with xenoestrogens. Marine Environmental Research; (46): 163-166.

Thomas P, Dong J. 2006. Binding and activation of the seven-transmembrane estrogen receptor GPR30 by enviromental estrogens: A potential novel mechanism of endocrine disruption. Journal of Steroid Biochemistry and Molecular Biology; (102): 175-179.

Thomas P, Dressing G, Pang Y F, Berg H, Tubbs C, Benninghoff A, Doughty K. 2006. Progestin, estrogen and androgen G-protein coupled receptors in fish gonads. Steroids; (71): 310-316.

Thorpe K L, Hutchinson T H, Hetheridge M J, Scholze M, Sumpter J P, Tyler C R. 2001. Assessing the biological potency of binary mixtures of environmental estrogens using vitellogenin induction in juvenile rainbow trout (*Oncorhynchus mykiss*). Environmental Science & Technology; (35): 2476-2481.

Thorpe K L, Hutchinson T H, Hetheridge M J, Sumpter J P, Tyler C R. 2000. Development of an in vivo screening assay for estrogenic chemicals using juvenile rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry; (19): 2812-2820.

Toft G, Baatrup E. 2003. Altered sexual characteristics in guppies (*Poecilia reticulata*) exposed to 17 beta-estradiol and 4-tert-octylphenol during sexual development. Ecotoxicology and environmental safety; (56): 228-237.

Toft G, Baatrup E. 2001. Sexual characteristics are altered by 4-tert-octylphenol and 17 beta-estradiol in the adult male guppy (*Poecilia reticulata*). Ecotoxicology and environmental safety; (48): 76-84.

Tokumoto T, Tokumoto M, Nagahama Y. 2005. Induction and inhibition of oocyte maturation by EDCs in zebrafish. Reproductive Biology and Endocrinology; (3).

Tollefsen K E, Finne E F, Romstad R, Sandberg C. 2006. Effluents from oil production activities contain chemicals that interfere with normal function of intra- and extra-cellular estrogen binding proteins. Marine Environmental Research; (62): S191-S194.

Tollefsen K E, Ingebrigtsen K, Olsen A J, Zachariassen K E, Johnsen S. 1998. Acute toxicity and toxicokinetics of 4-heptylphenol in juvenile atlantic cod (*Gadus morhua L.*). Environmental Toxicology and Chemistry; (17): 740-746.

Tong W D, Fang H, Hong H X, Xie Q, Perkins R, Anson J, Sheehan D M. 2003. Regulatory application of SAR/QSAR for priority setting of endocrine disruptors: A perspective. Pure and Applied Chemistry; (75): 2375-2388.

Toran-Allerand C D. 2004. Minireview: A plethora of estrogen receptors in the brain: Where will it end? Endocrinology; (145): 1069-1074.

Tran D Q, Klotz D M, Ladlie B L, Ide C F, McLachlan J A, Arnold S F. 1996. Inhibition of progesterone receptor activity in yeast by synthetic chemicals. BioChemical and Biophysical Research Communications; (229): 518-523.

Trapani G, Altomare C, Sanna E, Biggio G, Liso G. 2000. Propofol in anesthesia. mechanism of action, structure-activity relationships, and drug delivery. Current Medicinal Chemistry; (7): 249-271.

Tremblay L, Van der Kraak G. 1998. Use of a series of homologous in vitro and in vivo assays to evaluate the endocrine modulating actions of beta-sitosterol in rainbow trout. Aquatic toxicology; (43): 149-162.

Tsuchiya H. 2001. Structure-specific membrane-fluidizing effect of propofol. Clinical and Experimental Pharmacology and Physiology; (28): 292-299.

Tsuda T, Suga K, Kaneda E, Ohsuga M. 2000a. Determination of 4-nonylphenol, nonylphenol monoethoxylate, nonylphenol diethoxylate and other alkylphenols in fish and shellfish by high-performance liquid chromatography with fluorescence detection. Journal of Chromatography B: Biomedical Sciences and Applications; (746): 305-309.

Tsuda T, Takino A, Kojima M, Harada H, Muraki K. 1999. Gas chromatographic mass spectrometric determination of 4-nonylphenols and 4-tert-octylphenol in biological samples. Journal of Chromatography B: Biomedical Sciences and Applications; (723): 273-279.

Tsuda T, Takino A, Kojima M, Harada H, Muraki K, Tsuji M. 2000b. 4-nonylphenols and 4-tert-octylphenol in water and fish from rivers flowing into Lake Biwa. Chemosphere; (41): 757-762.

Tsuda T, Takino A, Muraki K, Harada H, Kojima M. 2001. Evaluation of 4-nonylphenols and 4-tert-octylphenol contamination of fish in rivers by laboratory accumulation and excretion experiments. Water reasarch; (35): 1786-1792.

Tyl R W, Myers C B, Marr M C, Castillo N P, Seely J C, Sloan C S, Veselica M M, Joiner R L, Van Miller J P, Simon G S. 2006. Three-generation evaluation of dietary para-nonylphenol in CD (Sprague-Dawley) rats. Toxicological Sciences; (92): 295-310.

Tyler C R, Jobling S, Sumpter J P. 1998. Endocrine disruption in wildlife: A critical review of the evidence. Critical reviews in toxicology; (28): 319-361.

Tyler C R, Santos E M, Prat F. 2000. Unscrambling the egg - Cellular biochemical molecular and endocrine advances in oogenesis. Proceedings of the 6th international symposium on the Reproduction physiology of fish:273-280,.

Tyler C R, Sumpter J P. 1996. Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries (6):287-318,

Tyler C R, vanAerle R, Nilsen M V, Blackwell R, Maddix S, Nilsen B M, Berg K, Hutchinson T H, Goksoyr A. 2002. Monoclonal antibody enzyme-linked immunosorbent assay to quantify vitellogenin for studies on environmental estrogens in the rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry; (21): 47-54

Uguz C, Togan I, Eroglu Y, Tabak I, Zengin M, Iscan M. 2003. Alkylphenol concentrations in two rivers of Turkey. Environmental Toxicology and Pharmacology; (14): 87-88.

Vaccaro E, Meucci V, Intorre L, Soldani G, Di Bello D, Longo V, Gervasi P G, Pretti C. 2005. Effects of 17 beta-estradiol, 4-nonylphenol and PCB 126 on the estrogenic activity and phase 1 and 2 biotransformation enzymes in male sea bass (Dicentrarchus labrax). Aquatic toxicology; (75): 293-305.

van Baal J, Hassing G A, Goos H J Th, Schulz R W. 2000. Modulatoru effects of 4-nonylphenol on LH production in the african catfish pituitary. Proceedings of the 6th international symposium on the Reproduction physiology of fish;373.

Van den Belt K, Berckmans P, Vangenechten C, Verheyen R, Witters H. 2004. Comparative study on the in vitro in vivo estrogenic potencies of 17 beta-estradiol, estrone, 17 alpha-ethynylestradiol and nonylphenol. Aquatic toxicology; (66): 183-195.

Van den Belt K, Verheyen R, Witters H. 2003. Comparison of vitellogenin responses in zebrafish and rainbow trout following exposure to environmental estrogens. Ecotoxicology and environmental safety; (56): 271-281.

VanRy D A, Dachs J, Gigliotti C L, Brunciak P A, Nelson E D, Eisenreich S J. 2000. Atmospheric seasonal trends and environmental rate of alkylphenols in the lower Hudson River Estuary. Environmental Science & Technology; (34): 2410-2417.

Veith G D, Kiwus L M. 1977. An exhaustive steam-distillation and solvent-extraction unit for pesticides and industrial chemicals. Bulletin of Enviromental Contamination and Toxicology (17): 631-636,.

Vetillard A, Bailhache T. 2006. Effects of 4-n-nonylphenol and tamoxifen on salmon gonadotropin-releasing hormone, estrogen receptor, and vitellogenin gene expression in juvenile rainbow trout. Toxicological Sciences; (92): 537-544.

Villeneuve D L, Villalobos S A, Keith T L, Snyder E M, Fitzgerald S D, Giesy J P. 2002. Effects of waterborne exposure to 4-nonylphenol on plasma sex steroid and vitellogenin concentrations in sexually mature male carp (*Cyprinus carpio*). Chemosphere; (47): 15-28.

Wahlberg C, Renberg L, Wideqvist U. 1990. Determination of nonylphenol and nonylphenol ethoxylates as their pentafluorobenzoates in water, sewage-sludge and biota. Chemosphere; (20): 179-195.

Waldock M J, Thain J E. 1986. Environmental concentrations of 4-nonylphenol following dumping of anaerobically digested sewage: A preliminary study of occurrence and acute toxicity. ICES CM; (E:16): 1-9.

Walsh D E, Dockery P, Doolan C M. 2005. Estrogen receptor independent rapid non-genomic effects of environmental estrogens on [Ca2+]i in human breast cancer cells. Molecular and Cellular Endocrinology; (230): 23-30.

Wang J L, Liu C S, Lin K L, Chou C T, Hsieh C H, Chang C H, Chen W C, Liu S I, Hsu S S, Chang H T, Jan C R. 2005. Nonylphenol-induced Ca2+ elevation and Ca2+-independent cell death in human osteosarcoma cells. Toxicology Letters; (160): 76-83.

Wang X, Han X, Hou Y, Yao G, Wang Y. 2003. Effect of nonylphenol on apoptosis of Sertoli cells in vitro. Bulletin of Environmental Contamination and Toxicology; (70): 898-904.

Ward A J W, Duff A J, Currie S. 2006. The effects of the endocrine disrupter 4-nonylphenol on the behaviour of juvenile rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences; (63): 377-382.

Watanabe H, Iguchi T. 2006. Using ecotoxicogenomics to evaluate the impact of chemicals on aquatic organisms. Marine Biology; (149): 107-115.

Watanabe H, Suzuki A, Goto M, Lubahn D B, Handa H, Iguchi T. 2004. Tissue-specific estrogenic and non-estrogenic effects of a xenoestrogen, nonylphenol. Journal of Molecular Endocrinology; (33): 243-252.

Watson C S, Bulayeva N N, Wozniak A L, Finnerty C C. 2005. Signaling from the membrane via membrane estrogen receptor-alpha: Estrogens, xenoestrogens, and phytoestrogens. Steroids; (70): 364-371.

Weber L P, Hill R L, Jr., Janz D M. 2003. Developmental estrogenic exposure in zebrafish (*Danio rerio*): II. Histological evaluation of gametogenesis and organ toxicity. Aquatic toxicology; (63): 431-446.

Weber L P, Kiparissis Y, Hwang G S, Niimi A J, Janz D M, Metcalfe C D. 2002. Increased cellular apoptosis after chronic aqueous exposure to nonylphenol and quercetin in adult medaka (*Oryzias latipes*). Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology; (131): 51-59.

Weltzien F A, Andersson E, Andersen O, Shalchian-Tabrizi K, Norberg B. 2004. The brain-pituitary-gonad axis in male teleosts, with special emphasis on flatfish (*pleuronectiformes*). Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology; (137):447-477,.

Wenzel A, Bohmer W, Muller J, Rudel H, Schroter-Kermani C. 2004. Retrospective monitoring of alkylphenols and alkylphenol monoethoxylates in aquatic biota from 1985 to 2001: Results from the German Environmental Specimen Bank. Environmental Science & Technology; (38): 1654-1661.

Wheeler T F, Heim J R, LaTorre M R, Janes A B. 1997. Mass spectral characterization of p-nonylphenol isomers using high-resolution capillary GC-MS. Journal of Chromatographic Science; (35): 19-30.

White R, Jobling S, Hoare S A, Sumpter J P, Parker M G. 1994. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology; (135): 175-182.

Whitlock K E, Illing N, Brideau N J, Smith K M, Twomey S. 2006. Development of GnRH cells: Setting the stage for puberty. Molecular and Cellular Endocrinology 254:39-50,.

Wieland K, Jarre-Teichmann A, Horbowa K. 2000. Changes in the timing of spawning of Baltic cod: possible causes and implications for recruitment. ICES Journal of Marine Science; (57): 452-464.

Willey J B, Krone P H. 2001. Effects of endosulfan and nonylphenol on the primordial germ cell population in pre-larval zebrafish embryos. Aquatic toxicology; (54): 113-123.

Wozniak A L, Bulayeva N N, Watson C S. 2005. Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-alpha-mediated Ca2+ fluxes and prolactin release in GH3/B6 pituitary tumor cells. Environmental Health Perspectives; (113): 431-439.

Xiao X Y, McCalley D V, McEvoy J. 2001. Analysis of estrogens in river water and effluents using solid-phase extraction and gas chromatography-negative chemical ionisation mass spectrometry of the pentafluorobenzoyl derivatives. Journal of Chromatography A; (923): 195-204.

Xie Z Y, Lakaschus S, Ebinghaus R, Caba A, Ruck W. 2006. Atmospheric concentrations and air-sea exchanges of nonylphenol, tertiary octylphenol and nonylphenol monoethoxylate in the North Sea. Environmental pollution; (142): 170-180.

Yadetie F, Arukwe A, Goksoyr A, Male R. 1999. Induction of hepatic estrogen receptor in juvenile Atlantic salmon in vivo by the environmental estrogen, 4-nonylphenol. Science of the Total Enviroment; (233): 201-210.

Yadetie F, Male R. 2002. Effects of 4-nonylphenol on gene expression of pituitary hormones in juvenile Atlantic salmon (*Salmo salar*). Aquatic toxicology; (58): 113-129.

Yamaguchi A, Ishibashi H, Kohra S, Arizono K, Tominaga N. 2005. Short-term effects of endocrine-disrupting chemicals on the expression of estrogen-responsive genes in male medaka (*Oryzias latipes*). Aquatic toxicology; (72): 239-249.

Yanagihara N, Toyohira Y, Ueno S, Tsutsui M, Utsunomiya K, Liu M, Tanaka K. 2005. Stimulation of catecholamine synthesis by environmental estrogenic pollutants. Endocrinology; (146): 265-272.

Yang F X, Xu Y, Hui Y. 2006. Reproductive effects of prenatal exposure to nonylphenol on zebrafish (*Danio rerio*). Comparative Biochemistry and Physiology C-Toxicology & Pharmacology; (142): 77-84.

Yao G H, Hou Y Y. 2004. Nonylphenol induces thymocyte apoptosis through Fas/FasL pathway by mimicking estrogen in vivo. Environmental Toxicology and Pharmacology; (17): 19-27.

Yao G H, Hu Y L, Liang J F, Hou Y Y. 2005. Nonylphenol-induced thymocyte apoptosis is related to Fas/FasL pathway. Life Sciences; (77): 3306-3320.

Yao G H, Yang L S, Hu Y L, Liang J, Liang J F, Hou Y Y. 2006. Nonylphenol-induced thymocyte apoptosis involved caspase-3 activation and mitochondrial depolarization. Molecular Immunology; (43): 915-926.

Ying G G, Williams B, Kookana R. 2002. Environmental fate of alkylphenols and alkylphenol ethoxylates - a review. Environment International; (28): 215-226.

Yokota H, Abe T, Nakai M, Murakami H, Eto C, Yakabe Y. 2005. Effects of 4-tert-pentylphenol on the gene expression of P450 11 beta-hydroxylase in the gonad of medaka (Oryzias latipes). Aquatic toxicology; (71): 121-1H, Seki M, Maeda M, Oshima Y, Tadokoro H, Honjo T, Kobayashi K. 2001. Life-cycle toxicity of 4-nonylphenol to medaka (Oryzias latipes). Environmental Toxicology and Chemistry; (20): 2552-2560.

You H J, Choi C Y, Jeon Y J, Chung Y C, Kang S K, Hahm K S, Jeong H G. 2002. Suppression of inducible nitric oxide synthase and tumor necrosis factor-alpha expression by 4-nonylphenol in macrophages. BioChemical and Biophysical Research Communications; (294): 753-759.

Zacharewski T. 1997. In vitro bioassays for assessing estrogenic substances. Environmental Science & Technology; (31): 613-623.

Zaroogian G, Gardner G, Horowitz D B, Gutjahr-Gobell R, Haebler R, Mills L. 2001. Effect of 17 beta-estradiol, o,p '-DDT, octylphenol and p,p '-DDE on gonadal development and liver and kidney pathology in juvenile male summer flounder (*Paralichthys dentatus*). Aquatic toxicology; (54): 101-112.

Zhai J, Wang Z, Wang N, Ingersoll C. 2007. Histological alternation and vitellogenin induction in adult rare minnow (*Gobiocypris rarus*) after exposure to ethynylestradiol and nonylphenol. Chemosphere; (66): 488-495.

Zhang D P, Trudeau V L. 2006. Integration of membrane and nuclear estrogen receptor signaling. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology; (144): 306-315.

Zhao M, vanderWielen F, deVoogt P. 1999. Optimization of a matrix solid-phase dispersion method with sequential clean-up for the determination of alkylphenol ethoxylates in biological tissues. Journal of Chromatography A; (837): 129-138.

Zhou H, Zhang X J, Wang Z S. 2003. Research development of endocrine disrupting chemicals (EDCs) in water in China. Biomedical and Environmental Sciences; (16): 62-67.

Zhou T, JohnAlder H B, Weis J S, Weis P. 2000. Endocrine disruption: thyroid dysfunction in mummichogs (*Fundulus heteroclitus*) from a polluted habitat. Marine Environmental Research; (50): 393-397.

Zilberstein Y, Cohen Y, Rosenfeld H, Elizur A, Yaron Z. 2000. Nonylphenol as a xenoestrogen in tilapia: Hypophyseal effects. Proceedings of the 6th international symposium on the Reproduction physiology of fish;374.

Zoller U. 2006. Estuarine and coastal zone marine pollution by the nonionic alkylphenol ethoxylates endocrine disrupters: Is there a potential ecotoxicological problem? Environment International; (32): 269-272.