Endocrine Disruption in Fish

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To Jenny
for
25 years in the wild and beautiful places of our planet.
May they remain for future generations.

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Preface

The last half-century has shown a dramatic increase in the standard of living of millions of people in Europe, North America and many parts of the Third World. This has, in many ways been brought about by scientific and technical developments which were initiated in the 1940s and 1950s. Promises were then made that nuclear energy would provide electricity so cheap that it would not need metering, pesticides would end malnutrition throughout the world and plastics and other synthetic chemicals would revolutionise our manufacturing industry and our way of life. Whilst some of these promises have been fulfilled, the problems of long-term health risks to humans and wildlife arising from the use, production and disposal of these products were either unknown or deliberately understated. Nuclear power is rendered economically unviable when the real cost of decommissioning and storage of waste for several millenia is included, and the effects on health of both humans and wildlife of early pest eradication programmes with organochlorine pesticides were well documented in Rachel Carson's "Silent Spring". Evidence of the effects of aerosols and refrigerants on depletion of the ozone layer has led to restriction on the use of CFCs, and there is now increasing evidence of climate change resulting from our profligate use of fossil fuels.

In his forward to Rachel Carson's "Silent Spring", published in 1962, Lord Shackleton remarked that "We are dealing with dangerous things and it may be too late to wait for positive evidence of danger" and he quoted a distinguished British ecologist who thought that "Silent Spring overstated some things now but in ten years time or less these could be understatements". His remark applies equally well to endocrine disruptors, which over thirty years after Silent Spring was published is now recognised as one of its facets

which was greatly understated. Although the birds returned and sang again in spring after the mass eradication programmes were terminated, pesticides continued to be used in increasing quantities. Many of the original organochlorines were found to be persistent and were banned in North America and Western Europe in the 1970s and replaced by organophosphates and other chemicals. The final resting point of all of these chemicals is the aquatic ecosystem, and residues of organochlorine pesticides used half a century ago still find their way into tissues of the fishes of the North Sea and Great Lakes. In addition to the organochlorine pesticides, other chemicals such as PCBs remain in the sediments and are still being taken up into fish tissues decades after their production was discontinued.

"Silent Spring" dealt predominantly with the mass mortality of wildlife, but egg shell thinning and other symptoms were also indicative of endocrine disruption. Endocrinology was at that time, however, still in its infancy and research in comparative endocrinology hardly even begun. During the last decade there has been increasing evidence that many of the pesticides are not only toxic at high concentrations, but can cause disruption of the endocrine system at levels which do not cause *obvious* ill-health. Motor vehicle exhausts may not only be contributing to global warming and respiratory problems, but the polyaromatic hydrocarbons which they contain may also be causing disruption of our endocrine systems. Numerous other chemicals which underpin the present lifestyle of Western society may be having similar effects. Both humans and wildlife, whether they live in our cities or in the Arctic wilderness, are now exposed to a bewildering array of chemicals in the food they eat, the air they breathe and the water they drink. The obsession of the Western world with cancer has to a great extent obscured the evidence that many chemicals may, at very low levels, cause endocrine disruption in both humans and wildlife. Only during the last few years has such evidence finally attracted the attention of the public authorities and funding been given to research. Endocrine disruption is now a rapidly expanding field of research, and has shown that some of the chemicals, such as alkyl phenolics, which have for long been claimed to be inert are in fact capable of causing widespread disruption of the endocrine systems of both laboratory animals and wildlife. Few of the chemicals to which we, and our wildlife, are exposed have, however, been tested for such activity, and toxicity testing now needs to be redesigned to include the risk of possible endocrine disrupting activity in both humans and wildlife.

Current safety testing of chemicals concentrates on lethal or carcinogenic effects which have little relevance to the potential long-term low level effects which characterise endocrine disruption, yet alone transgenerational effects which may not become apparent until the second or even third generation attains adulthood. Recent workshops have shown that the design of such tests

requires a detailed knowledge of normal endocrine function and some clear indication of which endocrine parameters might be best used as the endpoints of such tests. Fish, which are at the greatest risk from pollution since their natural habitat is the final resting point of most anthropogenic chemicals, are increasingly seen as providing suitable data on pollution of the aquatic environment, a suitable laboratory test animal, and an early-warning of potential long-term health effects in humans. Although fish differ from mammals in the detail of their endocrine systems, the underlying basis is essentially the same, so that chemicals which affect fish have a very high probability of having similar harmful effects in humans. Studies of endocrine disruption in mammals rely overwhelmingly on experimentation with laboratory animals since mammalian wildlife is not readily amenable to examination of low-level effects and they are available only in limited numbers. Studies into the effects of environmental pollution on female fertility is difficult to demonstrate in either wild or captive mammals since the eggs produced are both inaccessible and small in number. For this reason, recent studies on humans have concentrated on males which produce readily available sperm in large numbers. While a decrease in human male fertility makes good headlines, and undoubtedly raises the profile of research into endocrine disruption, it distracts attention from possible effects on female fertility as well as the potential disruption of other components of the endocrine system of both humans and wildlife. Fish provide an excellent biomonitor for such effects, and are also in their own right of major importance as a source of both protein and employment for millions of humans.

The increased activity in this field is now attracting toxicologists and many others who have little or no background in endocrinology as well as endocrinologists who are not aware of much of the early literature on the effects of xenobiotic chemicals on the endocrine system, much of which was carried out long before it was termed "endocrine disruption". The aim of this volume is therefore to review the current literature on endocrine disruption in fish and to set it in the context of normal endocrine function. The boundaries of endocrinology are, however, ever-expanding and now encompass a wide range of intra- and intercellular control mechanisms. These are not discussed in detail, but the boundaries of the new field of endocrine disruption will inevitably expand in the same way to include disruption of behaviour, of the immune system and of embryonic development as a result of xenobiotic interference with the basic cellular control and signalling mechanisms. Popular concern has focused over-much on the environmental estrogens and their possible effects in decreasing human sperm counts. The data presented in this volume shows that endocrine disruption covers a very much wider range of tissues and chemicals than this and that many of these chemicals are

fundamental to the present high living standards of western civilisation. Whether they are really essential, could be used in smaller quantities, or replaced by alternatives, it is ultimately our choice as to whether we are prepared to risk the long-term consequences to our own and to future generations. Technological advances will inevitably lead to release of many new chemicals into the environment, but past mistakes should teach us that these need a thorough testing for long-term endocrine disruption before such release takes place in large quantities. We should, perhaps, remember DDT and pause long enough to think of possible unknown long-term effects, and subject such chemicals to rigorous testing before it is too late. While politicians may be reluctant to take unpopular measures, manufacturers may minimise the risks to gain short-term profits, and the popular press may distort or exaggerate the scientific facts, the scientific community has the responsibility of initiating the necessary research and obtaining the evidence of possible long-term risks and alerting the wider community to the consequences of its actions. If this volume helps to stimulate such research it will have served its purpose.

I am indebted to many scientific colleagues for their help in providing me with reprints or data, and particularly to Thomas Braunbeck, Richard Cody and William Davis, Sylvia Gimeno, Michelle Gray, Rudolf Hofer, Alice Hontela, K. P. Joy, Pekka Vuorinen, Judith Weis and Piet Wester for parting with valuable original photographs for reproduction, and to Glynn Woods for photographic assistance and for producing many of the line diagrams.

As befits such a volume, it was written in two distinct habitats. Part was written in a modern industrial university city of half a million inhabitants with access to all the "necessities" of modern civilisation. The other was written here on the remote west coast of Scotland where less than 500 people occupy around 1000 square kilometres of land, the village shop is 20 km away and the supermarket in the nearest town a further 70 km. Water runs untreated off the hillside, and heat is from the wood we cut ourselves, but survival for more than a week or two at any significant level of comfort requires the full infrastructure of modern civilisation - from the PVC pipe which brings water almost a kilometre from the stream, to electricity which both provides light and powers the computer on which these words are typed. The land and the climate made self sufficiency here a hard life and the last crofter left many a decade ago - it is naive to believe that we can return again to a simple chemical free world. Food supplies in both habitats are now dependent on the same world-wide network of transport, suppliers and packaging which inevitably increases the pollution of all our habitats. The land and air even here is not immune from the aerial deposition of pesticides, heavy metals and industrial chemicals carried on the winds from their sources thousands of kilometres away and which are the consequences of the system we choose to

provide the necessities of life. The apparently untouched mountains, glens and lochs may just gives us pause to ponder our priorities. It is ultimately an ethical decision as to whether the present high standard of living of a minority of the world's population can justify pollution of the entire planet with chemicals which may in the long-term decrease the fitness of all of its human and wildlife population to survive and to reproduce.

David Kime
"Druimnich"

Chapter 1

Introduction

1.1 Environmental pollution

It is over 30 years since the publication of Rachel Carson's "Silent Spring" (Carson, 1962) alerted the general population to the environmental degradation resulting from widespread use of pesticides in "eradication programmes". The effects of such extensive use of pesticides first became apparent in the appearance of dead fish and birds after spraying and later in the ill health of exposed human populations. These eradication programmes co-incided with the growth of the chemical industry after World War 2 and the belief that application of chemicals could completely destroy all crop pests. Only later did it become apparent that both harmful and beneficial insects were equally efficiently destroyed and that the indiscriminate use of pesticides could actually make the problem worse. The publication of Carson's book probably co-incided with the recognition by the pesticide industry itself that the problem was more complex than initially envisaged, and although the book brought attention to a wider audience of the effects, industry itself was changing its use of the chemicals. Carson's book well summarises the known effects of the pesticides in use at the time, but it concentrates on the short term effects and, although hinted at, there was little evidence at the time of possible long term effects of very low concentrations. It was not, however, only the pesticide industry that was expanding rapidly in the 1950s. The plastics and petrochemical industries were also in their infancy, and use of the motor car was still limited to the wealthier members of society. Packaging, in plastics and paper, was basic in comparison with today and the volume of paper production relatively low. These industries have contributed immensely to the increased standards of living of most of Western

Europe and North America, and such an increase is now spreading to the developing world. This increased productivity has led to a concomitant increase in industrial wastes, much of which is disposed of through the rivers and seas on which the industries are predominantly situated, or emitted as effluent into the atmosphere from which it will eventually fall onto land or water. In a consumer orientated society, a significant proportion of this waste is also generated in private homes as either solid or liquid household waste. Leachings from tip sites, gases from incinerators and effluents from sewage treatment works direct many of the chemical constituents into our waterways. The final resting place of all waste is ultimately the oceans and lakes and it is in such aquatic ecosystems that the first harmful effects of these wastes are likely to be found. The problem is now vastly more complex than that described by Carson, since not only are the wastes themselves much more diverse and arise from a multiplicity of sources, but their production is inextricably linked to the type of consumer orientated society which dominates the Western world and which is the dream of the developing world.

There is increasing recognition of the need for sustainable development and that continuing uncontrolled release of pollutants will inevitably destroy our civilisation. The appearance of ozone holes and the recognition that the greenhouse effect may be causing climate change have clearly shown that time is short and that action must be taken in a politically very short time scale. The link between CFCs and the ozone hole enabled action to be taken to phase out use of these chemicals. The link between global warming and increased carbon dioxide production resulting from the extravagant use of fossil fuels is now becoming clear, but the required action to limit energy use, particularly of private and commercial motor vehicles, is politically unpalatable and action is delayed by multiple vested interests.

Although concern is expressed about aquatic pollution after episodes of mortality among marine mammals and fish or attempts to dump at sea, there is as yet little popular knowledge about the full implications of what is perhaps the most intractable of problems. In a recent volume concerned with pollution of the North Sea (Salomons *et al.*, 1988) only 12 pages out of 700 were devoted to the effects of pollutants on fish (Bucke and Waterman, 1988) and even this was devoted entirely to diseases and cancers. These authors reviewed evidence that these diseases may be a result of pollution, but offered no causal relationship. It is, however, well known that stress acting via the adrenal (or interrenal in fish) secretion of corticosteroids causes immunosuppression leading to increased incidence of disease, and pollutants (as stressors) have been shown to increase cortisol secretion (Donaldson *et al.*, 1984). There is a vast scientific literature available which clearly shows that the aquatic ecosystem is heavily polluted and that fish accumulate toxic materials in their tissues. Such effects in the North Sea are well surveyed by contributors to the volume of Salomons *et al.* (1988). What has been less well

recognised is that at even relatively low levels, at which fish may show no signs of stress or disease, there are a multitude of long term effects which provide a warning both of ultimate destruction of economic fisheries and of hazards to human health. The high incidence of disease in fish and the incidences of high mortality among aquatic mammals suggests that such low level long-term effects may be much more common than hitherto suspected.

1.2 Endocrine disruption

The endocrine system is the chemical communication system of the body which regulates such activities as body fluid homeostasis, management of stress, and perhaps most importantly, reproduction and fertility which are necessary for propagation of the species. It comprises glands such as the testes, ovaries, pituitary and hypothalamus, thyroid and liver. It has become increasingly apparent in the scientific literature over the last two decades that the endocrine system is particularly sensitive to very low levels of pollutants. Any anthropogenic chemical which disrupts normal activity of the endocrine glands, and the production of the hormones which they secrete, can cause profound disruption to the exposed animal. Recent concern in the popular press has focused almost entirely on "environmental estrogens" - industrial chemicals which may mimic the natural estrogens. Such reports generally refer to such chemicals as "female hormones", ignoring the fact that they also play an essential natural role in male sexual differentiation and function. Reflecting a male dominated society these reports have centred almost entirely on the threat to male fertility and ignored possible effects on the female. It is, however, important to realise that the estrogen mimics (hormone mimetic compounds) that are the centre of these stories are only a minute proportion of the pollutants released into the environment. While the possible role of such mimics may play a role, the absence of a hormone can be even more important than that of the wrong hormone and any chemical which disrupts one part of the chain of hormone biosynthesis can have serious consequences.

The concern about the possible effects of environmental estrogens on human male fertility (Colborn and Clement, 1992) has drawn attention to the sensitivity of the vertebrate reproductive system to disruption by environmental pollutants and their ultimate effects on wildlife and human populations. The recent findings of the estrogenic activity of alkylphenols, biodegradation products of the alkoxyphenol detergents used widely in industry, exemplifies the problem particularly well (Colborn and Clement, 1992; Sumpter and Jobling, 1993; Jobling and Sumpter, 1994; Purdom *et al.*, 1994). There was little reason to expect harmful effects of these wastes and no reason to test them until sexual abnormalities were found in wildlife. Such effects were found only after these materials had been used for several

decades and released into the ecosystem in enormous quantities. Subsequent to the discovery of their harmful effects in aquatic animals, their possible harmful effects on human fertility is now being questioned (Sharpe and Skakkebaek, 1993). It is quite probable that many of the other industrial products in widespread use today may have equally harmful long-term effects. The environmental estrogen story has shown that the detection of aquatic pollution is not simply a matter of counting dead fish since long-term effects, particularly by endocrine disrupting chemicals, only become apparent after exposure to very low doses for a long period of time. Such effects may even appear only in the offspring of the exposed generation.

While environmental estrogens have recently captured the popular imagination, it is important to realise that scientific evidence has been available for over two decades to show that the reproductive function of fish could be affected by a wide range of common pollutants (see Kime, 1995). The recent publication of "Our Stolen Future" (Colborn *et al.*, 1996), surveys the history of endocrine disruptors for the non-scientific reader and provides an excellent sequel to Carson's "Silent Spring" (1962). Evidence supporting this suggestion of pollution induced endocrine dysfunction in fish is surveyed in the subsequent sections of this volume.

The definition of an "endocrine disruptor" is not perhaps as clear as may be first thought. Clearly a compound that mimics a natural hormone, like the environmental estrogens, can be considered an endocrine disruptor. Equally well, a pollutant that affects synthesis or metabolism of a hormone, its binding to cellular receptors or its expression in gene transcription can be considered as a disruptor. Pesticides in particular are designed to disrupt the nervous system, or other vital function, of the target pest and in larger non-target species such as fish will be equally toxic at high doses. Some components of the endocrine system may, however, be particularly sensitive so that although only a small part may be affected at low doses, this can induce a much amplified disruption of the endocrine system as a whole. This of course includes neurotoxic effects if these are localised in the parts of the brain which regulate the hypothalamus. Many of the organochlorines, such as DDT, are now known to be estrogenic, while the DDT metabolite DDE has anti-androgenic activity. These compounds are, however, also highly toxic in their own right and it is a matter of debate as to whether disruption of the endocrine system by such compounds is caused by their estrogenic or anti-androgenic effects, or by their cytotoxic or neurotoxic activity. Loss of male characteristics, or induction of female ones could in some cases be due to either activity. Although environmental estrogens have been represented as a threat to male fertility, it is important to realise that the behavioural characteristics of male mammals are imprinted on the brain by estrogens not androgens, and that exposure of the developing female brain to estrogens can result in masculinized behaviour (See Section 4.8). Furthermore testes of the

boar secrete large amounts of estrogens which are essential for the maintenance of its full libido and the secretions of its accessory sex glands (Joshi and Raeside, 1973; Schwarzenberger *et al.*, 1993).

The frontiers of endocrinology have expanded greatly and are no longer so clearly defined as in the past. The original definition of a hormone was a compound which was released from an endocrine gland into the blood by which it was transported to its target tissue where it exerted its effect. It is now clear that some hormones act within the gland in which they are produced, and may even act on neighbouring cells or within the same cell without passing into the circulatory system. This applies especially to some of the gonadal hormones (see Chapter 4), but the field is now wide enough to encompass a range of intracellular messengers. Endocrine disruption can therefore apply, not only to the classical range of endocrine function, but also to this extended area of intracellular communication. A wide range of developmental processes are under hormonal control, as is obvious in pubertal development, but it is often not clear whether abnormal development is a result of a direct toxic effect on cellular differentiation and division, or the result of disruption of the more subtle effects of hormone action. Many of these developmental changes are certainly encompassed by the more liberal interpretation of endocrine action which covers intracellular communication. This is especially important during embryonic development, which is not only affected by classical hormones, such as steroids and thyroid hormones of embryonic or maternal origin, but also by intracellular messengers which determine the development of the embryo. Disruption of embryonic development resulting in mortality or malformation of the fry may, therefore, also be considered to be the result of endocrine disruption interpreted in its broadest sense.

Reflecting the increase in scientific, popular and political concern about environmental endocrine disruptors over the last few years, a number of workshops have been held to discuss the actions necessary to assess the safety of anthropogenic chemicals and to define more clearly an endocrine disruptor. Kavlock *et al.* (1996) broadly defined an endocrine disruptor as **"an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes"**. Another, perhaps simpler, definition put forward at the Weybridge workshop in late 1996 (European Commission, 1996) defined **"An endocrine disruptor is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function"**, while **"A potential endocrine disruptor is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism"**. Such definitions cover a large number, but not all, of the effects described in this volume. In many cases the

boundaries are not so clearly defined as may be first thought from such definitions, and these will be discussed in the introductions to individual Chapters. The reader, however, is invited to refer to these definitions and consider for him/herself which of the different effects of pollutants can be described as endocrine disruption - and whether it does in fact really matter. Since many toxicologists are not familiar with endocrinology, Chapter 4 gives a simplified outline of the endocrine control of fish reproduction for the general reader unfamiliar with the subject, while the later Chapters contain a brief introduction to the endocrine aspects of the specific tissues concerned.

1.3 The need for biomonitors

The diversity of pollutants is such that it is no longer possible to make an analysis of water and determine the concentrations of all pollutants. Concentrations of specific pollutants may be analysed and shown to be extremely low, but no chemical test is available which can detect all pollutants. While pesticides are now relatively easily detected, industrial wastes, by their sheer diversity are particularly intractable and many of these effluents have never been tested for endocrine toxicity. A "biomonitor" can be defined in a variety of ways. At the most simplistic it treats the animal as a "black box" organism which when put into the test water exhibits a readily demonstrable effect if toxic chemicals are present. A simple toxicity test is the dose which kills 50% of the test animals (LD_{50}). A more complex test for lower pollutant levels might be a behavioural or feeding response of the organism, or changes in activity of one of its enzymes. The limitation of such a black-box approach is that it can be treated only as an "indicator" which may or may not be relevant to long term hazards. In many cases the organisms used are invertebrates which may have limited relevance to aquatic and terrestrial vertebrates. Where the approach totally fails is in predicting whole animal effects which become apparent only after long term low-level exposure. It is through such effects, particularly those relating to vertebrate fertility and development, that pollutants probably pose the greatest threat. These effects can only be examined by use of a long-term integrated approach on animals of comparable physiology. It is, perhaps, worth distinguishing between the two approaches by using the terms "bioindicator" (for a single parameter) and "biomonitor" (for an integrated approach). Fish are the group of animals most at threat from aquatic pollution and with their physiological similarity to mammals, including ourselves, together with their long-term exposure in natural habitats, provide a suitable biomonitor for environmental pollution.

It is important to realise that aquatic life is exposed to a bewildering array of anthropogenic chemicals and that these may interact synergistically with

each other to produce effects not seen on exposure to one chemical alone. Laboratory toxicity tests usually examine the effects of a single pollutant for a relatively short time under controlled conditions. Wildlife is typically exposed to lower levels of complex mixtures over a much longer time scale. As such, it can provide a valuable biomonitor of environmental pollution.

1.4 Why use fish as biomonitors?

1.4.1 Environmental indicators.

The first visual sign of a grossly polluted aquatic ecosystem is usually the appearance of dead fish, but although the presence of apparently healthy fish is commonly used as an indicator of good quality water, this can be misleading (Figure 1.1). Fish accumulate pollutants preferentially in their fatty tissues, such as liver and gonads, but the effects may become apparent only when concentrations in such tissues attain a threshold level after several years. This may result in damaged immune systems, tumours, respiratory problems etc. which shorten the lifespan and will decrease the population both by the premature mortality and by the decreased number of spawning seasons in which the fish is productive. It may also affect the reproductive system and decrease the fertility of otherwise apparently healthy fish. Larval survival and development, and the fertility of surviving larvae may also be affected. In rivers the resultant decline in population may become apparent only several years after a pollution incident when the low numbers, or even complete absence, of a particular year-class is recorded. In seas and lakes the effects of accumulation of pollutants over long periods may only become apparent decades later as fishing catches decline. Effects caused by pollutant build-up in sediment are not easily reversed. The sediments of the North Sea and the Great Lakes still contain high PCB and DDT levels decades after cessation of their production. These long-term effects are not readily linked to a particular industry or pollutant, but overall the fish population can act as a biomonitor for a sustainable environmental policy. Fish monitor not only the direct effects of pollutants. Pollution also affects vegetation and invertebrates, which are the food source for fish, and can cause starvation, poor condition or mortality. Large long-lived carnivorous fish, such as the cod or eels, can act as monitors of the whole chain from sediments, plants, invertebrates through to small fish both by loss of food resources and by bioconcentration of the pollutants through their food chain. Top predator species, like the large carnivorous fish, seabirds and marine mammals, are likely to be the first to show effects of pollution. Recent mortalities of seals and whales, together with their high body concentrations of pollutants is indicative of similar problems affecting fish populations, a fact that is often obscured by the emotive appeal of the

marine mammals. Unlike the marine mammals which are washed onto beaches in populous areas, other top predators such as cod die unnoticed at sea.

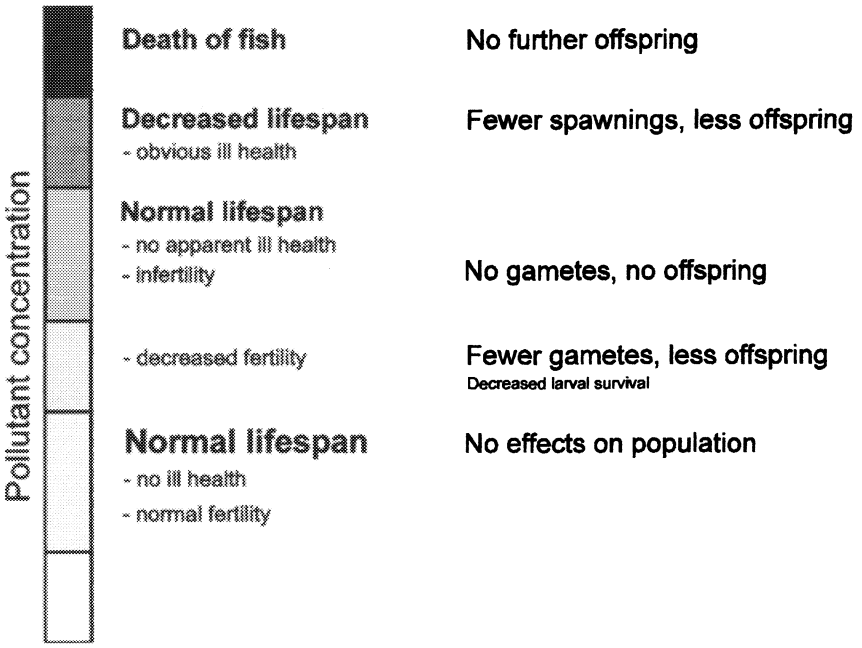


Figure 1.1. Effects of pollution on fish and their production of offspring

Fish are a major source of protein for human societies and fisheries are a major commercial activity. The decline in fisheries catches in the North Sea, Atlantic and other major areas has led to increasing political conflict as increasingly capital intensive fleets compete for the dwindling resources. Declining catches are generally attributed to overfishing, but it is probable that decreased fertility resulting from aquatic pollution may make a significant contribution to this decline in some areas. Clearly, the combination of increasingly efficient fishery equipment with an increasingly polluted habitat may be expected to result in a catastrophic decline in fish populations.

1.4.2 Long term effects.

Fish are particularly well suited as long-term biomonitors since they can be exposed to either a controlled quantity of pollutant in the diet or water in the laboratory, or to polluted ecosystems. They are unique amongst the vertebrates in that many species produce very large numbers of eggs (hundreds of thousands) during each reproductive cycle. Their fertility is

therefore very easily measured by the numbers and viability of their eggs and sperm, the fertilisation rate and the survival of their offspring. Mammals produce only a few oocytes during each reproductive cycle so that the effects of pollutants on female fertility is difficult to measure. While the motility of mammalian sperm following exposure of the male to a pollutant is readily determined, its fertilising efficiency and possible ill-effects transmitted to the resultant offspring can be determined only with difficulty. It is in the determination of such long-term effects of pollutants on fertility that fish provide the greatest advantage as biomonitors. Fish may produce several hundred thousand eggs during each reproductive cycle. The numbers of eggs produced, their fertilisation rate and the survival of the larvae through to sexual maturity may be followed with relative ease compared to mammals. The effects of exposure on males alone, or of their sperm may also be followed through to the offspring. Furthermore, the effects of pollution on the fertility of the offspring of exposed parents, and the possible genetic and fertility anomalies in second and third generations can be monitored. To date, there have been few such transgenerational studies, but the realisation that pollutants may have such long-term effects will undoubtedly lead to increasing numbers of such studies.

1.4.3 Economic relevance

Fish make a major contribution as a dietary protein source in many countries and in many coastal communities the economy is highly dependent upon the fishing industry and the supply of its products to inland areas. Such industries have expanded with the increased technological efficiency of their fishing fleets, but in many areas the economic base is now close to collapse as a result of dwindling fish catches. It is clear that for the long-term survival of such communities a sustainable fishery policy is essential and a pre-requisite of this is an understanding of the causes of the decline in fish populations and whether it is primarily due to overfishing or whether the underlying cause is the increased pollution of the aquatic environment over the last few decades. In the North Sea, for example, catches of cod have declined from 234,000 tonnes in 1983, to 98,000 tonnes in 1992. The survival of North Sea cod is now too low to give a recruitment that can maintain the stock, and less than 10% of the 1-year old fish live to become 3-year olds. In 1993, the spawning stock was estimated at 58,000 tonnes, which is the lowest ever registered and represents only one third of that which ICE'S Advisory Committee on Fishery Management (ACFM) recommends as the lowest acceptable spawning stock (Smedstad, 1994). Economic activity deriving from inland lakes, such as the Great Lakes, is similarly threatened. Many of the major rivers pass through large industrial areas and fish stocks downstream may be affected by pollution. In many cases rivers are used predominantly for sport fishing which

requires stocking with fish and the catch is strictly regulated. Such areas may be especially useful for biomonitoring. In some cases the rivers provide essential natural resources for aquaculture.

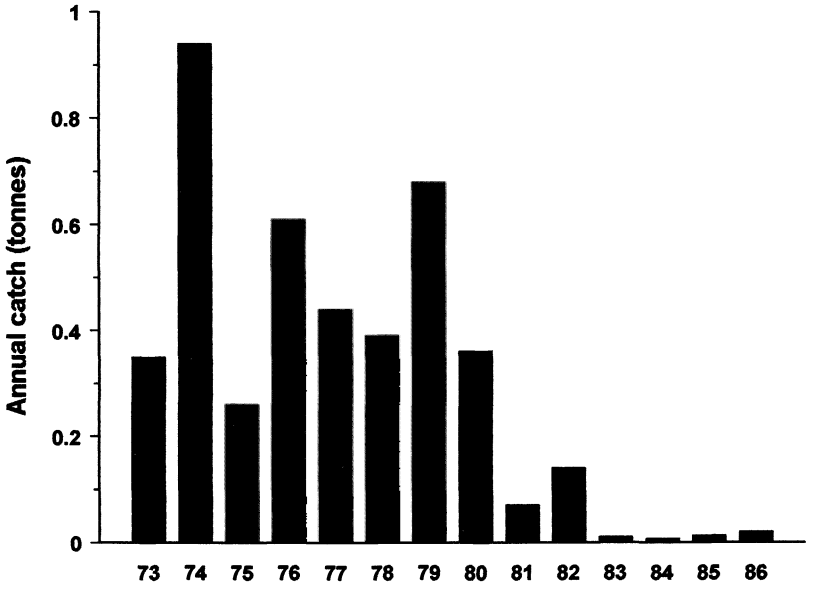


Figure 1.2. Decline in catches of glass eels in the River Yser, Belgium from 1973 to 1986. (Data from Denayer, 1994).

In Europe, the commercially important eel culture industry is based entirely on capture of glass eels from rivers since adults cannot be spawned in captivity and natural spawning takes place only after a marine migration to the Sargasso Sea. Glass eels in the River Yser in Belgium, however, have declined so rapidly that the catch in 1983-1986 was only 3% of that in the period 1973-1980 (Figure 1.2) (Bellepaire and Ollevier, 1987; Denayer, 1994). This, and similar declines in eel populations in other rivers threatens the whole basis of the eel culture industry. Furthermore there is a difficulty in obtaining male eels from rivers in Northern Europe and the suggestion has been made that this might be attributable to environmental factors of the rivers since sexual differentiation of the eel is very labile (Tesch, 1977; Beullens *et al.*, 1997). The possible effects of pollutants on the sex ratio of eels has not been examined, but lack of males on the spawning grounds would have serious repercussions for future river stocks of this species. The report of the European Inland Fisheries Advisory Commission, held in Rome 1994 noted that the yield of inland fisheries declined from 1989, but "the causes of

the decline, worsening environmental conditions, changing social and economic preferences, political upheavals or some combination of these remains to be established" (FAO, 1994).

1.4.4 Relevance to human health

Fish possess a similar physiological system to mammals, including humans, and many of the pollutant induced disturbances in fish are likely to have similar effects in humans. In particular, the reproductive endocrine system has a great deal of similarity within the vertebrates and effects found in fish may be readily applicable to other vertebrate systems. Carnivorous fish not only provide a suitable test model, they also provide a warning of the dangers to their consumer as the top predator. If bioaccumulation of pollutants in fish causes ill effects, that in fish-eating communities may be expected to extremely severe. Evidence is presented in a later section of the hazards of such accumulation by fish-eating human and animal communities (Chapter 11).

Human societies are exposed to a wide range of chemicals, in their water, air, household goods, and food. Pesticide and other residues remain in vegetables, meat and fish, and processed foods contain high levels of chemical additives. Many of these have not been rigorously tested for long-term effects - fish provide both a relevant model for such tests and warning of general environmental deterioration resulting from the general release of these chemicals.

The great expansion of output of the chemical industry, and widespread pesticide use began only in the 1950s. The generation born in this period absorbed these chemicals for 20-25 years before producing their first offspring in the 1970s. Any transgenerational effects of these chemicals will only become apparent as these offspring attain maturity. It is only now in the late 1990s that the effects of chemicals released 50 years ago would become apparent in the decreased fertility of the second generation. Although the study of Sharpe and Skakkebaek (1993) can only be considered a preliminary finding, and its validity has been questioned (Bromwich *et al.*, 1994) there are indications that human fertility may be decreasing, together with a concomitant increase in genital abnormalities. Fish provide a suitable model for monitoring such transgenerational effects both in the wild and in the laboratory. Any such effects found in fish might be expected to have much more serious consequences in humans in which the period of accumulation between generations is so much greater.

1.4.5 Fish as Biomonitors of Environmental Pollution

Our aquatic ecosystems have increasingly been the depository for pollutants for over half a century. Many of these pollutants remain in the sediments from which they will be absorbed into the aquatic organisms and hence up the food chain to large carnivorous fish and marine mammals. Some pollutants may either not be biodegraded to harmless materials, or may be degraded into more harmful products. The persistence of many such chemicals for decades after release shows that the problems of pollution would not disappear even if discharge stopped today. If the pollutants are harmful then, as with greenhouse gases and ozone depleting chemicals, steps must be taken to limit their release into the aquatic ecosystem.

The essential preliminary to the imposition of such limits is to determine whether present levels of a specific chemical are harmful and whether current levels of it's release are sustainable if life in seas and lakes is to be unaffected. This requires evidence on accumulation of pollutants by fish from the waters and sediments, whether the concentrations in their tissues are such that serious malfunction will occur and whether this will decrease either their viability and reproductive capacity or that of their offspring. An examination of such effects must progress stepwise (Figure 1.3). Firstly the pollutant must be identified and its site of action or localisation within the fish determined. Accumulation in muscle of edible fish has a clear implication for human health, while high levels in the gills will indicate respiratory problems. Lipid rich tissues such as the gonads and gametes are particularly liable to bioaccumulation of pollutants which will have adverse effects on reproductive potential. Accumulation in liver may affect reproduction, via vitellogenesis or altered metabolism of hormones, energy reserves and detoxification of other xenobiotics. At high concentrations pollutants will clearly cause fish mortality but at a lower level will result in stressed fish, increased prevalence of disease, general cellular damage or cancers, leading to premature mortality and a decreased number of spawning seasons. Such physical symptoms are readily visible and have been observed in polluted waters. At an even lower level, the fish may show no obvious signs of ill health, but the specific effects on the endocrine system may result in production of fewer gametes, decreased survival of larvae, and decreased ability to respond to stress. Furthermore, possible endocrine effects resulting from incorporation of pollutants into the eggs may become apparent only in the decreased fertility of the surviving offspring. These factors have, perhaps, the most serious implications for fish populations since they become apparent only when fish stocks show an "unexplained" catastrophic decline, by which time remedial action may be virtually impossible.

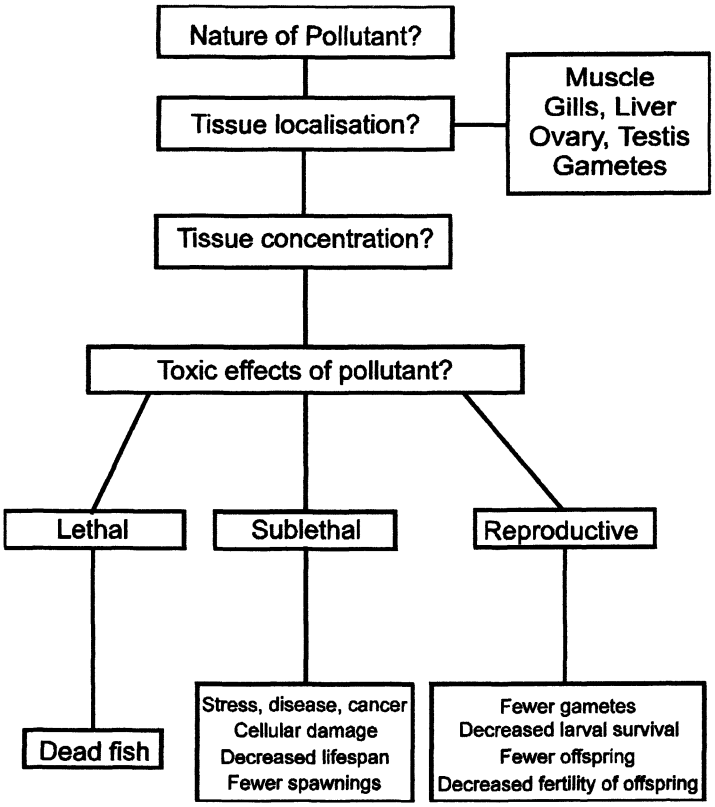


Figure 1.3. The levels for examination of the effects of pollutants on fish

In addition to the reproductive effects, pollutants can also modulate other parts of the endocrine system. Alterations to thyroid and interrenal activity can affect basal metabolism, energy utilisation and osmoregulation which can affect the long-term survival of the fish. While interrenal activity is normally elevated in conditions of stress, some pollutants can suppress this normal stress response and may decrease the ability of the exposed fish to cope with stressful situations.

These issues will be fully explored in later chapters of this volume.

Chapter 2

Sources of Aquatic Pollution

2.1 The origin of pollutants

Pollution of the aquatic environment arises from almost all current human activities, ranging from mining and processing of heavy metals, combustion of fossil fuels for transport, heating and electricity generation, pesticide use, food processing, production and disposal of plastics and a wide variety of miscellaneous industrial processes (Figure 2.1).

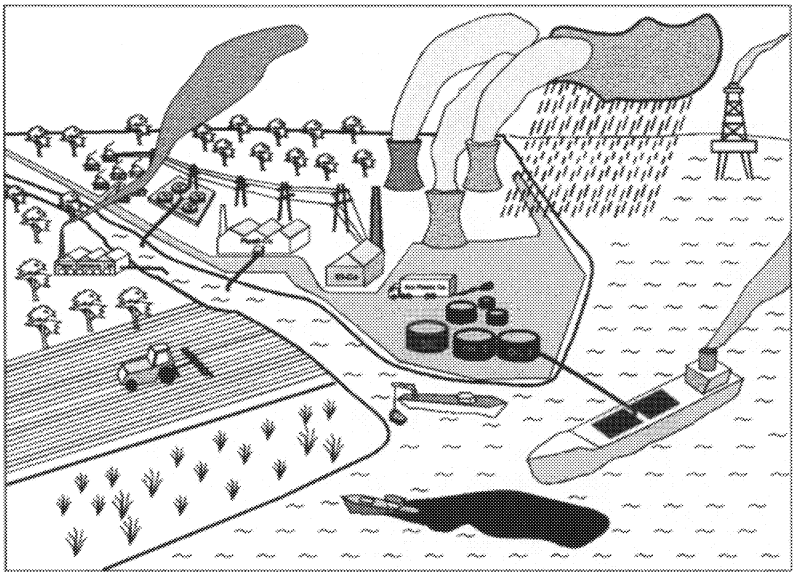


Figure 2.1. Pollution of the aquatic environment

The pollutants which have been examined for endocrine disrupting activity are predominantly pesticides, polychlorinated biphenyls, polyaromatic hydrocarbons, and heavy metals. There are a very large number of classes of chemicals which have so far received little or no attention, and even among pesticides, the bulk of the studies have concentrated on organochlorine and organophosphates, many of which are now banned or are being phased out. Most of the herbicides and fungicides now in use remain to be examined. Phthalates and alkylphenolics have received recent attention only because of a chance finding of their estrogenic activity, which suggests that many more chemicals currently considered safe on the basis of standard toxicity tests may in due course be found to have endocrine disrupting activity.

This Chapter aims to briefly outline the nature of the main pollutants discussed later in this volume. It is not meant to be fully exhaustive, but to indicate some of the main sources of each of the major pollutants described later. The data has been taken predominantly from literature cited in later chapters and from some of the many volumes devoted to structure, usage and metabolism of pesticides (White-Stevens, 1971; Green *et al.*, 1977; 1987; McEwan and Stephenson, 1979; Hassall, 1982; Hutson and Roberts, 1985). The diversity of pesticides described in such books shows very clearly that only a minute fraction of those used have been tested for endocrine disrupting activity. Since structure-activity relationships may be able to predict the general types of chemical which have endocrine disrupting activity, the structures of the main pollutants and their full chemical names are given for reference in the Figures and Tables of this Chapter.

2.2 Types of pollutant

2.2.1 Heavy metals

Pollution of aquatic ecosystems by heavy metals predates all other anthropogenic chemicals by several millennia. Limited metal processing began during the Iron and Bronze Ages but significant heavy metal pollution probably did not arise until mining and utilisation of lead became of major importance within the Roman Empire. The rapid spread of large scale mining and smelting during the Industrial Revolution has continued to increase to the present day, although improved processes and increased regulation has restricted emissions in Western Europe and North America. Aerial and aquatic emissions from mining and industrial processing remain unregulated in many parts of the world.

Arsenic is a natural trace element in many lakes and rivers, but locally elevated concentrations are found in the locality of mining and energy

development. Mining, milling, smelting and the burning of fossil fuels release significant amounts of the metal into the atmosphere, predominantly as arsenic trioxide. In the form of sodium arsenite, it is used as a herbicide to defoliate cotton before mechanical picking and to control aquatic vegetation. Arsenous oxide is used in a range of industrial products, feed additives and pesticides and concentrations are generally higher in aquatic than terrestrial animals. It reaches the aquatic environment by atmospheric fallout, release from industrial plants and improper use of herbicides and pesticides. Release from an Indian fertiliser factory, for example, led to concentrations of up to 2 mg/l in a local lake. It may, like mercury, be bacterially methylated to form the more toxic methyl and dimethyl arsenic compounds. It is lipophilic and accumulates in fatty tissue (Phillips and Russo, 1978; Nichols *et al.*, 1984; Shukla and Pandey, 1984b; Hwang and Tsai, 1993).

Cadmium occurs rarely in nature and has no biological function. It has chemical similarities to zinc and calcium and its toxicity and uptake into biological systems can be influenced by water hardness. It is released from lead-mine workings, but in other areas effluents from smelting and electroplating industries are the major source of cadmium into the aquatic environment (Phillips and Russo, 1978; James and Wigham, 1986). Contamination is usually restricted to inshore areas, especially mudflats, and levels of up to 5 mg/kg have been found in riverine, lacustrine and marine sediments where bottom-dwelling fish may receive significant exposure (Gill *et al.*, 1993). Annual inputs to the North Sea have been estimated at 46-52 tonnes from rivers, 40 tonnes from direct discharge, 14-380 tonnes from atmospheric deposition and 20 tonnes from dredgings (Kersten *et al.*, 1988).

Copper is an essential element in a number of biochemical processes and has relatively low toxicity compared to mercury and cadmium. It is widely used in electrical wiring and water pipes. It has been used as an algaecide and is a common constituent of acid mine drainage (Phillips and Russo, 1978).

Lead has been mined from pre-Roman times and is present in effluents and tailings from both abandoned mines and those in which metal extraction is continuing. While increasing use of non-leaded fuels has resulted in a significant decrease in the amount released from motor vehicles into both the atmosphere and via road run-offs, and legislation in many countries has decreased the amount used in lead based paints, large amounts remain in the environment as a result of high usage earlier this century. Snowmobile exhausts have contributed significantly to lead contamination in arctic regions (Phillips and Russo, 1978). Lead has also been widely used for roofing, water pipes and in production of electrical batteries. River inputs to the North Sea were estimated at 950 tonnes per year and atmospheric deposition between 1530 and 6400 tonnes (Kersten *et al.*, 1988).

Mercury in the environment results from the chlor-alkali and pulp and paper industries, alkylmercury fungicide treatment of seeds, burning of fossil

fuels and emissions from incinerators, mercurial catalysts in industry as well as natural weathering processes. Methoxyethyl mercuric chloride (Emisan) is used as a prophylactic treatment of seed potatoes, ethylmercury derivatives are manufactured by the pharmaceutical industry as an antiseptic and mercurials are widely used as slimicides and fungicides in the pulp and paper industry. Mercuric chloride is commonly used as a disinfectant for seeds, an anti-disease treatment for corn, tubers and bulbs and a fungicide for numerous vegetables and potatoes. In Finland alone the pulp industry used 65 tonnes per year of phenylmercury acetate between 1953 and 1967, before it was replaced by other products, and at the end of the 1960s the chlorine industry lost 4 tonnes per year in the waterways, while 76 tonnes of mercury was used as a seed dressing until such use was stopped in 1968. In North America the most severe aquatic contamination in the 1960s and early 1970s was attributed to the chlor-alkali and pulp and paper industries. For every tonne of chlorine produced, 150-200 g of mercuric chloride was estimated to have been released into U.S. waterways. The 35 chlor-alkali plants operating in the U.S. in 1965 produced 3 million tonnes of chlorine, resulting in release of 495-660 tonnes of inorganic mercury into the environment in a single year. Environmental release in India was estimated in 1980 at 180 tonnes per year, of which 92% came from caustic soda manufacture. Flooding of large areas to create reservoirs and hydro-electric dams has also been linked to increased exposure of aquatic life to mercury. Sewage sludge disposal at sea and dredge spoil has led to increased mercury levels in the Irish Sea. Imputs of mercury to the North Sea were estimated at 50-70 tonnes per year, comprising 20-21 tonnes from rivers, 10-30 tonnes from atmospheric deposition and 15 tonnes from dredging operations. Mercury was particularly high in the Firth of Forth area of the Scottish coast resulting from industrial discharges of 1 kg/day at Grangemouth. Human consumption of contaminated fish and shellfish from Minamata Bay in Japan in the 1950s, and cereals dressed with methylmercury fungicide in Iraq in the 1970s caused widespread and severe illness. Bacteria in natural waters convert mercury into methylmercury which is the form usually found in fish tissues (Heisinger and Green, 1975; Hattula *et al.*, 1978; Phillips and Russo, 1978; Ram and Sathyanesan, 1986a; Kersten *et al.*, 1988; Leah *et al.*, 1992; Bleau *et al.*, 1996). Controls of mercury release have been progressively introduced since 1970 in many countries, but municipal waste processing, fossil fuel consumption and resuspension of contaminated sediments continue to make it available to aquatic organisms. Bioavailability is affected by pH, dissolved organic carbon and temperature. Processes such as coal burning, waste incineration and ore refining contribute to the atmospheric dispersion of mercury and recent measurements suggest that from 1977 to 1990 the atmospheric concentrations in the Northern and Southern Hemispheres have increased by 1.46 and 1.17% per year

respectively (Slemr and Langer, 1992). Mercury is now a very widespread contaminant in the aquatic environment.

Tin is present in the aquatic environment predominantly in the form of organotin. Production increased from 5000 tonnes in 1955 to 35,000 tonnes in 1985 and may now have reached 50,000 tonnes. Organotins are used as pesticides and stabilisers in PVC (70% of production; dimethyl- dibutyl- and dioctyltin), catalysts for polyurethane and silicone elastomers (mono- and dibutyltin), timber, textile and leather protection (tributyltin, TBT), and as a disinfectant. Tributyltin was in widespread use from the 1970s as an antifouling paint on boats and marine installations, until evidence of its toxic effects on oysters and induction of imposex in gastropods became apparent. It's use in antifouling paints is now controlled or banned in many countries. 23% of world-wide production of organotin is used as agrochemicals and general biocides, and these are probably now the major sources of contamination of the aquatic environment. Such use includes fungicide treatment of potato blight, leaf spot on sugar beet and peanuts, scab on pecans, leaf spot and blight on carrots, and fungal diseases on soybeans, rice, cocoa and coffee. Metallic tin is less toxic than the organotins, but can be converted into methyltin by micro-organisms in marine sediments (Fent, 1966).

Zinc has a relatively low natural abundance, it is used extensively in alloys, galvanising and die castings, paints, cosmetics, pigments and other industrial products. It is an essential element for both plant and animals, but is toxic at high concentrations.

2.2.2 Pesticides

2.2.2.1 Organochlorines (Table 2.1, Figure 2.2)

Organochlorines have been used very extensively as pesticides since their introduction in the 1940s. The effects of overuse of these chemicals in post-war eradication programmes has been well documented (Carson, 1962). They are very persistent and remain in sediments for decades after release. They are lipophilic and bioaccumulate through the food chain in fatty tissues of both marine and terrestrial animals. Use of many of these pesticides has been banned in North America and Europe, where they have increasingly been replaced by organophosphates and other pesticides which are more readily degraded.

Table 2.1. Chemical names of common pesticides (From "Dictionary of Organic Compounds", 6th Edition, Chapman and Hall, 1996, from which names and structures of other chemicals may be obtained).

Common or Commercial Name	Chemical Name
Organochlorine pesticides	
Aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-*exo*-1,4:5,8-dimethanonaphthalene
Chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*H*-indene
Chlordecone (Kepone)	1,1a,3,3a,4,5,5a,5b,6-decachloroctahydro-1,3,4-methano-2*H*-cyclobuta(*cd*)pentalen-2-one
2,4-D	2,4-dichlorophenoxyacetic acid
p,p'-DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane
o,p'-DDD	1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl) ethane
p,p'-DDE	1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene
p,p'DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
Dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a -octahydro-1,4:5,8-dimethanonaphthalene
Endosulfan = Thiodan	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzadioxathiepin 3-oxide
Endrin (Hexadrin = 20% Endrin)	3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth[2,3b]oxirone
β-HCH	1α,2β,3α,4β,5α,6β-hexachlorocyclohexane
γ=HCH = γ-BHC = BHC = Lindane	1α,2α,3β,4α,5α,6β-hexachlorocyclohexane
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene
Linuron	*N'*-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea
Methoxychlor	1,1,1-trichloro-2,2-bis(4-methoxyphenyl) ethane
Mirex	1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorocyclo-octahydro-1,3,4-metheno-1*H*-cyclobuta[*cd*]pentalene
Toxaphene	2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane + related isomers
Organophosphorus pesticides	
Chlorfenvinphos = Birlane	2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate
Diazinon	*O,O*-diethyl-*O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl) phosphorothioate
Disulfoton	*O,O*-diethyl-*S*-(2-ethylthioethyl) phosphorodithioate
Fenitrothion = Sumithion	*O,O*-dimethyl-*O*-(3-methyl-4-nitrophenyl) phosphorothioate
Fenthion = Lebaycid	*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate
Glyphosate	*N*-(phosphonomethyl)glycine
Malathion	*S*-(1,2-dicarbethoxyethyl)-*O,O*-dimethyldithio phosphate

Common or Commercial Name	Chemical Name
Methylparathion = Paracid = Paramar M50 = Metacid 50	*O,O*-dimethyl *O*-(4-nitrophenyl) phosphorothioate
Mevinphos = Phosdrin	2-methoxycarbonyl-1-methylvinyldimethyl phosphate
Monocrotophos	Dimethyl 1-methyl-3(methylamino)-3-oxo-1-propenyl phosphate
Parathion	*O,O*-diethyl-*O*-(4-nitrophenyl) phosphorothionate
Phenthoate = Elsan	ethoxycarbonylbenzyl-*O,O*-dimethylphosphoro-dithioate
Quinalphos	*O,O*-diethyl-*O*-2-quinoxalinyl phosphorodithioate
Temephos	*O,O'*-(thiodi-4,1-phenylene)-*O,O,O',O'*-tetramethyl phosphorothioate
TEPA	tris(1-aziridinyl) phosphine oxide
Tetrachlorvinphos = Gardona	2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate
Carbamates and other pesticides	
Carbaryl = Sevin	1-naphthalenylmethyl carbamate
Carbofuran = Furadon	2,3-dihydro-2,2-dimethyl-7-benzofuranylmethyl carbamate
Atrazine	2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine

Aldrin together with the other cyclodiene insecticides, *dieldrin* and *endrin* (an isomer of dieldrin), developed in the late 1940's, were the most powerful general insecticides until synthetic pyrethroids appeared. They were particularly active where contact action and long persistence were required. They have been used against a wide range of insect pests, as a surface treatment of soil, seed dressing, root dipping, sheep dips and against the sucking and boring insects of many tropical crops. The volatility of aldrin while making it less persistent as an exposed deposit, increases the problem of atmospheric dispersion. The high toxicity of these compounds to mammals, ready absorption through the skin, extreme persistence in the environment and bioaccumulation in body fats has led to their withdrawal in many countries. In 1971 alone US production was estimated at around 5000 tonnes aldrin and 500 tonnes dieldrin. They were responsible for many of the harmful effects of the eradication programmes described in Carsons "Silent Spring" (1962). Registration of all three compounds was cancelled in the USA in 1974.

Chlordane, another persistent chlorinated cyclodiene, was first used commercially in the 1940s for agricultural and household use as well as termite control. From 1950 to 1970 the formulation consisted of 43-75% *cis*- and *trans*-chlordane with smaller quantities of heptachlor, nonachlor and chlordene isomers. From the 1970s until the cancellation of its pesticide registration in 1974 in the USA, a more concentrated formulation containing

over 95% *cis*- and *trans*-chlordane was used. Usage in the United States alone in 1971 was 1000 tonnes.

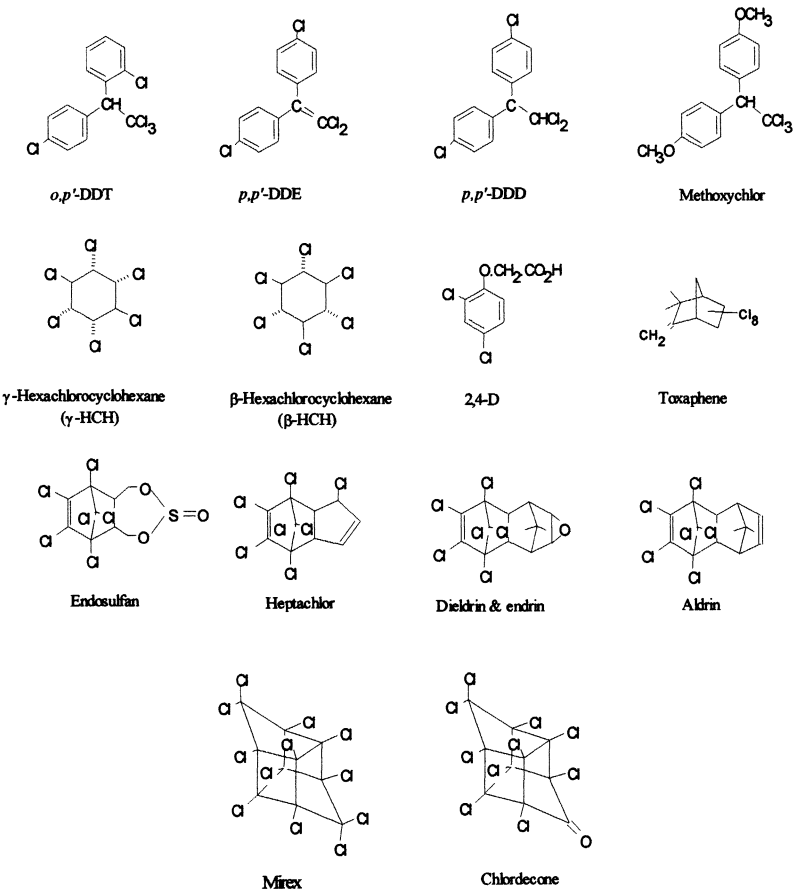


Figure 2.2. Chemical structures of organochlorine pesticides and some of their degradation products

Chlordecone (Kepone®) and *Mirex* were used as stomach poisons in baits, but are extremely persistent and lipophilic. There were health problems in workers at production factories and high pollution resulting from dumping in the James River and Chesapeake Bay where fish were affected. Mirex registration in the United States was cancelled in 1977.

2-4D is a selective herbicide widely used for weed control with apples, pears, barley, wheat, sorghum, oats, rice, sugar cane etc., with a usage in

1971 in North America of 25,000 tonnes which accounted for 20% of total herbicide use. It is not persistent and is readily metabolised by tolerant plants, soil bacteria and micro-organisms in the water. It acts by modifying the action of auxins, the natural plant hormones which regulate growth and root initiation. They are generally considered safe to animals although there was concern about the presence of teratogenic dioxins as impurities in the related 2,4,5-T which was used as the defoliant Agent Orange in the Vietnam war. U.S production of 2,4-D in the late 1970s was 20,000 tonnes/year.

DDT was used extensively as a pesticide from the late 1940's, before the hazards of overuse of pesticides and the persistence of organochlorines was fully appreciated. In 1963 alone 6.2 tonnes aldrin, 1.8 tonnes dieldrin and 57 tonnes DDT were used in the Wisconsin-Lake Michigan watershed. Only 10 tonnes of pollutant is sufficient to give a level of 2 ng/l in Lake Michigan and since organochlorines are stable and the average water retention time of the Lake is 30 years, there is a considerable time delay before even water-borne insecticides are cleared from the lake (Reinert, 1979). Attachment of the hydrophobic organochlorines to sediments would extend their availability in the lake considerably longer. Recent studies show that, more than 25 years after DDT insecticides were banned in the USA, high levels are still apparent in Great Lakes fish (see Chapter 3). Very high local levels can also result from dumping at sea. In San Pedro Bay, off the Californian coast, an estimated 2400 tonnes of DDT was discharged or dumped between 1947 and the early 1970s (Hose *et al.*, 1989). DDT exists in two forms *p,p'*- and *o,p'*- which are degraded to the respective DDE and DDD isomers. Commercial formulations contained 15-20% of the *o,p'*- and 80-85% of the *p,p'*-isomers.

Endosulfan (Thiodan®) is a moderately persistent cyclodiene insecticide developed by Hoechst in 1954 and is one of the most toxic pesticides for aquatic life with a 96 h LC_{50} as low as 0.01-40 μg/l depending on species. Disappearance from crops is believed to be primarily due to volatilisation. It is widely used in developing countries for pest control of jute, cotton, sugar cane and vegetable culture where it is commonly found as a contaminant of surface waters resulting from rainfall runoff and accidental spillage. 0.1-0.3 μg/l have been found in the Rhine where it has been implicated in fish kills (Arnold *et al.*, 1996a).

Heptachlor was used extensively against soil insects and particularly for fire ant control in the U.S.A. It is a volatile pesticide which is metabolised or degraded to its epoxide which bioaccumulates in animal tissue. It was banned in 1969 after concern about its effects on birds and its presence in milk.

Hexachlorocyclohexane (γ-HCH, lindane, benzenehexachloride, BHC) was introduced in 1949 and has been used extensively world-wide in control of wood inhabiting beetles, vector control (against *Triatoma*), and as a scabicide and pediculicide in pharmacological preparations. In India it is

widely used in fish-cum-paddy culture. LC_{50} ranges from 7 to 700 µg/l according to species (Braunbeck *et al.*, 1990a). Commercial lindane contains both the γ- isomer and 5-12% of the β-isomer which is formed during production processes. It is considerably more volatile and water soluble than DDT making it effective both as a fumigant and as a seed dressing.

Linuron was the first urea based organochlorine herbicide to be commercially produced and has a low accumulation in aquatic systems, but it has been listed as a potential carcinogen. It is used for control of germination in annual weeds among carrot, corn, cotton, potatoes, sorghum and soybean crops. It may be contaminated with both parent and breakdown products such as 3,4-dichloroaniline and 4-chloroaniline.

Methoxychlor replaced DDT in the late 1960s in blackfly control programmes in Canada in which it is injected into flowing waters where larvae develop to create a short pulse at a nominal concentration of 0.3 mg/l. Treatment times in Canada, however, correspond with spawning of white sucker (Holdway and Dixon, 1986a, b). It is relatively non-persistent and does not significantly accumulate in tissue of birds and mammals since it is detoxified by mixed function oxidases. Short term bioaccumulation may occur in some fish, but the residue is lost on return to clean water.

Pentachlorophenol is used primarily as a fungicide in pulp and paper processing and wood preservation and is a common contaminant in surface waters receiving effluents from forest products industries. 25,500 tonnes was used annually in North America in the early 1980s. Commercial formulations also contain dibenzodioxins and dibenzofurans as contaminants (Nagler *et al.*, 1986).

Toxaphene is an inexpensive broad spectrum pesticide introduced in 1948 and in 1982 was the most heavily used insecticide in the United States. In 1976 18 million tonnes were applied to US crops, 85% of which was cotton. It was also used against a variety of arthropod pests affecting livestock, sunflowers and ornamental plants, for the control of grey flies in Romania, alfalfa beetles in Hungary, cabbage crops in Europe as well as cotton in the USSR. Most of the pesticide is probably lost through the atmosphere by volatilisation and dust. Over 50% of the toxaphene applied to cotton plants either as an aerial emulsion or deposited on the soil was lost by evaporation within 8 weeks. Not surprisingly this results in wide dispersion, with levels of almost 3 ng/ m^3 in air over Arctic Canada and 0.6 ng/m^3 over the North Atlantic 1600 km from its nearest known use. It has only a short persistence in mammalian tissues and is rapidly lost when intake is stopped. The US Environmental Protection Agency cancelled its registration for many uses in 1982.

2.2.2.2 Organophosphates (Table 2.1, Figure 2.3).

The major action of phosphorothioates is inhibition of acetylcholine esterase activity in nervous and effector organs, but a prerequisite of such action is the biotransformation of the parent compound into the corresponding oxygen analogues by microsomal monooxygenases (Levi *et al.*, 1988) which are high in fish liver. Organophosphates are degraded much more readily than organochlorines which they have superseded in many areas as concern mounted over their persistence in the environment. Due to their low bioaccumulation they were also considered to be safer, but recently there have been suggestions of links with the illnesses affecting both Gulf War veterans and farmers using organophosphorus sheep dips.

Chlorfenvinphos (Birlane®) is highly toxic to mammals and has a narrow spectrum of activity as a soil insecticide against root maggots and corn rootworms.

Diazinon is used in the UK as a sheep dip to prevent and treat ticks, lice and scab. The main sheep dipping areas are close to upland streams which support salmonid populations, and can produce high transient peaks of up to 200 µg/l while dipping is in progress (Moore and Waring, 1996a). In developing countries it is used for control of household and soil insects, and pests on fruit, vegetables, field crops, lawns and ornamentals (Ansar and Kumar, 1988).

Disulfoton (Dimaz®, Di-syston®, Disytox®, Frumin®, Solvirex®) produced by Bayer is used as a broad-range systemic insecticide for tobacco, carrots, cucumber, parsley, peas and potatoes (Howard, 1991). It is classified by WHO as a product of extreme hazard because of its high risk to human health (WHO, 1986). Environmental contamination is primarily due to its use as an insecticide on crops and constant leaching and runoff during rain. It is short lived with a half-life of 5-12 h. It was released into the Rhine in large quantities as a result of an industrial accident in Basel in 1986 when river concentrations reached 0.6 mg/l leading to a major decrease in the eel population at least and exceeding the LC_{50} of the majority of fish species in the river.

Fenitrothion (Sumithion®) is widely used in Asia and Europe as a general purpose agricultural insecticide, but in North America is limited to spraying against spruce budworm. It is rapidly degraded by mammals and waters to *p*-nitrocresol and other polar metabolites.

Fenthion (Lebaycid, Baytex®) has a broad spectrum activity and is used against *Anopheles, Culex* and *Simulium* larvae in many tropical freshwaters (Kling, 1981) and control of fruit flies. It is highly toxic to birds and is persistent for several months.

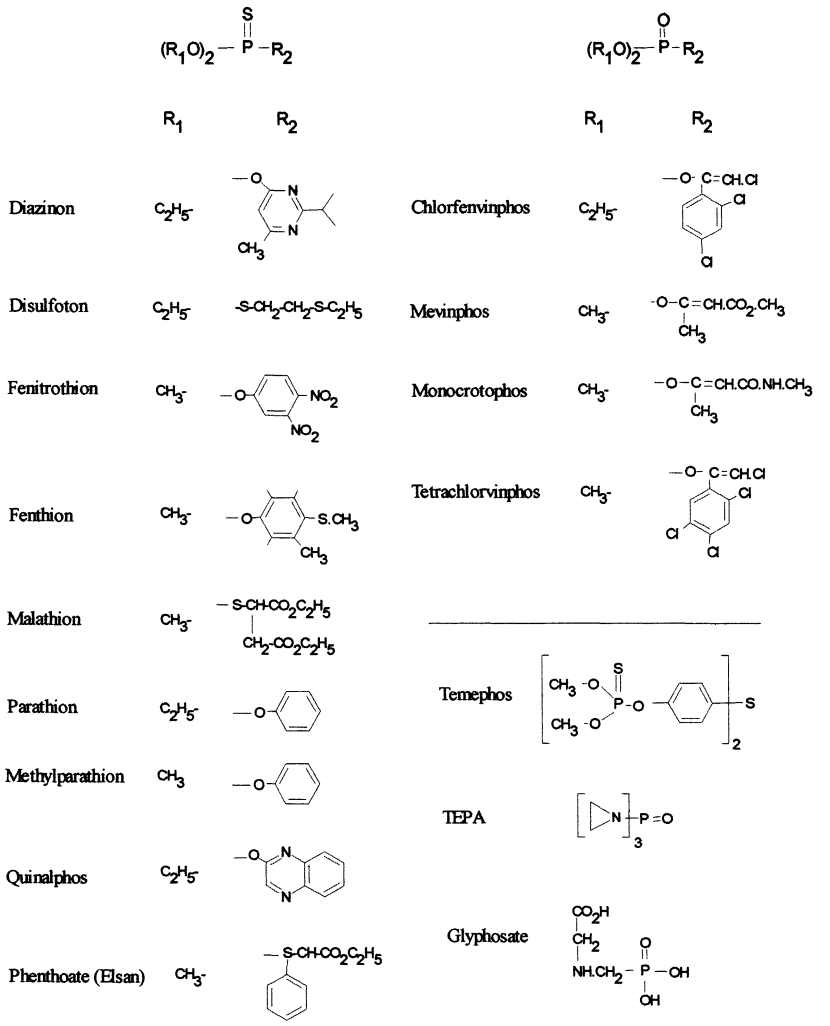


Figure 2.3. Chemical structures of organophosphate pesticides

Glyphosate (Roundup®) is already in very widespread use and such use is expected to increase very rapidly with the introduction of crops which have been genetically modified to have a resistance to this herbicide. Soybean was the first major genetically modified crop with herbicide resistance to be introduced, but it is likely that many other major crops worldwide will be similarly modified if licences are granted. Glyphosate is a glutamine synthetase inhibitor and unlike other organophosphates is not an inhibitor of

acetylcholinesterase. The active ingredient has low toxicity to fish and many of the harmful effects may be due to the formulation rather than the active ingredient. It has not been tested for endocrine disrupting activity in any animal.

Malathion (Cythion) has high toxicity to insects in which it is converted into the active malaoxon which is only slowly degraded, compared to mammals in which it is rapidly further metabolised. Due to it's low toxicity to mammals, it is used against many species of insects and mites, especially in the household and around livestock.

Methylparathion has similar activity to parathion and is used against insects, aphids and beetles. It has slightly lower mammalian toxicity so is used instead of parathion for citrus and cotton production. 13,000 tonnes were used in the United States in 1971.

Mevinphos (Phosdrin®) is a short-lived systemic and contact poison used on fruits and crops where insect control is required close to harvest time.

Monocrotophos (Azodrin®) is a broad spectrum insecticide for vegetable and cotton pests which persists for several months.

Parathion is one of the most toxic and widely used insecticides in North America. It can be quite persistent in soil, with measurable residues up to a year after application. It is a broad spectrum insecticide used against foliar pests and the adult stages of root maggots.

Temephos (Abate®) is an important insecticide for control of larvae of mosquitoes, black flies, midges and sand flies.

Tetrachlorvinphos (stirofos, Gardona®) has lower toxicity to mammals and is a less persistent soil insecticide than the related chlorfenvinphos. It is selectively toxic to Diptera, Coleoptera and Lepidoptera.

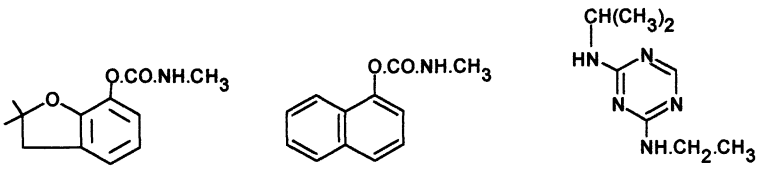
TEPA was one of the earliest aziridine compounds used for control of housefly and Mexican fruit fly and acts as a chemosterilant (Stock and Cope, 1969).

2.2.2.3 Other pesticides (Table 2.1, Figure 2.4)

Carbamates are often used as agricultural pesticides since they are considered safer than organophosphates and less persistent than the organochlorines. Like the organophosphates they are acetylcholinesterase inhibitors and are rapidly metabolised by plant and animal systems. The major products are the broad spectrum insecticides Carbofuran (Furadon®) and Carbaryl (Sevin®), which are used extensively on cotton, tobacco and vegetable crops and in paddy field culture.

Atrazine is a triazine based herbicide and one of the most widely used systemic herbicides. It is used for weed control in corn, orchards, sorghum and in non-cultivated areas and is found at levels up to 10μg/l in European

streams and ground water (Oulmi *et al.*, 1995b). It is degraded by micro-organisms in the soil where it has a half-life of 1-12 months.



Carbofuran Carbaryl Atrazine

Figure 2.4. Chemical structures of some other pesticides

2.2.3 Industrial

2.2.3.1 Polyaromatic hydrocarbons (PAHs) (Figure 2.5)

Even in 1979 it was estimated that 230,000 metric tonnes of PAHs entered the worlds aquatic environment every year (Neff, 1979). Sources include surface run-off, oil spills, recreational and industrial boating, municipal waste effluents and atmospheric deposition. In the Great Lakes region, aerial imputs are the major source and concentrations of PAH in the water from this source are 0.000025-0.003 mg/l (Neff, 1979; Eisenreich *et al.*, 1981). Input of petroleum hydrocarbons, of which only a small part are comprised of PAHs, into the oceans has been estimated as 3.2 million tons per year, of which 1.47 million tons arises from marine transport and 1.18 million tons from municipal and industrial wastes (McElroy *et al.*, 1989). In the urban area of Providence (Rhode Island, USA), the runoff of PAH from the Narragansett Bay watershed was estimated as 681 kg/year of which 360 kg originated from the highways and 228 kg from industry. The constituent hydrocarbons were rather different between the two main sources, with fluoranthene, chrysene and benz(*a*)anthrene predominating in highway runoff and fluoranthene, pyrene and phenanthrene in industrial wastes (Hoffman *et al.*, 1984). The majority of the urban PAHs are believed to arise from atmospheric deposition of hydrocarbons produced by combustion of fossil fuels (McElroy *et al.*, 1989) which suggests that the respiratory system of mammalian life in such urban areas may receive even greater exposure to these chemicals than those of fish in the surrounding waters, although biomagnification through the food chain may ultimately give the endocrine tissue of fish the greater exposure.

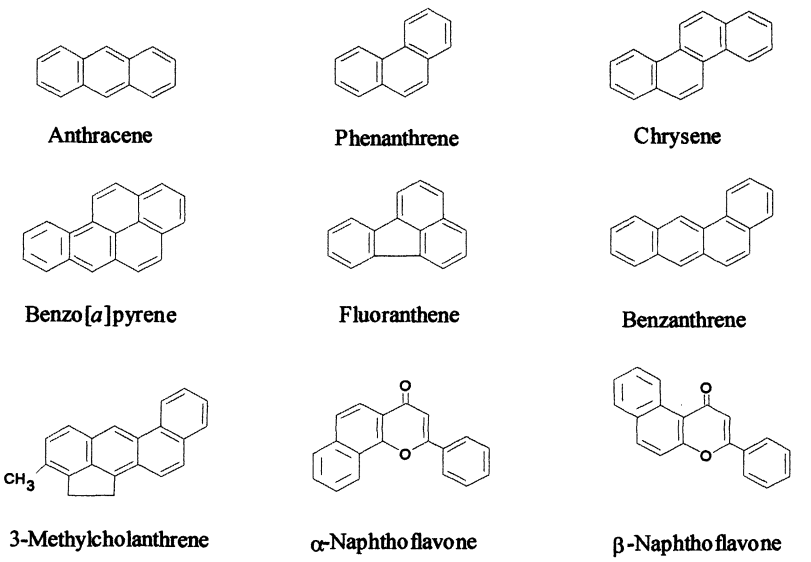
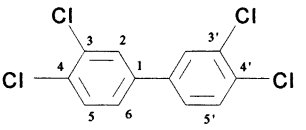


Figure 2.5. Chemical structures of some polyaromatic hydrocarbons (PAHs)

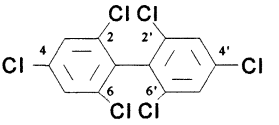
2.2.3.2 Polychlorinated biphenyls (PCBs) (Fig. 2.6)

PCBs, originally manufactured in the USA in 1929, were used in large quantities mainly as dielectric fluids in electrical equipment until restrictions on manufacture and use were introduced in the 1970s. Major PCB manufacturers in the USA, Japan, Germany, UK, France, Italy and Spain produced 1.2 million tonnes of PCBs between 1930 and 1980 (Bletchly, 1984). China, Latin America and Poland were also significant producers. Almost half of the PCB production was used in large long-lived capacitors and transformers, but they were also used as lubricants, hydraulic fluids, softeners, insulating fluids, and flame retardants. Incineration of plastics at temperatures below 1200° produces PCBs in the flue ash and atmospheric emissions. They also enter the environment by escaping from "closed systems" like small condensers, transformers or hydraulic systems and by accidental loss from open systems as in waste-oil incineration plants. The spatial distribution of PCBs in Atlantic and Baltic salmon suggests that dispersion is by continuous release into the atmosphere resulting in only small differences in concentrations between marine areas (Falandysz, 1994). Deposition of PCBs in the Great Lakes is also predominantly via atmospheric transmission rather than through direct runoffs from municipal and industrial sources. An input-output model of the lakes indicated an input of 8-9.7 tonnes

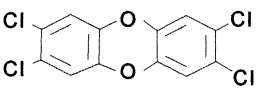
per year of which 6-8.3 tonnes was from the atmosphere, while output was estimated at only 1.1-1.7 tonnes per year (Hallett, 1988). The amount of PCBs entering the Baltic annually by atmospheric fallout was estimated at 6 tonnes (Kihlström and Berglund, 1978). PCB levels in the River Rhine in 1980 were 0.05-0.5 µg/l indicating a load of 5-10 tonnes per year entering the North Sea from this river alone (Hansen *et al.*, 1985).



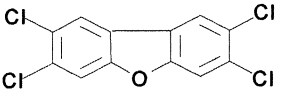
3,3',4,4'-Tetrachlorobiphenyl
(coplanar PCB)



2,2',4,4',6,6'-Hexachlorobiphenyl
(non-coplanar PCB)



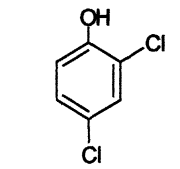
2,3,7,8-Tetrachloro-*p*-dioxin
(TCCD)



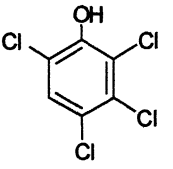
2,3,7,8-Dibenzofuran

Figure 2.6. Chemical structures of some polychlorinated biphenyls (PCBs) and related products

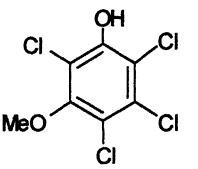
PCBs comprise a theoretical maximum of 209 chlorinated compounds (congeners), whose biological activity varies with the position and number of chlorine substituents. In the organism, PCBs are converted by cytochrome P-450 dependent mono-oxygenases into phenols and then conjugated with glucuronic acid. This transformation is dependent on the number and location of the chlorine atoms. Chlorinated biphenyls with a coplanar structure are particularly toxic since they bind to the cytosolic Ah(Aromatic hydrocarbon)-receptor complex which can penetrate the cell nucleus and influence gene expression, while di-*ortho*-substituted PCBs, in which the aromatic rings are forced out of coplanarity have low activity, probably as a result of low binding to this receptor. The biological activity of any PCB will therefore depend on the exact mix of congeners present (Gooch *et al.*, 1989). Highly chlorinated biphenyls containing 2,3,4-chloro substituents are also more resistant to metabolic breakdown and accumulate rapidly in body tissue (Safe *et al.*, 1985; Schlebusch *et al.*, 1989).



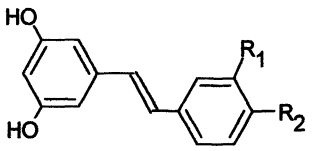
2,4-Dichlorophenol



2,3,4,6-Tetrachlorophenol

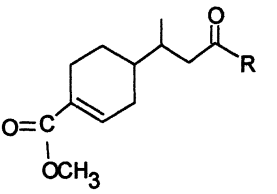


2,3,4,6-Tetrachloroguaiacol



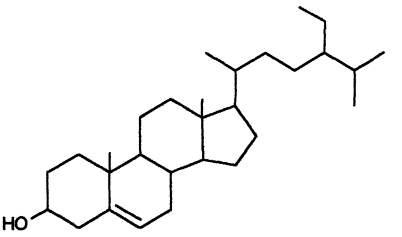
Isoharpontigenin (R_1=OCH_3; R_2=OH)

Pinosylvin (R_1=R_2=H)



Juvabione (R= -CH_2.$CH(CH_3)_2$)

Dehydrojuvabione (R= -C=$C(CH_3)_2$)



β-Sitosterol

Figure 2.7. Chemical structures of some constituents of pulp and textile mill effluents

2.2.3.3 Dioxins and related compounds (Figure 2.6)

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) originate from a wide variety of domestic and industrial process, including incineration of plastics, industrial processes where chlorinated hydrocarbons are used, combustion of fossil fuels and pulp mills using chlorine bleach

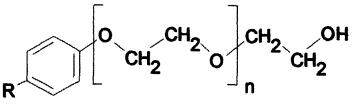
processes. As with PCBs, these can exist as a large number of possible congeners (75 PCDDs and 135 PCDFs). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) have been of particular concern with regard to incinerator emissions. Polychlorinated dibenzo-*p*-dioxins, dibenzofurans and tetrachloroxanthenes are present in approximately equal concentrations in sludge from pulp and paper mills (Kuehl *et al.*, 1987).

2.2.3.4 Pulp and textile mills (Figure 2.7)

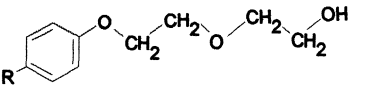
The paper industry uses predominantly the kraft process in which wood is pulped to its constituent fibres and then bleached to decolourise the lignin. If chlorine is used for this process a range of chlorinated phenolics (dioxins, tri- and tetrachlorinated phenols, guaiacols and catechols) and chlorinated organic acids will be released in the effluent, which will also contain a complex mixture of other chemicals from both degradation of the wood and the chemicals used in processing. Many of these are difficult to characterise (Owens, 1991). Natural wood breakdown products include β-sitosterol, isoharpontigenin, juvabione, dehydrojuvabione and pinosylvin.

2.2.3.5 Industrial detergents (Figure 2.8)

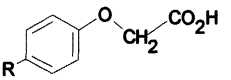
Alkyphenol polyethoxylates, introduced in the 1940's are one of the largest groups of non-ionic surfactants produced commercially and are in widespread use as detergents and as constituents of paints, herbicides, pesticides and many other formulated chemicals. Nonylphenol polyethoxylates account for about 80%, and octylphenol polyethoxylates 20% of the annual world production of over 300,000 tons. About 60% of this will end in the aquatic environment, mainly via sewage treatment works in which they are degraded to the stable hydrophobic metabolites (nonyl and octylphenol) which accumulate in sewage sludge and river sediment. Other metabolites, such as the short chain alkylphenoldiethoxylates and alkylphenoxycarboxylic acids are more soluble (Jobling *et al.*, 1996). The nonyl- and octyl substituents of these chemicals are always present as a mixture of isomeric forms and the degree of branching of the chain can influence the estrogenic activity - different preparations of the same alkylphenolic may therefore differ in the proportions of different isomers and therefore in estrogenic potency. The widespread use of these products, which are now known to be estrogenic, in pesticide formulations (Green *et al.*, 1987) may require a re-evaluation of data obtained using commercial products in which they have been described as "inert ingredients".



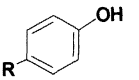
Alkylphenol polyethoxylate



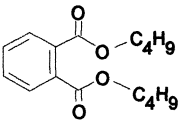
Alkylphenol diethoxylate



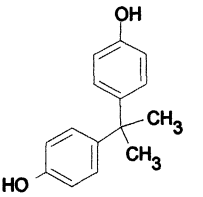
Alkylphenol acetic acid



Alkylphenol



Di-n-butylphthalate



Bisphenol-A

Figure 2.8. Chemical structues of some industrial products with estrogenic activity

2.2.3.6 Acid rain

Large amounts of sulphur dioxide are produced by combustion of sulphur containing fossil fuels for electricity production in all industrialised countries. Smelting works also release large amounts and the Sudbury area of Ontario which has a nickel smelting industry was responsible for release of 2.6 million tonnes of sulphur dioxide in 1969 - half of that produced by the whole of Canada and 10% of US production. Local lakes had a pH as low as 4.0. (Beamish, 1976). In northern latitudes and mountain regions there may be seasonal peaks in the acidity of aquatic systems co-incident with the spring snow melt.

2.2.3.7 Fertilisers and other agrochemicals

Extensive use of ammonia and nitrate based fertilisers on agricultural land can result in high levels of ammonia, nitrate, nitrite and nitrosamines in adjacent waterways as a result of rainwater runoff. In Eastern Europe such fertilisers are routinely added to many carp culture ponds.

2.2.3.8 Other industrial pollutants

Many industrial chemical products, their intermediates, by-products or degradation products are released into the environment during production, use or disposal. These are too numerous to list, and the following comprises only those so far found to have endocrine disrupting activity.

4-Chloroaniline is an intermediate in the production of azo dyes, antioxidants, pharmaceuticals and fertilisers. Global production is about 5000 tonnes per annum and although only a fraction directly enters the environment, larger amounts are released by the hydrolytic breakdown of phenylcarbamate, phenylurea and acylanilide herbicides. Aquatic concentrations of 1-2.2 μg/l have been found in German and Dutch rivers (Zahn *et al.*, 1993).

3,4-Dichloroaniline is used as an intermediate in the manufacture of dyestuffs and pigments (azo-dyes), antioxidants, pharmaceuticals and agricultural chemicals. The major source is release during hydrolytic breakdown of phenylcarbamate, phenylurea and acylanilide herbicides such as monolinuron, monuron and buturon and nitroanilide fungicides (Braunbeck *et al.*, 1990c).

4-Nitrophenol originates from degradation of parathion, methylparathion and nitrofen. It is an intermediate in the production of organophosphate pesticides, dyes, antipyretics, photographic materials and leather preservatives. Annual production in the USA was 18,600 tonnes in 1980. Fish toxicity ranges from 8-60 mg/l (Braunbeck *et al.*, 1989).

Bisphenol is an estrogenic plasticizer which can be released from polycarbonate flasks during autoclaving (Krishnan *et al.*, 1993).

Phthalates include butylbenzylphthalate which is used as a plasticizer especially in production of vinyl floor tiles, adhesives and synthetic leather and di-n-butylphthalate used as a plasticizer in food packaging, PVC, some elastomers and as an insect repellent. Both show estrogenic activity in some tests (Jobling *et al.*, 1995).

Polychlorinated naphthalenes (PCNs) such as Halowax 1014 have chemical and thermal properties similar to PCBs and have been used as plasticisers, pesticides, impregnating agents and seals for condensers.

Polybrominated diphenyl ethers (PBDEs) such as Bromkal 70-5DE are used as flame retardants in plastics, textiles, paints and hydraulic fluids (Holm *et al.*, 1994).

Phenol and *sulphide* are produced by municipal wastes, coal liquid and discharges from paper, pulp and jute mills and paint manufacture (Mukherjee *et al.*, 1991). Phenol is released from coal cooking plants, chemical plants, gas works, oil refineries, pesticide plants, and wood preservative and dye manufacturing industries (Razani *et al.*, 1986).

Chapter 3

Bioconcentration of Pollutants in Fish Tissue

3.1 Introduction

Any study of the effects of pollutants on the endocrine system must begin with some knowledge of the likely concentrations to which a particular tissue is exposed in fish in polluted habitats. This is not as simple as might first appear, since uptake of the pollutant, its distribution between different organs and its clearance rates differ not only between species and pollutants, but also between sexes and seasons and is influenced by a multitude of other factors. It will also depend upon whether it is administered via the holding water, or in the diet. These will all affect the biomagnification factor, the ratio of the concentration in tissue to that in the holding water. Exposing a top carnivore to the water concentration prevalent in its habitat is unlikely to mimic the effects in wild fish held in such waters since they consume a diet of smaller fish or invertebrates which in their turn have already accumulated the pollutant through their diet. It might be expected that concentrations of pollutants would be higher in predator than in prey fish, but while a number of studies (Tables 3.1-3.5) have examined concentrations in several species from the same locality, they have rarely provided any information on the diets of the different species which could explain the differences in xenobiotic uptake. Any exposure regime should therefore start with a knowledge about the basic biology of the fish and the most probable source from which it accumulates the chemical of concern. It is then posssible after exposure to compare the concentration in the tissues of interest with that in wild caught fish in the polluted habitat. With *in vitro* experiments, the situation is much more complex since it is not at all clear whether a tissue concentration of 10 μg/g, for example, in a fish from a polluted habitat equates to adding 10μg to

an incubation of 1 g of tissue or to 1 g of incubation medium, and in neither case does this relate to pollutant which may be localised within certain cellular components.

A further limitation on the data available in the literature on tissue concentrations is that the majority has been collected to monitor hazards to human health of consumption of the fish concerned. As a result there is a massive literature on concentrations in the whole carcass or in edible tissue such as muscle, relatively little on concentrations in liver, and even less on levels in the gonads, yet alone in the thyroid, kidney/interrenal, pituitary or hypothalamus. Of the many thousands of chemicals in the aquatic environment, only a minute fraction have been analysed - predominantly for heavy metals, PCBs and organochlorine pesticides. Although these give an indication of the relative pollutant burden in a particular fish, it is important to recognise that these are not the only xenobiotics present, nor necessarily the most toxic. It is also important not to attribute endocrine disruptive effects solely to the concentrations of pollutants that happen to have been measured in fish from a particular polluted habitat. Many fish in the North Sea and the Great Lakes have very high tissue concentrations of PCBs, and also show significant endocrine dysfunction. This does not mean that PCBs have caused the dysfunction since they may also have high concentration of, for example, alkylphenolics which are of very recent concern as endocrine disruptors but have been measured in very few specimens. Equally well they may contain high levels of some other chemical which is not even suspected of being an endocrine disruptor. The aim of this Chapter is, therefore, not to present a full review of the literature on tissue concentrations of xenobiotics in fish, but rather to present a sample, both from wild caught fish and from laboratory exposed specimens, so that these may be used as a basis for exposure regimes to study the effects of potential endocrine disrupting chemicals. The examples have been chosen to cover a range of different habitats and species, although the North Sea and Great Lakes predominate. The factors affecting uptake and distribution are not intended to cover the existing literature, but rather to illustrate the fact that uptake and distribution of xenobiotics is affected by a plethora of factors and that data on tissue concentrations should be interpreted with some caution. No attempt has been made to present water concentrations of pollutants in the various habitats. In many cases these are too low to be detectable by present methods, data are limited to a few pollutants which are present in highest amounts, and they are of doubtful relevance since uptake is frequently from dietary sources or aquatic uptake in close proximity to sediments. The data on levels in fish tissue represent accumulation over a considerable period, and experimental procedures are best designed to give similar tissue concentrations of the pollutants to those found in wild fish from polluted habitats as a result of dietary and/or branchial uptake.

Heavy metals were one of the earliest anthropogenic pollutants and are the most easily measured. It is therefore not surprising that it was with these pollutants that the earliest studies on bioconcentration were carried out. The predominant concern was with toxicity to human consumers of fish, particularly after the notable poisoning episodes in Japan resulting from mercury accumulation in fish and shellfish. Concentrations of heavy metals, particularly cadmium and mercury have been extensively studied in fish, but the majority of such studies have been limited to the edible tissues such as muscle and less commonly the liver. The early studies are well covered in the review of Phillips and Russo (1978) and are so extensive that they will not be repeated here in detail. Data on the concentrations of heavy metals in the non-edible tissues, such as gonads, gills and kidneys are less common, since they do not directly impact on the consumer. They may, however, be of major significance in the health of the fish and knowledge of such concentrations is essential in any discussion on the impact of heavy metal pollutants on reproduction, respiration, enzyme activities etc. The data summarised in Table 3.1 has been selected to cover a wide variety of habitats, from the heavily polluted rivers in close proximity to metallurgical complexes to the least polluted habitats of the Antarctic. Emphasis has been given to studies in which heavy metal concentrations have been measured in a range of tissues to show the distribution within the fish and so that extrapolations may be made from other studies in which only muscle was examined.

The concentrations of organic pollutants in fish tissues have been described rather less frequently than those for heavy metals. This is partly due to their more recent appearance as pollutants and partly due to the more sophisticated equipment necessary for their measurement. The data in Tables 3.2 - 3.5 provide information on a range of organic pollutants, species and geographical regions. The aim is to show the relation between exposure to a pollutant and its accumulation in different tissues. Since organic pollutants are known to be lipid soluble, there has been less concern than for the heavy metals with the health hazards arising from accumulation in the edible parts of the fish, and data has generally been collected for the liver and less frequently for muscle. During these studies the emphasis has also shifted to using pollutant concentrations as a means of monitoring the environmental ecosystem rather than primarily being concerned with human health, although a number of studies have been stimulated by concern for the impact of fish-borne pollutants on fish eating communities and wildlife. The data on organic pollutant levels in fish is now so extensive that it is impossible to include all studies, and the emphasis in the Tables is on providing values for a range of habitats and species. Not surprisingly the literature concentrates on commercial fish, such as cod and salmon, and polluted fisheries areas such as the North Sea, Atlantic, Baltic and the Great Lakes basin.

Wild fish are exposed to an array of pollutants and any observed pathological effects may be due to pollutants other than those measured. There is therefore, in addition to measurement of pollutants in wild-caught fish an increasing emphasis on laboratory exposure to specific xenobiotics of interest under controlled conditions. This has enabled investigation of the dynamics of uptake and how this might be affected by external factors such as salinity, pH, nature of the sediments etc, or by internal factors relating to different tissues which may explain variations in results between different species. It also shows more clearly which tissues accumulate the pollutant and how they are affected. Experimental data on tissue accumulation resulting from the exposure to artificially polluted water, diet or sediment, or by direct injection of pollutants is summarised in Tables 3.6 - 3.8.

Although early studies were concerned with the health of the fish consumer, there has more recently been increasing concern that the eggs and larvae represent a particularly sensitive stage of development and that their survival may be compromised by exposure to pollutants leading to decreased fish populations. Such concern, together with the possible use of egg and larval survival rates as a bioindicator of pollution has lead to studies on the uptake of pollutants by the eggs and larvae. The long-term effects of such exposure is covered in detail in Chapter 8, but the uptake of pollutants into early life stages is considered in Section 3.4 and data is summarised in Table 3.9.

3.2 Tissue Distribution

3.2.1 Heavy metals

The site of uptake of heavy metal pollutants may be dependent upon the means by which it is presented to fish - whether in the water or diet. For freshwater fish, which do not swallow water except with food, water borne pollutants have greatest contact with the gills. Exposure of tilapia (*Oreochromis aureus*) to mercuric chloride for two hours, gave a significant increase only in gill concentrations (Allen *et al.*, 1988). Cadmium uptake from water was also highest in gills of trout after 3 months exposure (Roberts *et al.*, 1979; Sangalang and Freeman, 1979) and radioactive ^{65}Zn or ^{109}Cd in water accumulated predominantly (>95%) in the gills of goldfish (Ebrahimi and Kime, Unpublished data). If cadmium was injected into brook trout over 16 weeks, however, uptake was highest in the posterior kidney (0.93 μg/g), while the anterior kidney had much lower uptake (0.18 μg/g) and gills and liver were comparable at 0.3 μg/g (Sangalang and Freeman, 1979). Extremely high levels of zinc (7700 μg/g) were also taken up by kidneys of the African catfish held in either dam water or sewage effluent (van den Heever and Frey,

1994). In a natural habitat, however, fish take in heavy metal pollutants both directly from the water via the gills and from their diet, which may already be enriched in the metal. This may account for many of the differences found between species (Table 3.1) since dietary intake may be expected to be greater for carnivores higher up the food chain than for herbivores. Overall the data suggests that the liver is the greatest accumulator of heavy metals while, with some notable exceptions, muscle concentrations are low. Gonadal concentrations have been described for few species and data is very variable, although zinc accumulation in particular is high. The tissue distribution of heavy metals is not as easily predicted as that for organic pollutants which from their hydrophobic chemical structure would be expected to accumulate predominantly in fatty tissues. Metal accumulation is more likely to be associated with binding to proteins, and particularly in the liver with metallothioneins. It must also be remembered that zinc and copper are essential metals in many biochemical processes and a deficiency may cause reproductive and endocrine dysfunction, while cadmium, mercury and lead play no role in normal biological functioning.

3.2.2 Organic pollutants

Of the vast number of organic pollutants present in the aquatic environment, data are only available for a very few, reflecting both the difficulty of measurement and the areas of greatest concern. PCBs and organochlorine pesticides therefore predominate in much of the literature reflecting the general concern about long-term persistence of these products in the aquatic ecosystem.

Polychlorinated biphenyl (PCB) concentrations are generally 10-100 times higher in liver than muscle (Table 3.2), although there are some exceptions in which similar values are obtained for both. In heavily polluted areas, such as the Baltic and North Seas and the Great Lakes, hepatic concentrations often exceed 10μg/g. Whole body values probably reflect these very high liver concentrations. In some samples, muscle concentrations exceed 1 μg/g, but unfortunately no data on liver concentrations are available for these samples. Gonadal concentrations are generally comparable to those in the liver, but there are few data available for direct comparison. Data is lacking for accumulation by the gills and kidneys.

Organochlorine pesticides show very low accumulation in the muscle, but concentrations in liver are in general 10-100-fold higher (Tables 3.3 and 3.4). Gonadal concentrations tend to be intermediate between liver and muscle. Their distribution therefore is very similar to that of PCBs. Distribution data can sometimes be misleading. In brook trout fed DDT, for example, testicular concentrations were apparently lower than for the whole body which, however, also included liver in which there was probably a very high

accumulation (Macek, 1968). This explanation is supported by the fact that over 50% of the whole body content consisted of DDT metabolites, while in the testis over 90% of DDT remained unmetabolised. Values for "whole body" should therefore be recognised as a composite of high liver and low muscle concentrations. The pattern of distribution varies little between the organochlorines, but the data for other organic pollutants is scarce. DDT and its metabolites frequently attain concentrations greater than 1μg/g in liver in the more polluted areas, but other organochlorines are generally present at less than one-tenth of this concentration. Data on gonadal concentrations of these chemicals are very sparse.

Organophosphorus pesticides are cleared relatively quickly from the vertebrate system, but in areas where agricultural use lasts for several weeks, or when it co-incides with rapid changes in the fish, as during the reproductive cycle, they may have serious consequences. Their site of accumulation, even for a short time, is of importance in determining such potential effects. Although organophosphates do not accumulate significantly in tissues over long periods, there is some evidence from mammals that their neurotoxic actions may be cumulative, and the possibility arises of long-term damage to neuroendocrine function as a result of repeated exposures.

Polyaromatic hydrocarbons (PAH) are non-polar chemicals with low water solubility which can remain attached for a considerable time to the sediments which act as reservoirs for uptake into marine animals. The presence of sediment burrowing organisms can have a considerable impact on their bioavailability, both by bringing them to the surface of the sediment and by ingesting and metabolising them so that they may be taken up by carnivorous fish. PAHs are converted into more polar products both by the ultraviolet radiation in sunlight and by metabolism in marine invertebrates and microorganisms (Cerniglia and Heitkamp, 1989; James, 1989; McElroy *et al.*, 1989). Such conversions generally involve addition of one or more hydroxyls to the aromatic ring, with eventual breakdown of one of the rings to a catechol. The rate at which these metabolites are formed increases both their bioavailability to fish and their toxicity. The metabolites, as planar polycyclic phenols also have a much closer structural similarity to the steroidal estrogens. Similar metabolism occurs in the livers of fish, and the rate of metabolism, or proportions of ingested metabolites will influence the distribution between the different tissues of the fish. The distribution also differs considerably between the different PAHs. After intragastric exposure to radioactive PAH, naphthalene was equally distributed between liver and ovary of English sole, while concentrations of its metabolites were five fold higher in ovary than liver (Figure 3.1). By contrast benzo[*a*]pyrene was much higher in ovary than liver, but its metabolites were preferentially concentrated in the liver (Varanasi *et al.*, 1989). The concentrations of PAHs in liver are

generally lower than that of PCBs and DDTs from similar locations (Table 3.5).

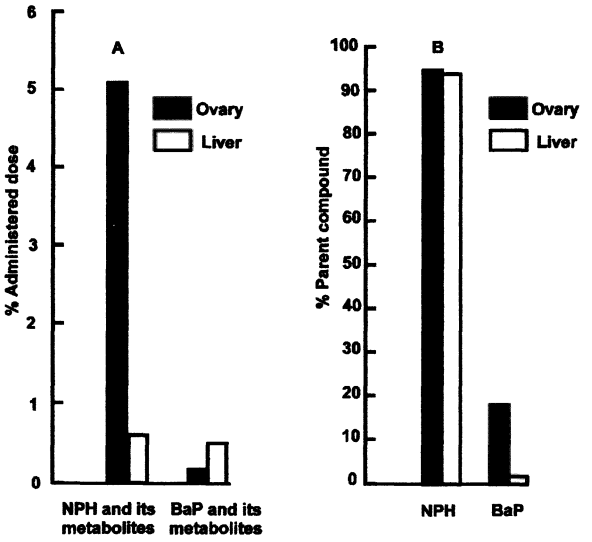


Figure 3.1. Percent of administered dose (A) and percent parent compound (B) in liver and ovary of gravid English sole exposed intragastrically to either [^{3}H]-naphthalene (NPH) or [^{3}H] benzo[*a*]pyrene (BaP). (Redrawn with permission from Varanasi, U., Stein, J. E. and Nishimoto, M. (1989). Biotransformation and disposition of polycyclic aromatic hydrocarbons (PAHs) in fish. In "Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment", (U. Varanasi, Ed.) pp. 93-149. Copyright CRC Press, Boca Raton, FL. Data from Varanasi U., Stein, J. E. and Hom, T., *Biochem. Biophys. Res. Comm.* **103**, 780 - 787, (1981) and Reichert, W. L. and Varanasi, U. *Environ. Res.* **27**, 316-324, (1982).

Apart from the studies on PCBs, organochlorine pesticides and PAHs, there is very little data on the many other industrial organic chemicals. Many phenolics and effluents from pulp mills may be considerably more water soluble and may have very different tissue distribution. The similar concentrations in gills and liver of mullets of the sodium salt of pentachlorophenol (Tachikawa *et al.*, 1987) could, for example, be due to such higher water solubility.

3.3 Factors affecting uptake of pollutants by fish

3.3.1 General

The levels of uptake of pollutants into a fish will be determined by a wide range of factors. These are discussed in many standard text books on ecotoxicology, and only a few examples are given here to indicate some of the

factors which may cause the high variability of the data shown in the Tables. The nature of the pollutant - whether it is a water soluble metal or a lipid soluble organic chemical - is clearly a major factor and this is discussed in greater detail in Sections 3.3.2 and 3.3.3 below. Also of importance is the life strategy of the species itself. For bioaccumulating chemicals, the life span is clearly a major factor and expression of the long-term exposure effects on the reproductive or immune sytems will increase with age. Also of major importance is the position of the species in the food chain. Small species with a diet of plants or invertebrates may have a relatively small exposure while the top carnivores will have an intake of food in which the pollutants have been successively bioaccumulated from the water or sediments into plants or aquatic invertebrates, and thence through increasingly larger fish. Such bioaccumulation up the food chain may increase pollutant levels several millionfold (Figure 3.2). Large long-lived fish such as cod will have exposure only a little less than the marine mammals, and problems associated with these emotive and high visible species are also likely to have similar effects on the major species of interest to commercial fisheries. Conversely the marine mammals, as consumers of these fish, will have similar problems to any other carnivorous mammal, including humans, which consumes large quantities of fish from polluted waters.

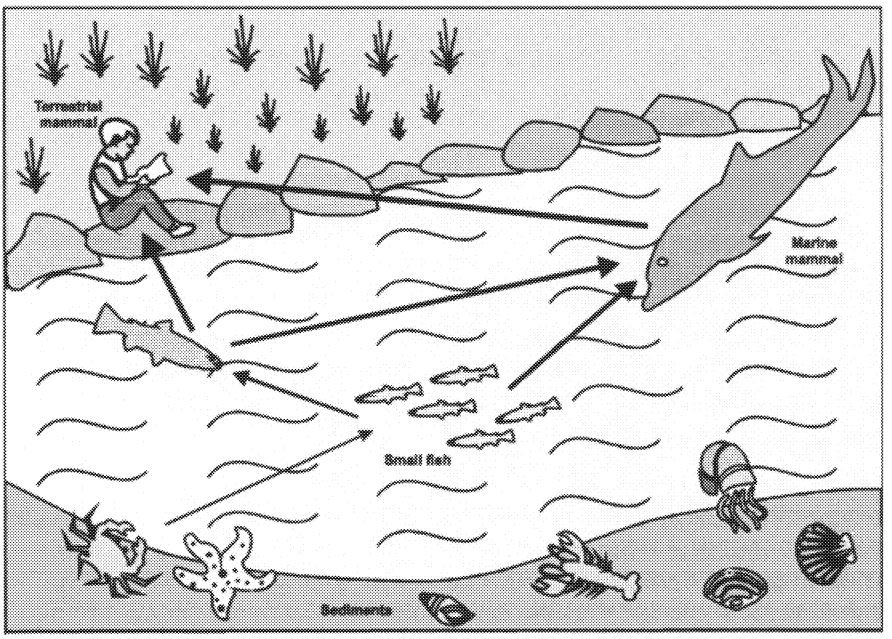


Figure 3.2. Biomagnification of pollutants through the food chain

3.3.2 Heavy metals

Uptake of heavy metals, unlike organic xenobiotics, is influenced by water hardness and acidity. Uptake of ^{65}Zn by sticklebacks, for example, was 3.5 times greater in hard water than in calcium free water during the first 5 h of exposure (Matthiessen and Brafield, 1977). Interactions in uptake have also been found between copper and cadmium (Pelgrom *et al.*, 1994), and zinc and cadmium (Wicklund *et al.*, 1988, 1990). Acidity affected lead accumulation in sunfish (*Lepomis gibbosus*) which was three times greater at pH 6.0 than at pH 7.5, although the tissue distribution was unaffected by pH (Merlini and Pozzi, 1977).

Uptake of heavy metals is influenced by the size of the fish. Mercury residues increased with increasing age and subsequently size in yellow perch (*Perca flavescens*), smallmouth bass (*Micropterus dolomieui*) and white bass (*Morone chrysops*) in Lake Erie (Kelso and Frank, 1974), in lake trout (Backe *et al.*, 1971) and in the Antarctic fish (*Pagothenia bernacchii*) (Mingati *et al.*, 1994). In flounder (*Platichthys flesus*) in the Loire estuary there was a significant positive correlation between age and whole body lead and copper content, but not of zinc or cadmium (Metayer *et al.*, 1982). Mercury accumulation was positively correlated with size in northern pike, largemouth bass, smallmouth bass, rockbass and yellow perch, but absolute levels were higher in predatory than prey fish (Wren and McCrimmon, 1986).

Temperature and season also exert an important influence on uptake of metals. The estuarine fish, *Gillichthys mirabilis* accumulated lead more readily at high temperatures and while the content of most tissue doubled between 10 and 22°, that of spleen and liver concentrations increased 20 fold (Somero *et al.*, 1977). This may be due to temperature dependent rate of flow through gill membranes, changes in membrane permeability, rate of respiration, or possibly the extent of complexing of lead to mucus (Somero *et al.*, 1977).

For migratory fish, and estuarine fish which move between fresh and sea water, uptake of pollutant may alter as they move between areas of high and low salinity. Accumulation of lead by the estuarine fish, *Gillichthys mirabilis*, for example, was inversely proportional to the salinity with similar effects in spleen, gills, fins, intestine, skin liver and muscle (Somero *et al.*, 1977). The high lead accumulation in tissues of this species which are characterised by the presence of mucus on their inner or outer surfaces may be due to complexing of the metal to mucus proteins, the greater loss of lead from mucus rich tissues being attributed to mucus turnover (Somero *et al.*, 1977).

Not unexpectedly, the highest levels of heavy metals in fish tissues are recorded in close proximity to metallurgical plants. Very high levels were found in fish from the Tigris River, Turkey which receives wastes from a copper plant. Sediment concentrations attained levels of 3433 mg/kg copper,

403 mg/kg nickel and 716 mg/kg zinc, while water levels were 0.04, 0.9 and 0.14 mg/kg respectively. In liver of *Cyprinus macrostomus*, concentrations of copper, nickel and zinc were 1497, 502 and 607 μg/g respectively and much higher than in muscle which contained 75, 8 and 47 μg/g respectively (Gümgüm *et al.*, 1994). The high levels of zinc and copper in catfish in South Africa (van den Heever and Frey, 1994) may also be associated with local mineral deposits.

3.3.3 Organic pollutants

3.3.3.1 General

The uptake of organic materials into fish has often been estimated from partition coefficients. Organic molecules partition between an aqueous phase and an organic phase, the partition coefficient depending upon the polarity of the organic chemical and that of the organic phase. Partition coefficients derived from water-organic solvent mixtures have been used to calculate the extent to which the contaminant will bioaccumulate in fatty tissues of an organism. Such an approach may be useful in some circumstances but is extremely simplistic and rather assumes that the tissue is a black bag of organic solvent (fat). The organs of most concern with respect to bioaccumulation, are far from such simple cases of partition. If the xenobiotic is to have an effect on the function of the organ it must first penetrate the cell wall and migrate to a suitable intracellular location with respect to key enzymes or genetic coding on the nucleus. Tissues such as the gonads are far from homogenous and contain many different cells between which the xenobiotic may have differential distribution. If it is stored in a fat globule it may not have any action until released as a pulse when fat stores are utilised, while ovarian accumulation could be within the developing oocyte which effectively removes it from the fish but passes it on to the next generation. Simple partitioning may no longer be a valid measure to estimate bioaccumulation for the organic pollutants which have hormone mimetic properties. Natural hormones are bound to specific plasma proteins which transport them to the target tissue, where they bind to specific receptor sites. Like the hormones they mimic, such xenobiotics may not obey simple partition rules within biological systems. Similarly both coplanar PCBs and PAHs bind to a cytosolic receptor complex (aromatic hydrocarbon = Ah-receptor) which can penetrate the cell nucleus and modulate the action of genes (Schlebusch *et al.*, 1989). Within the target cell, or after circulation through the plasma to the liver, natural hormones are deactivated, usually by reduction and conversion to water soluble conjugates which are rapidly excreted. Teleost gonads are unusual in that, unlike mammals, they are able to carry out these conversions and produce reduced and conjugated inactive metabolites. This may be one way in which the gonads are protected from

excessive amounts of the natural hormones. The plasma concentration of any hormone is a balance between production, deactivation and excretion. Negative feedback maintains a steady balance of the plasma hormone concentration (see Chapter 4). Although hormones excreted by one fish into an enclosed aquarium may be absorbed by another fish, they will not bioaccumulate since they will be steadily deactivated and will suppress natural synthesis. Synthetic hormones, such as 17-ethynylestradiol or 17-methyltestosterone, may also be absorbed from the aquarium water and elicit a response such as stimulation of vitellogenin or increased protein production. Such synthetic hormones, used as oral contraceptives or anabolic steroids, are more slowly metabolised (the 17α-ethynyl or methyl group is designed specifically to prevent metabolic conversion to the less active estrone or androstenedione) and have longer half-lives, but they will in due course be excreted. The greater activity of the synthetic steroids over the natural hormones is in great part due to this slower clearance. Unless the uptake continually exceeds excretion there will be only a temporary increase in levels. Since concentration in the plasma and tissue may exceed that in the surrounding water this can, in the short term, be considered bioconcentration, but since it behaves in a similar manner to the natural hormone, and will relatively quickly be excreted as water soluble conjugates, it will not bioaccumulate and levels will fall as soon as the external application is removed. Organochlorines, on the other hand, behave very differently. If they have endocrine activity, this may be because they bind (possibly irreversibly) to receptors or to plasma binding proteins. Their binding may, however, be less specific and they may, within the cell or organ, partition between aqueous and fatty areas. As such they may remain within the cells rather than pass back into the circulation. Even if they do so, they have little ressemblance to any natural product and the liver may be unable to metabolise and excrete them, or it may convert them to even more toxic metabolites. Many organochlorines cannot form water soluble conjugates and therefore metabolites are not likely to be so rapidly excreted. Of the many PCB congeners, the more heavily chlorinated are the most persistent since they are less readily metabolised. The hexachlorinated congeners IUPAC 138 (2,2',3,4,4',5'-hexachlorobiphenyl) and IUPAC 153 (2,2',4,4',5,5'-hexachlorobiphenyl) are the most commonly found PCBs in fish tissues (Luckas and Harms, 1987; Kelly and Campbell, 1994). Such pollutants, may therefore both bioconcentrate and bioaccumulate. In such cases tissue concentrations would be expected to increase with age and duration of exposure and may only very slowly decrease on transfer to freshwater. While the term *bioconcentration* could be applied to any substance which is taken up by the animal such that its concentration in tissue is greater than the surrounding water, *bioaccumulation* can only be used for a more permanent long-term accumulation in body tissues. *Biomagnification* occurs when

pollutants are accumulated through the food chain, where each successive predator increases its tissue concentration of the xenobiotic - a bioaccumulation through the food chain. Both bioaccumulation and biomagnification therefore refer to long term accumulation of pollutants into the organism rather than to short-term uptake.

3.3.3.2 Uptake dynamics.

The uptake of organic pollutants into tissues may alter with time reflecting redistribution between tissues. The temporal pattern of uptake of tetrachlorobiphenyl by fathead minnow (*Pimephales promelas*), for example, showed that within two hours of exposure liver content was ten times higher than the ovary, but after 5 days levels were similar and by 12 days ovarian levels were 2.5 times greater than the liver (Lindstrom-Seppa *et al.*, 1994). This suggests a transfer from liver to ovary, possibly associated with vitellogenin uptake by oocytes.

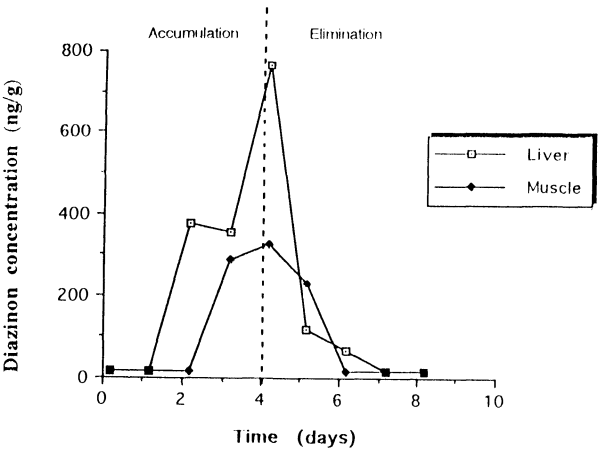


Figure 3.3. Uptake and clearance of the organophosphate, diazinon, from liver and muscle of the eel, *Anguilla anguilla*. (Reproduced from Sancho, E., Ferrando, M. D., Andreu, E. and Gamon, M. (1993). Bioconcentration and excretion of diazinon by eel. *Bull. Environ. Contam. Toxicol.* **50**, 578-585, Fig. 2, by kind permission of Springer-Verlag, Berlin and the author).

Unlike the organochlorines, organophosphates are rapidly removed from the tissues once exposure has ceased. For example, when eels were exposed to 0.056 μg/l diazinon, the concentration of the pesticide in the water decreased to zero by 3 days and concentrations in muscle and liver were 0.29 and 0.09 μg/g respectively (bioconcentration ratios of 1600 and 900), but by the second day after return to clean water the levels in the tissue were undetectable (Figure 3.3) (Sancho *et al.*, 1993). It is also important to note

that after 1 day of exposure the water concentration in the aquarium had fallen to only 1% of the initial level but that in a real polluted river, tissue concentrations would be expected to continue rising for rather longer since the aquatic source is no longer finite as diazinon may persist in the aquatic environment (Kanazawa, 1978; Ferrando *et al.*, 1992). A range of other organophosphates and carbamates showed similar bioconcentration factors and rapid excretion within a few days, although there were major differences between different pesticides and between species (Tsuda *et al.*, 1989, 1990, 1994) (Table 3.6). The results suggest that a short term exposure to sublethal levels of an organophosphate may not be harmful due to its rapid excretion, but if this coincided with a period of reproduction in which hormonal balances are changing rapidly as during final oocyte maturation the result could nevertheless be serious. A similar rapid excretion of diazinon and its metabolite pyrimidol was found in guppy and zebrafish (Keizer *et al.*, 1991), but there were differences between the two species in the extent of oxidative biotransformation of the pesticide which may affect both toxicity and rate of excretion. Alkylphenolics are also rapidly taken up by fish tissues but are completely excreted within several days on return to freshwater (McLeese *et al.*, 1981).

3.3.3.3 Uptake from sediment

Pollutants accumulate in the sediments of the rivers and seas. From there uptake into fish could be considered to occur by 1) desorption from sediment to water and uptake from water via gills, 2) uptake via resuspended particles containing pollutants and then ingestion or absorption and 3) feeding on invertebrates or plants that have absorbed the pollutant from the sediment. Eels, *Anguilla anguilla*, held for 77 days over sediment containing 1 μg/g PCB accumulated 2.5 - 8.9 μg/g if separated from the sediment by a net, but 7.2 - 14.1 μg/g if allowed to feed in the sediment which contained chironomids and tubificids (Larsson, 1984) indicating that a major source of PCB uptake was via water (which contained 0.1 - 0.7 μg/l) rather than from sediment or invertebrates (Larsson, 1984). Similar conclusions were obtained with the spot (*Leiostomus xanthurus*) in which half the PCB uptake from sediment was derived from the water while the rest derived from exposed worms (Rubinstein *et al.*, 1984). The results suggested, however, that after longer exposures the dietary contribution could be greater.

3.3.3.4 Effect of lipid.

Since chlorinated hydrocarbons are extremely oil soluble, the different fat content between species or tissues, or resulting from age or maturation will affect the amount that is accumulated. Differences in the levels of insecticides between species is considerably less if concentrations are calculated on the basis of oil. Levels of DDT and dieldrin in bloaters was three times greater

than in yellow perch, but on an oil basis there was little difference between the species. Similarly the higher DDT content in larger lake trout was related to their greater percentage of fat (Reinert, 1979).

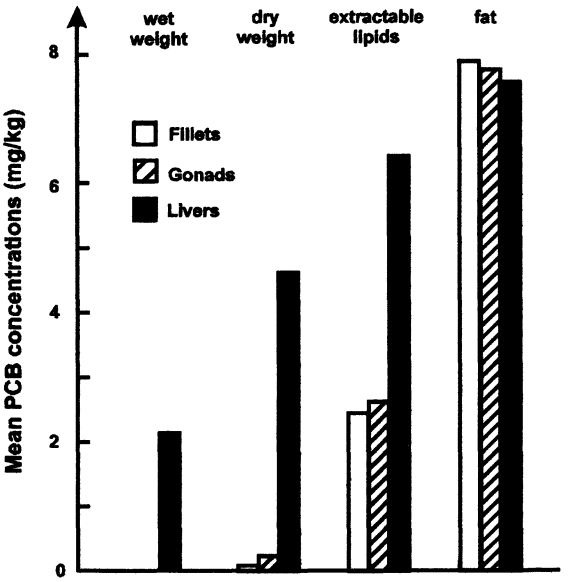


Figure 3.4. Mean PCB concentration in cod fillets (open bars), gonads (hatched bars) and livers (solid bars) normalized to wet weight, dry weight, extractable lipids (material soluble in 10% water in methanol), and fat (material soluble in n-hexane). (Redrawn from Schneider, R. (1982). Polychlorinated biphenyls (PCBs) in cod tissues from the Western Baltic: Significance of equilibrium partitioning and lipid composition in the bioaccumulation of lipophilic pollutants in gill breathing animals. *Meeresforsch.* **29**, 69-79 by kind permission of Verlag Paul Perey).

The lipophilic nature of PCBs is shown by its higher accumulation in the lipid rich red (22% lipid; 4.2 μg/g) than white muscle (3.2 % lipid; 0.4 μg/g) tissue of the female paddlefish (*Polyodon spathula*) in the Ohio River. Levels were highest in the lipid rich ovaries (43.6% lipid, 7.3 μg/g) and testes (54.8% lipid, 16.2 μg/g) (Gundersen and Pearson, 1992). In this study PCB levels were also lower in mature ovaries than in immatures which had a higher lipid content. There was a significant correlation between PCB content and percentage lipid in muscle and reproductive tissue. Similar effects of tissue fat content on accumulation were found in cod. On a simple weight basis, PCB concentrations were 15 times higher in liver than gonad, while muscle concentrations were only one-tenth that of gonads (Figure 3.4). On the basis of concentration in extractable fat, however, all three tissues contained very similar amounts of PCBs (Schneider, 1982). Clearly there will be significant

species differences in bioaccumulation in such tissues since their fat content will vary from species to species.

3.3.3.5 Effect of age, size and sexual maturity

If there is a steady bioaccumulation as a result of failure of the fish to deactivate and excrete the pollutant, accumulation would be expected to increase with time of exposure. In wild fish this would relate to age. In any population, larger fish would be expected to be the older ones, although in the species as a whole this may not be a valid assumption since pollutants might also affect growth.

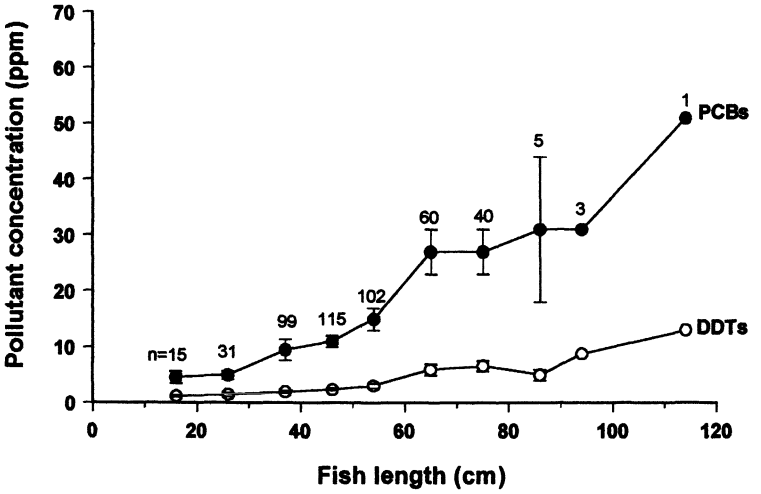


Figure 3.5. Relationship between concentration of PCBs and DDTs and length of cod. (Data from Falandysz, J. (1984). Organochlorine pesticides and polychlorinated biphenyls in livers of cod from the Southern Baltic, 1981. *Z. Lebensm. Unters. Forsh.* **179**, 311-314)

There is some evidence that organochlorines do increase with size of the fish. In Baltic cod, liver concentrations of DDTs and PCBs increased from 1.2 and 4.6 μg/g respectively in 11-20 cm cod to 6.0 and 27 μg/g in 70-80 cm fish (Figure 3.5) (Falandysz, 1984). The trend continued with larger fish but sample sizes were small. Similar results were obtained if calculation was made on a lipid weight basis rather than liver weight. No such correlation, however, was found in cod from the Northwest Atlantic (Hellou *et al.*, 1993). The relation also held for some, but not all, salmonines in the Great Lakes. Larger lake trout from Lake Superior had higher DDT content than smaller

fish, but much of this was attributed to the increase in fat content in larger fish and when calculated on an oil basis, equilibrium was reached when fish attained 25-30 cm (Reinert, 1979). Miller (1993) has drawn attention to the importance of capturing fish at a uniform time of their reproductive cycle since significant amounts of the pollutant burden can be lost in the eggs during spawning - particularly in semelparous salmonids such as the chinook salmon which loses an estimated 28-39% of its PCBs into developing eggs. The higher levels of PCBs in testes than ovaries of the paddlefish in the Ohio River was attributed to the lower weight of milt lost in males compared to eggs in females (Gundersen and Pearson, 1992). In the English sole (*Parophrys vetulus*) 24 h after injection with 80 mg/kg body weight benzo[*a*]pyrene, concentrations were much higher (165.5 μg/g) in semen than in testis (25.1 μg/g). While this may be good for the male fish, such concentration in close proximity to sperm may have more serious consequences for its ability to fertilise, and for the survival, health and fertility of the offspring. PCBs themselves may in fact increase both the hepatosomatic index and the liver lipid content of salmon, possibly acting via the thyroid gland (Leatherland *et al.*, 1979), leading to increased uptake.

3.3.3.6 Effect of salinity

Uptake of the pesticide chlordane differed between fish held in sea water and those that had been adapted to freshwater. After 24 h exposure of mullet (*Mugil cephalus*) to chlordane (0.050 mg/kg), freshwater acclimated fish had accumulated 4.2 μg/g in the liver while seawater fish had only 1.1 μg/g (Tachikawa *et al.*, 1987). A similar dfference was found for exposure to 0.2 mg/l pentachlorophenol-Na (PCP-Na), but the differences in liver concentrations were much smaller (20 vs 15 μg/g). Such differences in accumulation may be important factors for any fish which migrates between fresh and sea water, and it is important to note that the LC_{50} of PCP-Na was much greater in seawater (0.26 mg/l) than in freshwater (0.04 mg/l). Similar results had been found by these authors with the euryhaline tilapia (*Tilapia nilotica*). For euryhaline fish moving between the salt and fresh water of a polluted estuary, the proportion of time spent in the two different environments would have significant effects on tissue concentrations of pollutants even though the aquatic concentration might be the same.

3.3.3.7 Geographical distribution

Although there were differences between PCB levels in cod liver oil originating from fish in the Baltic, North Sea and North Atlantic, this was relatively small ranging from 7.9 μg/g in the Southern Baltic to 2 μg/g off Iceland, and Falandysz (1994) has suggested that this is due predominantly to the continuous release of these chemicals into the atmosphere and aerial dispersal over a wide area. Hellou *et al.* (1993), however, cites data showing

a wider range of values in liver tissue of cod, with lowest values in the Northwest Atlantic (0.15 μg/g), Northern Finland (0.26 μg/g) and Norway (0.36 μg/g), higher levels in Nova Scotia (1.71 μg/g) and the northern Baltic (1.1 μg/g), while the southern Baltic recorded extremely high levels (27 μg/g). DDTs showed a similar pattern of distribution ranging from 0.16 μg/g in the Northwest Atlantic to 6 μg/g in the Southern Baltic. Within the North Sea, the hepatic organochlorine content of Pleuronectiformes was high in areas of input from the major rivers, but high levels, especially of DDE, were also present in fish from the area of the mid-North Sea used for waste incineration. Lindane was particularly high in fish captured along the Dutch and German coasts (Knickmeyer and Steinhart, 1990). Residues of DDT and its metabolites were high in fish from the Great Lakes, reflecting the extensive use of this pesticide during the 1950s.

The primary pollutant is, in many cases, transformed to a number of metabolites in the aquatic environment. γ-HCH (lindane) is transformed to α-HCH, while DDT is converted firstly to DDE and thence to DDD. The ratio of the primary pollutant to its metabolites in fish tissue can be used to determine the time spent by the pollutant in the environment since it was discharged (de Boer, 1989; Hellou *et al.*, 1993). Data on liver from cod captured in the Northwest Atlantic indicated that the pollutants had been transported a considerable distance (Hellou *et al.*, 1993). The presence of significant amounts of DDT in fish from Michigan Rivers in 1990, 20 years after its use ceased in North America was also indicative of very long range atmospheric transport from areas of the world where it is still in use (Giesy *et al.*, 1994a).

3.3.3.8 Are concentrations of pollutants decreasing?

Use of DDT ceased in the Baltic States and much of N. America in the early 1970s, and that of PCBs has been restricted for a similar period. It might therefore be expected that concentrations of these pollutants in fish tissue would now be showing signs of a decrease. These chemicals are, however, very persistent and high levels remain in the sediments from previous usage, while there is also considerable atmospheric deposition from areas of the world in which they are still in use (Kihlström and Berglund, 1978; Falandysz, 1994).

Levels of a range of organochlorines in cod off Nova Scotia, including PCBs, DDTs, HCBs showed little change from 1972 to 1980, apart from a temporary decline in PCB and DDT from 1972 to 1975 (Freeman *et al.*, 1984a). PCB levels in Baltic cod liver oil maintained a steady state condition between 1971 and 1989 (Falandysz, 1994). DDT in Baltic herring started to decline between 1969 and 1972, and PCBs between 1975 and 1978 (Olsson and Reutergardh, 1986). In five species of Great Lakes salmonine the proportions of PCBs, chlordane and dieldrin remained constant between

samplings in 1985 and 1990 while muscle concentrations of all three pollutants showed a decline of about 30%, except for the chinook salmon in which no change occurred (Miller *et al.*, 1993). Organochlorine pollutants such as PCBs and chlordane changed little from 1982-1992, and PCBs levels were in excess of the U.S. Food and Drug Administration's (FDA) action limit in 31% of channel catfish sampled between 1978 and 1986. Levels in excess of the FDA limit were still being consistently detected in the Ohio River in 1992 (Gundersen and Pearson, 1992). The data overall suggests that organochlorine pollutants have attained a steady state in fish tissues, reflecting the great persistence of these pollutants in the sediments and their continued dispersion, albeit in reduced quantities, into the aquatic ecosystem by atmospheric and terrestial pathways. The absence of any significant downward trend in their concentrations in fish tissues since use of these chemicals was discontinued over twenty years ago suggests that their effects will be exerted for some considerable time to come.

3.4 Uptake of pollutants into eggs and larvae

Eggs can derive their pollutant burden from two sources, the parent or the aquatic environment. Maternal intake will reflect that of the parent during the period of vitellogenesis when the yolk is laid down in the oocyte. Direct uptake by the egg may be restricted to certain phases while it retains a membrane permeability, except for binding of metals to the chorion which is not dependent upon such permeability. Such uptake may be affected by similar environmental factors to that of uptake by the parent. Using ^{65}Zn, Wedermeyer (1968) showed that uptake of the metal by eyed coho salmon eggs was affected by pH and temperature. 70% of the metal was firmly bound to the chorion, while 26% was found in the perivitelline fluid. Exposure of rainbow trout eggs to 10 mg/l cadmium for 22 h also showed that accumulation was predominantly in the chorion (860 μg/g) compared to the embryo body (16 μg/g) or yolk (2 μg/g) (Beattie and Pascoe, 1978). Organic pollutants may be passed unmetabolised from parent to egg and only become metabolised when the larvae attain an age at which hepatic enzymes are functional. When adult brook trout were fed 2 μg/g DDT for 156 days prior to spawning, concentrations in both eggs and fry were around 3 μg/g and while the pesticide was largely unmetabolised in the eggs, fry had converted 43% into DDE and DDD (Macek, 1968). Uptake of PAHs and PCBs by eggs and larvae directly from the water may have serious consequences on fish stocks if the spawning grounds are in areas affected by marine pollution or offshore oil fields. Using radiolabelled naphthalene, phenanthrene, benzo[*a*]pyrene and 2,4,5,2',4',5'-hexachlorobiphenyl (PCB), Solbakken *et al.* (1984) found that maximum accumulation into 1 day eggs, yolk-sac larvae

and 9 day larvae occurred with phenanthrene, while naphthalene accumulated only slowly and was more rapidly eliminated. The low uptake of PCB by eggs may be a result of it's difficulty in penetrating the chorion.

3.5 Tissue concentrations as a bioindicator of aquatic pollution

Measurement of tissue concentrations of pollutants can give not only an estimate of the extent of pollution of the habitat in which the fish lives but also some indication as to whether the populations are under threat from accumulation of such toxins and their subsequent disruption of vital systems such as reproduction or respiration. Fish are exposed to a wide range of pollutants, but assay methodology permits only measurement of a very limited number. Clearly, high concentrations of organochlorines or PAHs are indicative of heavy industrial and/or agricultural pollution, while elevated heavy metal concentrations indicate the presence of mining activity or metallurgical processes. Such data provides a general indication of a polluted ecosystem. Further extrapolation has dangers since pollutant uptake is affected by a large number of variables which have been discussed in this Chapter. Species and tisssues vary in their lipid content and therefore ability to sequester organic pollutants, while uptake is affected by both the diet of the fish and a number of physical variables which relate to transfer from the sediment to the fish, such as temperature and water quality. Between species, there may be extensive variation in the susceptibility of the vital systems to a given level of pollutant, and what may be safe in one species may have serious consequences for another. It must also be remembered that since high tissue concentrations of PCB, for example, are only *indicative* of industrial pollution, there is not necessarily a cause-effect relationship between high PCB and toxic effects on reproduction, egg survival, or enzyme activity since these may well be caused by other unmeasured pollutants which are present in the same area. The numbers of chemicals released into the environment is enormous, and very few of these are measured during tissue analysis. The data given in the Tables and elswhere in the literature for PCBs, pesticides etc represent only the chemicals that have been measured. These values can also be indicative of the tissue uptake of pollutants with similar chemical properties, so that a high PCB content for example will also reflect high concentration of other lipophilic chemicals present in the same sediments or diet. Cluster analyses have been used to analyse the behaviour of different groups of chemicals within tissues and between species (Hellou *et al.*, 1995) and this may provide some information on the chemicals likely to be found together. Its application to other industrial chemicals which have so far received little attention would be of considerable interest.

Table 3.1. Tissue concentrations of heavy metals in wild captured fish in µg/g (ppm) wet weight. For comparison, literature values in dry weight have been converted on the basis of 1 g dry tissue = 5g wet tissue. (m) and (f) indicate male and female fish. Year indicates capture date

				Concentration (µg/g) wet weight					
	Species	Exposure site (mg/l)	Year	whole	muscle	liver	gonad	kidney	gills	Reference
Cadmium	*Abramis brama*	Industrial water, UK.			2.0	1.7	1.6	1.1	2.2	Badsha and Goldspink, 1982
	Alepes djeddaba	Red sea coast	1991		0.17	1.56		3.21	1.96	Al-Mohanna, 1994
	Caranx melamphygus	Red sea coast	1991		0.16	3.84		0	1.74	Al-Mohanna, 1994
	Lenthrinus lentjan	Red sea coast	1991		2.53	0.5		1.0	0.16	Al-Mohanna, 1994
	Lepomis macrochirus	urban runoff, Florida	1991-92	0.006						Campbell, 1994
	Lepomis microlophus	urban runoff, Florida	1991-92	1.64						Campbell, 1994
	Lutjanus johni	Red sea coast	1991		1.56	2.53		0	0.66	Al-Mohanna, 1994
	Micropterus salmoides	urban runoff, Florida	1991-92	3.16						Campbell, 1994
	Platichthys flesus	Loire estuary	1979		0.002	0.18		0.09	0.02	Metayer, *et al.*, 1982
		Elbe estuary (N. Sea)				0.29				Luckas and Harms, 1987
	Rutilus rutilus	Industrial water, UK.			1.3	32	3.1	51	0.7	Badsha and Goldspink, 1982
	Scarus strongylocephalus	Red sea coast	1991		0.2	2.37		0	1.41	Al-Mohanna, 1994
	Solea solea	Loire estuary	1979		0.002	0.10		0.06	0.02	Metayer, *et al.*, 1982
Copper	*Alepes djeddaba*	Red sea coast	1991		0.13	5.56		1.13	0.13	Al-Mohanna, 1994
	Caranx melamphygus	Red sea coast	1991		0.15	3.7		0.55	0.92	Al-Mohanna, 1994

Table 3.1 (continued)

Species	Exposure site (mg/l)	Year	Concentration (μg/g) wet weight whole	muscle	liver	gonad	kidney	gills	Reference
Carassius auratus (f)	Bologna	1989-90		0.5	4.1	1.4	2.1		Carpene *et al.*, 1994
Catastomus commersoni (f)	metal contam. lake	1987				3.8			Munkittrick and Dixon, 1989
Catastomus commersoni (m)	metal contam. lake	1987				3.1			Munkittrick and Dixon, 1989
Clarias gariepinus	S. Africa sewage effluent	1991		7	891		100		van den Heever and Frey, 1994
Cyprinion macrostomus	Cu-plant waste-0.04Cu			75	149 7				Gümgüm *et al.*, 1994
Cyprinus carpio (f)	Bologna	1989-90		0.3	3.8	0.7	1.2		Carpene *et al.*, 1994
Garra rufa	Cu-plant waste-0.04Cu			87	111 4				Gümgüm *et al.*, 1994
Ictalurus melas (f)	Bologna	1989-90		0.34	4.5	1.2	1.4		Carpene *et al.*, 1994
Lenthrinus lentjan	Red sea coast			0.17	3.2		1.3	0.59	Al-Mohanna, 1994
Lepomis macrochirus	urban runoff, Florida	1991 92	2.08						Campbell, 1994
Lepomis microlophus	urban runoff, Florida	1991-92	3.81						Campbell, 1994
Lutjanus johni	Red sea coast	1991		0.19	3.4		1.7	0.65	Al-Mohanna, 1994
Oncorhynchus kisutch	Washington, USA	1993	1.91						Felton *et al.*, 1994
Platichthys flesus	Loire estuary	1979		0.4	18	3.6	0.8	0.4	Metayer *et al.*, 1982
Scarus strongylocephalus	Red sea coast	1991		0.57	2.2		0.51	0.47	Al-Mohanna, 1994
Solea solea	Loire estuary	1979		0.2	34	70	0.8	0.4	Metayer *et al.*, 1982

Table 3.1 (continued)

	Species	Exposure site (mg/l)	Year	Concentration (µg/g) wet weight whole	muscle	liver	gonad	kidney	gills	Reference
Lead	*Abramis brama*	Industrial water, U. K.			2.1	2.1	1.0	4.1	3.5	Badsha and Goldspink, 1982
	Alepes djeddaba	Red sea coast	1991		1.8	15		0	4.4	Al-Mohanna, 1994
	Cyprinion macrostomus	Cu-plant waste-0.5Pb			nd	0.4				Gümgüm *et al.*, 1994
	Garra rufa	Cu-plant waste-0.5Pb			nd	0.4				Gümgüm *et al.*, 1994
	Lenthrinus lentjan	Red sea coast	1991		1.9	5.0		7.8	4.0	Al-Mohanna, 1994
	Lepomis microlophus	urban runoff, Florida	1991-92	16						Campbell, 1994
	Lepomis macrochirus	urban runoff, Florida	1991-92	0.77						Campbell, 1994
	Lutjanus johni	Red sea coast	1991		1.9	25		0	3.9	Al-Mohanna, 1994
	Micropterus salmoides	urban runoff, Florida	1991-92	12						Campbell, 1994
	Platichthys flesus	Loire estuary	1979		0.2	1.6		1.2	0.6	Metayer *et al.*, 1982
		Elbe estuary (N. Sea)				0.12				Luckas and Harms, 1987
	Rutilus rutilus	Industrial water, U. K.			0.6	18	2.0	21	9	Badsha and Goldspink, 1982
	Scarus strongylocephalus	Red sea coast	1991		2.7	27		0	4.2	Al-Mohanna, 1994
	Solea solea	Loire estuary	1979		0.3	3		5	0.6	Metayer *et al.*, 1982
Mercury	*Abramis brama*	Finnish lake	1972-74	0.34						Hattula *et al.*, 1978
	Alepes djeddaba	Red sea coast	1991		1.4	7.8		0	7.4	Al-Mohanna, 1994

Table 3.1 (continued)

Species	Exposure site (mg/l)	Year	Concentration (μg/g) wet weight whole	muscle	liver	gonad	kidney	gills	Reference
Biardiella chrysura	S. Carolina	1972-74	3.06						Reimold and Shealy, 1976
Caranx melamphygus	Red sea coast	1991		0.95	7.4		0	2.2	Al-Mohanna, 1994
Carassius carassius	Japanese rivers (0.00003)		0.3						Matsunaga, 1975
	Finnish lake	1972-74	0..27						Hattula *et al.*, 1978
Coregonus albula	Finnish lake	1972-74	0.42						Hattula *et al.*, 1978
Coregonus lavaretus	Finnish lake	1972-74	0.42						Hattula *et al.*, 1978
Esox lucius	Finnish lake	1972-74	1.07						Hattula *et al.*, 1978
Esox lucius (m/f)	Canadian lake	1970		1.0	1.6	0.4/0.6	2.2	0.5	Lockhart *et al.*, 1972
Leuciscus idus	Finnish lake	1972-74	0.35						Hattula *et al.*, 1978
Lenthrinus lentjan	Red sea coast	1991		1.6	3.0		3.3	4.7	Al-Mohanna, 1994
Lota lota	Finnish lake	1972-74	1.51						Hattula *et al.*, 1978
Lutjanus johni	Red sea coast	1991		1.4	6.3		0	5.4	Al-Mohanna, 1994
Micropogonias undulatus	S. Carolina	1972-74	0.80						Reimold and Shealy, 1976
Osmerus eperlanus	Finnish lake	1972-74	0.42						Hattula *et al.*, 1978
Pagothenia bernacchii	Antarctica	1989-90		0.09					Minganti *et al.*, 1994
Platichthys flesus	Elbe estuary (N. Sea)			0.32					Luckas and Harms, 1987
	N. Wales/NW England			0.23					Leah *et al*;., 1992
Rutilus rutilus	Finnish lake	1972-74	0.50						Hattula *et al.*, 1978
Salvelinus namaycush	New York Lake	1970	0.5						Backe *et al.*, 1971
Scarus strongylocephalus	Red sea coast	1991		1.7	6.8		0	6.0	Al-Mohanna, 1994

Table 3.1 (continued)

	Species	Exposure site (mg/l)	Year	Concentration (µg/g) wet weight whole	muscle	liver	gonad	kidney	gills	Reference
	Stellifer lanceolatus	S. Carolina	1972-74	0.43						Reimold and Shealy, 1976
	Tribolodon hakonensis	Japanese rivers (0.00005)		0.7						Matsunaga, 1975
		Japanese river (0.000004)		0.012						Matsunaga, 1975
Nickel	*Cyprinion macrostomus*	Cu-plant waste-0.9Ni			47	607				Gümgüm *et al.*, 1994
	Garra rufa	Cu-plant waste-0.9Ni			66	483				Gümgüm *et al.*, 1994
	Lepomis macrochirus	urban runoff, Florida	1991-92	0.156						Campbell, 1994
	Lepomis microlophus	urban runoff, Florida	1991-92	5.32						Campbell, 1994
	Micropterus salmoides	urban runoff, Florida	1991-92	2.46						Campbell, 19943
Zinc	*Abramis brama*	Industrial water, U. K			3.0	71	86	95	73	Badsha and Goldspink, 1982
	Alepes djeddaba	Red sea coast	1991		7.3	34		0	63	Al-Mohanna, 1994
	Caranx melamphygus	Red sea coast	1991		2.8	73		22	16	Al-Mohanna, 1994
	Carassius auratus (f)	Bologna	1989-90		8.7	16	61	44		Carpene *et al.*, 1994
	Catastomus commersoni (m/f)	metal contam. lake	1987				18/23			Munkittrick and Dixon, 1989
	Clarias gariepinus	S. Africa sewage effluent	1991		213	3385		7117		Van den Heever and Frey, 1994

Table 3.1 (continued)

Species	Exposure site (mg/l)	Year	Concentration (μg/g) wet weight whole	muscle	liver	gonad	kidney	gills	Reference
Cyprinion macrostomus	Cu-plant waste-0.14Zn			47	607				Gümgüm *et al.*, 1994
Cyprinus carpio (f)	Bologna	1989-90		5.6	67	110	186		Carpene *et al.*, 1994
Garra rufa	Cu-plant waste-0.14Zn			66	483				Gümgüm *et al.*, 1994
Ictalurus melas (f)	Bologna	1989-90		4.5	17.1	55	21		Carpene *et al.*, 1994
Lenthrinus lentjan	Red sea coast	1991		4.1	240		5.6	7.6	Al-Mohanna, 1994
Lepomis macrochirus	urban runoff, Florida	1991-92	17						Campbell, 1994
Lepomis microlophus	urban runoff, Florida	1991-92	42						Campbell, 1994
Lutjanus johni	Red sea coast	1991		11	16		0	19	Al-Mohanna, 1994
Micropterus salmoides	urban runoff, Florida	1991-92	30						Campbell, 1994
Platichthys flesus	Loire estuary	1979		10	28	119	53	25	Metayer *et al.*, 1982
Rutilus rutilus	Industrial water, U. K.			18	122	124	288	112	Badsha and Goldspink, 1982
Scarus strongylocephalus	Red sea coast	1991		2.7	10		0	38	Al-Mohanna, 1994
Solea solea	Loire estuary	1979		10	18	151	20	9	Metayer *et al.*, 1982

Table 3.2. Tissue concentrations of PCBs in wild captured fish in μg/g (ppm) wet weight. For notes, see Table 3.1

Species		Exposure site	Year	Concentration (μg/g) wet weight whole	muscle	liver	gonad	Reference
Clupea harengus		Clyde	1991		0.11			Kelly and Campbell, 1994
		Firth of Forth	1991		0.02			Kelly and Campbell, 1994
		N. North Sea	1991		0.05			Kelly and Campbell, 1994
	(f)	Baltic Sea	1979		0.9	0.2	0.1	Hansen *et al.*, 1985
	(f)	S. Baltic	1979				0.12	Von Westernhagen *et al.*, 1987
Cyprinus carpio		Michigan rivers	1990	6.0				Giesy *et al.*, 1994
Esox lucius		Finnish lake	1987		6.2/3.0			Koistinen *et al.*, 1989
Gadus morhua		Clyde	1990			5.1		Kelly and Campbell, 1994
		Firth of Forth	1991			1.3		Kelly and Campbell, 1994
		N. North Sea	1991			0.5		Kelly and Campbell, 1994
		Liverpool Bay			0.003	5.6		MAFF, 1991
		S. North Sea			0.008	1.6		Harms and Kerkhoff, 1988
		Nova Scotia, Canada	1980			1.71		Freeman *et al.*, 1984a
	(f)	NW Atlantic	1991		0.001	0.15	0.05	Hellou *et al.*, 1993
		W. Atlantic	1983			0.85		Uthe *et al.*, 1984
		Baltic	1978/84				0.05	Von Westernhagen *et al.*, 1987
		Baltic	1977-83		0.09	7.6		Huschenbeth, 1986
		Baltic	1983		0.058			Falandysz, 1986
		Baltic	1981			27		Falandysz, 1984
		N. Baltic	1980			2.3		Wickstrom and Pyysalo, 1981
		N. Baltic	1980			1.1		Haati and Pertilla, 1988
		W. Baltic	1977		0.023			Schneider, 1982
		W. Baltic	1984			2.3		Staveland and Marthinsen, 1989
		Finland	1978		0.032			Miettinen *et al.*, 1985
		N Finland	1985-9			0.26		Paasivirta and Rantio, 1991

Table 3.2 (continued)

Species	Exposure site	Year	Concentration (μg/g) wet weight whole	muscle	liver	gonad	Reference
	Norway	1982			0.5		Skare *et al.*, 1985
	Norwegian fiord (4 weeks)	1990			0.23[a]		Goksøyr *et al.*, 1994
	Norway	1988			0.36		Koistinen *et al.*, 1989
Gadus morhua (liver oil)	North Sea (UK)	1982			4.6		Falandysz, 1994
	Norwegian Sea (Norway)	1983			5.1		Falandysz, 1994
	N. Atlantic (Iceland)	1984			2.0		Falandysz, 1994
	Baltic Sea	1971			8.0		Falandysz, 1994
	Baltic Sea	1980			17		Falandysz, 1994
	Baltic Sea	1985			10		Falandysz, 1994
Genyonemus lineatus (f)	S. California	1985-6			1.35	1.67	Cross and Hose, 1988
Hippoglossoides platessoides	North Sea	1987			0.82[a]		Knickmeyer and Steinhart, 1990
Limanda limanda	North Sea	1987			0.83[a]		Knickmeyer and Steinhart, 1990
	Liverpool Bay			0.066	2.2		MAFF, 1991
Merlangus merlangus	Liverpool Bay			0.012	6.1		MAFF, 1991
(f)	Dutch N. Sea	1984			3.95	0.13	Von Westernhagen *et al.*, 1987, 1989
(m)	Dutch N. Sea	1984			4.53	0.09	Von Westernhagen *et al.*, 1989
Oncorhynchus kisutch	Lake Ontario			5.3			Leatherland and Sonstegard, 1982
	Pacific Ocean			0.17			Leatherland and Sonstegard, 1982
	Lake Michigan	1985		1.10			Miller *et al.*, 1993
	Lake Michigan	1990		0.76			Miller *et al.*, 1993
Oncorhynchus mykiss	Lake Michigan	1985		1.08			Miller *et al.*, 1993
	Lake Michigan	1990		0.66			Miller *et al.*, 1993
	Michigan rivers	1990	3.9				Giesy *et al.*, 1994

Table 3.2 (continued)

Species	Exposure site	Year	Concentration (µg/g) wet weight whole	muscle	liver	gonad	Reference
Oncorhynchus tshawytscha	Lake Michigan	1985		1.05			Miller *et al.*, 1993
	Lake Michigan	1990		1.05			Miller *et al.*, 1993
Platichthys flesus	Elbe estuary	1977-83		0.21	2.5		Huschenbeth, 1986
	Liverpool Bay			0.01	0.88		MAFF, 1991
	Elbe estuary (N. Sea)			0.28			Luckas and Harms, 1987
	S. Baltic (f)				0.36	0.16	Von Westernhagen *et al.*, 1981
	S. Baltic (f)	1978-84				0.16	Von Westernhagen *et al.*, 1987
Platichthys stellatus	San Francisco Bay	1986-7			12.9[a]		Spies *et al.*, 1990
Pleuronectes platessa	Liverpool Bay			0.02	1.9		MAFF, 1991
	North Sea	1987			1.72		Knickmeyer and Steinhart, 1990
Polyodon spathula (m/f)	Ohio River	1988-9		0.7/0.4		16.2/7.3	Gundersen and Pearson, 1992
Salmo namaycush	Lake Michigan	1985		3.71			Miller *et al.*, 1993
	Lake Michigan	1990		2.44			Miller *et al.*, 1993
Salmo salar	N. Baltic	1986		0.345			Koistinen *et al.*, 1989
Salmo trutta	Lake Michigan	1985		1.99			Miller *et al.*, 1993
	Lake Michigan	1990		0.77			Miller *et al.*, 1993
Salvelinus fontinalis	Lake Michigan	1985		0.90			Miller *et al.*, 1993
Solea solea	Liverpool Bay				0.39		MAFF, 1991
Stellifer lanceolatus	Georgia USA	1972-4	0.51				Reimold and Shealy, 1976
Stizostedion vitreum	Michigan rivers	1990	3.5				Giesy *et al.*, 1994
	Finnish Lake	1987		13.7			Koistinen *et al.*, 1989

[a] expressed as µg/g in extractable lipid.

Table 3.3. Tissue concentrations of DDT and its metabolites in wild captured fish in μg/g (ppm) wet weight. For notes see Table 3.1

				Concentration (μg/g) wet weight				
	Species	Exposure site	Year	whole	muscle	liver	gonad	Reference
DDTs	*Alosa pseudoharengus*	Lake Michigan	1965-68	3.89				Reinert, 1979
	Clupea harengus	Clyde	1991		0.036			Kelly and Campbell, 1994
		Firth of Forth	1991		0.003			Kelly and Campbell, 1994
		N. North Sea	1991		0.013			Kelly and Campbell, 1994
	(f)	Baltic Sea	1979		0-0.4	0-0.1	0.25	Hansen *et al.*, 1985
	(f)	S. Baltic	1979				0.025	Von Westernhagen *et al.*, 1981
	Coregonus artedii	Lake Michigan	1965-68	6.71				Reinert, 1979
	Coregonus hoyi	Lake Michigan	1965-68	8.61				Reinert, 1979
	Cyprinus carpio	Michigan rivers	1990	0.77				Giesy *et al.*, 1994
	Esox lucius	Michigan rivers	1990	0.28				Giesy *et al.*, 1994
	Gadus morhua	Clyde	1990			0.46		Kelly and Campbell, 1994
		Firth of Forth	1991			0.16		Kelly and Campbell, 1994
		N. North Sea	1991			0.11		Kelly and Campbell, 1994
		Nova Scotia,	1980			0.53		Freeman *et al.*, 1984a
		S. North Sea			0.003	0.75		Harms and Kerkhoff, 1988
		S. Baltic	1981			6.6		Falandysz, 1984
		Baltic	1977-83		0.08	5.2		Huschenbeth, 1986
	(f)	Baltic	1978-84				0.05	Von Westernhagen *et al.*, 1981
	Genyonemus lineatus (f)	S. California	1985-6			1.52	2.10	Cross and Hose, 1988
	Merlangus merlangus (f)	Dutch N. Sea	1984				0.12	Von Westernhagen *et al.*, 1987
		Liverpool Bay				1.6		MAFF, 1991
	Micropogonias undulatus	S. Carolina	1972-4	0.035				Reimold and Shealy, 1976
	Oncorhynchus mykiss	Michigan rivers	1990	0.98				Giesy *et al.*, 1994
		Lake Michigan	1990		0.38			Miller *et al.*, 1993
	Oncorhynchus mykiss (f)	New Zealand	1966		0.21		0.50	Hopkins *et al.*, 1969

Table 3.3 (continued)

	Species	Exposure site	Year	Concentration (μg/g) wet weight whole	muscle	liver	gonad	Reference
	Oncorhynchus tshawytscha	Michigan rivers	1990	0.36				Giesy *et al.*, 1994
		Lake Michigan	1990		0.48			Miller *et al.*, 1993
	Osmerus mordax	Lake Michigan	1965-68	2.31				Reinert, 1979
	Perca flavescens	Lake Michigan	1965-68	3.22				Reinert, 1979
	Platichthys flesus	Baltic	1977-83		0.03	0.44		Huschenbeth, 1986
		Elbe estuary (N. Sea)			0.34			Luckas and Harms, 1987
		S. Baltic (f)	1978/84				0.05	Von Westernhagen *et al.*, 1981
	Platichthys stellatus	San Francisco Bay	1986-7			1.7		Spies *et al.*, 1990
	Salmo namaycush	Lake Michigan	1990		1.83			Miller *et al.*, 1993
	Salmo trutta	Lake Michigan	1990		0.36			Miller *et al.*, 1993
	Salvelinus fontinalis	Lake Michigan	1985		0.41			Miller *et al.*, 1993
	Stellifer lanceolatus	S. Carolina	1972-4	0.01				Reimold and Shealy, 1976
	Stizostedion vitreum	Michigan rivers	1990	0.72				Giesy *et al.*, 1994
DDE	*Limanda limanda*	North Sea	1987			0.5[a]		Knickmeyer and Steinhart, 1990
	Hippoglossoides platessoides	North Sea	1987			0.24[a]		Knickmeyer and Steinhart, 1990
	Pleuronectes platessa	North Sea	1987			0.12[a]		Knickmeyer and Steinhart, 1990

[a] expressed as μg/g in n-hexane extractable lipid.

Table 3.4. Tissue concentrations of other organochlorines in wild captured fish in μg/g (ppm) wet weight. ND = not detectable. For other notes see Table 3.1

				Concentration (μg/g) wet weight				
	Species	Exposure site	Year	whole	muscle	liver	gonad	Reference
Chlordanes	*Cyprinus carpio*	Michigan rivers	1990	0.04				Giesy *et al.*, 1994
	Gadus morhua (f)	NW Atlantic			ND	0.16	0.019	Hellou and Payne, 1993
	Hippoglossoides platessoides(m)	NW Atlantic	1991-3		0.012	0.056	0.001	Hellou *et al.*, 1995
	Oncorhynchus kisutch	Lake Ontario			0.16			Leatherland and Sonstegard, 1982
		Lake Michigan	1985		0.13			Miller *et al.*, 1993
	Oncorhynchus mykiss	Lake Michigan	1985		0.13			Miller *et al.*, 1993
		Lake Michigan	1990		0.12			Miller *et al.*, 1993
		Michigan rivers	1990	0.06				Giesy *et al.*, 1994
	Oncorhynchus tshawytscha	Lake Michigan	1985		0.13			Miller *et al.*, 1993
		Lake Michigan	1990		0.14			Miller *et al.*, 1993
	Platichthys stellatus	San Francisco Bay	1986-7			0.08[a]		Spies *et al.*, 1990
	Reinhardtius hippoglossoides (f)	NW Atlantic	1991-1993		0.021	0.035	0.001	Hellou *et al.*, 1995
	Salmo namaycush	Lake Michigan	1985		0.37			Miller *et al.*, 1993
		Lake Michigan	1990		0.32			Miller *et al.*, 1993
	Salmo trutta	Lake Michigan	1985		0.15			Miller *et al.*, 1993
		Lake Michigan	1990		0.07			Miller *et al.*, 1993
	Salvelinus fontinalis	Lake Michigan	1985		0.11			Miller *et al.*, 1993
Dieldrin	*Alosa pseudoharengus*	Lake Michigan	1967-8	0.11				Reinert, 1979
	Clupea harengus	Clyde	1991		0.012			Kelly and Campbell, 1994

Table 3.4 (continued)

Species		Exposure site	Year	Concentration (μg/g) wet weight whole	muscle	liver	gonad	Reference
		Firth of Forth	1991		0.003			Kelly and Campbell, 1994
		N. North Sea	1991		0.002			Kelly and Campbell, 1994
	(f)	Dutch North Sea	1979				0.004	Von Westernhagen *et al.*, 1987
Coregonus artedii		Lake Michigan	1967-8	0.20				Reinert, 1979
Coregonus hoyi		Lake Michigan	1967-8	0.23				Reinert, 1979
Cyprinus carpio		Michigan rivers	1990	0.06				Giesy *et al.*, 1994
Gadus morhua		Clyde	1990			0.1		Kelly and Campbell, 1994
		Firth of Forth	1991			0.03		Kelly and Campbell, 1994
		N. North Sea	1991			0.02		Kelly and Campbell, 1994
		S. North Sea			0.003	0.15		Harms and Kerkhoff, 1988
		Baltic			0.003	0.03		Huschenbeth, 1986
	(f)	Baltic	1978/84				0.004	Von Westernhagen *et al.*, 1987
		Nova Scotia	1980			0.06		Freeman *et al.*, 1984a
Oncorhynchus kisutch		Lake Michigan	1985		0.05			Miller *et al.*, 1993
Oncorhynchus mykiss		Lake Michigan	1985		0.06			Miller *et al.*, 1993
		Lake Michigan	1990		0.07			Miller *et al.*, 1993
		Michigan rivers	1990	0.11				Giesy *et al.*, 1994
Oncorhynchus tschawytscha		Lake Michigan	1985		0.05			Miller *et al.*, 1993
		Lake Michigan	1990		0.08			Miller *et al.*, 1993
Osmerus mordax		Lake Michigan	1967-8	0.06				Reinert, 1979
Perca flavescens		Lake Michigan	1967-8	0.08				Reinert, 1979
Platichthys flesus		Elbe estuary	1977-80		0.002	0.04		Huschenbeth, 1986
	(f)	Baltic	1979			0.005	0.025	Von Westernhagen *et al.*, 1981

Table 3.4 (continued)

	Species	Exposure site	Year	Concentration (μg/g) wet weight whole	muscle	liver	gonad	Reference
	Platichthys stellatus	San Francisco Bay	1986-7			0.22[a]		Spies *et al.*, 1990
	Salmo namaycush	Lake Michigan	1985		0.21			Miller *et al.*, 1993
		Lake Michigan	1990		0.13			Miller *et al.*, 1993
	Salmo trutta	Lake Michigan	1985		0.14			Miller *et al.*, 1993
		Lake Michigan	1990		0.06			Miller *et al.*, 1993
	Salvelinus fontinalis	Lake Michigan	1985		0.04			Miller *et al.*, 1993
HCB	*Gadus morhua*	S. North Sea			0.002	0.03		Harms and Kerkhoff, 1988
		Nova Scotia,	1980			0.02		Freeman *et al.*, 1984a
	Hippoglossoides platessoides	North Sea	1987			0.008[a]		Knickmeyer and Steinhart, 1990
	Limanda limanda	North Sea	1987			0.011[a]		Knickmeyer and Steinhart, 1990
	Platichthys flesus	Elbe estuary (N. Sea)			0.27			Luckas and Harms, 1987
	Pleuronectes platessa	North Sea	1987			0.007[a]		Knickmeyer and Steinhart, 1990
HCHs (Lindane)	*Gadus morhua*	S. North Sea			0.003	0.06		Harms and Kerkhoff, 1988
	Hippoglossoides platessoides	North Sea	1987			0.018[a]		Knickmeyer and Steinhart, 1990
		Nova Scotia	1980			0.06		Freeman *et al.*, 1984a
		S. Baltic	1981			0.30		Falandysz, 1984
		Baltic	1977-83		0.001	0.08		Huschenbeth, 1986
	Limanda limanda	North Sea	1987			0.032[a]		Knickmeyer and Steinhart, 1990
	Platichthys flesus	Elbe estuary			0.08			Luckas and Harms, 1987

Table 3.4 (continued)

	Species	Exposure site	Year	Concentration (μg/g) wet weight whole	muscle	liver	gonad	Reference
	Platichthys stellatus	San Francisco Bay	1986-7			0.04[a]		Spies *et al.*, 1990
	Pleuronectes platessa	North Sea	1987			0.032[a]		Knickmeyer and Steinhart, 1990
Heptachlors	*Cyprinus carpio*	Michigan rivers	1990	0.013				Giesy *et al.*, 1994
	Gadus morhua	Nova Scotia	1980			0.17		Freeman *et al.*, 1984a
	Oncorhynchus mykiss	Michigan rivers	1990	0.015				Giesy *et al.*, 1994
	Platichthys stellatus	San Francisco Bay	1986-7			0.05[a]		Spies *et al.*, 1990
Mirex	*Oncorhynchus kisutch*	Lake Ontario			0.27			Leatherland and Sonstegard, 1982
	Platichthys stellatus	San Francisco Bay	1986-7			0.12[a]		Miller *et al.*, 1993
OCS[b]	*Platichthys flesus*	Elbe estuary (N. Sea)			0.15			Luckas and Harms, 1987
PCDDs[c]	*Clupea harengus*	Baltic Sea	1987		0.0077[d]			Bergqvist *et al.*, 1989
	Gadus morhua	Baltic Sea	1987		ND			Bergqvist *et al.*, 1989
	Gadus morhua (f)	NW Atlantic			ND	0.0012	ND	Hellou and Payne, 1993
PCDFs[e]	*Gadus morhua* (f)	NW Atlantic			ND	0.0066	ND	Hellou and Payne, 1993
Toxaphene	*Salmo salar*	N. Baltic	1986		0.12			Koistinen *et al.*, 1989

[a] expressed as μg/g in n-hexane extractable lipid; [b] OCS = octachlorostyrene; [c] polychlorinated dibenzo-p-dioxins; [d] expressed as TCDD equivalents; [e] polychlorinated dibenzofurans

Table 3.5. Tissue concentrations in µg/g (ppm) wet weight of miscellaneous industrial chemicals in wild captured fish. For notes see Table 3.1

				Concentration (µg/g) wet weight					
	Species	Exposure site	Year	whole	muscle	liver	gonad	gills	Reference
PAHs	*Gadus morhua*	Norwegian fiord 4 weeks	1990			1.45[a]			Goksøyr *et al.*, 1994
	Mugil dussumieri	United Arab Emirates		3.62					Khan *et al.*, 1995
	Siganus canaliculatus	United Arab Emirates		2.0					Khan *et al.*, 1995
Dibenzo-thiophene	*Gadus morhua*	Norwegian fiord 4 weeks	1990			0.032[a]			Goksøyr *et al.*, 1994
Dibenzo-thiophene (alkylated)	*Gadus morhua* (f)	Northwest Atlantic	1990			0.019	0.026		Hellou *et al.*, 1994
Fluorene	*Gadus morhua*	Norwegian fiord 4 weeks	1990			0.045[a]			Goksøyr *et al.*, 1994
	(f)	Northwest Atlantic	1990			0.014	0.018		Hellou *et al.*, 1994
Naphthalene	*Gadus morhua*	Norwegian fiord 4 weeks	1990			0.09[a]			Goksøyr *et al.*, 1994
Naphthalene/ anthracene (alkylated)	*Gadus morhua* (f)	Northwest Atlantic	1990			0.029	0.028		Hellou *et al.*, 1994
Nonylphenol	*Barbus barbus*	Swiss river	1984-5		0.38	0.98	0.09	<0.03	Ahel *et al.*, 1993
Nonylphenol-monoethoxyate	*Barbus barbus*	Swiss river	1984-5		3.1	0.88	0.15	0.06	Ahel *et al.*, 1993
C1-Phenan-threne	*Gadus morhua*	Norwegian fiord 4 weeks	1990			0.14[a]			Goksøyr *et al.*, 1994

Table 3.5 (continued)

	Species	Exposure site	Year	Concentration (μg/g) wet weight whole	muscle	liver	gonad	gills	Reference
Tetrachloro-dibenzofuran	*Gadus morhua* (f)	NW Atlantic			ND	0.005	ND		Hellou and Payne, 1993
	Reinhardtius hippo-glossoides (f)	NW Atlantic	1991-93		0.003	0.004	ND		Hellou and Payne, 1993

[a] as μg/g of hepatic lipid

Table 3.6. Tissue concentrations in μg/g (ppm) wet weight in fish exposed to polluted water under laboratory conditions. For comparison, literature values in dry weight have been converted on the basis of 1 g dry tissue = 5g wet tissue

	Species	Exposure mg/l	Time (days)	Concentration (μg/g) wet weight whole	muscle	liver	gonad	kidney	gills	Reference
Heavy metals										
Cadmium	*Lepomis macrochirus*	0.0084	28			0.52	9.5			Cope *et al.*, 1994
	Oncorhynchus mykiss	0.027	90			1.1		1.5	3.6	Roberts *et al.*, 1979
	Oreochromis aureus (m/f)	0.1	7		0.1	12.8	0.8/1.2	39.6	1.2	Allen, 1995
	Oreochromis niloticus	0.01Hg, 0.1Cd, 1Zn	14	5.1						Cuvin-Aralar, 1994
	Salmo trutta	0.027	90			1.1		1.1	1.8	Roberts *et al.*, 1979
	Salvelinus fontinalis	0.001	0		0.006		0.02	0.05	0.15	Sangalang and Freeman, 1979
			93		0.022		0.08	0.92	0.92	Sangalang and Freeman, 1979
		0.0034	266		0.02	2.0	0.6	10	0.6	Benoit *et al.*, 1976
Copper	*Ictalurus nebulosus*	0.027	30			18		2.5	4.4	Brungs *et al.*, 1973
	Lepomis macrochirus	0.16	730		1	480	5	44	13	Benoit, 1975
	Lepomis macrochirus	0.04	730		1	61	5	12	5	Benoit, 1975
Lead	*Anabas testudineus* (f)	5	30				1.1			Tulasi *et al.*, 1989
	Oreochromis aureus (m/f)	0.1	7		0.34	3.8	2.1/0.4	116	28	Allen, 1995
Mercury	*Oncorhynchus mykiss*	0.01	42		0.47	0.9	0.6	7.7	4.2	Walczak *et al.*, 1986
		0.0001	30		0.23	2.6		5.3	6.9	Ribeyre and Boudou, 1984

Table 3.6 (continued)

	Species	Exposure mg/l	Time (days)	Concentration (µg/g) wet weight whole	muscle	liver	gonad	kidney	gills	Reference
	Oreochromis aureus	1	0.08			0		0	7.7	Allen *et al.*, 1988
	Oreochromis niloticus	0.01Hg, 0.1Cd, 1Zn	14	1.8						Cuvin-Aralar, 1994
	Notopterus notopterus	0.088	30		1.48	2.16	2.10			Verma and Tonk, 1983
Mercury (methyl)		0.0001	30		3.0	11.0		13.7	16.3	Ribeyre and Boudou, 1984
Tin (phenyl)	*Oncorhynchus mykiss*	0.006	28		11.5	16.1	-	9.0	3	Fent, 1996
Tin (tributyl)	*Oncorhynchus mykiss*	0.0004	15		0.49	4.8	4.3	3.7	1.4	Martin *et al.*, 1989
Zinc	*Gasterosteus aculeatus*	1	1	1		3.8	0.8	3.2	5.1	Matthiessen and Brafield, 1977
	Oreochromis niloticus	0.01Hg, 0.1Cd, 1Zn	14	38.1						Cuvin-Aralar, 1994
Organochlorines										
Chlordane	*Mugil cephalus* freshwater	0.05	1			4.2			1.5	Tachikawa *et al.*, 1987
	Mugil cephalus seawater	0.05	1			1.1			1.1	Tachikawa *et al.*, 1987
DDT	*Anguilla vulgaris*	0.001	1		1.1	10.7	2.0		3.3	Hilmy *et al.*, 1983a
	Mugil cephalus	0.001	1		1.2	10.8	2.1		3.1	Hilmy *et al.*, 1983a
Endrin	*Anguilla vulgaris*	0.001	1		0.8	2.3	1.1		2.5	Hilmy *et al.*, 1983a
	Mugil cephalus	0.001	1		0.3	1.2	0.5		1.5	Hilmy *et al.*, 1983a

Table 3.6 (continued)

	Species	Exposure mg/l	Time (days)	Concentration (µg/g) wet weight whole	muscle	liver	gonad	kidney	gills	Reference
PCB	*Salvelinus fontinalis*	0.2	21		33					Freeman and Idler, 1975
Pentachloro phenol-Na	*Mugil cephalus* freshwater	0.2	1			20			15	Tachikawa *et al.*, 1987
	Mugil cephalus seawater	0.2	1			15			13.5	Tachikawa *et al.*, 1987
TCB[a]	*Morone americana* (f)	1µg/g inj x3	84			3	15			Monosson *et al.*, 1994
		5µg/g inj x3	84			18	40			Monosson *et al.*, 1994
	Pimephales promelas (f)	0.65	5		0.5	4	5			Lindstrom-Seppa *et al.*, 1994
Organophosphates										
Diazinon	*Anguilla anguilla*	0.056	3		290	90				Sancho *et al.*, 1993
	Danio rerio	0.4	2		28	34			55	Keizer *et al.*, 1991
	Cyprinus carpio	0.002	3		0.025	0.071		0.156		Tsuda *et al.*, 1990
	Gnathopogon caerulescens	0.0029	2	0.25						Tsuda *et al.*, 1989
	Poecilia reticulata	0.4	2		36	37			24	Keizer *et al.*, 1991
Chlorpyri-phosmethyl	*Gnathopogon caerulescens*	0.0008	3	0.84						Tsuda *et al.*, 1994
Edifenphos	*Gnathopogon caerulescens*	0.0026	3	0.13						Tsuda *et al.*, 1994
Ethopro-phos	*Gnathopogon caerulescens*	0.0018	3	0.027						Tsuda *et al.*, 1994

Table 3.6 (continued)

	Species	Exposure mg/l	Time (days)	Concentration (µg/g) wet weight whole	muscle	liver	gonad	kidney	gills	Reference
Endosulfan	*Danio rerio*	0.0003	21	0.81						Toledo and Jonsson, 1992
Fenitro-thion	*Cyprinus carpio*	0.0023	3		0.048	0.111		0.223		Tsuda *et al.*, 1990
	Gnathopogon caerulescens	0.003	2	0.39						Tsuda *et al.*, 1989
IBP	*Cyprinus carpio*	0.0066	3		0.0063	0.025		0.034		Tsuda *et al.*, 1990
	Gnathopogon caerulescens	0.0062	2	0.04						Tsuda *et al.*, 1989
Malathion	*Cyprinus carpio*	0.0051	3		0.009	0.005		0.020		Tsuda *et al.*, 1990
	Gnathopogon caerulescens	0.0057	2	0.03						Tsuda *et al.*, 1989
Methylpara-thion	*Gnathopogon caerulescens*	0.0017	3	0.122						Tsuda *et al.*, 1989
Vamido-thion	*Gnathopogon caerulescens*	0.0005	3	0.81						Tsuda *et al.*, 1989
Carbamates										
Bendiocarb	*Gnathopogon caerulescens*	0.0033	3	0.116						Tsuda *et al.*, 1994
Pirimicarb	*Gnathopogon caerulescens*	0.0048	3	0.025						Tsuda *et al.*, 1994
Industrial										
p-sec-Butyl-phenol	*Salmo salar* (juv)	0.83	1	28.3						McLeese *et al.*, 1981

Table 3.6 (continued)

	Species	Exposure mg/l	Time (days)	Concentration (μg/g) wet weight whole	muscle	liver	gonad	kidney	gills	Reference
p-Hexyl-phenol	*Salmo salar* (juv)	0.24	1	46.4						McLeese *et al.*, 1981
p-Nonyl-phenol	*Salmo salar* (juv)	0.31	1	72.7						McLeese *et al.*, 1981
p-Dodecyl-phenol	*Salmo salar* (juv)	0.24	1	17.8						McLeese *et al.*, 1981

[a] TCB = 3,3',4,4'-tetrachlorobiphenyl

Table 3.7. Tissue concentrations in µg/g (ppm) wet weight in fish fed contaminated diets or injected with pollutants. For comparison, literature values in dry weight have been converted on the basis of 1 g dry tissue = 5g wet tissue. (m) = male;(f) = female

		Exposure	Concentration (µg/g) wet weight						
	Species	(µg/g, days)	whole	muscle	liver	gonad	kidney	gills	Reference
Cadmium	*Poecilia reticulata* (f)	210 in midge larvae, 61d		0.19	33	0.16	43	4	Hatakeyama and Yasuno, 1987
	Oncorhynchus mykiss	10,000, 28d		0.008	1.21		0.46	0.51	Handy, 1993
	Salvelinus fontinalis	1 µg/g bw /week inj., 112d		0.022	0.33	0.025	0.93	0.3	Sangalang and Freeman, 1979
Copper	*Carassius auratus*	commercial diet		0.34	4.48	1.24			Carpene *et al.*, 1994
	Cyprinus carpio	commercial diet		0.28	3.7	0.68			Carpene *et al.*, 1994
	Ictalurus melas	50-80Zn/2-150Cu		5.4	4.2	1.4			Carpene *et al.*, 1994
	Oncorhynchus mykiss	10,000, 28d		0.42	85.5		1.85	0.41	Handy, 1993
Lead	*Gillichthys mirabilis*	2.65 in 100% SW, 36d		0.5	1.8	0.5		14	Somero *et al.*, 1977
		2.65 in 25% SW, 36d		0.95	2.8			24	Somero *et al.*, 1977
Zinc	*Carassius auratus*	commercial diet		4.6	17.6	55.2			Carpene *et al.*, 1994
	Cyprinus carpio	commercial diet		5.6	67	110			Carpene *et al.*, 1994
	Ictalurus melas	50-80Zn/2-150Cu		8.8	15.6	60.8			Carpene *et al.*, 1994
DDTs	*Oncorhynchus kisutch*	Lake Michigan trout 2.3, 190d	2.4						Leatherland and Sonstegard, 1982
	Salvelinus fontinalis	2 /week, 156d	7.6			0.5			Macek, 1968
Mirex	*Oncorhynchus kisutch*	50, 90d		9.6					Leatherland *et al.*, 1979
		5, 90d	1.6						Leatherland *et al.*, 1979
PCBs	*Gadus morhua* (m)	1, 165d		0.03	10.1	0.06	0.8		Freeman *et al.*, 1982
		10, 165d		0.29	81	0.51	0.7		Leatherland *et al.*, 1979
	Morone americana	1x inj./3 wks, 84d			0.6	2.8			Monosson *et al.*, 1994
		10x inj/3 wks, 84d			3.4	7.6			Monosson *et al.*, 1994

Table 3.7 (continued)

	Species	Exposure (µg/g, days)	Concentration (µg/g) wet weight whole	muscle	liver	gonad	kidney	gills	Reference
	Oncorhynchus kisutch	500, 90d		43					Leatherland *et al.*, 1979
		50, 90d		14					Leatherland *et al.*, 1979
		Lake Michigan trout 5.8, 190d	6.3						Leatherland and Sonstegard, 1982
Benzo[*a*]-pyrene	*Parophrys vetulus* (m/f)	1 x 80 mg/kg inject-				25.1/ 32.2			Hose *et al.*, 1981

Table 3.8. Tissue concentrations in µg/g (ppm) wet weight in fish held in water containing PCB contaminated sediment

Species	Sediment (µg/g)	Exposure (days)	Concentration (µg/g) whole	muscle	Reference
Anguilla anguilla	1	77		14.1	Larsson, 1984
Leiostomus xanthurus	5.2	60	0.5		Rubinstein *et al.*, 1984

Table 3.9. Concentration of pollutants in eggs and larvae µg/g (ppm) wet weight. For comparison, literature values in dry weight have been converted on the basis of 1 g dry tissue = 5g wet tissue

Pollutant	Species	Stage	Exposure mg/l	days	Stage measured	Tissue Conc.	Reference
Cadmium	*Oncorhynchus mykiss*	egg	0.01	1.5	egg	0.5	Beattie and Pascoe, 1978
	Salmo salar	egg	0.1	78	egg	2	Rombough and Garside, 1982
Copper	*Catastomus commersoni*	parent	contam. lake	life	egg	2.1	Munkittrick and Dixon, 1989
		parent /egg	contam. lake	life	larvae	102	Munkittrick and Dixon, 1989
	Oncorhynchus kisutch	parent	Lake Ontario	life	egg	5.2	Morrison *et al.*, 1985a
		parent	Lake Erie	life	egg	6	Morrison *et al.*, 1985a
		parent	Lake Michigan	life	egg	6.1	Morrison *et al.*, 1985a
Mercury	*Oryzias latipes*	eggs	0.0015	16	egg	29	Heisinger and Green, 1975
Zinc	*Catastomus commersoni*	parent	contam. lake	life	egg	16.7	Munkittrick and Dixon, 1989
		parent /egg	contam. lake	life	larvae	102	Munkittrick and Dixon, 1989
	Oncorhynchus kisutch	parent	Lake Ontario	life	egg	62	Morrison *et al.*, 1985a
		parent	Lake Erie	life	egg	80	Morrison *et al.*, 1985a
		parent	Lake Michigan	life	egg	71	Morrison *et al.*, 1985a
PCB	*Oncorhynchus kisutch*	parent	Lake Ontario	life	egg	3	Morrison *et al.*, 1985a
		parent	Lake Erie	life	egg	1	Morrison *et al.*, 1985a
		parent	Lake Michigan	life	egg	3	Morrison *et al.*, 1985a
	Pleuronectes americanus	parent	Rhode Island	life	egg	8	Black *et al.*, 1988
	Salmo salar	parent	New Brunswick	life	egg	1.9-13.7	Zitko and Saunders, 1979
	Salvelinus alpinus	parent	Lake Geneva 1981	life	egg	0.1-0.5	Monod, 1985
	Salvelinus fontinalis	parent	0.2	21	egg	78	Freeman and Idler, 1975

Table 3.9 (continued)

Pollutant	Species	Stage	Exposure mg/l	days	Stage measured	Tissue Conc.	Reference
	Salvelinus namaycush	parent	Lake Michigan	life	egg	0.4-4.5	Mac and Schwarz, 1992
DDTs	*Oncorhynchus kisutch*	parent	Lake Ontario	life	egg	0.45	Morrison *et al.*, 1985a
		parent	Lake Erie	life	egg	0.1	Morrison *et al.*, 1985a
		parent	Lake Michigan	life	egg	0.57	Morrison *et al.*, 1985a
	Oncorhynchus tshawytscha	parent	L. Michigan/ Superior	life	egg	4.55	Miller, 1993
	Pleuronectes americanus	parent	0.002	varied	egg	1.1-3.7	Smith and Cole, 1973
	Salmo salar	parent	New Brunswick	life	egg	0.7-3.1	Zitko and Saunders, 1979
	Salvelinus alpinus	parent	Lake Geneva 1981	life	egg	0.07-0.17	Monod, 1985
	Salvelinus namaycush	parent	L. Michigan/ Superior	life	egg	2.24	Miller, 1993
Dieldrin	*Pleuronectes americanus*	parent	0.002	varied	egg	0-1.7	Smith and Cole, 1973
HCB	*Salmo salar*	parent	New Brunswick	life	egg	0.09-0.16	Zitko and Saunders, 1979

Chapter 4

Introduction to Fish Reproduction

4.1 Introduction

Pollutants may affect an extremely wide range of physiological parameters and manifest their effects at a variety of different loci in the reproductive system. These effects cannot be properly understood without at least a basic understanding of the mechanism by which the endocrine system controls all aspects of reproduction. This Chapter is therefore intended to give the non-specialist a simple introduction to the control of reproduction in the normal teleost fish and to show how such control may be disrupted by environmental pollutants at a multiplicity of sites. It also provides a basic knowledge of endocrine processes which forms the basis for understanding the endocrinology of other glands such as the interrenal and thyroid, as well as showing some of the approaches which can be used to investigate the action of endocrine disrupting chemicals in fish. Since there are over 20,000 species of teleosts, only a small fraction of extant species have been investigated and, since teleost fish occupy a more diverse range of habitats than any other vertebrate, it cannot be expected that a single simple pattern will pertain for all species. Much of the information available therefore comes from the even smaller numbers of species which are of commercial importance, readily available in the geographical areas of the major research laboratories, and easily kept in captivity. Studies of the salmonids (salmon and trout), cyprinids (goldfish and carp), and to a much lesser extent, silurids (catfish) and cichlids (tilapia) thus predominate.

4.2 Seasonal cues for reproduction

The majority of teleost fish of commercial importance, which are predominantly from the temperate regions of Europe and North America, undergo a seasonal reproductive cycle. This comprises two distinct phases, gonadal recrudescence and spawning, each of which has a separate control mechanism (Figure 4.1).

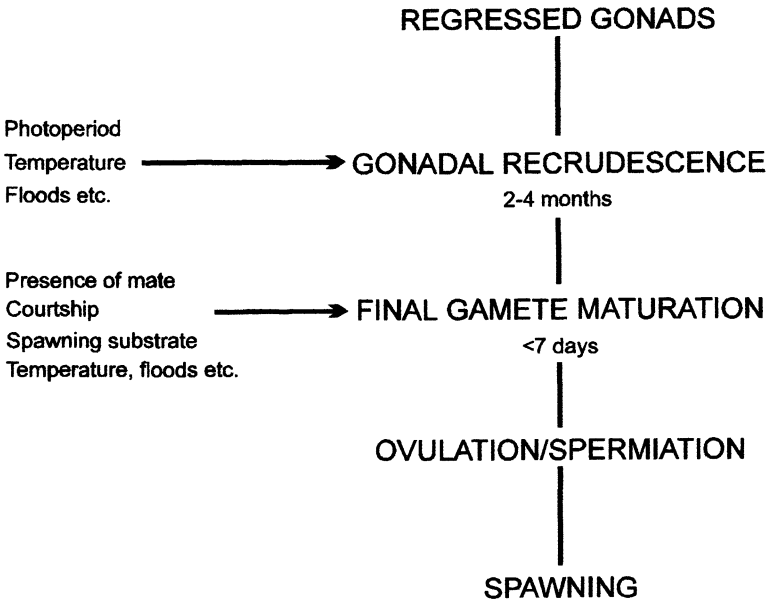


Figure 4.1. The reproductive cycle of teleost fish.

Gonadal recrudescence is the growth of the gonad from the regressed state, comprising less than 0.1% of the body weight (GSI = 0.1; gonadosomatic index (GSI) = gonad weight x 100/body weight), to a fully mature state comprising around 5-10% of the body weight in males (GSI = 5-10) or 20-30% in females (GSI = 20-30). Such growth, which requires significant amounts of energy and takes place over a period of several months is usually initiated by photoperiodic, temperature, or other environmental cues which are reliable indicators of seasonality. This phase is complete when a batch of gametes are fully grown. The second phase, gamete final maturation and spawning, is of short duration (hours, or days) and occurs when the necessary local factors, such as the presence of a mate and his correct courtship behaviour, spawning substrate, food, a permissive minimum temperature, floodwater etc., are present that will ensure fertilisation and give maximum chance of survival for the eggs and larvae (Lam, 1983; Stacey, 1984). The

temporal sequence must be such that spawning occurs at the most suitable time of the year for survival of the eggs and fry. In most temperate animals spring/early summer represents the most suitable time for production of young and, as in mammals, this must be anticipated well in advance so that the gonads may have sufficient time for development. In higher latitudes photoperiod is the most reliable seasonal cue and is often also combined with temperature which may, however, show significant short-term variations. In equatorial climates reproductive seasonality may be less pronounced, and reproductive development may be regulated by factors such as temperature or rainfall. In mammals, birds and reptiles, the pineal gland in the brain, may act either directly as a photoreceptor or may mediate retinal signals. This results in a diurnal pattern of secretion by the pineal of the hormone melatonin during hours of darkness. This is further interpreted by the brain and the hypothalamus as a seasonal signal leading to changes in the reproductive endocrine system which initiates gonadal development (Bye, 1984; Whittier and Crews, 1987; Bromage *et al.*, 1990; Sumpter, 1990). In fish the evidence for involvement of the pineal and melatonin in the transduction of photoperiodic signals as cues for reproductive development is less clear. Photoperiod is, however, the major environmental signal in salmonids and gonadal recrudescence may be either delayed or advanced by application of modified photoperiods such as shorter or longer days or by compressed or advanced seasonal photoperiodic cycles. In some cases it is the rate of change of day length rather than absolute length which is important. In cyprinids, both photoperiod and temperature are important for regulating gonadal recrudescence. The exact signal is species dependent and reflects its particular reproductive strategy. Trout, for example, begin gonadal development in autumn, spawn in winter and lay large eggs in protected nests so that they hatch after several months in early spring, while in cyprinids gonadal recrudescence may begin much later since they lay small eggs in spring/early summer which hatch in only a few days.

Whatever the specific external cues are for controlling the processes from gonadal recrudescence to spawning in a particular species, it is modulated by the reproductive endocrine system which shows surprisingly little general interspecific differences.

4.3 The teleost endocrine system

While the teleost endocrine system has many similarities to that of other vertebrates, there are also important differences. Details of the vertebrate endocrine system can be found in standard text books of comparative endocrinology (Bentley, 1982; Matty, 1985; Chester Jones *et al.*, 1987) for those so interested.

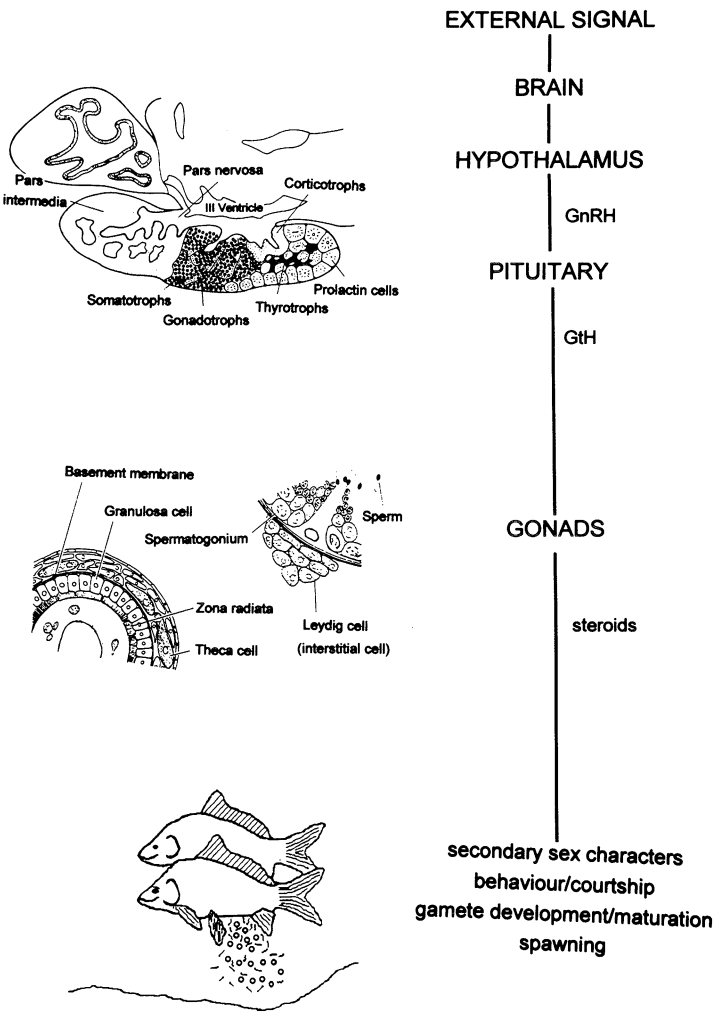


Figure 4.2. The pituitary-hypothalamic-gonadal system and control of reproduction in fish, showing the structure of the pituitary and the gonads (*Anguilla anguilla* pituitary reproduced from Olivereau, M. (1967). Observation sur l'hypophyse de l'anguille femelle en particulier lors de la maturation sexuelle. *Z. Zellforsch. Mikrosk. Anat.* **80**, 286-306, Fig. 1, by kind permission of Springer-Verlag, Berlin (Copyright 1967) and the author; gonads reproduced from Hoar, W. S. and Nagahama, Y. (1978). The cellular sources of sex steroids in teleost gonads. *Ann. Biol. Anim. Biochem. Biophys.* **18**, 893-898 by kind permission of I.N.R.A, Versailles). The somatotrophs, gonadotrophs, thyrotrophs, corticotrophs and prolactin cells are located in the pars distalis of the adenohypophysis.

The following is intended as a simple introduction to cover only the basics required for an understanding of the effects of pollutants on reproduction of fish. Up-to-date reference to the latest advances in the field are available in Proceedings of the Symposia on Reproductive Physiology of Fish (Richter and Goos, 1982; Idler *et al.*, 1987; Scott *et al.*, 1991; Goetz and Thomas, 1995).

Signals from the brain, resulting from the external seasonal or local cues, are modulated by the hypothalamic-pituitary system as changes in hormone secretion (Figure 4.2). Releasing hormones from the hypothalamus (gonadotrophin releasing hormone, GnRH; corticotrophin releasing hormone, CRH; thyrotrophin releasing hormone, TRH) cause release of hormones from secretory cells in the pituitary gland. The structure of the teleost pituitary shows some significant differences to other vertebrates (Matty, 1985; Batten and Ingleton, 1987), but this need not concern us for the purposes of this study since we need only consider the pars distalis of the pituitary gland and its secretions of gonadotrophin (GtH, from the gonadotrophs), and to a lesser extent, adrenocorticotrophin (ACTH, from the corticotrophs) and thyrotrophin (TSH, from the thyrotrophs). These are peptide hormones which act respectively on the gonad, adrenal and thyroid glands to stimulate secretion of their respective hormones. Other hormones secreted by the somatotrophs, prolactin cells and the pars nervosa will be discussed in later Chapters. Of most concern in the context of reproduction is gonadotrophin (GtH). In mammals, the balance between the two gonadotrophins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), is of key importance in regulating the reproductive cycle. There has been considerable controversy as to the number of gonadotrophins in fish (Swanson, 1991). Two such hormones are certainly present in some, but not all species, and are generally referred to as GtH-I and GtH-II. GtH-I predominates during vitellogenesis or spermatogenesis and may be involved in both steroid synthesis, and in the uptake of vitellogenin by the oocyte (Tyler *et al.*, 1991), while GtH-II is involved in gamete final maturation when it may stimulate synthesis of the maturation inducing steroid and initiate migration of the germinal vesicle. For our purposes, however, they can usually be simply considered as "gonadotrophin". The main role of gonadotrophin is to stimulate the gonads and their secretion of steroids. These in turn initiate changes in secondary sexual characteristics (if present), behaviour and courtship patterns, and in development of the gametes, their maturation and eventually spawning.

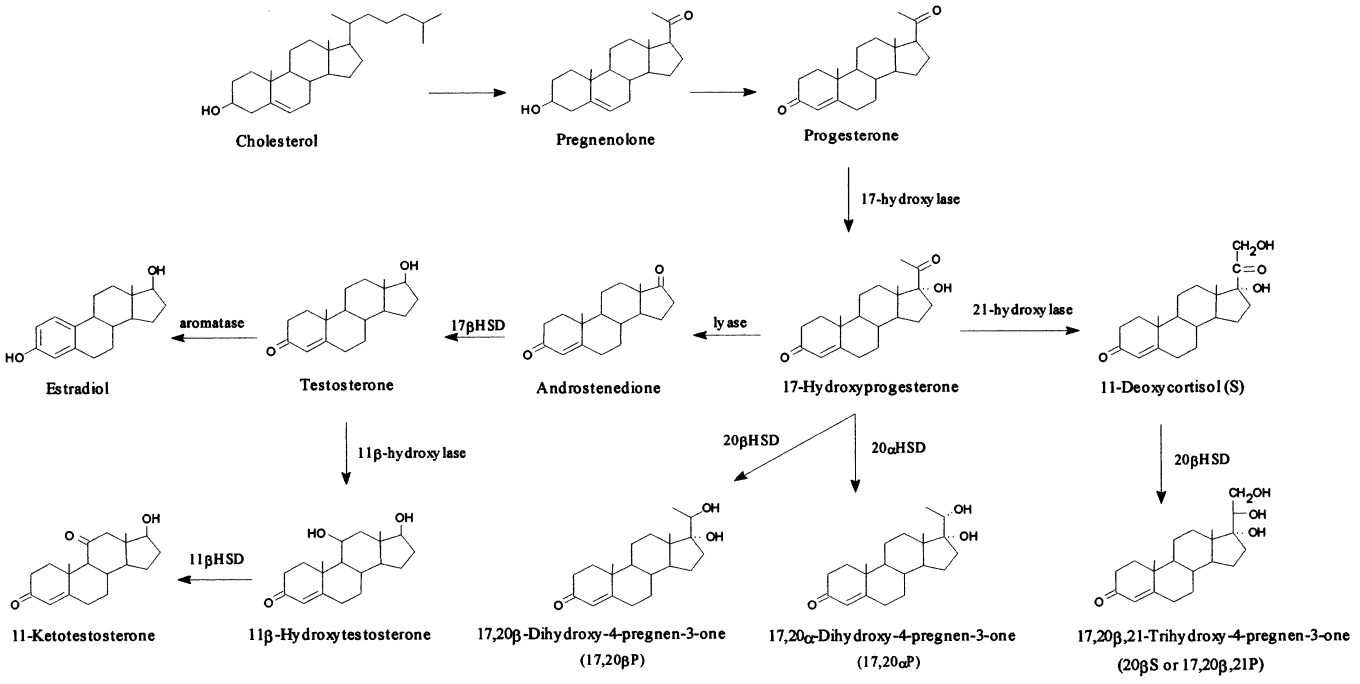


Figure 4.3. Steroid biosynthesis in teleost fish. (Systematic nomenclature: cholesterol, 3β-hydroxy-5-cholestene; pregnenolone, 3β-hydroxy-5-pregnen-20-one; progesterone, 4-pregnene-3,20-dione; estradiol, 1,3,5(10)-estratriene-3,17β-diol; testosterone, 17β-hydroxy-4-androsten-3-one; androstenedione, 4-androstene-3,17-dione; 17-hydroxyprogesterone, 17-hydroxy-4-pregnene-3,20-dione; 11-deoxycortisol, 17,21-dihydroxy-4-pregnene-3,20-dione; 11-ketotestosterone, 17β-hydroxy-4-androstene-3,11-dione; 11β-hydroxytestosterone, 11β,17β-dihydroxy-4-androsten-3-one).

It is in the steroids that fish show a major difference to mammals. For reference the structures of the main teleost steroids are shown in Figure 4.3, together with their interrelationship through the biosynthetic pathway and the major enzymes involved. For brevity this figure shows only the major pathways and neither shows all of the intermediates nor possible alternative pathways such as the 5-ene pathway in which pregnenolone is converted to androstenedione via 17-hydroxypregnenolone and dehydroepiandrosterone.

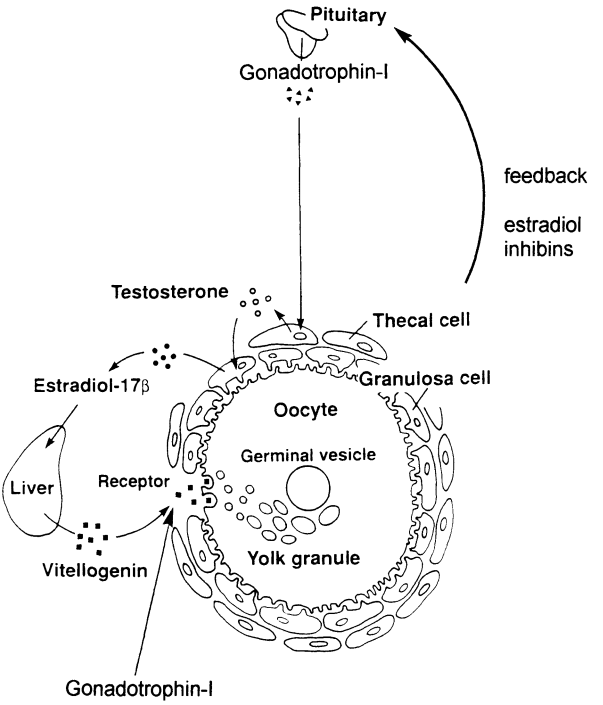


Figure 4.4. Control of vitellogenesis in fish. (Adapted from Nagahama, Y. (1994). Endocrine regulation of gametogenesis in fish. *Int. J. Dev. Biol.* **38**, 217-229, by kind permission of U.B.C. Press, Vizcaya and the author).

In male fish, 11-ketotestosterone is the predominant hormone responsible for secondary sexual characteristics and behaviour, as well as gonadal development, while testosterone (the "male" hormone in mammals) is secreted by both sexes although its function is still far from clear. Estradiol, the major hormone of female fish, is produced in the follicle of the developing oocyte. This follicle consists of two parts, an outer theca layer which produces testosterone under the influence of gonadotrophin, and an inner granulosa layer adjacent to the oocyte which converts testosterone to estradiol (Figure 4.4; Nagahama, 1983). The role of estradiol is to stimulate the liver to produce the yolk protein vitellogenin which is then incorporated into the

developing oocyte and is responsible for the major increase in gonadal weight during recrudescence (Figures 4.4 and 4.5). It is also important to note that the liver of male fish has the necessary receptors for estradiol and may also be stimulated by estrogens to produce vitellogenin, even though estradiol is not normally present in the male. As in mammals, the gonadal steroids, estradiol and testosterone, feedback to the pituitary-hypothalamic system to regulate release of gonadotrophin.

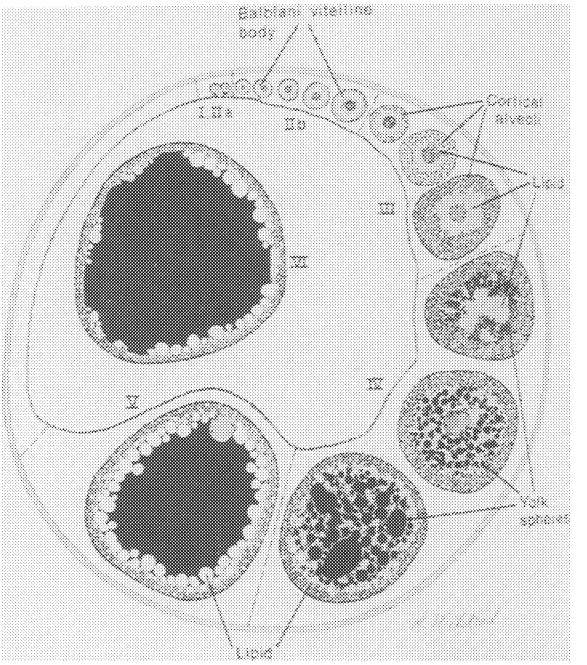


Figure 4.5. Cross-sectional diagram of the ovary of the pipefish depicting ovarian organization and phases of follicular development: I, oogonia; IIa, chromatin-nucleolus oocytes; IIb, perinucleolar oocyte; III, cortical alveoli oocytes; IV, vitellogenic oocytes; V. maturing oocyte; VI, ovulated oocyte (in ovocoele). (Reproduced from Begovac, P. C. and Wallace, R. A. (1988). Stages of oocyte development in the pipefish, *Sygnathus scovelli. J. Morphol.* **197**, 353-369, by kind permission of Wiley-Liss, New York).

Progesterone plays no known role in any fish, but a number of related progestogens are important during gamete final maturation leading to spawning. Two of these, 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) and 17,20β,21-trihydroxy-4-pregnen-3-one (20β-reduced 11-deoxycortisol, 20βS or 17,20β,21P), have clear roles in this respect but other steroids may also be involved (Kime, 1993). These progestogens have some structural similarity to the corticosteroids which can also induce oocyte maturation in some species (Sundararaj and Goswami, 1966). In the female, oocyte final maturation

involves migration of the germinal vesicle to the periphery of the oocyte under the influence of a gonadotrophin surge, usually initiated by male courtship. Gonadotrophin then stimulates the thecal layer of the follicle to produce 17-hydroxyprogesterone which is converted to the maturation inducing steroid (MIS) by the granulosa cells (Nagahama, 1983). The teleost gonad is also unusual in that it converts both androgens and the maturation inducing steroids to 5α- and 5β-reduced metabolites and their conjugates (Kime, 1993) - a process which in mammals occurs predominantly during hepatic catabolism. This may be because the maturation inducing steroid acts locally on the oocyte as a paracrine regulator and deactivation is necessary to prevent action on neighbouring oocytes which may not have attained a sufficient stage of maturity. This may be particularly important in batch spawners in which several hierarchies of oocytes are present at different stages of development. The maturation inducing steroid causes germinal vesicle breakdown (GVBD) which is shortly followed by ovulation. In male fish an analogous change takes place, with gonadotrophin stimulating production of a progestogen which induces final maturation of sperm and changes in the composition of the milt.

In addition to its vitellogenic function, the liver is also, as in all vertebrates, the major site of catabolism (or deactivation) of the steroid hormones. Such catabolism, as in mammals, gives mainly reduced and conjugated steroids, but in fish these products may also be produced in large quantities by the gonads themselves. In some species either the gonadal steroids or their reduced or conjugated metabolites have pheromonal activity (Canario and Power, 1995) and are used as a means of signalling sexual status. The essentials of the teleost endocrine system are summarised in Figures 4.1 - 4.5, but it must be emphasised that these are generalisations and different species of fish may differ in specific aspects. The two cell models of steroid secretion, in particular, are derived from salmonids in which the theca and granulosa layers are easily removed from the oocyte, but there is little reason to suspect that it does not apply to other species.

4.4 Seasonal changes in hormones and gonads

Since the pituitary gland regulates the gonad in response to external environmental cues, it is to be expected that the secretions of the gonads will change with season. In addition, the nature of the hormones secreted by the gonad change with maturational changes within the gonads themselves. Numerous studies have related these hormonal changes to gonadal histology. Plasma concentrations of the gonadal steroids and the estradiol stimulated production of vitellogenin may thus be used to monitor the reproductive stage

of fish. In females, plasma estradiol increases during the vitellogenic phase of the oocyte and then declines as oocytes complete vitellogenesis (Figure 4.6).

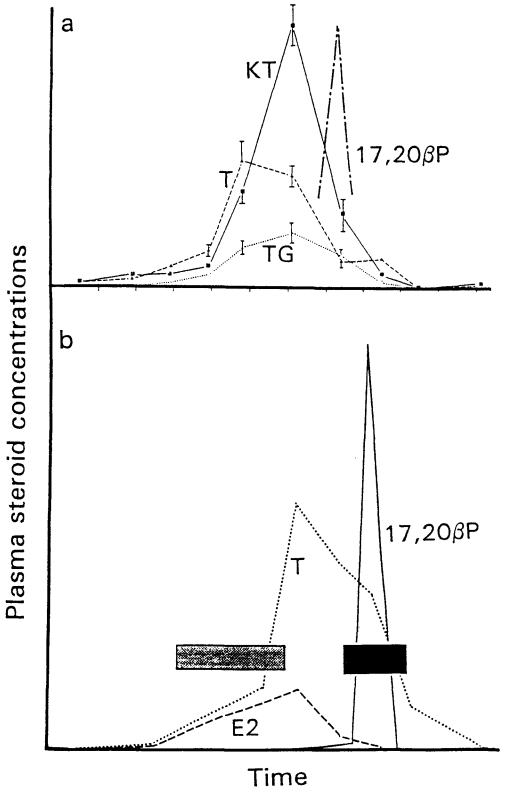


Figure 4.6. The seasonal hormonal cycles in a) male and b) female teleosts (Reproduced from Kime, D. E. (1993). "Classical" and "non-classical" steroids in teleost fish. *Rev. Fish Biol. Fisheries.* 3, 160-180, by permission of Chapman and Hall, London).

There is then a holding phase during which the aromatase enzyme is switched off and the follicle secretes testosterone. When the necessary conditions are present for a successful spawning, a gonadotrophin surge is initiated which leads to migration of the germinal vesicle to the periphery of the oocyte and stimulates the follicle to produce the maturation inducing progestogen (17,20βP or 17,20β,21P). This in turn causes the germinal vesicle to break down (GVBD) and the ovulatory process to begin. In males, a similar sequence occurs, with testosterone and/or 11-ketotestosterone secretion during gonadal recrudescence and a progestogen during the period of spermiation when hydration of the sperm occurs (Figure 4.6). Prostaglandin $F_{2\alpha}$ which is involved in the final stages of ovulation and

possibly spermiation (Goetz *et al.*, 1987), may also have pheromonal activity in some species.

4.5 Courtship behaviour and spawning

Courtship behaviour serves a number of functions. It aids recognition of conspecifics who in some cases may not be visually different from other closely related species but have a recognisably different courtship programme and it helps to select the best mate. This may involve one sex showing the other a nest site, or guarding and holding a territory which can provide either a suitable substrate for the spawned eggs or a food supply for the parents. It also helps to release the eggs into the most suitable environment - in cod for example the courtship "dance" leads to release of the eggs into the upper currents of the sea where they will flow with the plankton which ensures future food supply; salmonids court in a gravel nest and the eggs released fall between the pebbles which offer protection etc. (Keenleyside, 1979). In addition to the obvious pattern of a courtship ritual there is increasing recognition that fish communicate by release of semiochemicals or pheromones (Stacey *et al.*, 1986; Stacey, 1991; Canario and Power, 1995; Stacey and Cardwell, 1995). A male who can detect which female is about to ovulate clearly has a selective advantage over other males. Since the hormonal pattern of the female changes rapidly during the final maturational phase of germinal vesicle migration, breakdown and ovulation, release of the hormones responsible or their metabolites either from ovarian fluid or via the urine would be a means by which the female could signal reproductive readiness. 17,20βP, its conjugates, and prostaglandin $F_{2\alpha}$ have all been shown to be detected by male fish and in some cases to stimulate his secretion of pituitary gonadotrophin leading to increased milt production. In some species such as the herring, pheromones in the milt stimulate other fish in the region to release eggs and sperm in a mass spawning (Sherwood *et al.*, 1991).

4.6 Reproductive strategies

Among the 20,000 or more species of teleost, almost all conceivable reproductive strategies have been adopted (Keenleyside, 1979). The numbers of eggs may range from a very few guarded well by the parent in a nest or held internally in the body cavity, to the millions released by cod into the plankton with no further parental care. Eggs may be spawned into moving oceans currents which will bear them thousands of kilometres before hatching, or they may be carefully laid on vegetation, between pebbles, inside crevices or even in the cavities of live mussels. Spawning may be an annual event as in many temperate freshwater fish, may be continuous throughout the year or

may, as in eels and Pacific salmon, be the final act of their lives followed by inevitable death. Particularly in such species it may be the culmination of a long spawning migration involving movement between fresh and sea water with the inevitable requirement for changes in osmoregulatory activity. Spawned eggs may contain little yolk and hatch within days as in spring and summer spawning cyprinids or they may, as in trout, contain sufficient yolk in the large eggs for the hatch to take place several months after spawning.

4.7 Fecundity and Fertility

The ultimate measure of reproductive success is the number of surviving progeny that carry genes derived from the parents, and who will in turn reproduce successfully. In addition to the species-specific strategies discussed above, fish within a given species may regulate the numbers and sizes of the eggs they produce in response to external environmental stimuli. Fecundity, the numbers of eggs produced, determines only the numbers of *potential* offspring. There is, however, a trade-off with size since larger eggs, which provide greater nutritional reserves, may have a better chance of survival than smaller eggs. Fecundity may be determined either before the spawning season (determinate fecundity), or may be increased during gonadal development by recruitment of additional oocytes into vitellogenesis or reduced by atresia (indeterminate fecundity) (Hunter and Macewicz, 1985). The number of eggs will also usually increase with size (age) of the fish. The balance between large numbers of smaller eggs and small numbers of larger eggs may be affected by water temperature, salinity, food availability, and stress (Tanasichuk and Ware, 1987; Contreras-Sanchez *et al.*, 1995; Wilson *et al.*, 1995). Since fecundity is age related, it is clear that early mortality, resulting from either pollution or fishing activity will have an effect disproportionate to the number of spawning seasons lost. Although larger eggs give an increased amount of nutrient to the developing embryo they also allow for increased incorporation of pollutant from the mother and consequent greater exposure of the embryo during development. Species that produce large eggs may therefore be more at risk of embryonic deformities arising from maternally transmitted xenobiotics.

Reproductive strategies of fish have evolved to give a balance between numbers of eggs produced and survival rates of the offspring. It is a fallacy to believe that there can be no problems with fish stocks since fish produce millions of eggs and some will always survive. They produce millions so that at least two may survive and maintain stocks. Fewer eggs, lower fertilisation rates or decreased survival of the larvae and offspring, even at a relatively low level, will inevitably threaten this balance. Furthermore ultimate reproductive success is measured not only by survival of the offspring, but by their

reproductive success and that of their progeny. Large numbers of offspring may indeed survive but, if they have reduced fertility, few genes will be passed on to later generations. Reproductive success is therefore a measure both of numbers of offspring surviving to adulthood and the numbers of their offspring that survive to produce fertile progeny i.e. the number of grandchildren and great-grandchildren. Since pollution can affect lifetime fecundity, survival of the offspring to adulthood, and the fertility of these offspring it is essential that any indicator of its effects on fish stocks considers the totality of reproductive success and does not rely on a simplistic measure such as dead fish, GSI, numbers of eggs produced etc., however valuable these may be as individual indicators

4.8 Sex determination and reversal

In mammals, sex is genetically determined by the XX or XY combination of chromosomes. During fetal development testicular tissue, determined by the Y chromosome, secretes both an anti-Müllerian factor and testosterone which together destroy the basic female pattern of development and superimpose the male pattern of reproductive ducts and glands. During the neonatal period, a further secretion of testosterone by the testis and its conversion to estradiol (often considered as the *female* sex steroid) in the brain imposes the male pattern of non-cyclic pituitary hormone release and pre-programmes a number of key enzymatic and behavioural patterns which are only expressed at puberty (Short, 1982; Vom Saal *et al.*, 1992). A failure, or decreased ability to synthesise or secrete these hormones at the critical period of development results in either female development or incomplete male differentiation with lost or decreased fertility of the adult. It is important to note both that female differentiation does not require any hormone and arises simply from the *lack* of testicular secretion, and that these effects are irreversible and cannot be changed by hormone administration in later life. Any external factor which leads to altered hormonal exposure at this critical stage of fetal life will have similar effects and is well exemplified by the case of stilbestrol administration during pregnancy in the 1960s (Colborn and Clement, 1992). In mammals there is no such thing as a functional hermaphrodite and no amount of hormonal administration can give functional sex reversal.

In fish, sex determination is much less clear than in mammals and the XY chromosomal pattern is far from universal. In some species, even the temperature at which eggs are hatched can determine the proportions of male and female offspring and may be due to the effect of temperature on the synthesis of steroid hormones (Strüssman and Patiño, 1995). Sex may also be reversed by administration of hormones during certain critical periods of embryonic development (Hunter and Donaldson, 1983). Furthermore, such

phenotypically reversed females can produce sperm which may be used to fertilise normal females and produce eggs which are genetically 100% female.

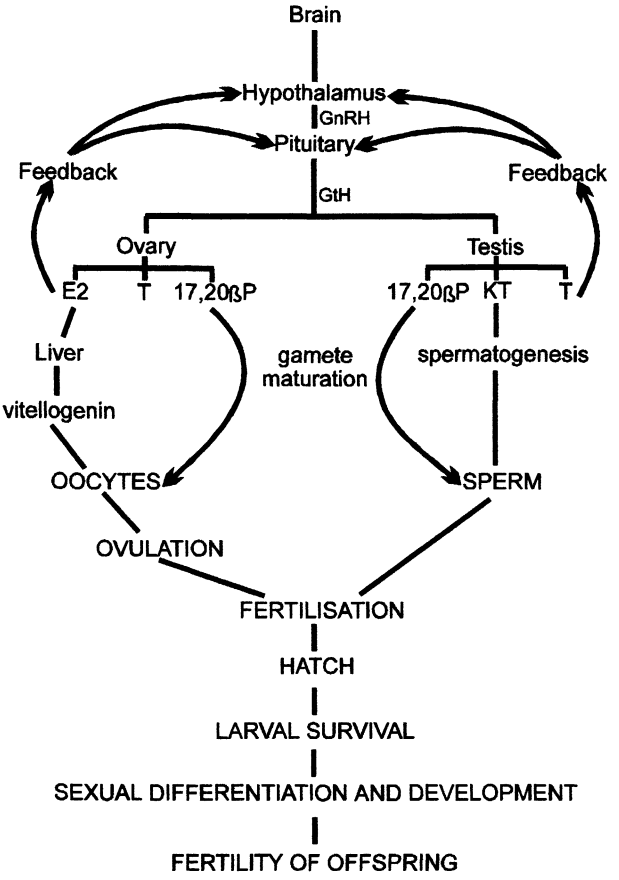


Figure 4.7. The reproductive system of fish and possible sites of action of pollution (GnRH=gonadotrophin releasing hormone; GtH = gonadotrophin; E2 = estradiol; T = testosterone; KT = 11-ketotestosterone; 17,20βP = 17,20β-dihydroxy-4-pregnen-3-one).

In some species of fish, especially the sparids and serranids, natural sex reversal occurs as part of the normal life cycle. In protandrous species, all fish hatch and develop as normal males but, after several reproductive cycles, they undergo a change involving regression of the testis and growth of an ovary. In protogynous fish the reverse occurs and the all-female younger fish change into males. Frequently such species adopt a hierarchical colony of a dominant male (or female) and a number of functional or non-functional females (or males). Removal of the dominant fish leads to the rapid sex change of the next in the hierarchy. Clearly aggressive interactions (and probably interrenal activity) play a role, but the mechanisms of regulation and

the hormonal involvement of androgens and estrogens in sex reversal is still far from clear.

4.9 Impact of pollutants

The aim of the foregoing was to briefly outline the reproductive control in fish, and to demonstrate some of the very wide diversity to be found in their reproductive patterns. It should be immediately clear that reproduction involves a complex chain of hormonal and developmental events (Figure 4.7) and that even minor disruption at any site can have a severe impact on the reproductive process as a whole.

Hormones act at extremely low concentrations and are present in plasma at typical concentrations of 1-10 ng/ml (1-10 ppb). For a hormone mimetic pollutant, a concentration of 1 mg/l (1 ppm) in plasma represents 100-1000 times the amount that is naturally present, so even an activity of 1/1000 of the natural hormone can be important. What is really important is the concentration at the receptor site, and for a pollutant which concentrates in the target tissue local concentrations could be even greater than this.

Many insecticides act on chemical processes of the brain and such effects may clearly modify both the neural interpretation of environmental signals indicative of seasonal cycles and the behavioural patterns necessary for courtship, foraging, mate selection etc. It is, however, unlikely that such parts of the brain would be selectively affected and damage is most likely to upset a whole range of neural activity leading to early mortality. At very low levels, however, the general effects on neural activity may not be harmful to general functioning, but disruption of neurones involved in interpretation of environmental and behavioural signals and innervation of the hypothalamus might have a disproportionate effect on the endocrine system which acts as an amplifier of these signals. Direct disruption of enzyme activities at the cellular level may disrupt the synthesis and release of hypothalamic releasing hormones, pituitary hormones, gonadal steroids and their hepatic catabolism, as well as hepatic vitellogenin synthesis. Clearly there is also a cascade effect since inhibition of hormone production at the hypothalamus or pituitary will inevitably lead to decreased gonadal steroid production which in turn will result in decreased gonadal development and lower GSI. In many of the studies which will be discussed later in this volume a single parameter, such as the GSI, has been chosen for ease of measurement. Although this informs us that reproduction is most probably inhibited, it is not clear whether the primary site of action is the gonad itself, by blocking receptors or inhibiting steroidogenesis, or whether it is due to primary failure of secretion of the pituitary or blockage of the neurotransmitters in the hypothalamus or the brain itself.

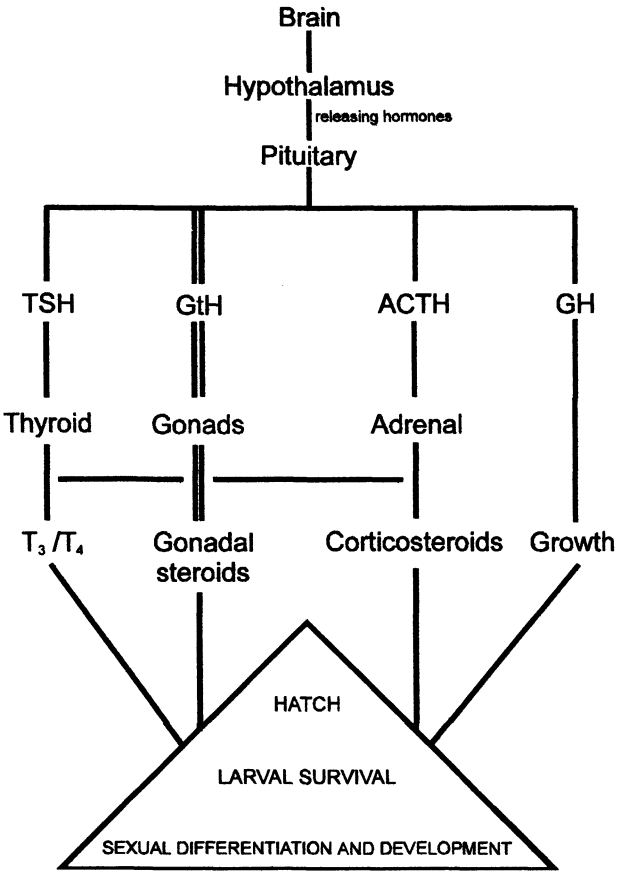


Figure 4.8. Other endocrine effects on reproduction. TSH = thyroid stimulating hormone; GtH = gonadotrophic hormone; ACTH = adrenocorticotrophin; GH = growth hormone; T_3/T_4 = triiodothyronine/thyroxine.

Although gonadotrophin and the gonadal steroids play the major role in reproductive development, their actions may be modulated by a range of other endocrine factors (Figure 4.8). Stress, for example, has a clear inhibitory effect on reproduction (Kime *et al.*, 1980; Campbell *et al.*, 1992, 1994), probably acting via adrenocorticotrophin (ACTH) or cortisol secreted by the interrenal gland (homologous to the mammalian adrenal gland). Some of the reported effects of pollution on reproduction could be simply a stress effect. The thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), also affect both reproduction and larval development, and any perturbation of the maternal thyroid gland by pollution could affect both ovarian development and, since maternal thyroid hormones are incorporated into the oocyte, larval development. Action at the pituitary and hypothalamus might also affect

secretion of growth hormone which in turn would affect development of both the exposed fish and its offspring.

In addition to direct effects on the reproductive endocrine system itself, it is also likely that other biochemical processes are affected. Once the eggs have been spawned, they are under the influence of both external pollutants, and most importantly, of pollutants laid down into the egg itself during vitellogenesis. Such exposure may affect ontogenesis, resulting in malformed and unviable larvae, or lead to death of either egg or larvae as critical stages of development are blocked. At lower concentrations development may be normal, but any inhibition of steroidogenesis in the embryo will impact on the hormonal profile and may lead to changes in sexual differentiation and/or development. Such effects may result in decreased fertility when the fry attain reproductive maturity. As yet little is known of such possible second generational effects which, if demonstrated, would be the most dangerous and unpredictable effect of pollution on long term viability of fish stocks.

Overall, each species has adapted to its specific habitat and reproductive strategy so that it is in such a balance as to be able to increase numbers as the opportunity arises from availability of fresh habitats, and to replenish numbers removed by predatory activities or disease. Competition for limited food supplies ensures survival of only a small proportion of the remainder. For a stable population only two out of possibly over two million eggs (0.0001%) spawned over a lifetime need to survive and reproduce. Species with a low reproductive rate and a strategy of production of small numbers of eggs require a much higher survival rate, but this is generally ensured by parental care to obviate the high losses associated with young fry. Such a balance is easily upset by pollutants (Figure 4.9).

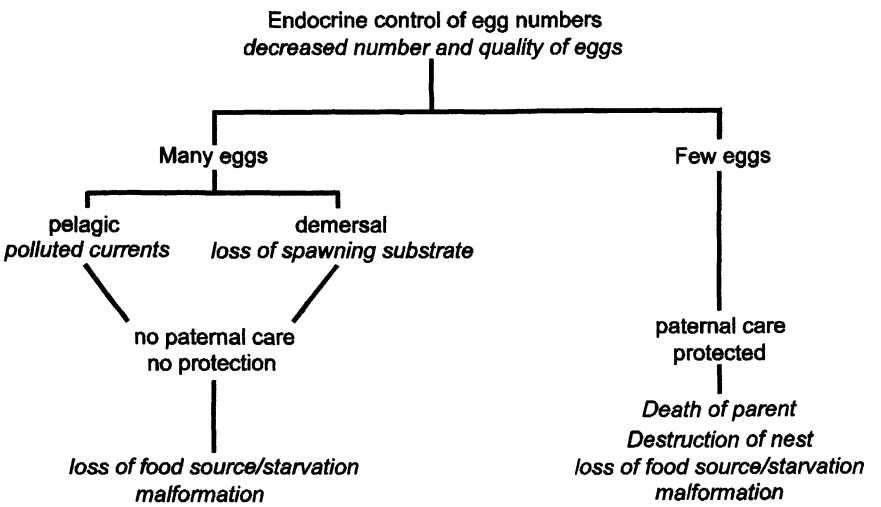


Figure 4.9. Factors affecting fecundity and fish populations.

Changes in hormonal equilibrium could decrease both the numbers and quality of the eggs spawned. Spawned pelagic eggs may have low survival rates if swept into polluted ocean currents. Vegetation which is necessary as a spawning substrate for many demersal eggs and as nest building material for some species exercising parental care may be affected by pollution. Absence of such vegetation may either decrease the survival of the eggs or even prevent spawning if its presence is an essential visual cue which either triggers synthesis of the maturation inducing steroid or courtship behaviour. For all species loss of food, both animal and vegetable, can lead to starvation, poor health and decreased fertility or premature death.

Controlled fisheries taking a limited number only of those fish that have had the opportunity to reproduce at least once may have a relatively small effect on population numbers. Uncontrolled fishery with use of smaller mesh sizes for capture of immature fish, together with larger numbers of unwanted non-commercial species can have a more serious impact. If this is combined with high pollution levels which both decrease the fecundity of the remaining fish and shorten their lifespan and therefore their number of spawnings, recruitment can no longer match losses and stocks will rapidly decline.

4.10 Mechanisms of Action

Pollutants can act at a range of sites within the reproductive system (Figure 4.10). Pesticides, in particular, can damage the nervous system and so might be expected to damage the neurones involved in reproductive behaviour, transmission of retinal or pineal signals of seasonal or diurnal cyclicity or in regulation of the hypothalamus. There have been, as yet, no studies to determine whether these neural functions are affected to any greater extent than any other area of the brain. Pollutants may also inhibit the enzymes involved in brain aromatisation of testosterone which may be important in feedback signalling from the gonads. They can affect the pituitary by altering its ability to synthesise and release gonadotrophin (GtH), thyrotrophin (TSH), adrenocorticotrophin (ACTH) and growth hormone (GH) which in turn will lead to decreased stimulation of the gonads, the thyroid and interrenal glands and general growth. Such effects might be exerted by causing morphological damage to the pituitary, by affecting the hormone synthesising mechanism or by inhibiting the feedback regulation by gonadal and thyroid hormones. Within the gonad, thyroid, interrenal and liver, pollutants can affect the activities of biosynthetic enzymes. Cytochrome P450 enzymes, which are involved in many steps of both gonadal synthesis and hepatic catabolism, are sensitive to a range of pollutants (see Chapter 9). Morphological changes may also result from pollutant exposure, but it is necessary to distinguish between primary causes in which the pollutant directly affects the cellular integrity of

the gonad, and secondary causes which result from under or over secretion of pituitary gonadotrophin. Pollutants can also affect a range of developmental processes within the embryo and larvae.

BRAIN/HYPOTHALAMUS	Neural damage, GABA receptors etc GnRH release, aromatisation Behaviour patterns (courtship)
PITUITARY	GtH, TSH, GH, ACTH synthesis and release Feedback mechanism/aromatisation
GONAD	Enzyme inhibition, GtH receptors Intracellular messengers Hormone mimic Morphological damage Vitellogenin incorporation into oocyte Gamete development & maturation
THYROID/ADRENAL	T_3, T_4 and corticosteroid synthesis
LIVER	Hormone catabolism Estrogen receptor, Vitellogenin synthesis
EMBRYO/LARVAE	Growth and Development Sexual differentiation Hatch, Survival Reproductive success as adult
ENVIRONMENTAL	Decreased food resources Loss of spawning substrate

Figure 4.10. Sites of action of pollutants on reproduction.

In addition to their toxic effects, some pollutants may act as hormone agonists or antagonists. To act, a hormone must bind to a receptor. In the case of large peptide molecules such as gonadotrophin, these are located in the cell membrane, but cause concentration changes within the cell of second messengers such as c-AMP or calcium. Small molecules such as steroids pass through the membrane and bind to receptors within the cell, which then initiate translation of relevant genes within the DNA of the nucleus. Such receptors may recognise a pollutant, such as an environmental estrogen, bind it and act in the same way as if they had bound the natural hormone. Such

pollutants will act as hormone mimics. Alternatively the receptor may bind a pollutant, but fail to elicit the same changes that occur when it binds to the natural hormone, or it may be bound so strongly that it cannot be displaced by the natural hormone (Figure 4.11). The anti-androgenic action of the DDT metabolite DDE in mammals (Kelce *et al.*, 1994, 1995) may result from such a mechanism where the receptor site is blocked and cannot bind the natural hormone.

In both cases, the relative affinity of the pollutant and the natural hormone for the receptor site will determine the activity. In the case of membrane receptors for peptide hormones such as gonadotrophin, pollutants could affect either binding of the hormone to it's receptor or the production and action of the second messenger. Inhibition of adenyl cyclase or changes in calcium balance could be particularly important in this respect.

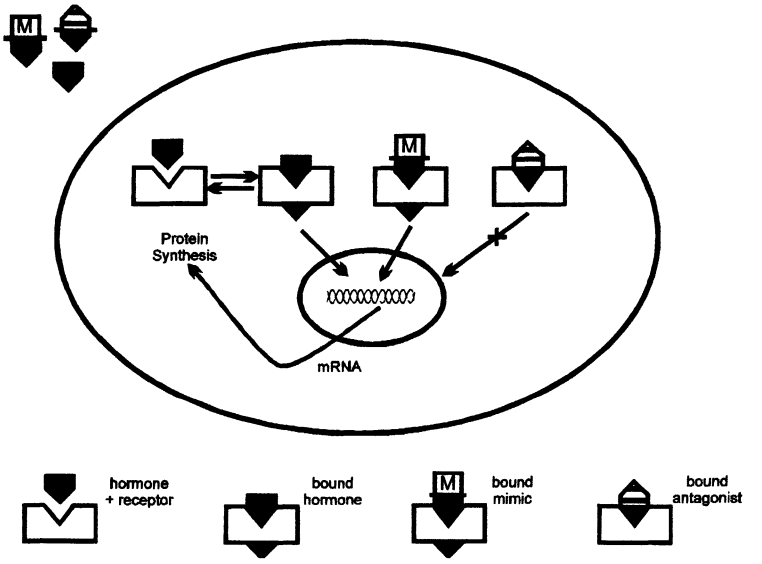


Figure 4.11. Mechanism of action of hormones, hormone mimics and anti-hormones.

In addition to these direct factors affecting the endocrine part of the gonad, pollutants may affect gamete development by disruption of hormonal balance, nutrition and growth. In the ovary, pollutants may be transferred into the oocytes where they can affect normal development of the larvae. Such development may be regulated by thyroid hormones, growth hormones and a range of intracellular messengers. Sperm motility, and hence fertilisation, can be affected by developmental abnormalities resulting from either faults in the hormones from the interstitial (Leydig) cells, or by nutritional abnormalities resulting from disturbance of the Sertoli cells.

The liver plays an important role in metabolising steroids, and hence in maintaining the plasma levels. A decreased rate of metabolism will lead to higher plasma levels and, through negative feedback, decreased pituitary secretion of gonadotrophin. The liver is also the target tissue for estrogens and in response produces vitellogenin which is incorporated into the developing oocyte.

This Chapter has aimed at giving a simple introduction to reproductive endocrinology of fish, but the full picture is vastly more complicated and endocrinology now encompasses a vast array of intracellular messengers which interact at a variety of levels including embryonic development and functioning of the immune system. Study of such factors is yet in its infancy and very little information is available as to how they might be affected by pollutants.

4.11 Experimental Approaches

The above discussion has centred on the various sites of the reproductive system that might be affected by environmental pollutants. Many of the references cited in the Appendix and in the following Chapters show that the reproduction of fish is affected by pollution, with the result that the gonads are abnormally small, there are few eggs, or the larvae fail to survive. Far fewer studies have examined how these pollutants act within the endocrine system or how such studies can lead to determination of the sites most sensitive to pollutants or to a prediction of harmful levels that will affect fecundity. Two major investigative approaches can be envisaged - *in vivo* and *in vitro.*

4.11.1 *In vivo* experiments.

Treatment of the whole animal is the most realistic approach to that pertaining in polluted environments. In such experiments fish are exposed to sublethal levels of suspected pollutants for an appropriate length of time and the effects on different organs, tissues, cells or hormones examined. A fish may, for example, be put into 1 μg/l DDT for three weeks and measurements taken of plasma concentrations of estradiol, vitellogenin, gonadotrophin and 11-ketotestosterone and histological sections taken from the pituitary, liver and gonads. Radioimmunoassay (RIA) and Enzyme Linked ImmunoSorbent Assay (ELISA) methods are available to measure the plasma hormones. The definition of "sub-lethal" or "safe" requires some care since there is the temptation to use the highest dose consistent with the fish surviving over the period of the experiment so that a harmful effect is found which can be published. If treated fish are in worse condition than untreated controls, the

experiment is probably of little real application since the fish would not survive long enough to spawn, and the changes observed may be attributable simply to stress effects. Ideally a sufficiently wide range of doses should be used to show the maximum dose that has no effect on the parameters measured, and evidence produced that the treated fish differ in no other way from the controls, i.e. they do not differ in rates of disease and mortality. Experiments in which a single dose of two different pollutants are compared have little real value. Some of the harmful effects which have been demonstrated *in vivo* undoubtedly arise from secondary effects of stress rather than direct pollutant inhibition of reproductive function. It has clearly been shown in fish as well as other vertebrates that stress is a major inhibitor of reproduction (Kime *et al.*, 1980; Pickering *et al.*, 1987; Campbell *et al.*,1992, 1994).

It is also important to realise that the timing of the experiment is important, such that exposure during gonadal recrudescence will have different effects to those carried out during final gamete maturation. A major difficulty in reproductive studies is that a dynamic situation is being monitored, and the timing of the start, duration and termination of the experiment will all affect the outcome and make comparison with other studies very difficult. This is a particular problem with annual spawners such as trout in which studies can only be performed during a relatively short window each year. Small fish which can be spawned in the laboratory throughout the year, such as danios, minnows or medaka have advantages in this respect since fish can always be available at defined stages of their reproductive cycle.

In vivo studies can also be extended to the offspring with measurements of the survival rates and hatchability of the eggs and larvae, or the eggs themselves may be exposed to pollutants. Exposure of the female parent has the additional advantage that measurements may be made of actual incorporation of the pollutant into the egg and correlation of such concentrations with survival rate. In exposed males, measurements may also be made of the sperm motility, or fertilisation rate. One of the major difficulties is in choosing an appropriate exposure regime; whether to expose to polluted water, to administer a contaminated diet, or to inject the xenobiotic directly into the peripheral circulation. Choice of species is also important, since insufficient plasma for hormone analysis may be available from small species, while it is difficult to hold larger species sufficiently long or in large enough numbers to examine intergenerational effects or population dynamics.

While it may superficially appear attractive, the comparison of fish from polluted and unpolluted water has a number of difficulties. It is not always easy to ensure that the appropriate controls are used in such experiments. In the case of a point-source effluent, such as a factory or sewage works, fish may be taken from above and below the effluent for comparison only if they

do not migrate between the two areas. If they do not migrate the possibility then arises that they may be genetically different and be at a different stage of gonadal maturity. It is also possible that the effluent may be at a different temperature to the river and that the observed effect may be thermally rather than chemically induced. During the breeding period many fish spawn at a spacific time of the day, e.g. dawn, and it is particularly important that sampling at different sites is done at the same time of the day so that diurnal changes in hormones or gamete quality and quantity are not falsely attributed to differences between sites. Such sampling is, however, valuable in that it can lead to further *in vitro* and *in vivo* studies of the components of the effluents and has provided evidence for reproductive disruption by pulp mills and sewage effluents. Marine species are more difficult to examine in the wild and samples taken from polluted and unpolluted estuaries and bays can only be used if migratory, spawning and genetic information is available to substantiate the comparison between the populations.

Another approach is to measure a range of parameters, such as plasma hormone levels, fecundity etc. from wild fish and relate this to tissue levels of a particular pollutant. Similarly, laboratory fish can be exposed to a range of pollutant concentrations and accumulations of the pollutant in various tissues compared with those in fish from polluted ecosystems. Direct comparison of treated and untreated laboratory fish may then be extrapolated to predict effects on wild fish with the same concentrations of the pollutant. The major problem with such an approach is that the wild fish may contain accumulations of many other pollutants in addition to the one of concern and there may therefore be no apparent correlation between the tissue levels of a specific xenobiotic and the measured parameter.

Suitable endpoints for monitoring the effects of xenobiotics *in vivo* include: steroid and pituitary hormone levels; gonadosomatic index; gonadal, pituitary and hepatic histology; courtship behaviour; sperm quality and quantity; egg size and numbers; hatch rate; sex ratio of offspring; sexual development and differentiation of offspring; fecundity and behaviour of the offspring (the F_1 generation); fecundity and behaviour of the F_2 generation.

While the *in vivo* approach is useful for studying the integral effect in the whole animal it gives little information on the site of action or the cellular mechanism of action. For such information it is necessary to use *in vitro* studies of individual tissues.

4.11.2 *In vitro* experiments

While an *in vivo* experiment may show, for example, that estradiol and GSI are decreased and that there is degeneration of the ovary, such observations are consistent with decreased GtH secretion resulting from pituitary damage, damage to ovarian GtH receptors, morphological damage to

the ovary and its steroidogenic cells, decreased activity of the steroidogenic enzymes or to increased hepatic clearance of estradiol. Suitably designed incubations of isolated tissue can resolve such possible mechanisms of action. The ovary may be incubated alone or with GtH, and with a range of doses of the pollutant to determine whether the GtH stimulation of estradiol is affected, or the liver (or hepatocytes) may be incubated with estradiol and a pollutant to determine whether estradiol stimulated vitellogenesis is affected. The characteristics of binding sites for estradiol in liver, or GtH in ovary might also be examined with a range of pollutant concentrations. Individual steroidogenic enzymes may be examined by incubation of gonadal tissue with exogenous (usually radiolabelled) precursor and the rate of production of metabolites examined.

In vitro studies are also valuable as screening tools for detecting potential endocrine disruptors, in that large numbers of xenobiotics may be routinely tested using the minimum numbers of animals since a carp or salmon gonad can provide sufficient tissue for several hundred experiments. Specific cell lines or tissue cultures have rarely been used in fish, but are commonly used for regulatory testing in mammals.

One of the major drawbacks of the *in vitro* system is that it looks at specific tissues or cells in isolation from other influences. Many xenobiotics are metabolised by the liver or other tissues so that it is a metabolite, rather than the xenobiotic itself, that comes into contact with the gonad. *In vitro* experiments are also usually short term (1-6 h) so that effects consequent to long-term pollution-induced changes in the gonads are not readily detected. These disadvantages can be partly overcome by combining the two approaches and using *in vitro* studies on fish which have been exposed to different concentrations of pollutants (the *ex vivo* approach). This approach also has the advantage that it can be used to examine the effect of pollutants which have bioaccumulated within the gonad, and the end point related to the level of accumulation. This avoids one of the major difficulties in designing *in vitro* studies which is the choice of concentration of pollutant to use.

Suitable end points for *in vitro* or *ex vivo* experiments would include: steroid hormone production with and without GtH stimulation; identity of steroid profiles using radiolabelled precursors; binding characteristics of receptors or plasma binding proteins; gene expression. As with *in vivo* studies it is important that the exact stage of maturity of the gonad used is carefully defined.

4.11.3 Synergisms.

In both *in vitro* and *in vivo* studies it is important to recognise that pollutants do not act in isolation and that most fish will be exposed to a cocktail of chemicals. Although each may act individually they can also have

a synergistic effect such that the total effect is not simply the sum of individual effects. One pollutant may, for example, cause hepatic damage which in turn will diminish the efficiency of the liver to catabolise a second pollutant and remove it from the organism, leaving more of it free to cause damage to other parts of the system. The two pollutants will thus be considerably more harmful than one on its own. In other cases one pollutant may diminish the harmful effects of another. Such effects are more commonly found with heavy metal pollutants in which zinc, for example, can diminish the harmful effects of other metals (see Kime *et al.*, 1996). Many pollutants, such as PAHs, PCBs, or alkylphenols, contain a mixture of chemical isomers and congeners, all of which may have different potencies, and which usually occur in different proportions in different areas. The proportions may be important in quantifying any effect of such products.

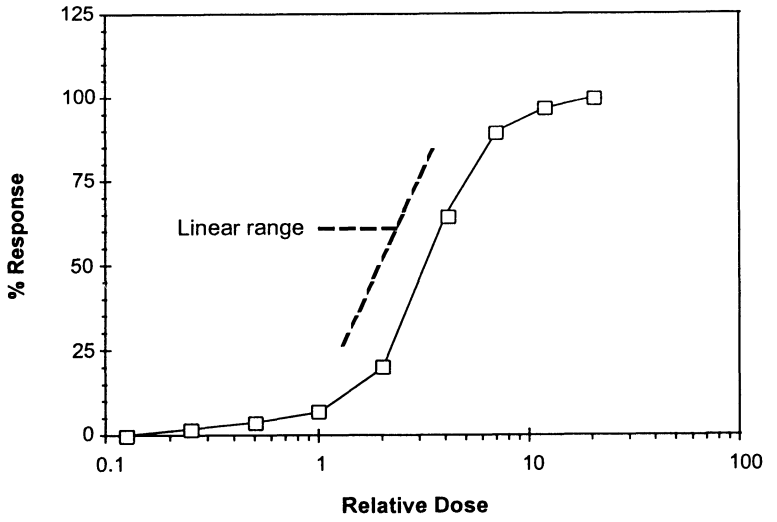


Figure 4.12. Schematic figure of a typical dose response curve. The graph shows that the linear response occurs over a narrow dose range. The lower dose range of the curve is highly transient and use of doses within this range may favour a synergistic interpretation of the data. For example, a relative dose of 1 produces a 5% response, whereas a relative dose of 2 produces a 25% response (Reproduced from Zacharewski, T., Campbell, P., Routledge, E., Cooper, R., Kime , D., Wenzel, A. and Huet, M-C. (1997). Current approaches for the use of *in vitro* tests in identifying the hazard of endocrine modulating chemicals to wildlife., In "SETAC-Europe/OECD/EC Expert Workshop on Endocrine Modulators and Wildlife - Assessment and Testing (EMWAT), Veldhoven, The Netherlands 10-13 April 1997." (L. Tattersfield, P. Matthiessen, P. Campbell, N. Grandy and R. Lange, Eds.), pp.41-58. by kind permission of SETAC-Europe, Amsterdam).

Synergistic effects have so far received little study, but they are essential to any understanding of the effects of pollutants on fish. In any study of

synergism it is important that it be validated by suitable dose response curves for both of the xenobiotics used. Many dose-response curves are non-linear and a true synergistic effect is only demonstrable within the linear part of the curve (Zacharewski *et al.*, 1997). For example if two xenobiotics A and B both cause an identical 5% response at a dose of 1 unit, a response of 25% (rather than 10%) from exposure to 1 unit of A plus 1 unit of B is not synergism if 2 units of A or 2 units B also gave this response due to a non-linearity of the dose response curve (Figure 4.12). As with all studies it is important to use a range of concentrations.

4.11.4 An integrated approach

It is essential to keep all experimental approaches within an appropriate context. Each parameter measured provides information on a particular system or pollutant, but what really matters is the fish population as a whole. The inhibition of ovarian steroidogenesis, for example, at 10 mg/l of pollutant is irrelevant if embryonic mortality occurs at 0.01 mg/l and respiration ceases at 1 mg/l. To provide such information it is therefore essential to examine the whole life cycle of the animal and determine the most sensitive component to the pollutant in terms of the population as a whole. This can be done by exposing small populations of fish to the pollutant and determining firstly the dose that has no obvious effect on the general health of the animal or on it's life span. This should then be followed by increasingly smaller doses until only one or two components are affected. Although this may locate the most sensitive component, it may not necessarily have the greatest impact on the population as a whole. It is thus important to consider the relative sensitivities of each component and use these to model the effects of the disruption on the eventual population of the species. It is also important to consider the different aspects of a particular pollutant. The recent concern with environmental estrogens has often obscured the fact that many of these pollutants are toxic in the own right. Such chemicals may well be endocrine or reproductive disruptors, but this could be due to toxicological as well as hormone-mimetic effects. Stimulation of vitellogenin may be a good measure of estrogenic activity, but the question must be asked whether this actually harms the fish. Does the pollutant decrease testis size and activity because it is an estrogen or because it causes morphological or neurological damage elsewhere in the reproductive system as a result of its toxicological activity? For the weakly estrogenic organochlorine pesticides whose primary aim is to disrupt normal biochemical function this is a particularly important question which remains to be answered.

4.11.5 A basis for regulatory testing

The issue of endocrine disruption is very recent and the majority of regulatory tests are not designed to examine this aspect of toxicity. Although current tests for reproductive dysfunction may pick out some potential endocrine disruptors, others may well escape detection. The data presented in this volume, and particularly in the Appendix, suggest that a very wide range of chemicals in current use can be considered as potential endocrine disruptors. There is, however, little consistency in the way the data has been obtained, and little attempt to define lowest observable adverse effect concentrations (LOAEC). Although many of these studies could form the basis for regulatory testing there are, at the time of writing, no nationally or internationally harmonised methods for testing for endocrine disruptors in wildlife, including fish. Recent discussions suggest that structure-activity relationships, *in vitro* and *in vivo* testing, and field surveys can form the basis for development of such a regulatory framework (Tattersfield *et al.*, 1997). The data presented in this volume will give some indication of the measurable parameters which might form the basis for such tests.

Chapter 5

Disruption of Hypothalamic and Pituitary Function

5.1 The Hypothalamic-pituitary system in fish

Since the hypothalamus and the pituitary glands together regulate the whole of the endocrine system, any pollutant which affects their function will inevitably affect the function of the entire organism. The hypothalamic-pituitary system in fish shows close similarities to the mammalian homologue (Batten and Ingleton, 1987). The hypothalamus, the main integrative centre controlling synthesis and release of the pituitary hormones is innervated from the brain from which it receives information about body homeostasis and external environmental cues. The pituitary consists of two parts with distinctly different embryological origins, the neurohypophysis and the adenohypophysis. The neurohypophysis is stimulated via direct neuronal imput from the hypothalamus and secretes isotocin and arginine vasotocin (AVT) which are involved in salt and water balance. The pars distalis (part of the adenohypophysis) of the pituitary is stimulated by releasing factors secreted by the hypothalamus which in turn initiate the release of adrenocorticotrophic hormone (ACTH), thyrotrophin (TSH), and the gonadotrophins (GtH-I and GtH-II) from the corticotrophs, thyrotrophs and gonadotrophs respectively. ACTH, TSH and GtH in turn act respectively on the interrenal, thyroid and gonads (see Chapters 6, 7 and 10) (Figure 5.1). The pituitary also secretes growth hormone (somatotrophin), melanocyte stimulating hormone (MSH) and prolactin which are regulated from the hypothalamus by both inhibiting and releasing factors.

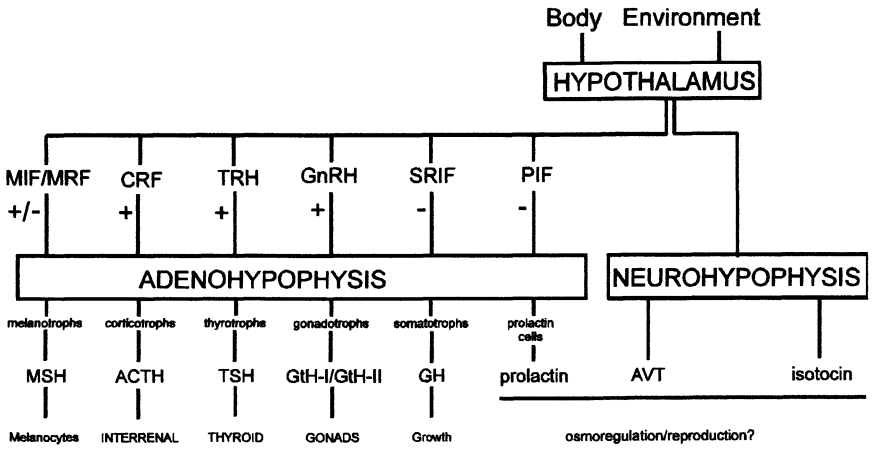


Figure 5.1. The hypothalamic-pituitary system in fish and its hormones. MIF/MRF = melanotroph inhibiting/releasing factor; CRF = corticotrophin releasing factor; TRH = thyrotrophin releasing hormone; GnRH = gonadotrophin releasing hormone; SRIF = somatotrophin release inhibiting factor; PIF = prolactin inhibiting factor; MSH = melanocyte stimulating hormone; ACTH = adrenocorticotrophin; GtH-I/GtH/II = gonadotrophins I and II; GH = growth hormone; AVT = arginine vasotocin.

Many of the inhibitory effects of pollutants on reproduction of intact fish could be mediated via the pituitary and hypothalamus rather than acting directly on the gonad itself. It is, however, much harder to isolate the pituitary-hypothalamic system than the gonad or liver, so it has received relatively little attention. Studies of the effects of pollution on the pituitary have related predominantly to the gonadotrophins, adrenocorticotrophin and thyrotrophin. Other hormones have received very little attention.

5.2 Effects of pollutants on the hypothalamus

The hypothalamus, which is functionally part of the brain, is particularly difficult to examine in isolation. There is little reason to suspect that its neural function is disrupted by pollutants in any way that differs from that of a neurotoxin which will destroy any nervous tissue and lead to a general malfunction of the animal. Pesticides which are designed to damage the nervous system of target species would clearly be expected to cause such disruptions. There might, however, be a quantitative difference in the effect on the animal since the endocrine system acts as an amplifier of the signal and relatively minor disruption of the neural imput could result in profound disturbance of endocrine function. Pollutants might therefore affect neurosecretory function within the hypothalamus at levels lower than that which induces general neural damage and this in turn could disrupt the secretion of releasing hormones into the pituitary. There is some evidence

that this may occur with mercury since *Channa punctatus* held for 6 months in water containing 0.01 mg/l mercuric chloride, or 0.2 mg/l of the mercurial fungicide Emisan (methoxyethylmercuric chloride) showed no apparent signs of toxicity, yet the ovaries contained no vitellogenic oocytes whereas the controls were fully ripe with mature vitellogenic Stage IV oocytes (Ram and Joy, 1988). Examination of the hypothalamo-neurohypophysial complex indicated that in exposed fish the neurones of the nucleus preopticus, which are responsible for GnRH release, showed signs of cellular pyknosis and nuclear necrosis, particularly in the Emisan treated group. Five-month exposure of *Clarias batrachus* to lead nitrate (Katti and Sathyanesan, 1986), caused accumulation of neurosecretory material in the anterior neurohypophysis, with degenerative changes in both the nucleus preopticus and the nucleus lateralis tuberis (Katti and Sathyanesan, 1986). The resultant failure to secrete GnRH and hence gonadotrophin was shown by the absence of vitellogenic oocytes in ovaries and sperm in testes together with involution of the Leydig cells, while in control fish the gonads contained fully vitellogenic oocytes, sperm and large Leydig cells within the testes.

Since dopamine acts as a gonadotrophin release inhibiting factor (Chang and Peter, 1983; Chang *et al.*, 1984), the finding that whole brain dopamine levels were elevated after exposure to 0.01 mg/l cyanide for 12 days suggests that the resultant decrease in gonadotrophin release may be responsible for the decreased gonadal development in the exposed fish (Szabo *et al.*, 1991).

The effect of exposure to pollution on the hypothalamic production of GnRH has been investigated indirectly by injecting unexposed fish with a hypothalamic extract from exposed fish. ^{32}P uptake into the ovary was then used as a bioassay of the amount of pituitary GtH released by the GnRH in the extract (Singh and Singh, 1982a). Hypothalamic extracts from catfish exposed to cythion, paramar, aldrin or hexadrin were significantly less effective than those from untreated fish in stimulating ovarian phosphorus uptake. The decreased potency of hypothalamic extracts in releasing pituitary GtH *in vitro* has also been demonstrated using radioimmunoassay in the murrel (*Channa punctatus*) after Metacid-50 or Carbaryl exposure (Ghosh *et al.*, 1990).

5.3 Effects of pollutants on the pituitary

5.3.1 Introduction

The pituitary itself, is much more amenable to investigation than the hypothalamus. Morphologically it can be readily removed and methods are available for the measurement of its hormones. It is, however, a relatively small gland and even in large species only a small amount of tissue is

available which restricts *in vitro* studies. Plasma concentrations of pituitary hormones and histological examination are therefore the most common means by which the effects of pollutants have been examined. Ideally an examination of both of these aspects should be carried out in parallel with examination of steroid or thyroid hormones so that the locus of the endocrine malfunction can be identified.

Histological examination of the pituitary can provide evidence for the mechanism by which its function, and hence secretion of hormones is affected by xenobiotics. It will show, for example, whether the pituitary is responding by normal hyperactivity to loss of feedback from the thyroid, interrenal or gonad, or whether there is degeneration resulting from either loss of releasing hormones from the hypothalamus or from toxic action of the pollutant on the gland itself. It will also show whether there is specific action at the level of the thyrotrophs, gonadotrophs or corticotrophs, which will imply secondary effects on the thyroid, gonads or interrenal.

The morphology of the pituitary gland may be affected by exposure during the very early stages of life. Exposure of the American flagfish *Jordanella floridae* to cyanide at 0.065-0.087 mg/l from fertilisation to hatching reduced the size of the pituitary gland but did not affect its morphology. Sexual maturity was attained 14 days later than control fish and when sexually mature they produced 30% fewer eggs and had a shorter reproductive period, factors which might be attributable to decreased pituitary activity (Cheng and Ruby, 1981). Exposed embryos, however also exhibited abnormalities of the eye and skeleton and in the wild may not have survived to adulthood. A second exposure at sexual maturity did not result in further effects which suggested that the embryonic stage was the most sensitive for pituitary development.

5.3.2 Effects mediated via the gonadotrophs

Exposure to both organochlorine (DDT and BHC) and organophosphate (malathion) insecticides at 4 mg/l for 20 days affected the pituitary of adult tilapia, *Oreochromis mossambicus* (Shukla and Pandey, 1984a). Acidophils and cyanophils of the proximal pars distalis were severely damaged and there was less neurosecretory material in the pars intermedia. In sexually maturing rainbow trout exposed to 10 μg/l cyanide there was also a loss of basophils in the proximal pars distalis which might be the cause of inhibited spermatogenesis (Ruby *et al.*, 1993a). Similar damage together with vacuolisation of both gonadotrophs and thyrotrophs, and damaged nuclei was observed in endosulfan treated tilapia at only 0.001 mg/l (Shukla and Pandey, 1986a).

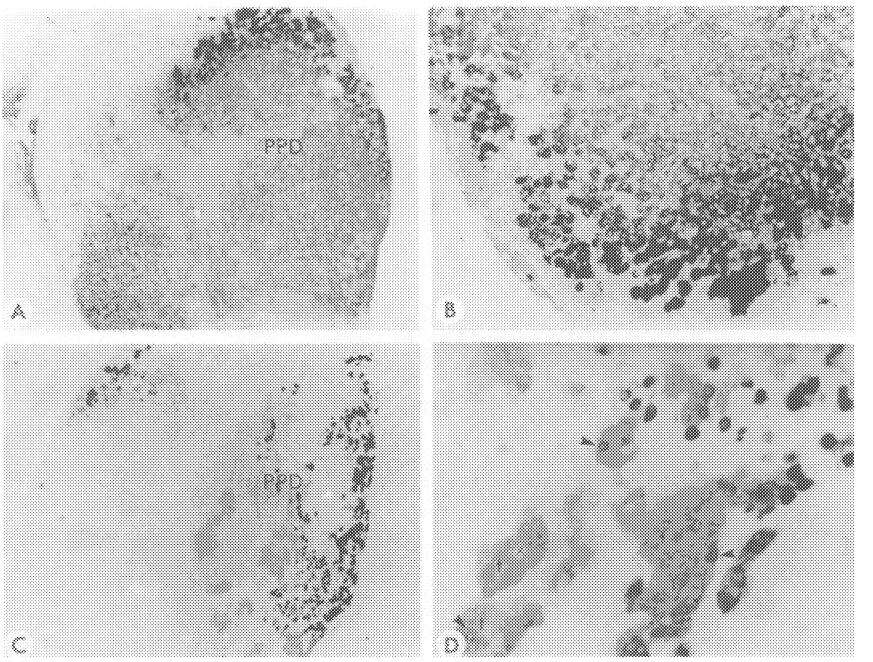


Figure 5.2. Sagittal sections of the pituitary of *C. batrachus* stained with PAP method using *Clarias* anti a, b GtH (1:5000) as the primary antibody A) Control fish in the spawning season with enlarged proximal pars distalis (PPD). The GtH cells were extensively degranulated and vacuolated (x 80), B) The PPD of a control fish showing degranulated and vacuolated GtH cells and groups of TSH cells (x 730), C) The pituitary of a $HgCl_2$ treated fish showing the small size of the PPD and reduced number of GtH cells (x 80), and D) The PPD of a $HgCl_2$ treated fish showing degranulated TSH cell. Arrow heads show cells with atrophic nuclei. The empty space (x) shows a dissoluted TSH cell. The dark cells are GtH cells. (x 730). (Reproduced from Joy, K. P. and Kirubagaran, R. (1989). An immunocytochemical study on the pituitary gonadotropic and thyrotropic cells in the catfish, *Clarias batrachus* after mercury treatment. *Biol. Struct. Morphol.* **2**, 67-70)

Pituitary gonadotrophs were both less active and reduced in number in *Channa punctatus* after 6 months exposure to 0.2 mg/l of the mercurial fungicide emisan or to 2 mg/l of the organophosphate cythion (Ram and Sathyanesan, 1987a, b) leading to correlative decreases in gonadal activity. Both thyrotrophs and gonadotrophs were decreased in size in *Puntius ticto* treated for 30 days with 7.7 mg/l cadmium (Pundir and Saxena, 1992), but the effect was gradually reversible on return to clean water. In the closely related *Puntius sarana,* hypertrophy, extensive vacuolation and degranulation of the gonadotrophs occurred by 30 days at 5 or 20 mg/l but not at 2 mg/l or less (Kumari and Dutt, 1991). Direct morphological damage to the gonads occurred at the same concentration and may have been aggravated by loss of

pituitary stimulation. Both tissues returned to normal after exposure to clean water. Fewer and smaller inactive gonadotrophs were also found in *Clarias batrachus* exposed to organic or inorganic mercury for 180 days (Figure 5.2) (Joy and Kirubagaran, 1989). The gonadotrophs of *Poecilia reticulata* were, however, stimulated after exposure to the lindane impurity β-HCH. This action was attributed to the estrogenic activity of the impurity mimicking positive feedback (Wester *et al.*, 1985). This might also account, at least in part, for the observation that goldfish exhibiting epizootics of ulcerative disease taken from Lake Ontario in which organochlorine content is high, showed no seasonal changes in stage of egg development, frequency of mature ova or in pituitary gonadotrophs which maintained high activity throughout the year (Munkittrick and Leatherland, 1984). These effects could also, however, be attributed to non-functional ovarian gonadotrophin receptors. Pituitary gonadotroph histology of coho salmon differed between the Great Lakes (Leatherland and Sonstegard, 1980a, 1981). Lake Erie salmon had fewer and less active gonadotrophs compared with salmon from the Lakes Ontario, Michigan and Superior and this was related to other reproductive dysfunctions.

Using ovarian ^{32}P uptake in hypophysectomised catfish as a measure of GtH, Singh and Singh (1981, 1982a) showed that pituitary glands from catfish exposed to the organochlorines, aldrin (0.85 mg/l) and hexadrin (0.008 mg/l), or to rather higher levels of the organophosphates, cythion (35 mg/l) and Paramar M50 (32 mg/l) had significantly lower GtH than those from control fish. Using a similar bioassay, plasma levels of GtH were shown to be decreased in both male and female catfish after exposure to hexadrin and cythion (Singh and Singh, 1981, 1982a, 1982a,b). The similarity of this response to the effects on hypothalamic activity (see Section 5.3.1) suggests that the pollutants act on both the pituitary and hypothalamus, since action at the hypothalamus alone would decrease GnRH and could result in increased pituitary GtH content. This dual locus of action is supported by the morphological evidence (see Section 5.2).

More recent studies have used a direct assay for gonadotrophin. In goldfish exposed to γ-HCH, plasma GtH concentration was decreased at 0.1 mg/l in males and 0.01 mg/l in females (Singh *et al.*, 1994) (Figure 5.3). Murrel (*Channa punctatus*), exposed to Metacid-50 (0.11 μg/l) or Carbaryl (1.7 mg/l) had decreased serum GtH, and pituitary GtH content (Ghosh *et al.*, 1990). The effect increased from 7 to 30 days exposure. The similarity of effect on GtH and GnRH (see Section 5.3.1) suggests action at both pituitary and hypothalamic levels. In an experimental paddy field sprayed under normal culture conditions so that water concentrations of Metacid-50 and Carbaryl were 0.24 μg/l and 3.7 mg/l respectively there was a larger decrease during the first two days after application, but no further decrease by 7 days, possibly due to the disappearance of the pesticides from the water. In neither

laboratory nor field studies did the fish show any physiological or behavioural distress. The widespread use of these pesticides at such concentrations in paddy-cum-fish culture in the Indian sub-continent may have long-term consequences for fertility of the exposed fish.

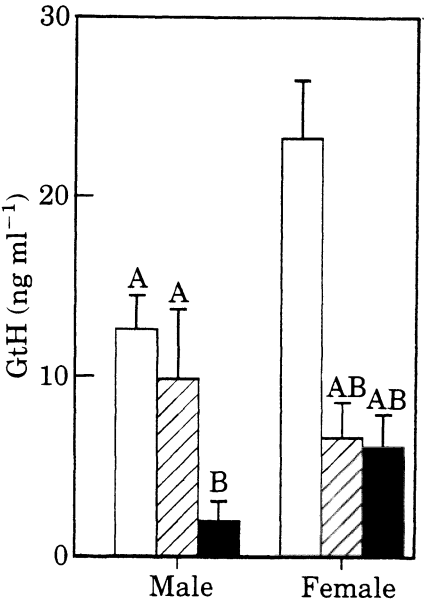


Figure 5.3. Plasma gonadotrophin (GtH) concentrations in male and female goldfish held in freshwater (□), 0.01 (▨), or 0.1 (■) mg/l γ-hexachlorocyclohexane. Bars indicate mean ± SEM (n=5). Bars with the same letter are not significantly different (P>0.05). (Reproduced from Singh, P. B., Kime, D. E., Epler, P. and Chyb, J. 1994. Impact of γ-hexachlorocyclohexane exposure on plasma gonadotropin levels and *in vitro* stimulation of gonadal steroid production by carp hypophyseal homogenate in *Carassius auratus*. *J. Fish Biol.* **44**, 195-204 with kind permission of the Fisheries Society of the British Isles).

Industrial pollutants have similar effects to pesticides. Plasma GtH-II in white sucker (*Catastomus commersoni*) was 30-50-fold lower in both sexes after exposure to bleached kraft pulp mill effluent (BKME) than in unexposed populations (Van der Kraak *et al.*, 1992). A single injection of sGnRH-A increased plasma GtH in male and female fish at both sites although the response was greatly reduced in the BKME exposed fish. Coho salmon from Lake Erie had lower pituitary and serum GtH than comparable Lake Ontario salmon (Leatherland *et al.*, 1982) which correlated well with morphological studies of the pituitary (see Section 5.2).

In contrast to the inhibitory effects of the pesticides on pituitary function, cadmium can be stimulatory. Exposure of Atlantic croaker (*Micropogonias undulatus*) during early gonadal recrudescence to 1 mg/l cadmium for one month resulted in precocious ovarian growth, increased ovarian steroids and

vitellogenesis. The pituitary glands produced higher GtH *in vitro* than those from control fish, both on their own and in the presence of LHRH (Thomas, 1993) (Figure 5.4). This reflects other anomalous effects of cadmium in its effects on the gonads (see Chapters 6 and 7) and may be caused by the chemical similarity of the metal with calcium and its disruption of calcium transport systems and calcium-dependent ATPases.

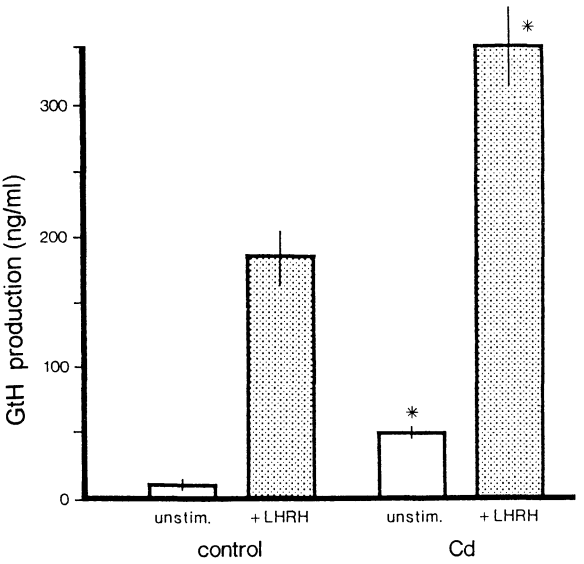


Figure 5.4. The effect of exposure of Atlantic croaker to 1mg/l cadmium for one month on gonadotrophin (GTH) secretion by hemipituitaries after incubated in vitro for 24 h. Unstim. = incubation alone; +LHRH = incubated with 100 ng/ml LHRHa. Vertical bars represent means ± SEM (n=12). * denotes significant difference between exposed and control fish ($P<0.05$). (Reprinted from *Mar. Environ. Res.* **35**, Thomas, P., Effects of cadmium on gonadotropin secretion from Atlantic croaker pituitaries incubated *in vitro,* pp. 141-145, Copyright 1993, with kind permission from Elsevier Science Ltd. The Boulevard, Langford Lane, Kidlington, OX5 1GB, UK).

Although there has been little effort to investigate the mechanism of pituitary and hypothalamic disruption, a recent study (M. Sokolowska-Mikolajczyk, J. Chyb and P. Epler, Unpublished data) suggests that the GABA receptors which regulate GnRH release from the hypothalamus and GtH from the pituitary may be the site of disruption of some pollutants. Although zinc is essential for normal reproduction and plays an important role in neural functioning, dietary administration at a level of 60 mg/g feed during the reproductive season affected both type A and type B GABA receptors in carp pituitaries. Plasma GtH-II levels were lower in fish which had been held in 2.5 mg/l zinc than in controls (K. Bieniarz, M. Sokolowska-Mikolajczyk and P. Epler, Unpublished data). The effects of other classes of pollutants on

these receptors would be of considerable interest since several pesticides affect GABA and its receptors in invertebrates and mammals (Ghiasuddin and Matsumura, 1982; Coccini *et al.*, 1993; Cole *et al.*, 1993).

Another possible mechanism whereby pollutants may affect the pituitary is indicated by the induction of P4501A1 in rainbow trout pituitaries by β-naphthoflavone (Andersson *et al.*, 1993). P4501A1 was identified by double immunostaining in the gonadotrophs containing GtH-II, but there was no co-localisation with GtH-I or growth hormone. Plasma GtH-II and testosterone were higher in the β-naphthoflavone treated fish than in controls suggesting that the effects on the gonadotrophs resulted in a hormonal imbalance. Although induction of the cytochrome P450 isozymes in the liver is associated with detoxification (see Chapter 9), the function of these enzymes in the pituitary is poorly understood, but they may somehow alter the steroid feedback mechanism possibly by affecting the steroid aromatase enzyme. Since many of the effects of pollutants on the gonads appear to originate from altered pituitary function, further studies of their mechanism of action at the level of the hypothalamus and pituitary would be timely.

Steroid hormones may feedback to modulate the action of the hypothalamus and pituitary and it is in this field that endocrine disruptors might play an important role. The brains of fish contain an aromatase that converts testosterone into estradiol, and estrogens and their metabolites, catecholestrogens, may be important in modulating feedback action to the hypothalamus and pituitary (Callard *et al.*, 1981; Timmers and Lambert, 1987; Andersson *et al.*, 1988a). Hormone-mimetic pollutants or anti-hormones might, therefore, affect either such brain transformations or the binding of steroids to receptor sites within the brain, hypothalamus and pituitary. β-hexachlorocyclohexane, in addition to its estrogenic effect in stimulating vitellogenesis, also stimulates the pituitary gonadotrophs while retarding testicular development (Wester *et al.*, 1985). This is in accord with previous studies showing a positive feedback on the pituitary of estrogen in juvenile fish (Sage and Bromage, 1970; Olivereau and Olivereau, 1979; Crim *et al.*, 1981). The incorporation of vitellogenin into the oocytes of the β-HCH exposed fish also indicates that the pituitary was secreting GtH-I on which this process is dependent (Tyler *et al.*, 1991).

5.3.3 Effects on pituitary thyrotrophins

Thyrotrophs and gonadotrophs showed similar decreases in activity after exposure to cadmium (Pundir and Saxena, 1992) and endosulfan (Shukla and Pandey, 1986a). Although organic and inorganic mercury had an inhibitory effect on pituitary gonadotrophs, the TSH cells of *Clarias batrachus* were highly stimulated after 180 days exposure (Figure 5.2) (Joy and Kirubagaran, 1989). Thyrotrophs were also hypertrophied in catfish exposed to 5 mg/l lead

nitrate for 150 days (Katti and Sathyanesan, 1987a). In both cases the hypertrophy was attributed to low plasma thyroxine as a result of inhibited iodine uptake by the thyroid.

5.3.4 Effects on pituitary corticotrophins

The corticotrophs and their secretion of ACTH may be affected by pollutants in two distinct fashions. A simple stress response to the xenobiotic would activate the corticotrophs as a result of hypothalamic stimulation via increased corticotrophin releasing hormone, leading to increased ACTH secretion and elevated interrenal secretion of cortisol. Direct endocrine disrupting effects of a pollutant could either alter the activity of the hypothalamus or the corticotrophs, or could alter the activity of the interrenal which in turn would alter the feedback response of the hypothalamic-pituitary system. These activities can be distinguished by measuring both ACTH and cortisol levels and examination of pituitary and interrenal morphology.

Direct stress effects result in increased pituitary function, which is apparent in the presence of large pituitary corticotrophs in response to normal capture stress. Similar corticotrophs are found in fish subjected to chemical stressors. Catfish (*Clarias batrachus*) exposed to mercuric chloride, emisan-6 or methylmercuric chloride for 90 days had very active corticotrophs, but at longer exposure times, especially with organic mercury, there was evidence of necrosis (Kirubagaran and Joy, 1991) which was attributed to the effect of long-term overstimulation resulting from the lack of negative feedback caused by primary action of mercury on interrenal biosynthesis. In fish captured from waters containing high levels of PAHs, PCBs and mercury, however, the pituitary corticotrophs were atrophied, leading to low plasma cortisol levels and inhibited stress response (Hontela *et al.*, 1992).

Many of the exposure regimes used, while sublethal, may also be stressful to the fish and it would not be surprising if this affected pituitary corticotrophs and their secretion of ACTH. The suppressed secretory activity of gonadotrophs, associated with follicular atresia, in acid stressed brook trout has been attributed to the elevated ACTH and cortisol secretion, hyperglycemia, acidosis and hyponatremia (Tam *et al.*, 1990). Stress effects rather than direct action of the pollutant on the pituitary may well be the cause of inhibited gonadotroph activity with other species and pollutants. This emphasises the importance of clearly defining the level of pollutant used so that it can be shown that it acts at levels which do not lead to a stress response.

5.3.5 Effects on prolactin secretion

Although prolactin is affected by pollutants, this appears to be rather an attempt at re-establishing homeostasis than of a primary effect on the pituitary cells. The increase in prolactin cell activity during the first 4 days exposure of tilapia to 10μg/l cadmium appeared to be a mechanism for counteracting cadmium-induced hypocalcaemia (Fu *et al.*, 1989) rather than direct stimulation. In rainbow trout, intra-peritoneal injection of 4.4 or 7.7 mg/kg, exposure to tank water (0.5-10 mg/l) or incubation of pituitary glands in medium containing 50 mg/l cadmium had no consistent effect on prolactin cell activity (James and Wigham, 1986). Although several attempts have been made to correlate changes in plasma prolactin or prolactin cell activity with exposure to acid and/or aluminium (Notter *et al.*, 1976; Fryer *et al.*, 1988; Flik *et al.*, 1989; Balm *et al.*, 1995; Waring *et al.*, 1996a), the results were inconsistent and probably due to a secondary stress effect rather than to true endocrine disruption.

5.3.6 Effects on the neurohypophysis

There have been very few studies on the effects of pollution on neurohypophysial hormone production. Brain and pituitary levels of arginine vasotocin (AVT) were elevated 7 days after exposure of brook trout to sublethal conditions of pH 5.6, but was not further increased by addition of 300μg/l aluminium (Hontela *et al.*, 1991). The differential effects of aluminium and acidity were demonstrated in the strong correlation between urotensin I and pH, and between urotensin II and aluminium in the urophysis (Hontela *et al.*, 1989). Some caution must, however, be used in interpreting such data since the effect of acidity on AVT was modulated by body length and by season (Hontela *et al.*, 1993a, b). It is also not clear whether this is a primary endocrine disruption or an attempt by the pituitary and urophysis to restore body fluid homeostasis.

5.4 Conclusions

There is a paucity of information on the effects of pollutants on the hypothalamic-pituitary system which reflects the difficulties of working with a small and relatively inaccessible gland. Information, such as exists, suggests that both the hypothalamus and the pituitary can suffer morphological damage as a result of exposure to pollutants which is reflected in a decreased secretion of their respective hormones, GnRH and GtH. This in turn leads to inhibition of gonadal function, and it is important to consider that many of the effects discussed in Chapters 6 and 7 may be due, at least in part, to dysfunction at

the hypothalamic-pituitary centre. The effects on thyrotrophs and TSH secretion are similar to that resulting from disruption to the gonadotrophs, but some caution must be exercised in interpreting effects on the corticotrophs. Many of the stimulatory effects on the corticotrophs are undoubtedly a result of general stress, while others like those affecting prolactin and AVT secretion are normal responses to a disturbance in body fluid homeostasis.

There appears to be little qualitative difference between different classes of pesticides and industrial pollutants. Organochlorine pesticides, however, cause pituitary disruption at concentrations several orders of magnitude lower than the organophosphates and carbamates. Cadmium has an anomalous action on the gonadotrophs which parallels its action at other centres of the reproductive system.

Many of the studies described have examined the pituitary and hypothalamus in isolation, and more integrated studies which relate the changes at this level to those occurring in the target glands of the pituitary hormones and determine the primary, or most sensitive, site of action would add greatly to our knowledge of the mechanisms by which xenobiotics cause endocrine disruption.

Chapter 6

Disruption of Male Reproductive Function

6.1 Introduction

A decreased gonadosomatic index resulting from exposure of fish to xenobiotics has been described by many authors, but it is frequently not clear whether this is due to disruption via the pituitary-hypothalamic system or to direct action on the testis itself. Such general results will not be discussed in detail in this Chapter, but are listed in the Appendix. Direct effects on the testis can be considered either as cytotoxicological, where the disruption is caused by damage to the cellular integrity or function of gonadal cells in general, or endocrine in which the function of specific cells are disrupted due to an endocrine malfunction. The latter could be due to altered pituitary secretion or to alterations in the chain of enzymes within the testis which lead to synthesis of the testicular hormones. Such disruption of steroidogenesis can have secondary effects on the testicular somatic and germ cells which are dependent upon the correct hormonal milieu for normal functioning. Furthermore other functions of the male, such as secondary sexual characteristics and courtship behaviour are hormonally dependent and can be disrupted in the absence of normal circulating hormone levels. The recent concern with environmental estrogens and their effect on male fertility (Colborn and Clement, 1992; Purdom *et al.*, 1994; Sharpe and Skakkebaek, 1993) is particularly relevant in this section. Many organochlorine pesticides and phenolics are known to be weakly estrogenic and dysfunction of male reproduction has been attributed (Colborn and Clement, 1992) to estrogenicity of these pollutants. Pesticides are, however, designed specifically as toxic agents and if they disrupt the cellular functions of pests they are also likely to have similar effects on non-target organisms such as fish or

mammals. Reproductive tissues may well be affected, but such action may have nothing to do with estrogenic activity. It is therefore important to compare the effects on the male which are caused both by estrogenic and non-estrogenic pollutants so that the true cause of the damage may be ascertained. It is possible, for example, to expose the fish or its tissue to a level of estradiol, or the synthetic analogue ethynylestradiol, that has equivalent estrogenic potency to the pollutant in, for example, stimulation of vitellogenesis. The concentration of the estrogen will, perhaps, be less than 1/1000 of that of the pollutant and its effects can be considered purely as estrogenic without any toxicological side effects. Any effects caused by the pollutant which are not apparent with the estrogen will therefore be due to toxic rather than estrogenic activity. The relative estrogenic and toxic activities will also vary from one pollutant to another which should be reflected in the nature of the disruption caused to the reproductive system. Popular concern with environmental estrogens may well be misdirected if it leads to restriction only of pollutants which are estrogenic, while compounds which actually cause more damage to the reproductive system through their toxicological activity continue in use. Even nonylphenol, which is of recent concern as an environmental estrogen has a 96 h LC_{50} of only 0.12 mg/l in salmon (McLeese *et al.*, 1981), a level lower than some pesticides. Furthermore, restrictions on one compound will inevitably lead to increased use of other chemicals which may be more toxic. It is important to realise that "environmental estrogens", "endocrine disruptors" and "disruptors of reproduction" are not synonymous and that the latter term is what really matters when the effect on the population as a whole is considered. Although some of the toxic effects found for xenobiotics on the testis, or sperm, do not fit clearly within the definition of "endocrine disruptor", their effects on the fertility of the affected fish are similar and use of strict definitions is irrelevant. The important factor to consider is whether the testis is affected at concentrations at which the rest of the fish is unharmed. A similar consideration of course applies to female reproduction.

Organochlorines pose a particular danger to the testis since they are both lipid soluble and bioaccumulate, and are only very slowly biodegradable to inactive material. Other chemicals may pose dangers if they are permanently present in the aquatic ecosystem, either free in the water, or as part of the diet. Furthermore, release into the water for only a short period can cause serious damage if it is co-incident with a crucial phase of testicular activity. This is especially important during the final stages of sperm maturation and courtship where synchrony with female development is essential, and there may be direct effects on the viability of sperm released into the water during spawning or held within the sperm ducts ready for release. The use of organophosphate sheep dips during the spring spawning season may be particularly hazardous in this respect.

The effects of pollutants on the male are for convenience divisible into those effects that are apparent in changes of the structure of the testis and developing sperm, those that result in changes in the hormones secreted, and those that directly affect the essential role of the male in provision of sperm capable of fertilising the eggs produced by the female. These three types of effect are discussed separately in this Chapter, although quite clearly all three are interdependent.

6.2 Effects on testicular morphology

6.2.1 Heavy metals

Exposure of *Colisa fasciatus* for 30 days to 2 mg/l arsenic, the concentration of effluent from a fertiliser factory, had little effect on testicular structure, but at 14 mg/l there were degenerative changes in the lobules and dissolution of their walls, and significant reductions in diameters of the interstitial cells (Shukla and Pandey, 1984b). The nucleic acid content of the testis was also decreased by arsenic exposure possibly by generating chromosomal abnormalities or inhibition of DNA repair (Shukla and Pandey, 1984c).

Cadmium caused extensive damage to the structure of testes of the brook trout after only 24 h exposure to concentrations as low as 10 μg/l (10 ppb) (Sangalang and O'Halloran, 1972, 1973). Seven days after a 24 h exposure to 25 μg/l of cadmium there was evidence of testicular damage with extensive discolouration suggestive of massive haemorrhage. Histological examination showed extensive haemorrhage, necrosis and disintegration of the lobule boundary cells, which had pyknotic nuclei, although there was no detectable damage to the primordial germ cells.

Other non-salmonid studies have used much higher concentrations of cadmium which would probably have been toxic to a salmonid. 225 mg/l of cadmium, which was considered a safe level for *Lebistes reticularis,* caused an arrest of spermatogenesis, a decrease in numbers of spermatogenic cysts, necrosis of interstitial cells and hyperplasia of Sertoli cells after 30 days (Sehgal and Pandey, 1984; Sehgal *et al.*, 1984). In *Puntius sarana,* exposure to 20 mg/l cadmium for 10 days gave a disorganisation of the testicular lobules and an accumulation of a yellow-brown pigment. The vascular bed was severely damaged at lower concentrations (2 mg/l) than that which caused damage to the pituitary gonadotrophs suggesting that the primary effect was on the testis rather than the pituitary (Kumari and Dutt, 1991). Damage to the cytoplasm and nucleus of interstitial cells, vacuolisation and destruction of spermatogonia and primary spermatocytes, lobular rupture and sperm duct damage were found in the related *Puntius ticto* after 4 days

exposure to 26 mg/l cadmium at different periods of the reproductive cycle (Pundir and Saxena, 1990). Injection of *Clarias batrachus* with 0.5 µg cadmium chloride per 100 g body weight arrested spermatogenesis at the secondary spermatocyte level and caused vacuolisation and degeneration of the Sertoli cells, damage to the lobular walls, and pycnosis of the interstitial cells (Ahsan and Ahsan, 1974).

Lead caused similar structural damage to the testis. Exposure of *Colisa fasciatus* to 15 mg/l lead for 4 days caused reduced spermatogenic activity, dilation and rupture of the testicular blood vessels, and collapse of the lobules as a result of necrosis, shrinkage and dissolution of the germinal epithelium (Srivastava, 1987). Dilation of testicular blood capillaries with necrosis and disintegration of the seminiferous tubules was also found in *Puntius conchonius* exposed to 0.1 mg/l of lead or 11 mg/l zinc for 4 months. In rainbow trout the transformation of spermatogonia to spermatocytes was inhibited after a 12 day exposure to 10 µg/l zinc (Ruby *et al.*, 1993a).

Copper caused only a transient arrest in spermatogenesis in *Puntius conchonius* during the first two months exposure to 0.2 mg/l (Kumar and Pant, 1984), while in *Lebistes reticularis* at 0.5 mg/l damage was similar to that caused by 225 mg/l cadmium (Sehgal *et al.*, 1984) although it also caused an agglutination of sperm heads.

Testes of *Channa punctatus* exposed to 0.01 mg/l methyl mercury for six months during gonadal recrudescence showed only secondary spermatogonia in their testes while control fish had mature sperm. There was no evidence of morphological damage and the inhibition of development in exposed fish was correlated with inactivity of pituitary gonadotrophs (Ram and Sathyanesan, 1983). Both organic and inorganic mercury at 0.04 - 0.05 mg/l decreased the GSI of *Clarias batrachus*, inhibited the transformation of spermatids into functional spermatozoa, decreased the size of the seminiferous tubules, and caused degeneration of the interstitial cells (Kirubagaran and Joy, 1992). The differences between these two studies in stage of spermatogenic arrest is probably attributable to differences in the exact stage of the cycle at which fish were exposed.

6.2.2 Pesticides

This class covers a very wide range of pollutants including the organochlorines, organophosphates and carbamate pesticides.

Exposure of tilapia (*Oreochromis mossambicus*) to 2 mg/l benzene hexachloride for 10 days produced vacuolated cells and necrosis in the seminiferous tubules, atrophy of the interstitial cells, and thickening of the wall of the sperm duct (Pandey and Shukla, 1980). At 0.02 and 0.005 mg/l this pesticide had no effect on gonadal morphology of male medaka but disrupted ovarian function (Hirose, 1975). DDT at only 0.001 mg/l caused

deformation of the seminiferous lobules, degeneration of the germinal cells, primary and secondary spermatocytes and atrophy of the interstitial cells of tilapia (*Oreochromis mossambicus*), but this damage was reversed after 30 days in clean water (Bhattacharya and Pandey, 1989).

The organochlorine chlordecone (Kepone) at 0.02 mg/l for 20 days, or 0.04 mg/l for 4 days caused flattening, degeneration and desquamation of the germinal epithelium of the seminiferous tubules, thinning of the intertubular epithelium, cytolysis of spermatids and sperms and atrophy and vacuolisation of the interstitial cells in *Heteropneustes fossilis* (Srivastava and Srivastava, 1994). No effect was observed at 0.008 mg/l for 60 days. Whether these effects are due to the toxic effect of this pesticide or to the estrogenic effects which have been demonstrated in mammals (Eroschenko and Palmiter, 1980) is not clear.

In a comparison of the effects of the organochlorine endosulfan (0.00075 and 0.001 mg/l) and the carbamate carbaryl (10 and 20 mg/l) on testes of *Channa punctatus*, rupture of the peritoneal lining and walls of the seminiferous lobules, failure of spermatogenesis at the spermatid stage, necrosis of interstitial cells, fibrosis of connective tissue and reduced GSI were observed with both pesticides (Arora and Kulshrestha, 1984). The effects of the two pesticides were progressive with exposure, from 2 to 30 days and with increased concentration, and differed in extent rather than in the nature of the damage caused. Since the chemical structures of these two pesticides differ so greatly and no estrogenic activity has been demonstrated for carbamates, it is more probable that these effects and those of chlordecone and other organochlorines are toxicological rather than estrogenic. A similar study in which vitellogenin levels were also compared as an indicator of estrogenic activity would be of great interest.

The low toxicity of carbamide (urea) which is released from fertiliser factories was demonstrated by Shukla and Pandey (1984d) in which doses of 2,500 mg/l for 15 days were without effect on the testes of *Colisa fasciatus*, and only after 30 days did dissolution of the germinal epithelium and necrosis and degeneration of the interstitial cells occur. Carbamide derivatives such as carbofuran which are used as pesticides are considerably more toxic and at 0.5 mg/l caused disintegration of the walls of the seminiferous tubules and clumping of spermatids in testes of *Clarias batrachus* after 30 days exposure, while the organophosphate malathion was without effect at this concentration (Sadhu and Mukhopadhyay, 1985). At higher concentrations both carbofuran (5 mg/l) and the organophosphate fenitrothion (1.5 mg/l) caused testicular damage in *Channa punctatus* after 120 days (Saxena and Mani, 1985, 1987). Malathion at 2 or 4 mg/l also caused histophysiological damage in *Oreochromis mossambicus* after 10 days (Pandey and Shukla, 1982). Cythion at 2 mg/l caused similar damage in *Channa punctatus* after 6 months (Ram and Sathyanesan, 1987b), while parathion at 0.01 mg/l caused

degeneration of all categories of germinal cells in the testis only after 40 days (Billard and de Kinkeln, 1970). While most of these pesticides affected both sexes, male *Poecilia reticulata* are more sensitive than females to the organophosphate TEPA which at 24 mg/l caused inhibition of testicular but not ovarian function (Stock and Cope, 1969).

Both β-HCH and nonylphenol induce intersex in medaka which are exposed from hatch, and can stimulate vitellogenin production in juvenile male guppies (Wester and Canton, 1986; Wester, 1991; Gray and Metcalf, 1997) (see Chapter 8), but there are no reports of the effects of these estrogenic pollutants on the testicular morphology of adult males.

6.2.3 Industrial and other chemicals

Despite the wide variety of industrial pollutants, few have been examined for possible morphological damage to the reproductive system of male fish.

Bleached kraft mill effluent decreased testicular weight and maturity but did not cause any damage to germ cells or reproductive tissues (Sandstrom *et al.*, 1988). Testicular size was also decreased in white suckers below kraft mill effluents in Canada (McMaster *et al.*, 1992; Gagnon *et al.*, 1994a).

In *Mystus vittatus*, thiourea decreased the size of the testicular lobules and decreased the number of sperm mother cells and spermatids while increasing the numbers of primary and secondary spermatocytes and increasing the thickness of interlobular septa (Misra and Pandey, 1985). Cytolysis, degeneration and reduced diameter of the interstitial cells also occurred which may be due to suppression of pituitary function leading to decreased steroidogenesis.

Cod (*Gadus morhua*) fed a diet of herring contaminated with PCBs (1-50 μg/g) had similar liver and testis PCB concentrations to those found in some cod and herring caught in the North Sea and Baltic Sea (Chapter 3) and exhibited severe testicular damage (Sangalang *et al.*, 1981; Freeman *et al.*, 1982). Abnormalities were present only in fish at either functional maturity or in a stage of rapid spermatogenic proliferation, and not in testes that were immature or regressed. Damage included disorganisation of the lobules and spermatogenic elements, inhibition of spermatogenesis, fibrosis of lobule walls, fatty necrosis and in one case a total disintegration of the elements in many lobules. The fatty degeneration of the spermatogenic elements mimicked the pathological response of the liver to chlorinated hydrocarbons.

Injection of 25 mg/kg body weight of the PCB Arochlor 1254 into rainbow trout and carp gave no observable changes in testicular morphology by light microscopy, but under the electron microscope there was evidence of some alterations in sub-cellular organelles (Sivarajah *et al.*, 1987a). Spermatozoa were damaged in the head region but this was attributed to the decreased androgen level rather than to a direct effect on the spermatozoa.

Despite stories in the popular press, there is no scientific evidence that adult male fish can be turned into females as a result of exposure to environmental estrogens, although intersex fish may result from such exposure during embryonic or juvenile development (Chapter 8). Although Gimeno *et al.* (1998) found evidence of an early ovotestis in mature male carp exposed to estradiol, the levels used (1 μg/l) caused greater testicular atrophy and vitellogenin stimulation than even 1 mg/l of 4-*tert*-pentylphenol. and suggests that environmentally unrealistic levels of 100 mg/l of the alkyl phenol would be needed to even initiate sex reversal.

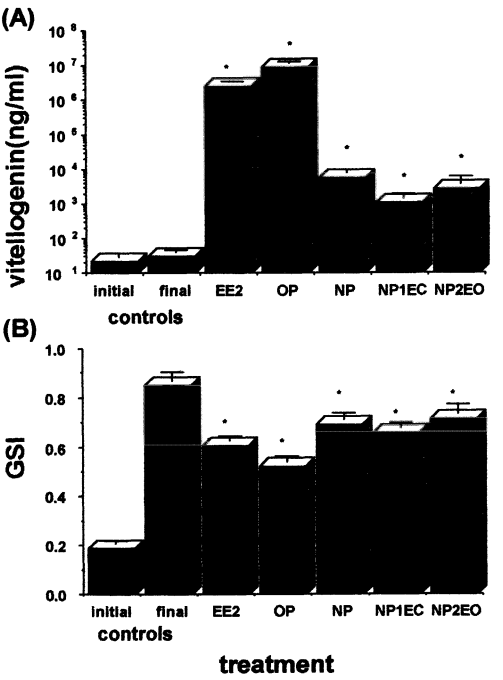


Figure 6.1. Effect of estrogenic alkylphenolic compounds (0.03 mg/l) and ethynylestradiol (EE2; 2 ng/l) on (A) plasma vitellogenin and (B) testicular growth in male rainbow trout exposed for 3 weeks. * is significantly different from the final control values. (OP = octylphenol; NP = nonylphenol; NP1EC = nonylphenoxycarboxylic acid; NP2EO = nonylphenoldiethoxylate). (Reprinted with kind permission from *Environmental Toxicology and Chemistry*, 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals, by Jobling, S., Sheahan, D., Osborne, J. A., Matthiessen, P. and Sumpter, J. P. Volume 15, pp. 194-202. Copyright Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL, 1996).

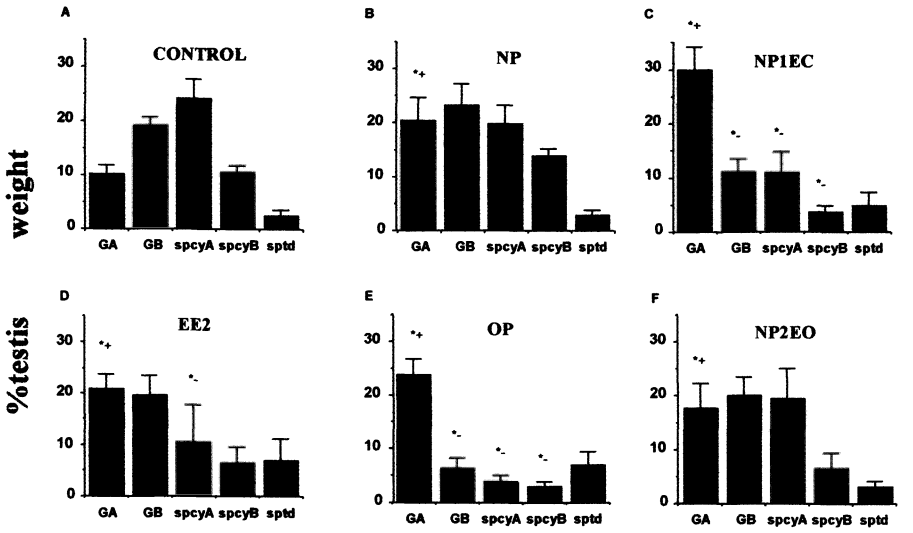


Figure 6.2. The effect of alkylphenolic compounds on spermatogenesis. Data from testes of control rainbow trout and those exposed to octylphenol (OP), nonylphenol (NP), nonylphenoxy carboxylic acid (NP1EC) and nonylphenoldiethoxylate (NP2EO) at approximately 30 µg/l, or ethynylestradiol(EE2) at 2 ng/l for 3 weeks are shown. The five germ cell stages were:spermatogonia A (GA), spermatogonia B (GB), spermatocyte A (spcyA), spermatocyte B (spcyB), and spermatid (sptd). The proportional distribution of these cell types are illustrated. *+ denotes significantly higher than control; *- denotes significantly lower than control ($P<0.05$) (Reprinted with kind permission from *Environmental Toxicology and Chemistry*, 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals, by Jobling, S., Sheahan, D., Osborne, J. A., Matthiessen, P. and Sumpter, J. P. Volume 15, pp. 194-202. Copyright Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL, 1996).

While the earlier studies (Jobling and Sumpter, 1994; Purdom *et al.*, 1994) with environmental estrogens, such as the alkylphenolics, concentrated on the stimulation of hepatic vitellogenesis (Chapter 9), a more recent study has shown that a wide range of alkylphenolics can also affect testicular development at levels similar to that which stimulates vitellogenin (Jobling *et al.*, 1996). In rainbow trout exposed for three weeks to 0.03 mg/l octylphenol, nonylphenol, nonylphenoldiethoxylate and nonylphenoxycarboxylic acid towards the end of gonadal recrudescence, testicular growth was inhibited to a similar extent to that induced by 0.000002 mg/l (2 ng/l; 0.002 ppb) ethynylestradiol. Of the alkylphenolics, octylphenol was the most potent in both inhibiting testicular growth and in inducing hepatic vitellogenin synthesis (Figure 6.1). This decreased gonadal growth resulted from increased numbers of the early stages of gamete development such as spermatogonia A and decreased numbers of spermatocytes, a pattern which was mimicked by

exposure to estradiol (Figure 6.2). It is not clear whether these compounds acted directly on the testis or whether they acted indirectly via hypothalamic or pituitary receptors and inhibited gonadotrophin secretion. Similar inhibitory effects on spermatogenesis were found in mature male carp exposed to 4-*tert*-pentylphenol (Gimeno *et al.*, 1998).

While these compounds are undoubtedly estrogenic, as shown by their stimulation of vitellogenin synthesis, it is important to note that their effect on the testis, in arresting spermatogenesis at early stages, is comparable to many of the other pollutants discussed in this Chapter, many of which have no estrogenic activity. Even cyanide, which has not the slightest resemblance to an estrogen, decreased the transformation of spermatogonia into spermatocytes, probably acting via the pituitary (Ruby *et al.*, 1993a), while low pH decreased the numbers of mature sperm and development of testicular cysts (Ruby *et al.*, 1978).

6.3 Effects on testicular hormone production

6.3.1 *In vitro* studies

Studies of *in vitro* steroidogenesis by the testis can provide valuable information on how plasma steroid hormone concentrations might be affected in the intact animal exposed to pollutants. They must, however, be used with some discretion and followed up by confirmatory studies *in vivo*. *In vitro* incubations are an artificial system and it is difficult to relate the concentrations of xenobiotic added to an incubation flask to those that might be present in the tissue of exposed fish. Even in unexposed fish, results obtained *in vitro* do not necessarily relate to those pertaining in the intact animal. Furthermore, *in vitro* studies examine only the pollutant itself, whereas *in vivo* the effects could be due to products formed by hepatic or peripheral metabolism.

It is important to differentiate between the two types of *in vitro* study which use exogenous and endogenous precursors. In the simplest case, tissue is incubated with a suitable exogenous steroid precursor (e.g. 17-hydroxyprogesterone) and the effects of addition of different amounts of added pollutant on the pattern of metabolites monitored. For simplicity of detection and identification, radiolabelled precursors (^{14}C or ^{3}H) are frequently used in such experiments. It is also possible to compare incubations of tissue taken from fish which have been exposed to different concentrations of pollutants (the *ex vivo* approach). Such a study may show which enzyme is most sensitive to pollutant challenge. For example if addition of a pollutant caused decreased 11-ketotestosterone, but increased testosterone production this is indicative of inhibition of the 11β-hydroxylase

enzyme (see Figure 4.3). Studies of this type require use of radioisotopes and specialised instrumentation and separation techniques which are not generally available. Such studies only indicate changes affecting the precursor chosen, even though earlier steps in the biosynthetic sequence could also be affected. Use of endogenous precursors by incubating testes in the presence of gonadotrophin and assaying the steroids produced would determine whether any stage of the biosynthesis, or its stimulation by gonadotrophin is affected, but it is important to remember that products other than those which are assayed could confuse the picture. The major advantage of *in vitro* studies is that they permit comparison of a range of pollutants and concentrations on identical tissue without using large numbers of fish.

6.3.1.1 Heavy metals

Apart from cadmium, the effects of few heavy metals have been examined *in vitro*. Testes from brook trout which had been exposed to 25 μg/l cadmium and showed morphological damage (see Section 6.2) did not convert [^{14}C]pregnenolone into 11-ketotestosterone, and production of 11β-hydroxytestosterone and testosterone were lower than in unexposed fish (Freeman and Sangalang, 1977a; Freeman *et al.*, 1984b; Sangalang and O'Halloran, 1972; 1973). Addition of 10, 100 and 1000 μg cadmium/g testis from unexposed fish also resulted in inhibition of 11-ketotestosterone production and decreased conversion of radiolabelled pregnenolone. These results indicate that the 11β-HSD enzyme (conversion of 11β-hydroxytestosterone to 11-ketotestosterone) was preferentially affected, although other steps in the conversion of pregnenolone to testosterone were also partially inhibited. Cadmium also affected endogenous production of 11-oxygenated androgen production in testes of rainbow trout, but in this case the effect was stimulatory (Kime, 1984) possibly due to the effects of cadmium on calcium flows as discussed for pituitary function (Section 5.3.1).

Another approach to steroidogenesis is to determine 3βHSD activity in tissue. This requires less sophisticated technology than that described above but provides less information. 3βHSD is a key enzyme in conversion of pregnenolone to progesterone (Figure 4.3) and decreases in enzyme activity are assumed to correlate with decreased steroidogenic activity. Exposure to 0.05 and 0.5 mg/l methylmercuric chloride and emisan-6 (6% methoxyethyl mercuric chloride) respectively reduced 3βHSD activity in interstitial cells of *Clarias batrachus* (Kirubagaran and Joy, 1988a).

6.3.1.2 Pesticides

The effects of γ-hexachlorocyclohexane (lindane) has been extensively studied *in vitro*. With testes of the roach *Rutilus rutilus* it had only a small impact on the metabolism of radiolabelled 17-hydroxyprogesterone, predominantly by inhibiting glucuronidation of 17,20βP, while with

endogenous precursors it significantly inhibited 11-ketotestosterone and 17-hydroxyprogesterone production while stimulating that of testosterone and its glucuronide (Kime and Singh, 1996). In spermiating goldfish there was also little effect on the metabolism of exogenous radiolabelled precursors while production of deoxycortisol and testosterone from endogenous precursors was stimulated (Kime and Singh, 1996). These results suggest that the pesticide acts predominantly on the early stages of steroidogenesis, rather than on the enzymes that convert 17-hydroxyprogesterone. Much clearer results were obtained with incubations of testes from goldfish which had been exposed to 0.01 mg/l γ-HCH for 4 weeks (Singh *et al.*, 1994). Testosterone, testosterone glucuronide and 11-ketotestosterone were all significantly suppressed while production of 11-deoxycortisol increased. The results suggest an inhibition of androgen synthesis, possibly due to inhibited side chain cleavage (17,20-lyase) activity at the testicular level, but whether the lower GSI in exposed fish was a cause or effect of inhibited steroidogenesis is not clear.

After 20 days exposure of *Oreochromis mossambicus* to 0.001 mg/l DDT 3β and 17β-HSD activity was absent from interstitial cells of the testes but after 30 days exposure to freshwater, activity had returned concomitant with repair to the morphological damage (Bhattacharya and Pandey, 1989). These enzyme activities were also significantly decreased in testes of *Clarias batrachus* exposed to 0.025 mg/l of the organophosphorus quinalphos for 4 days (Bagchi *et al.*, 1990). 3βHSD activity was also partially reduced in testes of carp exposed to 0.3 mg/l fenitrothion (Kapur *et al.*, 1978).

Cythion and hexadrin exposed *Heteropneustes fossilis* had a lower testicular ^{32}P uptake than unexposed fish which might be indicative of decreased steroidogenesis (Singh and Singh, 1980a). The carbofuran and fenitrothion induced changes in testicular cholesterol and phospholipids in *Channa punctatus* may also have had a similar cause (Saxena *et al.*, 1986).

6.3.1.3 Industrial

The only industrial pollutant which has received significant attention *in vitro* is PCB. Unlike γ-HCH, PCB exposure of brook trout (*Salvelinus fontinalis*) stimulated production of 11β-hydroxytestosterone from [^{14}C]pregnenolone by testes *in vitro* but did not affect yields of testosterone or 11-ketotestosterone (Freeman and Idler, 1975). Plasma 11-ketotestosterone was unaffected by exposure, but unfortunately 11β-hydroxytestosterone was not measured so it is difficult to corroborate *in vivo* and *in vitro* data. In cod fed a diet of herring containing 1-10 μg/g of the PCB Arochlor 1254 for 5½ months conversion of [^{14}C]progesterone to 11-ketotestosterone was inhibited (Freeman *et al.*, 1980, 1982).

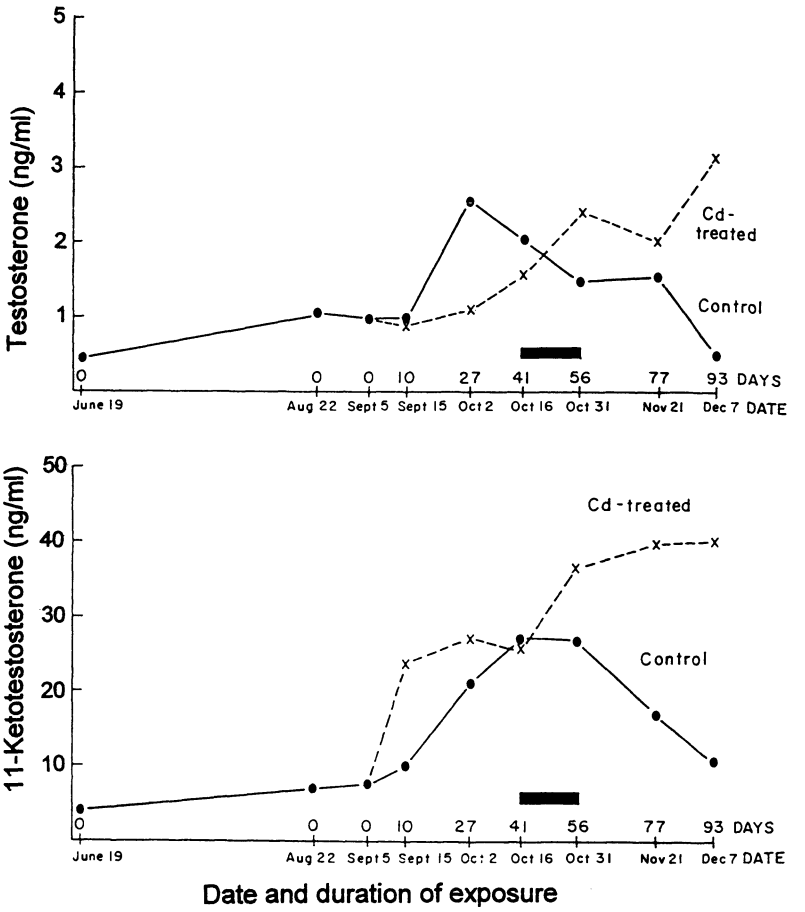


Figure 6.3. The effect of 1µg/l cadmium on peripheral plasma levels of testosterone and 11-ketotestosterone in male brook trout. Solid bar indicates peak period of functional maturity. (Reproduced from Sangalang, G. B. and Freeman, H. C. 1974. Effects of sublethal cadmium on maturation and testosterone and 11-ketotestosterone production *in vivo* in brook trout. *Biol. Reprod.* **11**, 429-435 by kind permission of the Society for the Study of Reproduction).

6.3.2 Circulating hormones

Measurement of the response of circulating plasma gonadal hormones in response to pollution exposure has been very patchy. Cadmium is the only heavy metal examined and there are no reports of effects of pesticide exposure, while PCBs, effluents of pulp mills and waters of the Great Lakes have been the most extensively studied. As with *in vitro* studies, availability of assay methodology in close proximity to a pollution problem is the main explanation for such a distribution of studies. The increasing use of simple ELISA (Enzyme Linked ImmunoSorbent Assay) methods may extend the

availability of such hormone assays to regions in which use of radiochemical assays, and their associated need of sophisticated counting equipment, is not possible.

6.3.2.1 Heavy metals

In normal male brook trout, plasma concentrations of testosterone and 11-ketotestosterone correlated well with gonadal recrudescence and then declined rapidly after spawning (Sangalang and Freeman, 1974). By contrast fish that had been exposed to 1 μg/l of cadmium since early recrudescence showed earlier testicular regression, but their androgen levels continued to rise for over a month (Figure 6.3). Unfortunately at the time of this study the role of the switch from 11-ketotestosterone to 17,20βP during spermiation was not known so this latter hormone was not measured. The result suggests that the switch-off of 17,20-lyase which occurs in normal fish at spawning may be inhibited by cadmium and may provide a useful tool to examine this mechanism.

6.3.2.2 Industrial

The effect of effluent from bleached kraft pulp mills has received extensive attention by Canadian scientists. Male white suckers collected from below the mill during the prespawning season had only half the serum concentrations of testosterone, 11-ketotestosterone and 17,20βP as fish from a reference site which may explain the increased age to maturity, smaller gonads and lack of secondary sexual characteristics of the exposed fish (McMaster *et al.*, 1991, Munkittrick *et al.*, 1991; Gagnon *et al.*, 1994a). 17,20βP levels, however, were not significantly different during the spawning season and were higher in exposed males than in reference fish in the July post-spawning period even though gonads had fully regressed. This suggests a disturbance of the temporal factors rather than direct inhibition of 20βHSD. A similar reduction in plasma testosterone occurred in lake whitefish (*Coregonus clupeaformis*) (Munkittrick *et al.*, 1992). Effects on plasma testosterone and 11-ketotestosterone of white suckers were similar at a larger number of mills throughout Canada (Munkittrick *et al.*, 1994). Although the testosterone response was rather variable, that of 11-ketotestosterone was consistently lower below pulp mills (Figure 6.4). Non-chlorinated mills were not consistently less harmful than chlorinated mills and the harmful effects had not been decreased by installation of secondary treatment since the earlier study.

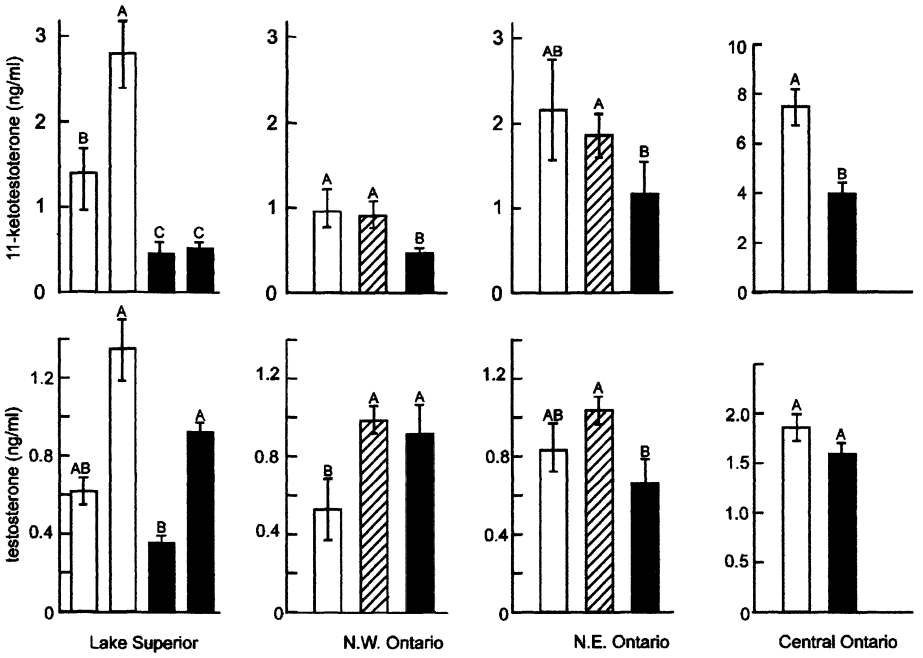


Figure 6.4. Plasma levels of 11-ketotestosterone and testosterone in male white sucker collected downstream of bleached-kraft mills (solid bars), non-chlorinated mills (hatched bars) and reference sites (open bars). Comparisons were made only to reference sites within the same geographic region. Bars sharing an alphabetical letter were not significantly different ($p>0.05$). (Redrawn with kind permission from *Environmental Toxicology and Chemistry,* 1994. Survey of receiving-water environmental impacts associated with discharges from pulp mills. 2. Gonad size, liver size, hepatic EROD activity and plasma sex steroid levels in white sucker by Munkittrick, K. R., Van der Kraak, G. J., McMaster, M. E., Portt, C. B., Van den Heuvel, M. R. and Servos, M. R.. Volume **13**, pp. 1089-1101. Copyright Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL, 1994).

Part, at least, of these effects may be due to lowered response of the gonad to pituitary secretion since fish below the effluent showed a lowered response to Ovaprim (GnRH analogue + dopamine receptor antagonist) (Gagnon *et al.*, 1994b). The relatively small suppression of 11-ketotestosterone and lack of effect on testosterone could, however, also be attributable to the slightly smaller gonads of the exposed fish, or to failure of the pituitary to respond to GnRH. Conjugation of testosterone was almost entirely suppressed in white suckers exposed to BKME (Van der Kraak *et al.*, 1992). McMaster *et al.* (1994) have drawn attention to the need to avoid stress during sampling of fish since acute handling stress or overnight confinement eliminated the site difference for 17,20βP. A recent study (MacLatchy *et al.*, 1997) has shown that β-sitosterol, a natural wood product released in BKME has strong endocrine disrupting activity, and would explain the endocrine disruption

apparent in fish exposed to effluent from mills in which chlorination is no longer used and in which dioxins and other chlorinated aromatics are less likely to be present. β-sitosterol decreased both plasma levels of androgens and the response to gonadotrophin (hCG), while GtH levels remained unchanged. Although β-sitosterol has been shown to be estrogenic in some binding studies (Tremblay *et al.*, 1995; Mellanen *et al.*, 1996), the effects *in vivo* differed markedly from those of estradiol. Gonadal cholesterol was significantly decreased in β-sitosterol treated fish, but not in those exposed to estradiol. The authors conclude that β-sitosterol can cause endocrine disruption either by decreasing the availability of cholesterol to the P_{450scc} enzyme that converts cholesterol to pregnenolone, or by decreasing the activity of this enzyme. Such a mechanism appears much more probable than that due to any estrogenic activity given the close structural similarity between β-sitosterol and cholesterol. It also warns against assuming too readily that the action of an endocrine disruptor is due to its estrogenic activity if this has been demonstrated using screening procedures involving binding affinities.

The field studies in Canada have been supported by laboratory studies on effluent from Finnish pulp mills. Mature prespawning male rainbow trout exposed to 0.5-0.75% BKME for 2 weeks had plasma testosterone levels 50% lower than control fish (Lindström-Seppä and Oikari, 1989a). Intraperitoneal injection of 2μg/kg body weight of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCCD), a constituent of pulp mill effluent did not significantly affect plasma testosterone, but fish in this second experiment did not respond either to BKME exposure which suggests that 11-ketotestosterone or 17,20βP might have been a more appropriate hormone to measure at this stage of maturity.

There were significant differences in plasma steroid levels in coho salmon between the Great Lakes consistent with the thyroid dysfunctions discussed in Chapter 11. Plasma 11-ketotestosterone was significantly lower in Lake Erie stock than stocks of male salmon from Lakes Ontario and Michigan sampled at a comparable stage of sexual development (Figure 6.5) and may explain the poor development of secondary sexual characteristics in fish from Lake Erie (Morrison *et al.*, 1985a). Testosterone concentrations did not differ between the lakes. Slight differences in sampling time may explain the differences between this and an earlier study which also showed a depression of 11-ketotestosterone in Lake Michigan fish (Leatherland *et al.*, 1982). These and other studies cited elsewhere (Chapters 7 and 11) clearly demonstrate a major endocrine disruption of salmon in the Great Lakes in general, and in Lake Erie in particular. The possible relation between these dysfunctions and the burden of pollutants in these lakes has been discussed in detail by Leatherland (1993).

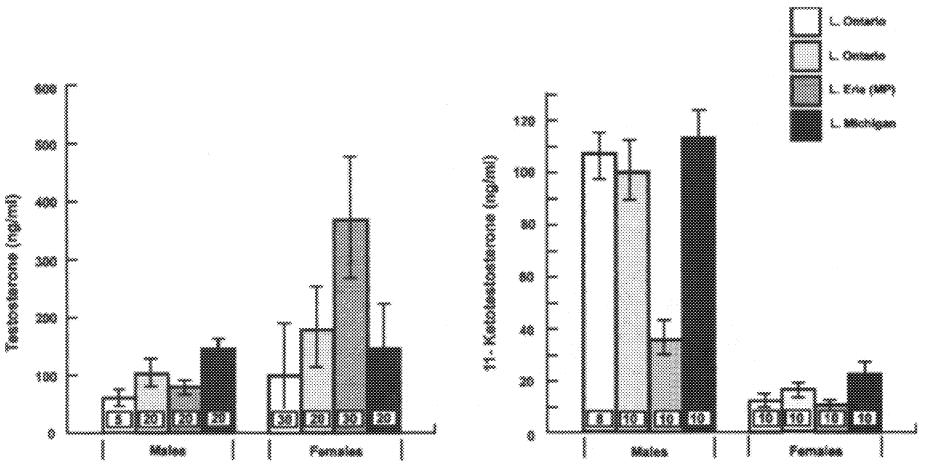


Figure 6.5. Plasma testosterone and 11-ketotestosterone in coho salmon collected from Lakes Ontario (2 sites), Erie or Michigan Vertical bars indicate 95% confidence limit, and numbers of samples are shown at the base of the bars. (Redrawn from *Comp. Biochem. Physiol.* **80A,** Morrison, P. F., Leatherland, J. F. and Sonstegard, R. A. Plasma cortisol and sex steroid levels in Great Lakes coho salmon (*Oncorhynchus kisutch* Walbaum) in relation to fecundity and egg survival, pp. 61-68, Copyright 1985, with kind permisssion from Elsevier Science.

The Great Lakes, like the North Sea is contaminated by high levels of PCBs and several laboratory studies have examined the effects of these pollutants on steroid levels in male fish. Intraperitoneal administration of 25 mg/kg body weight Arochlor 1254 to rainbow trout or common carp at weekly intervals for 4 weeks resulted in a steady decrease in testosterone compared with control fish (Sivarajah *et al.*, 1978). Carp given a single intraperitoneal administration of Arochlor 1248 also had lower plasma testosterone than control fish after 6 days (Yano and Matsuyama, 1986). Feeding experiments with PCBs did not give conclusive results with male cod, but androgen levels remained low in fish which had accumulated high levels of PCBs in their testes (Freeman *et al.*, 1984b).

Exposure of male salmon (*Salmo salar*) and flounder (*Pleuronectes platessa*) to crude oil generally caused a decrease in testosterone and 11-ketotestosterone but the results for the salmon were not consistent. Conjugation of the androgens may have been preferentially affected in the flounder and in both species 11-hydroxylation was particularly sensitive (Truscott *et al.*, 1983). In further studies with the flounder using sediment containing crude oil (Truscott *et al.*, 1992), free androgens were not affected, but the glucuronide levels were significantly decreased although it is not clear whether this was due to decreased UDP-glucuronyl transferase activity in the testis or liver. The increased liver size in exposed fish, however, indicated that this organ had been affected by the pollutant.

Plasma androgens did not differ between carp held in outflows from sewage lagoons and those held in a reservoir (Sivarajah *et al.*, 1979), but this study took place before the widespread use of alkylphenolics and other potential estrogens which have recently been shown to affect fish below such discharges (Purdom *et al.*, 1994).

Atlantic salmon in the acidic Westfield River (pH 4.7) had significantly lower testosterone and 11-ketotestosterone than those in the less acid Medway River (pH 5.6) (Freeman *et al.*, 1983), but rainbow trout exposed in the laboratory to a range of pHs from 4.5 to 7.1 did not show any differences (Weiner *et al.*, 1986). This suggests that other factors may have caused the suppression in the Westfield River fish.

6.4 Effects on sperm viability

6.4.1 Sperm motility

For fertilisation of eggs it is clearly essential for the male to produce motile sperm that can both locate the eggs and then the micropyle for entry into the oocyte. The motility and morphology of fish sperm can be affected by xenobiotics in several ways: 1) by alteration of the internal hormonal environment during it's development, 2) by alteration in the function of the Sertoli cells which nurture the developing germ cells, 3) by changes in the seminal fluid or sperm maturation as a result of changes in 17,20βP synthesis and 4) by direct effects on the sperm itself which can result in either cytological damage or changes in the efficiency of mitochondrial energy production. Measurement of sperm motility therefore assesses the totality of the impact of any pollutant on the male reproductive system as a whole.

Fish sperm differ from those of mammals in three important aspects, all related to the prevalence of external fertilisation. Firstly they are immotile within the testis or sperm ducts and attain motility only on ejaculation when the milt is diluted with water. Secondly, this motility is of only short duration and rarely exceeds 2 minutes. Thirdly they do not penetrate the oocyte wall, but enter via a special channel, the micropyle. The rapid decrease in velocity of the sperm within such a short time makes motility measurement very difficult. Fish milt is a viscous liquid and is only mixed with water with some difficulty under a microscope. The two step dilution used by Billard and Cosson (1992), involving initial dilution with an extender solution, in which sperm remain immotile, followed by a second dilution with pure water on the microscope stage itself to initiate motility has greatly facilitated such studies. Furthermore this technique permits the exposure of sperm for a longer period. The initial dilution may be made with extender to which pollutant has been added and the final dilution and observation made 24 h later since little loss of

motility occurs during this period in extender. Such exposure would mimic exposure of sperm to pollutants in the seminal fluids which may reflect body burdens within exposed fish and the results may be compared with observations made on sperm to which the final dilution is made with polluted solution analogous to that which occurs during spawning in polluted water (Kime *et al.*, 1996).

Early studies on sperm motility used a subjective measure of motility such as the time taken for most sperm to stop moving or by rating activity on a scale of 1 to 5. This gave a very rough and ready assessment since not all sperm within a sample have the same behaviour and all do not cease swimming at the same instant. In such experiments it is usually necessary for several observers to give independent assessments. Using a scale from 1 (poor motility with 0-20% spermatozoa moving slowly) to 5 (excellent motility, 80-100% sperm moving rapidly) the motility score was significantly lower (3.1) for sperm from white sucker exposed to bleached kraft mill effluent than for control fish (4.0) (McMaster *et al.*, 1992). Milt volume, spermatocrit, mineral content and osmolarity did not differ significantly. There was no difference in fertilisation rate (>95%) by sperm from the two populations which suggests that the relatively small differences in motility were not important provided that sufficient milt was produced. Ratings of 3 and 4 (40-60% rapid vs. 60-80% very rapid sperm) are also very difficult to differentiate objectively. By use of a similar 4 point scale Khan and Weis (1987a, b) found that motility of mummichog sperm was decreased when exposed to 0.05 mg/l of either mercuric chloride or methylmercury. This study used only a single dilution step since mummichog sperm normally remains motile for 10-20 min after dilution.

The duration of sperm motility has also been used by several groups as a measure of motility, but it is not clear from such studies how many of the sperm were initially moving and how precise such measurement is since individual sperm cease movement at a wide range of times and one or two sperm still move very fast some time after the majority have stopped (Ebrahimi and Kime, Personal observations). pH had very different effects on sperm motility in two esocids, the chain pickerel and the northern pike (Duplinsky, 1982). The duration of motility of northern pike sperm decreased by 50% as pH decreased from 8.0 to 5.5, while that of chain pickerel decreased sharply above 7.0 and more gradually to pH 5.0 (Figure 6.6), but still retained a high duration of motility down to pH 4.0. By contrast white sucker sperm was little affected by pH and motility time decreased linearly from 77 sec at pH 7.0 to 52 sec at pH 3.0 (Mohr and Chalanchuk, 1985). Duration of motility in the Atlantic salmon also decreased gradually to pH 4.5, but then declined so that there was no motility at pH 4.0 (Daye and Glebe, 1984).

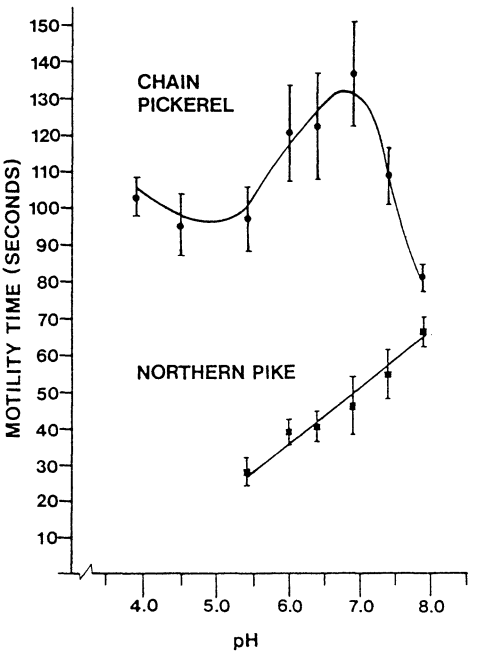


Figure 6.6. Sperm motility times for two esocids at various pH values. (Reprinted from Duplinsky, P. D. (1982). Sperm motility of northern pike and chain pickerel at various pH values. *Trans. Am. Fish. Soc.* **111**, 768-771 with kind permission of the American Fisheries Society).

Reports on the direct effects of other pollutants on sperm motility are rare, but an early study (Mann and Schmid, 1961) showed that a range of detergents decreased both the motility and fertilisation rate of exposed sperm. Although recent concern has been expressed about the use of alkylphenolic detergents, the minimum effective concentration (10 mg/l) of nonylphenol-30-AcO did not differ from that of dodecylbenzylsulphonate, coconut oil alcohol sulphate or oleic acid-20-AcO. A comparison of these detergents, and their metabolites and breakdown products, on the gonadal development, androgen levels and sperm quality in fish exposed for longer periods would be of considerable importance in the context of replacing alkylphenolics with non-estrogenic substitutes.

During the last few years, the availability of computer assisted sperm analysis (CASA) has enabled such studies to be put onto an objective and quantitative basis. This usually involves videotaping sperm movement under a phase contrast or dark field microscope, followed by interpretation of the videotape using sophisticated computer software. The separation of video recording and interpretation makes it possible for observations to be made in the field or in a collaborators' laboratory and interpretation carried out later in a centralised facility or partners' laboratory holding the more expensive

tracker. A number of trackers are available for such studies, ranging from those capable of tracking individually tagged sperm on a frame-by-frame basis to those which are capable of simultaneously tracking several hundred sperm during a real-time run of the video tape. Most instruments produce a large number of parameters, of which the most useful are straight-line velocity (VSL; the straight-line distance between the start and end points of the track divided by the time of the track), curvilinear velocity (VCL; the sum of the incremental distances moved in each frame along the sampled path divided by the total time of the track) and angular path velocity (VAP; a derived path based on an average number of points and divided by the time of the track), and percentage of sperm that is motile.

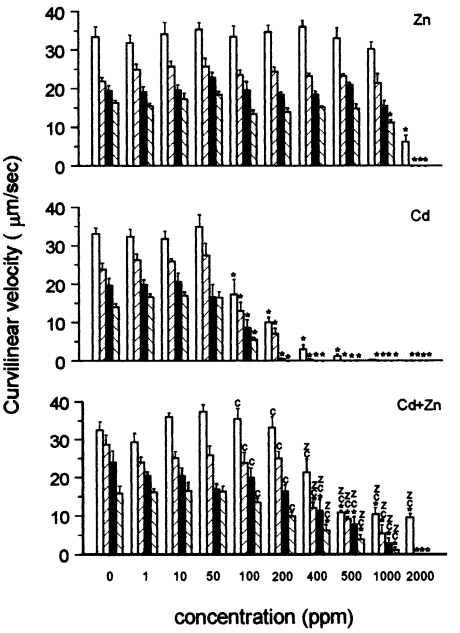


Figure 6.7. The effect of 24 h exposure of catfish sperm to zinc, cadmium and zinc+cadmium on curvilinear velocity (VCL, µm/s) at 20-35, 35-50, 50-65 and 65-80 seconds after final dilution. Vertical lines indicate S. E. M. s. * = significantly different from unexposed sperm ($P<0.001$); c= significantly different from Cd alone ($P<0.001$); z = significantly different from Zn alone ($P<0.05$). (Reprinted from *Aquat. Toxicol.* **36**, Kime, D. E., Ebrahimi, M., Nysten, K., Roelants, I., Moore, H. D. M. and Ollevier, F. Use of computer assisted sperm analysis (CASA) for monitoring the effects of pollution on sperm quality of fish; application to effects of heavy metals, pp. 223-237. Copyright, 1996 with kind permisssion of Elsevier Science - NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands)

Such a methodology has recently provided some interesting information on the toxicity of sperm to heavy metal toxicants (Ebrahimi *et al.*, 1995; Kime *et al.*, 1996). When sperm of the catfish (*Clarias gariepinus*) was exposed to heavy metal pollutants in extender for 24 h, motility was suppressed by 100 mg/l cadmium or 2000 mg/l of zinc (Figure 6.7). Surprisingly, combinations of zinc plus cadmium were much less toxic than those of cadmium alone. Similar effects were obtained on sperm in which the metal was added directly to the water only during the final dilution to induce motility, but the shorter exposure time was reflected in the higher concentrations required to decrease motility. More recent studies (Ebrahimi and Kime, Unpublished data) have shown that the protective effect of zinc may due to competition of zinc and cadmium for binding sites on the sperm so that the toxicity of cadmium may be due, at least in part, to its displacement of essential zinc from sperm-binding sites. Furthermore, since calcium is also displaced by both cadmium and zinc, these heavy metals could disrupt intracellular calcium flows. The role of calcium as a second messenger in hormone action might thus be particularly sensitive to such disruption. These interactions between zinc, cadmium and calcium may also be important in other toxic effects of heavy metals in which action is too rapid for the protection of metallothioneins to be implicated.

While cadmium had a relatively low toxicity when added during the final dilution to induce motility and would be unlikely to cause any effect on sperm if only present in river water, mercury was much more toxic. Addition of only 0.001 mg/l to the final diluent was sufficient to cause a decrease in motility (Figure 6.8) and longer exposure (6 h in extender) had little additional effect (Rurangwa *et al.*, 1998) suggesting that sperm damage occurred within a very short time of exposure. Mercury is absorbed in the form of methylmercury and exposure of guppies (*Poecilia reticulata*) to only 0.0018 mg/l of this form of the metal caused moderate sperm necrosis (Wester, 1991; Wester and Canton, 1992). These studies suggest that mercury at concentrations of only a few μg/l can cause serious reproductive problems in male fish. Although mercury is present in many areas of the North Sea at concentrations of only 5-30 ng/l (Kersten *et al.*, 1988; MAFF, 1991), fish in Japanese rivers containing 4 or 30 ng/l had mean whole body concentrations of 0.5 and 2 mg/kg respectively (Matsunaga, 1975) which is comparable to the body concentrations of 0.05-0.4 mg/kg found in fish in many polluted waters (Table 3.1). This suggests that in many fish the sperm is exposed within the testes to concentrations which are several orders of magnitude greater than that which causes a decrease in motility on instant contact. Of particular relevance to the disappearance of eels from many European rivers (Bellepaire and Ollevier, 1987) is the very high concentration of mercury (>1mg/kg) in a substantial proportion of eels in the Elbe (Dethlefesen and Tiews, 1987). There was also histological evidence of damage in the swim bladder, kidney

and liver at similar concentrations, but unlike in mammals there was no morphological evidence of neurotoxicity (Wester, 1991; Wester and Canton, 1992). The concentrations of mercury which cause these effects are present in the water of many polluted areas and are comparable to the permitted levels in drinking water (0.001 mg/l; 1 ppb).

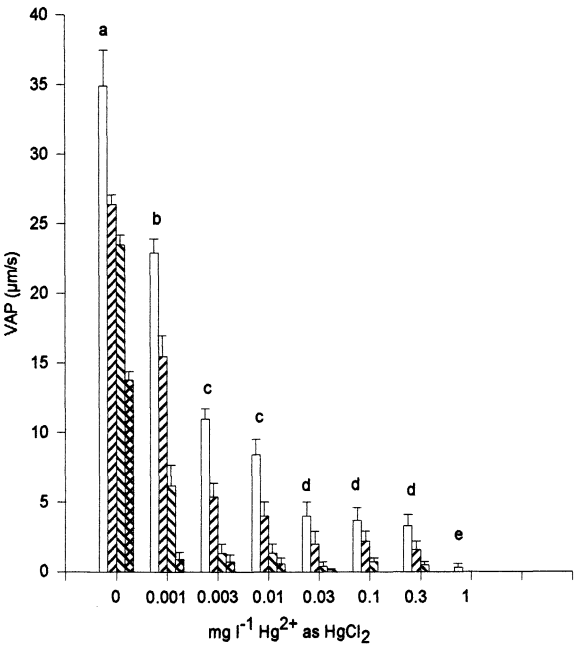


Figure 6.8. Average Path Velocity (VAP-µm/s) of African catfish sperm exposed to $HgCl_2$ at different exposure times: 21-35 (open bars), 36-50 (hatched bars, bottom left to top right), 51-65 (hatched bars, top left to bottom right), and 66-80 (crossed bars) seconds after activation. The values are the means ± SEM (n=6). Means for 21-35 seconds after activation with different characters are significantly different by Tukey HSD test after one-way ANOVA ($P<0.05$). (Reproduced from Rurangwa, E., Roelants, I., Huyskens, G., Ebrahimi, M., Kime, D. E. and Ollevier, F. 1998. The minimum effective spermatozoa to egg ratio for artificial insemination and the effects of mercury on sperm motility and fertilization ability in the African catfish (*Clarius gariepinus*, Burchell 1822). *J. Fish Biol.* **53**, 402-413, with kind permission of the Fisheries Society of the British Isles).

Although scanning electron microscopy did not show any effect of mercury on the morphology of mummichog sperm at 0.05 mg/l (Khan and Weis, 1987a, b), other studies (Ebrahimi and Kime, Unpublished data) show that mercury, cadmium and copper caused immediate and irreversible

morphological damage to carp sperm. Modern advances in computer technology now make quantitative measurement of such morphological changes possible, and will be particularly useful in assessing the effects of endocrine disruptors on the development of sperm as a result of action either on the Sertoli cells or on the hormonal support during spermatogenesis. Such motility and morphology measurements may be used either to compare the sperm from wild fish populations in polluted and non polluted environments, or that of laboratory exposed and control fish. This would be of particular interest if it were also related to changes in the morphology of the gonads and pituitary and their secretion of hormones.

Not all of the effects of pollutants may be apparent from a simple examination of sperm. Macek (1968) found that the cumulative mortality for brook trout fry from parents in which only the male was exposed to DDT was greater than in controls even though the fertilisation rates did not differ. Although motility was not directly measured, sperm volume was unaffected by the organochlorine insecticide. It is not clear whether mortality resulted from chromosomal damage or to other biochemical changes in the sperm, but the rapid increase in mortality 7 weeks after fertilisation would suggest that some key developmental stage had been affected.

6.4.2 Fertilising ability

While sperm motility measurements can provide a rapid and readily quantifiable parameter for potentially harmful effects of pollutants without the need for direct exposure of experimental animals, only the much more labour intensive fertilisation of eggs by sperm can at present show how this actually affects fertility. As yet there have been no reports of parallel studies on sperm motility and fertilising capability, but a number of studies have clearly shown that fertilising ability is affected by similar pollutants to those which decrease motility.

Exposure of male brown trout to bleached kraft mill effluent at 0.2-0.5% concentration had no significant effect on the fertilising ability of their milt although the eggs produced by exposed females were less well fertilised at the same concentration (Vuorinen and Vuorinen, 1985). This suggests that in this case, eggs rather sperm are the most sensitive to the pollutant. In contrast, using *in vitro* exposure of gametes for 40 min, Billard and Roubaud (1985) found that the toxicity of chromium, iron, cyanide and zinc was greater for sperm than ova of the rainbow trout. Sperm of the mummichog were also more sensitive than eggs to mercuric chloride and methyl mercury (Khan and Weis, 1987a, b). Fertilisation success was decreased after exposure of sperm for 2 min to 0.01 mg/l of the pollutant, while eggs exposed to 0.5 mg/l for 25 min were unaffected. The difference between these studies may reflect

different pollutants, or that the male and female gametes may have different relative sensitivities during development (*in vivo* exposure) and when fully mature (*in vitro* exposure). Methylmercury at 1 mg/l also decreased fertilisation by steelhead trout (*Oncorhynchus mykiss*) sperm (McIntyre, 1973). Copper at 0.11 mg/l decreased fertilisation by exposed sperm by 50%, but higher exposures of embryos and larvae were required to affect their viability (Anderson *et al.*, 1991).

The percentage hatch rates of rainbow trout eggs was lower when fertilised by sperm from males exposed to ambient pH 4.5 to 5.5 than to pH 6.5-7.1 although steroid hormone levels and other physiological parameters in the exposed males were unaffected (Weiner *et al.*, 1986). The means by which this was effected is not clear since, unlike other pollutants, acid water does not contain bioaccumulating material and under laboratory conditions the effects could not have been caused by changes in water mineral content induced by high acidity. Stress during critical stages of gamete formation is perhaps the most likely explanation.

In none of these reports is any data given on the sperm:egg ratio used, but clearly if sperm is present in say a 100 fold excess, then 99% could be rendered unviable and still leave sufficient for complete fertilisation. Such an excess of sperm is more likely to be present during *in vitro* fertilisation procedures than in the wild and these experiments may well underestimate the toxicity of the pollutants used. A necessary pre-requisite of such experiments should, therefore, be a determination of the minimum sperm:egg ratio required for complete fertilisation of the eggs. A recent such study (Rurangwa *et al.*, 1998) has shown that the spermitoxicity of a pollutant, such as mercury, on fertilisation rate is in fact very dependent upon the sperm:egg ratio. This study showed that increasing the sperm:egg ratios above 3000:1 had no effect on the rate of fertilisation and hatch of catfish eggs, while the rates decreased rapidly if less sperm were used. This was reflected in the toxicity of mercury in that fertilisation and hatching rates were unaffected by addition of 1 mg/l mercuric chloride to the extender in which sperm was added to the eggs if a sperm:egg ratio of 9000:1 was employed, while at 3000:1 there was a significant decrease and at 1500:1 fertilisation and hatch rates were effectively zero (Figure 6.9). This study shows that if an excess of sperm is present, then it doesn't matter if the pollutant destroys the viability of large numbers of sperm, provided that the minimum number remain sufficiently motile to ensure fertilisation of the eggs, but if only this minimum is present initially even very low losses will be reflected in a decreased fertilisation rate.

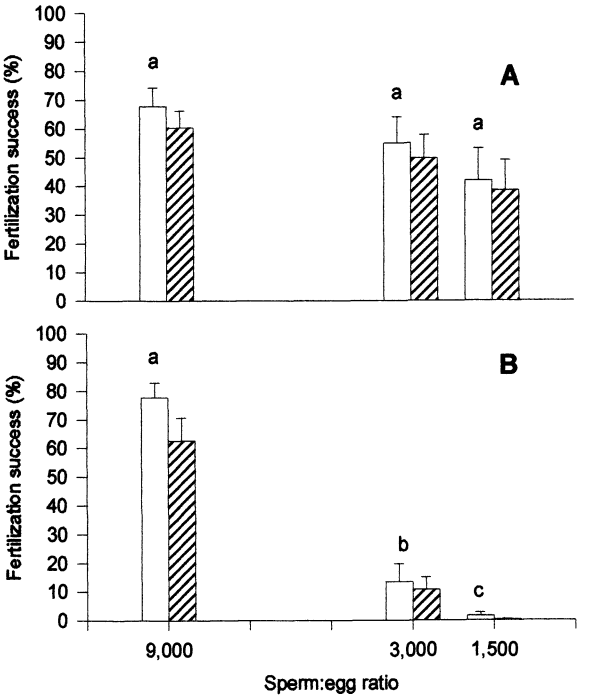


Figure 6.9. Fertilization (open bars) and hatching (hatched bars) rates of eggs fertilised with (A) non-exposed and (B) 1 mg l^{-1} Hg^{2+}-exposed catfish sperm at different sperm to egg ratios. Values are the means ± SEM (n=12). Means with different characters are significantly different (P<0.05). (Reproduced from Rurangwa, E., Roelants, I., Huyskens, G., Ebrahimi, M., Kime, D. E. and Ollevier, F. 1998. The minimum effective spermatozoa to egg ratio for artificial insemination and the effects of mercury on sperm motility and fertilization ability in the African catfish (*Clarius gariepinus*, Burchell 1822). *J. Fish Biol.* **53**, 402-413, with kind permission of the Fisheries Society of the British Isles).

Although all of these studies show that pollutants can affect the ability of sperm to fulfil its role of fertilisation, leading to hatch of viable offspring, they provide little information on the levels which affect their ability to do so in a natural habitat since the fertilisation of eggs with sperm in close proximity within a fertilisation bowl differs greatly from that in a flowing river or open water where contact between eggs and sperm may be decreased. The use of small fish, such as zebrafish, which can be allowed to spawn naturally in captivity, may provide a useful experimental model for such studies in which fertilisation rates of exposed and unexposed fish may be compared directly. Very little is known about whether, in a natural habitat,

male fish release more sperm than is required to fertilise the eggs of the female, and if so how great an excess is released. Recent studies on coral reef fish (Warner *et al.*, 1995; Warner, 1997), however, suggest that males can regulate the amount of sperm that they release to correspond to the size of the female, or to the number of females with whom they intend to mate. They may accept a fertilisation rate slightly lower than maximal in order to conserve sufficient sperm to fertilise an increased number of females. This suggests that the amount of sperm released is not in excess, and that the *in vitro* fertilisation tests using a minimal sperm:egg ratio may be a good approximation to the situation in wild fish. In group spawning species, in which sperm competition between males may play an important role, the situation may be different and sperm may be produced in greater excess by dominant males to increase their competitive advantage. In such cases, pollutants might be expected to alter the relative fertilising success among competing males. They may also differentially alter the reproductive success of different species within the same ecosystem if there are marked interspecific differences in the amount of sperm released during spawning. In such cases pollution might have marked effects on the population ecology of the habitat. There have been no studies on sperm release in the species which are under the greatest threat in polluted habitats. Such studies are, however, essential to understand the vulnerability of the male and how his ability to fertilise eggs may be affected by pollutants. Recent concern about the sustainability of North Sea cod has concentrated solely on the effects of overfishing (Cook *et al.*, 1997), but if the cod is a species which does not produce a sufficient excess of sperm to compensate for the effects of pollution, then the outlook for the cod population is indeed bleak.

6.5 Other effects on the male

6.5.1 Courtship behaviour.

Courtship patterns are controlled by both hormonal and neural factors, and are an essential preliminary to spawning in many species. Both lindane and water from the Munich wastewater purification plant modified the behaviour of male guppies (*Poecilia reticulata*) which, surprisingly, led to greater reproductive success of the exposed fish than controls (Schröder and Peters, 1988). Breeding behaviour in tilapia was delayed by exposure to 0.5 μg/l endosulfan, a concentration which did not cause morphological changes to the gonads or significant changes in gonadal steroid production (Matthiessen and Logan, 1984). While brook trout were unaffected by 3.4 μg/l cadmium during the 24 week prespawning period, the males suddenly began swimming erratically, lost equilibrium, lapsed into paralysis and died

within minutes once the females began their nest building activity in the gravel substrate (Benoit *et al.*, 1976). Although the cause of this sudden mortality was not clear it cautions against the notion of a safe dose as this clearly can become lethal as a result of social interactions.

There is increasing recognition that in addition to visible behaviour, fish also use olfactory signals (pheromones) as cues (Canario and Power, 1995). Such cues not only signal the stage of sexual maturation of the female, but directly stimulate the male endocrine system. Of the ovarian pheromones, the most studied are the steroids and the prostaglandins. The effects of pollution on ovarian steroid synthesis will be discussed in Chapter 7, but there have been no studies on how prostaglandin synthesis might be affected although it would also presumably be affected by ovarian dysfunction. To detect such pheromones the male must possess functional olfactory receptors and be able to interpret their response by an elevation of his own hormones. Recent studies have shown that such olfactory function can be disrupted by pollutants. The organophosphate pesticide diazinon at 1μg/l significantly decreased the electrophysiological response of the olfactory epithelium to prostaglandin $F_{2\alpha}$ (Moore and Waring, 1996a, b) and exposure to the pesticide for 5 days significantly reduced the levels of plasma 17,20βP, testosterone and gonadotrophin II after priming with urine from ovulated female salmon which is known to play a role in synchronising reproductive physiology and behaviour of the sexes. Carbofuran at a concentration of only 0.001 mg/l also impaired the ability of male Atlantic salmon to detect and respond to the reproductive priming pheromone, prostaglandin $F_{2\alpha}$ which is released by the female at ovulation (Waring and Moore, 1997). 0.003 mg/l of the pesticide significantly reduced plasma concentrations of 17,20βP, testosterone and 11-ketotestosterone, and the volume of expressible milt after priming with this pheromone. The acidity of holding water also has a deleterious effects on the males ability to detect important odorants and pheromones which may be important mediators of reproductive physiology and behaviour (Moore, 1994). It is not clear whether such pollution induced dysfunction is due to direct action on the function of the olfactory receptors themselves or whether it is due to disruption to the transmission and interpretation of the signal.

6.6 Conclusions

Morphological damage to the testis can be caused by a wide range of pollutants, such as heavy metals, organochlorines, organophosphates, carbamates and a range of industrial chemicals. The literature is extremely patchy, covering a wide range of pollutants, species, doses and exposure times, but there have been few concerted attempts to compare the relative

cytotoxicity and endocrine disrupting potential of different pollutants under standardised conditions. Such a study requires choice of a suitable species and then examination of a number of pollutants over a wide range of concentrations. Morphological changes caused by the different xenobiotics should then be examined to determine whether they really differ in their basic nature or whether such differences are simply quantitative and reflect the different potencies of the pollutants and would disappear if different relative doses were used. While morphological data clearly shows disruption of testicular function, it does not indicate whether the testis is the primary site or whether changes in its structure are a secondary effect of pituitary-hypothalamic dysfunction, or of the failure of key steroidogenic enzymes. A truly integrative study would combine morphological criteria with measurement of pituitary and steroidogenic activity and could provide invaluable data on the mechanism of action of xenobiotics as well as information on the safest chemical for the designated use. Clearly, comparisons also have to be made between species to determine whether sensitivities to all effects change in similar proportions from species to species. Furthermore, for practical application to pesticide choice and usage it is necessary to relate the toxic effects on the fish to the concentrations that need to be used on the target pest.

While studies of the effects of pesticides and heavy metals are very patchy, those for the large numbers of industrial pollutants which are released into the waterways are notable by their absence, apart from some studies on pulp mill effluents and on fish from the Great Lakes. Although studies on morphology and endocrine function may provide explanations for reduced fertility, the bottom line is whether the sperm is capable of fertilisation and whether the male is capable of delivering it to the female. Determination of fertilisation rates are very labour intensive, and a simple method for relating sperm motility to its fertilising capability would be of great potential use. Study of the effects of pollution on courtship behaviour, and especially of the release and detection of pheromones is in its infancy, but is vitally important in ensuring delivery of sperm to eggs.

Chapter 7

Disruption of Female Reproductive Function

7.1 Introduction

Pollution can affect female fish in very much the same way as males. It can cause morphological disruption of the ovary, delayed oocyte development, and inhibition of steroid hormone synthesis. As with the testis it may be difficult to differentiate whether morphological changes are the cause or result of changes in hormonal production, and whether the effect originates from disruption at the gonadal or hypothalamo-pituitary levels. With the female, unlike the male, a significant amount of the body burden of a pollutant can be passed on into the gamete and may be incorporated into the developing offspring which use maternally derived yolk protein for early stages of development and may consequently be particularly susceptible to xenobiotic disruption. It is therefore not only necessary to consider the effects of pollution on the ability of the female to produce and spawn eggs, but also to consider the fertilisation rate of the eggs, their hatch rate, and the survival of offspring until adulthood. Furthermore, since sexual differentiation is determined during the very early life stages, xenobiotic disruption at this stage may result in changes in the sex ratios, abnormal sexual differentiation or changes in fertility of the offspring when they attain maturity. In this Chapter only such effects derived from exposure of the parent are considered. Effects resulting from direct exposure of eggs or larvae to pollutants are discussed in Chapter 8.

7.2 Effects on ovarian morphology

7.2.1 Effects of heavy metals

Arsenic at 14 mg/l for 30 days inhibited development of *Colisa fasciatus* oocytes beyond Stage II, and caused both an increase in intra-follicular spaces and increased atresia (Shukla and Pandey, 1984a). It is not clear whether this was due to direct action on the growth of the follicle, inhibition of estrogen synthesis and consequent lack of vitellogenin, or to indirect action via the pituitary. At this concentration fish showed fast movement and increased mucus secretion probably indicative of generalised stress, but no effect on the ovary was observed at lower exposures. Although reproductive dysfunction of the female may occur at such concentrations, resulting from accidental release of arsenic from local fertiliser factories, any long term exposure to such concentrations is more likely to lead to early mortality.

Cadmium at 3 mg/l caused disorganised ovarian lamellae, inhibition of oogenesis, necrosis, fibrosis of lamellar walls and disintegration of ovarian structure in *Monopterus albus* (Singh, 1989). It would be of interest to know whether long-term low level exposure of this protogynous fish to the metal had any effect on its sex reversal. Cadmium is much more toxic to the cyprinid, *Lepidocephalichthys thermalis*, with a 96h LC_{50} of 1.3 mg/l, while after 20 days at 0.2 mg/l there was vacuolation of the oocortex, necrosis of the oolemma and hypertrophy of the follicular cells (Victor *et al.*, 1986). The presence of shrunken and less yolky mature oocytes suggested that production of vitellogenin or its transfer across the oolemma might be inhibited. By contrast cadmium at 1 mg/l increased ovarian growth in the Atlantic croaker (Thomas, 1990a) as a result of increased steroidogenesis.

At 0.017 mg/l, mercuric chloride significantly decreased the GSI of female *Channa punctatus*, which had smaller and fewer Stage II and III oocytes, while the numbers of Stage I oocytes increased (Ram and Sathyanesan, 1983; Dey and Bhattacharya, 1989). Six months exposure of this species to 0.2 mg/l of the organic mercury fungicide, emisan, however also caused changes in the structure of Stage I oocytes which had cytoplasmic proteinaceous inclusion bodies and intranuclear ring-like bodies which enlarged and destroyed the oocyte (Ram and Sathyanesan, 1987b). The failure of mature oocytes to develop may be due to inhibition of steroidogenesis resulting from the decreased activity of the pituitary gonadotrophs of these fish, but could also result from direct effects on the ovary itself since specific binding for mercury has been found on the oocyte membrane (Ghosh *et al.*, 1991). Mercuric chloride, methylmercuric chloride and emisan-6 (at 0.03-0.05 mg/l active ingredient) also inhibited ovarian recrudescence in the catfish *Clarias batrachus* after 180 day exposure, so that ovaries contained only Stage I pre-

vitellogenic oocytes (Kirubagaran and Joy, 1988b). Exposure of mature *Lepidocephalichthys thermalis* to 0.1 mg/l mercuric chloride for only 20 days led to atresia of vitellogenic oocytes and damage to young oocytes similar to that observed for cadmium (Victor *et al.*, 1986). Unlike cadmium, vitellogenin incorporation was not affected. The stage at which exposure to mercury occurs seems to be important in determining its effects.

Lead contaminated food at 0.2 mg/kg body weight probably decreased ovarian growth in Atlantic croaker due to inhibition of pituitary and steroidogenic function (Thomas, 1990a). Nine-month exposure of *Clarias batrachus* to 5 mg/l lead retarded ovarian maturation and development of oocytes beyond Stage I (Katti and Sathyanesan, 1987a). Intranuclear and extranuclear inclusion bodies, similar to those observed for mercury were also found. Ovarian regression of fully mature female *Anabas testudineus* resulted from exposure to 1-20 mg/l lead (Tulasi *et al.*, 1989) while 0.1 mg/l lead or 0.2 mg/l copper caused atresia predominantly of Stage III, IV and V oocytes of *Puntius conchonius* after 4 months exposure (Kumar and Pant, 1984). Copper, however, can enhance vitellogenesis and maturation in other species (Khosa and Chandrasekhar, 1972). Zinc exposure of *P. conchonius* also caused atresia, but unlike copper and lead affected predominantly younger oocytes (Kumar and Pant, 1984).

7.2.2 Effects of pesticides

Although DDT was one of the earliest pesticides to be used, there have been few studies of its effects on reproduction even though it is one of the major pesticides in tissues of many species of fish as a result of bioaccumulation from sediments decades after its use ceased in many parts of the world (see Chapter 3). Exposure of tilapia, *Oreochromis mossambicus* to 0.001 mg/l DDT for 20 days resulted in decreased GSI, fibrogenesis and thickening in the ovarian tunic and structural disintegration with wide spaces between oocytes (Shukla and Pandey, 1985). Immature and maturing oocytes developed vacuolisation and a granular texture, while mature oocytes were deformed and had yolk with a granular texture. These abnormalities began to decrease after fish had been returned to fresh water for 10 days.

While use of DDT is now limited to only a few parts of the world, it has been replaced in many regions by other organochlorines which can cause very similar ovarian damage. Endosulfan at 0.001 mg/l for 45 days caused necrosis and fibrosis of the connective tissue, dilation of blood vessels and decreased mean diameter and deformity of the oocytes in the carp minnow *Rasbora daniconius* (Rastogi and Kulshrestha, 1990). There was also damage to the yolk vesicles and an increase in interfollicular spaces. Very similar effects were observed in *Channa striatus* after exposure to this concentration for 30 days (Kulshrestha and Arora, 1984). These studies

suggest that endosulfan and DDT have similar toxicities towards teleost ovaries and even concentrations as low as 0.001 mg/l (1 ppb) may cause serious ovarian damage.

The numbers of atretic oocytes in ovaries of *Puntius conchonius* increased steadily with exposure for 2 to 4 months to aldrin (0.05 mg/l) and methoxychlor (0.007 mg/l) (Kumar and Pant, 1988). Increased atresia, decreased oocyte diameter and increased interfollicular spaces were again the symptoms of exposure of the Indian catfish to chlordecone (Srivastava and Srivastava, 1994). Although this pesticide is estrogenic in birds and mammals (Eroschenko and Palmiter, 1980), its toxicity to gonads of both sexes is more likely attributable to its inhibition of cellular energy production by mitochondria (Desaiah, 1982). γ-Benzenehexachloride (γ-BHC) at 0.005 mg/l also increased atresia in the medaka (Hirose, 1975). Exposure to β-hexachlorocyclohexane, an impurity in the organochlorine insecticide lindane, at 0.32 mg/l decreased oocyte maturation, giving abnormal yolk formation with non-fusion of yolk droplets and the occurrence of exogenous yolk droplets in perinucleolus Stage-I oocytes along with an increase in endogenous yolk (Wester *et al.*, 1985). Together with hepatic changes, these effects were attributed to the estrogenic activity of the organochlorine, although no direct comparison was made with fish exposed to estradiol.

The use of organophosphates as alternatives to the organochlorines has led to an increasing number of studies into their effects on the female reproductive system, and although a number of papers have compared the effects of an organophosphate with an organochlorine there is little in the way of systematic comparison to determine which is the safest to use in terms of balance between wanted effect on the pest, unwanted effect on the exposed fish and long term biodegradation.

The organophosphate, monocrotophos at 53 μg/l increased numbers of atretic oocytes in *Puntius conchonius* after 2 months and in general terms caused changes similar to that of organochlorines used at 1/1000 th of this concentration (Kumar and Pant, 1988). As in many of these studies only a single concentration was used for each pollutant and no attempt was made to determine the minimum effective dose. Similarly methyl parathion and the carbamate carbofuran at 0.1 mg/l caused comparable damage to ovarian tissue to 0.001 mg/l endosulfan in *Rasbora daniconius* (Rastogi and Kulshrestha, 1990).

Organophosphates such as Lebaycid (fenthion) are more often used against malarial mosquitos instead of the persistent organochlorines in many tropical countries where tilapia are an important protein source. The effect on reproduction of this species has been studied by using five 24 hour exposures to 7μg/l separated by 2-day periods in clean water (Kling, 1981) to mimic the spraying regime. Exposed fish had decreased GSI and ovarian degeneration

with total follicular atresia and were unable to spawn for at least nine weeks after return to clean water.

Most other studies, however, have used a constant level of exposure over several weeks which more closely parallels general agricultural use where different fields are sprayed in succession and the pollutant leaches into the water courses and thence into lakes. Sumithion (fenitrothion) at 1 mg/l caused decreased GSI in *Garra mullya* after 30 days, resulting from atresia of the mature oocytes (Pawar and Katdare, 1983). In *Channa punctatus*, cythion at 2 mg/l for 6 months also retarded growth of the ovaries which had an absence of Stage III and IV oocytes due to atresia (Ram and Sathyanesan, 1987b). Proteinaceous "inclusion bodies" were also found in Stage I oocytes of exposed fish indicative of lack of hormonal support for continuation of development, probably resulting from the decreased gonadotroph activity observed in these fish. Elsan (phenthoate) exposure of *Channa punctatus* at 0.2 mg/l for 91 days (Dey and Bhattacharya, 1989), and malathion exposure of *Oreochromis mossambicus* to 2 mg/l for 20 days (Shukla *et al.*, 1984) also caused atresia of the more mature oocytes. The relative toxicity of four organophosphorus insecticides, birlane (chlorfenvinphos; 0.003 mg/l), gardona (tetrachlorvinphos; 0.15 mg/l), phosdrin (mevinphos; 0.00012 mg/l) and malathion (2.5 mg/l) was compared in *Mystus vittatus* (Haider and Upadhyaya, 1985). Only Stage I oocytes were present in all pesticide treated fish, while control fish contained mainly Stages II and III and the absence of 3βHSD activity suggested that the results were due to loss of steroid induced vitellogenesis. Since this study used only a single concentration for each pesticide, with a 20,000 fold concentration range between chemicals it provides little information on their relative toxicities to fish compared to that on insects. The inhibition of oocyte maturation and resultant atresia caused by organophosphates may be a result of decreased ovarian RNA and DNA content due to either inhibited synthesis or increased degradation (Ansari and Kumar, 1987). Similar effects were caused by carbofuran (5 mg/l), carbaryl (2 mg/l) and fenitrothion (1.5 mg/l) in *Channa punctatus* (Mani and Saxena, 1985; Saxena and Garg, 1978), carbaryl (10 mg/l) in *Channa striatus* (Kulshrestha and Arora, 1984) and carbofuran (0.7 mg/l) in *Colisa lalia* (Sukumar and Karpagaganapathy, 1992).

The inhibition of ovarian function is of particular interest in protogynous species in which ovarian atresia occurs as part of the natural sex-reversal although its endocrine basis is still not understood. During seven months none of the rice field eels (*Monopterus albus*) exposed to 3 mg/l malathion underwent sex reversal while 12 out of 18 females in the control group showed structural changes associated with sex reversal (Singh, 1993). Gonads of the exposed fish had partial to complete atrophy of ovarian tissue and degeneration of the testicular lobules of fish which were in the intersex or male phase at the beginning of exposure. Since ambisexual fish undergo a

type of "sexual indecision" with some reversing and some returning to their original sex during the postspawning period of gonadal regression (Kime *et al.*, 1991), exposure of fish at this critical time to low concentrations of pesticide might be of some interest and reflect relative sensitivities of the male and female parts.

7.2.3 Effects of industrial pollutants

A range of industrial pollutants, predominantly PCBs and PAHs, have also been shown to affect ovarian development. Although PCBs are no longer manufactured in the United States they continue to be found in tissue of fish as they accumulate from sediments which reflect past industrial use, and of the chemicals measured in fish tissue they have the highest levels (Chapter 3). Injection of 0.2 to 5 mg/kg body weight of the planar PCB 3,3',4,4'-tetrachlorobiphenyl (TCB) into female white perch (*Morone americana*) at 3 week intervals starting 3 months before the spawning season decreased the numbers of mature and maturing fish (by 60% at the highest dose), and of those that did mature the GSI was inversely related to the TCB dose and its accumulation into the liver and ovary (Monosson *et al.*, 1994). Levels of accumulation were comparable to those found in wild fish in the Great Lakes and the North Sea which suggests that the fertility of such natural populations of fish might be similarly compromised. Steroid and vitellogenin levels in this study were related to the stage of maturity rather than to exposure, and it is not possible to separate cause and effect. The authors do not describe ovarian morphology in sufficient detail to determine whether there was atresia, as found for organochlorine pesticides, which might indicate that the xenobiotic had decreased steroidogenesis and thence deprived the developing oocyte of vitellogenin. It is possible, however, that by simply decreasing pituitary secretion it could result in an overall slower development of the ovary. The presence of a significant population of mature fish even at high dose levels suggests that there is a considerable variation in susceptibility within the population and that in the wild this may result in selection, leading to a more PCB-tolerant strain. Atlantic croaker fed 0.24 mg Arochlor 1254 per day per 70 g fish for 30 days via their diet had lower GSI than control fish but did not have decreased estradiol (Thomas, 1988), although in later experiments in which they were fed only 0.05 mg/kg body weight for the same period both GSI and plasma estradiol decreased (Thomas, 1990a) suggesting that disruption may be quite complex.

Polyaromatic hydrocarbons are also common pollutants in industrial waters in which benzo[*a*]pyrene is a common constituent. Twice weekly injections of this hydrocarbon into Atlantic croaker decreased both GSI and circulating steroid levels (Thomas, 1988, 1990a). Both PAHs and PCBs are present in waters off the west coast of the USA and it is therefore not

surprising to find evidence of ovarian dysfunction in these waters. These compounds are, however, indicative of generalised industrial pollution and such dysfunction could therefore be caused, at least in part, by other contaminants. White croaker (*Genyonemus lineatus*) collected from a contaminated site off Los Angeles had high liver and ovarian PCB and DDT content, had significantly fewer early oocytes and higher numbers undergoing atresia than those from a reference site 80 km further away from the city (Cross and Hose, 1988; Hose *et al.*, 1989). Inhibition of ovarian development was also found in English sole (*Parophrys vetulus*) from contaminated sites further north along this coast in Washington Bay (Johnson *et al.*, 1988; Collier *et al.*, 1992). Ovarian development did not proceed beyond the perinuclear or early previtellogenic stage and the prevalence of this type of inhibition was higher in sole which had higher hepatic aryl hydrocarbon hydroxylase (AHH). There was also an increased incidence of inflammatory lesions and macrophage aggregates in ovaries of fish from the more contaminated sites (Johnson *et al.*, 1988). All sites contained both PCBs and PAHs, and in all probability a wide range of other pollutants so although AHH (as a measure of PAH) was shown to be the best predictor of ovarian dysfunction, the role of other contaminants cannot be discounted.

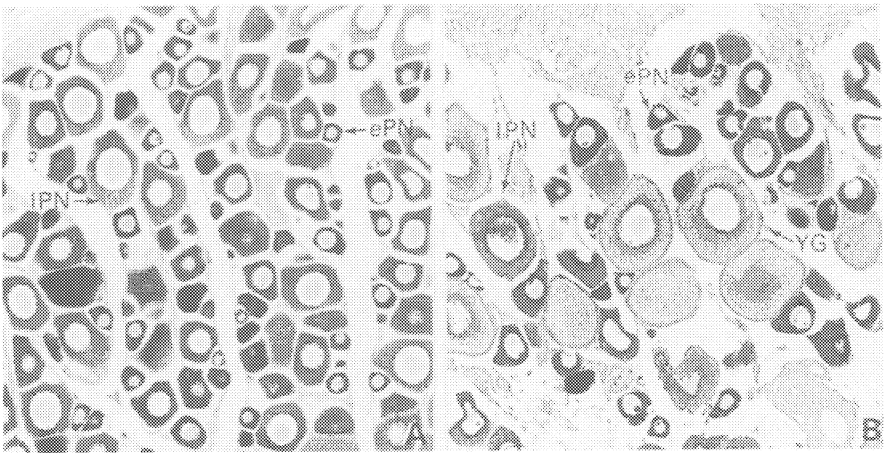


Figure 7.1. Ovarian development in the flounder, *Pleuronectes flesus* from the mesocosm systems (HE, x90). a) Ovary from control mesocosm in previtellogenesis (late perinucleolus); developing oocytes in primary growth phase are situated in the ovarian lamellae. b) Ovary from polluted mesocosm in premature vitellogenesis; oocytes in yolk-granule (YG) stage are present as are the oocytes in primary growth phase. (Reproduced from Janssen, P. A. H., Lambert, J. G. D. and Goos, H. J. Th. 1995. The annual ovarian cycle and the influence of pollution on vitellogenesis in the flounder, *Pleuronectes flesus*. *J. Fish. Biol.* **47**, 509-523, by kind permission of the Fisheries Society of the British Isles).

One difficulty with such studies is that although effects may be related to levels of specific pollutants in fish from each locality, reproduction could also be affected by different non-pollutant environmental factors in each area. In addition the populations are often distinct strains, or there may be migration between sampling sites. A more controlled method of determining the effects of pollutants is by use of a mesocosm. Such a mesocosm study using the flounder, *Pleuronectes flesus*, showed that fish exposed for 3 years to the sediment from Rotterdam harbour had advanced oocyte maturation compared with those that had been exposed to clean sediment from the Wadden Sea (Janssen *et al.*, 1995, 1997) (Figure 7.1). This data contrasts with the inhibition of oocyte development in fish off the west coast of the USA and may well reflect the wider variety of pollutants which have accumulated from the Rhine effluents. Although vitellogenin levels were elevated in the female flounder exposed to sediments, it was not detectable in either the control or exposed males (Janssen *et al.*, 1997). This suggests that the vitellogenin was synthesised in response to the elevated estradiol secreted by the more advanced ovarian follicles in exposed females. Benzo[*a*]pyrene, a major constituent of the sediment, induced EROD activity in flounder which resulted in a decreased rate of steroid catabolism (Janssen, 1996). This in turn may have elevated plasma estradiol further, but the mechanism by which premature vitellogenesis was induced is not fully clarified.

Specific industrial effluents have also been examined. Laboratory exposure of *Heteropneustes fossilis* to textile mill effluent caused vacuolisation in the cytoplasm, decreased cytoplasmic RNA and protein content, and atresia of previtellogenic oocytes (Murugesan and Haniffa, 1992). Ovarian recrudescence was also inhibited, with a low frequency of occurrence of Stage III oocytes, in *Channa punctatus* exposed to 5% vegetable oil effluent in the laboratory (Saxena and Bhatia, 1983). The degree of inhibition of ovarian recrudescence in Baltic perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) was related to the distance from the pulp mill at which fish were captured (Sandström *et al.*, 1988). Phenol and sulphide are two of the contaminants of pulp mills. Exposure of carp, *Cyprinus carpio*, for 30 days to either of these chemicals at levels much lower than those found in effluent decreased the GSI, but caused an increase in both hepatic and ovarian cholesterol which may have been a result of inhibited steroidogenesis (Kumar and Mukherjee, 1988). Pentachlorophenol (PCP), used as a fungicide in pulp and paper processing caused significant atresia of the Stage II oocytes in rainbow trout (Nagler *et al.*, 1986).

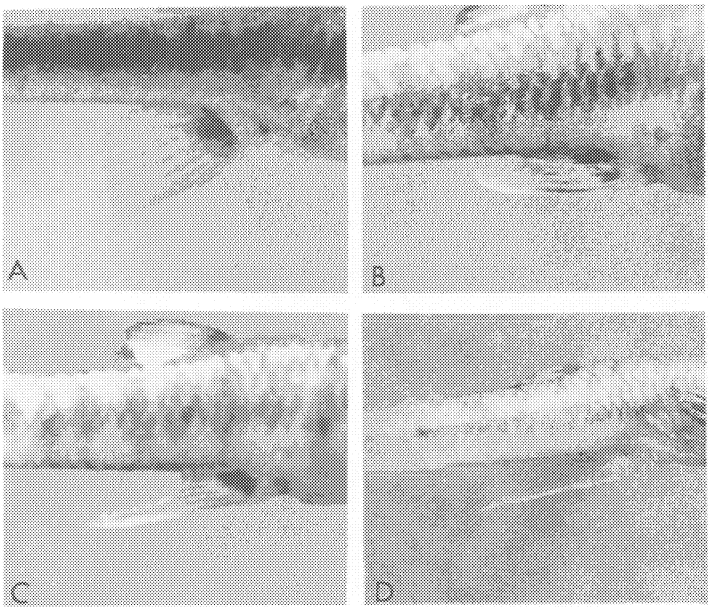


Figure 7.2. The effect of kraft pulp mill effluent in the Fenholloway River, Taylor County, Florida on gonopodial development in female least killifish (*Heterandia formosa*). Normal fish were from Etonia Creek, Putnam County, Florida. Pictures show the anal fins of A) a normal female, B) a partially masculinised female, C) a female with advanced masculinisation and D) a normal male. (Unpublished photographs kindly provided by R. Cody and W. Davis, University of West Florida).

While the studies cited have all been concerned with ovarian morphology, there is an interesting example where changes in morphology of the secondary sexual characteristics has resulted from industrial pollution. Recent concern with the effects of environmental estrogens and their effects on the male have obscured an example, first reported as long ago as 1980 by Howell *et al.* of masculinisation of females resulting from pollution. Mosquitofish, *Gambusia affinis holbrooki* inhabiting a stream below the discharge from a pulp mill in Florida showed masculinisation of the females, which had both the secondary sexual characteristics and reproductive behaviour of males. Males exhibited precocious development of their secondary sex characters and reproductive behaviour. In normal males of this species the anterior anal fin rays are elongated to form a gonopodium which acts as a copulatory organ to inseminate females. Such development is absent in normal females, but is present in those exposed to synthetic androgens or to pulp mill effluent. Subsequent studies (Bortone and Davis, 1994; Bortone and Drysdale, 1981; Bortone *et al.*, 1989; Davis and Bortone, 1992; Drysdale and Bortone, 1989) have shown that mosquitofish and another Poeciliidae, the least killifish *Heterandia formosa*, captured below other pulp mills or collected from

unexposed areas and treated with pulp-mill effluent in the laboratory were also masculinised (Figure 7.2). Some, at least, of these effects may be due to bacterial processes associated with the kraft mill process. Mosquito fish exposed to phytosterols mixed with the bacterium *Mycobacterium smegmatis* exhibited the same masculinisation of the anal fin as mill effluent (Denton *et al.*, 1985; Howell and Denton, 1989). These studies suggest that mosquito fish and least killifish may be a useful species to monitor environmental androgens.

While industrial pollution is generally associated with chemical effluents, excessive heat pollution can also cause reproductive problems. Many fish require specific or permissive temperature signals to induce gonadal recrudescence so that spawning may be seasonally synchronised with food supply of the fry. Both colder and warmer water than is normal for the species can therefore disrupt normal function. Warmer water in early season may advance spawning in some species while if the temperature is too high gonadal activity may be decreased. There may also be a disjunction between photoperiodic and thermal cues which might lead to abnormal function. There is increasing evidence that excessive use of hydrocarbon fuels and increased carbon dioxide emission is leading to global warming and such pollution induced temperature changes could have serious effects on the reproductive capability of temperature sensitive species. On a more local scale, power stations frequently use water bodies for cooling, leading to locally increased temperatures. Such local heating by power stations of the water used for breeding has been shown to affect the ovarian development in *Abramis brama* (Luksiene, 1978; Luksiene and Kontautus, 1988), *Rutilus rutilus* (Luksiene, 1981), and *Perca fluviatilis* (Luksiene, 1982).

Acid water is another frequent consequence of fossil fuel combustion and may also suppress reproductive performance. Increased acidity from pH 6.7 to 4.5 for 20 days progressively decreased the ability of flagfish (*Jordanella floridae*) oocytes to form mature eggs due to their loss of ability to deposit yolk within the cytoplasm (Ruby *et al.*, 1977). Low pH also increased follicular atresia in maturing brook trout and fathead minnows, but the elevated ACTH, cortisol and hyperglycaemia in the trout suggested that this was a generalised stress effect rather having any specific action on the gonads (McCormick *et al.*, 1989; Tam *et al.*, 1990).

Ammonia at 16 mg/l caused similar ovarian damage to elsan and mercury (see above) (Dey and Bhattacharya, 1989), but it is not clear whether this is a direct pollutant effect or simply a stress effect resulting from the alkaline waters. Although cyanide is a well known toxicant, it is released in sublethal amounts during many industrial processes. At 0.01 mg/l it caused abnormalities in the cytoplasm during early oocyte development, delayed secondary yolk deposition and caused increased frequency of atresia which

may be related to an inability to produce vitellogenin (Lesniak and Ruby, 1982).

7.3 Effects on steroidogenesis

7.3.1 Introduction

A large number of malfunctions of ovarian development were described in the previous section. Characteristics which dominate are the inability of early Stage I and II oocytes to progress through to vitellogenesis, atresia of vitellogenic oocytes, and increased interfollicular spaces resulting from the decreased size of the oocyte. Together these contribute to the reduced GSI which is the most readily measured indicator of pollution induced dysfunction. The symptoms of such dysfunction are a general failure to proceed, or continue with incorporation of the yolk protein vitellogenin. While this could undoubtedly arise from liver malfunction, it is more probably due to a failure either of the ovarian follicle to produce estradiol which is the essential stimulus for hepatic vitellogenesis, or to a failure of the oocyte to incorporate the vitellogenin produced. While few of the studies cited have measured vitellogenin, there is substantial evidence that many pollutants cause decreased hepatic vitellogenin production (Chapter 9), but none that vitellogenin accumulates in the plasma as a result of lack of incorporation by the oocytes. Failure of estradiol synthesis is therefore the most probable cause of the malfunction, and the nature of the ovarian degeneration suggests that in most cases this failure is a cause rather than an effect of ovarian changes. An electron microscopic examination of the effects of pollutants on the thecal and granulosa cells in which the steroids are produced would be useful in confirming such a hypothesis. It is, however, far from clear whether inhibition of steroidogenesis is due primarily to enzymatic disruption within the follicle, or to a secondary effect resulting from decreased GtH secretion as a result of xenobiotic disruption of the hypothalamo-pituitary axis (Chapter 5). The evidence for pollution induced disruption of ovarian steroidogenesis is surveyed in the following sections.

As with the testis, two approaches have been employed - *in vitro* and *in vivo*. Some caution must be exercised in interpretation of the data available. Decreased plasma steroid levels, or *in vitro* production by ovaries from fish in polluted water might be the result of inhibition of enzymes by the pollutant, but if the ovary is at a less mature stage the differences could simply be due to the different stages of maturity resulting from a completely different action of the pollutant. Direct effects on steroidogenic enzymes are best examined by culture of ovarian tissue from normal fish in the presence of different levels of pollutant. The majority of the data cited, however simply compares control

and treated fish and while this may confirm that decreased estrogen synthesis is the cause of lack of availability of vitellogenin for oocyte development it rarely defines whether the cause lies within the ovary or the pituitary and hypothalamus. This section concerns only estradiol and testosterone synthesis; that of the progestogens will be dealt with under Section 7.4 since these steroids are more involved in oocyte final maturation leading to ovulation and spawning.

7.3.2 Effects of heavy metals

As with some studies on the testis and pituitary, cadmium is unusual in having a contradictory effect on the ovary. In *Monopterus albus,* cadmium exposure decreased both estradiol and testosterone leading to lower vitellogenin secretion (Singh, 1989), while in Atlantic croaker it had a stimulatory effect on the ovarian secretion of estradiol (Thomas, 1989, 1990a). The increased secretion in the croaker was related to the higher spontaneous *in vitro* secretion of GtH by pituitaries from treated fish. The differences in the two species might therefore be related to their differential action at the pituitary level. A dose response curve, with measurement of both GtH and ovarian steroids might also determine whether at some concentrations cadmium increased GtH secretion while decreasing ovarian steroidogenesis.

Lead also decreased plasma testosterone and estradiol in the Atlantic croaker, but stimulated the estradiol secretion from ovarian tissue *in vitro* showing that steroidogenic tissue had not been adversely affected (Thomas, 1988, 1990a). This supports the hypothesis that primary dysfunction occurs at the hypothalamic-pituitary level rather than at the ovary.

7.3.3 Effects of pesticides

The impact of pesticides on steroidogenesis in fish ovaries has been widely investigated, predominantly by Indian scientists. While there have been numerous attempts to compare the toxicity of different pesticides these studies suffer from the criticism made earlier, in that many lack both true dose response data and relevance to the doses applied in the field.

Exposure to 0.001 mg/l DDT for 20 days inhibited both ovarian 3β- and 17β-HSD activities in *Oreochromis mossambicus* (Shukla and Pandey, 1985) which would decrease androgen and estrogen output. γ-BHC at the very much higher sub-lethal level (fish in distress) 16 mg/l for 4 weeks suppressed plasma concentrations of both testosterone and estradiol and their precursor 17-hydroxyprogesterone *Heteropneustes fossilis* (Singh and Singh, 1991). Normal plasma levels were restored by injection with 10-20μg/fish of either LHRH (mammalian GnRH) or *Mystus*-GtH. This suggests inhibition at the

level of pituitary secretion rather than at the response of the ovarian GtH receptors. Parallel measurement of plasma gonadotrophin concentrations together with a dose response for control as well as treated fish to GtH/LHRH may have provided such valuable information and confirmed the earlier data on pituitary-hypothalamic inhibition provided by this group (Chapter 5). Plasma testosterone, estradiol and estrone levels were also significantly decreased in *Clarias batrachus* after 4 weeks by sub-lethal doses of 4 mg/l cythion or 8 mg/l γ-BHC in which fish were in apparent distress (Singh and Singh, 1987a). At the more relevant safe levels, at which fish showed no symptoms of stress (1 and 2 mg/l respectively), testosterone levels were not significantly lower than controls, and estradiol was significantly lower only with cythion. Interestingly estrone was decreased more with BHC, which did not significantly affect estradiol, suggesting that there might be some impairment of 17β-HSD activity. Sub-lethal exposure to malathion and γ-BHC in *Heteropneustes fossilis* (Singh and Singh, 1992a; Singh *et al.*, 1993), and malathion and hexachlorocyclohexane (γ-BHC) in *Clarias batrachus* had similar effects (Singh and Singh, 1987b). Not surprisingly the pesticides were more effective in suppressing steroid levels when normal levels were high during the previtellogenic phase, rather than during the postspawning phase when levels were low and probably not being stimulated by the pituitary - perhaps further evidence that inhibition is primarily at the pituitary level. In all cases the effects on estrone were again particularly pronounced. Some caution must, however, be used in interpreting data from sub-lethal exposure and the depression of steroids could perhaps be profitably compared with what might result from other forms of stress (hypoxia, handling etc.). Measurement of plasma cortisol or glucose as an indicator of stress (Pickering, 1981) could also be informative and might help to determine whether the effects are due to specific chemical action of the pesticide within the reproductive system, generalised cellular or biochemical dysfunction, or mediated via a generalised stress response acting via ACTH. Studies at the sub-lethal level may be relevant to the situation where fish are exposed to pesticide for a short 2-4 week spraying season, but have little relevance to long-term exposure in which stress induced mortality would be high.

In vitro studies showed little effect of γ-HCH on the metabolism of 17-hydroxyprogesterone in goldfish (Kime and Singh, 1996), but the production of testosterone and 11-deoxycortisol in the presence of carp pituitary extract was higher in the presence of 1, 10 and 20 mg/l of the pesticide than in control incubations. Tissue from goldfish which had been exposed to the lower concentrations of γ-HCH (0.01 and 0.1 mg/l) for four weeks also had higher 11-deoxycortisol glucuronide production than control fish in response to carp pituitary extract *in vitro*, but both the free steroid and testosterone were decreased, although in exposed male fish elevated *in vitro* deoxycortisol was observed (Singh *et al.*, 1994). Although the data clearly indicates that

steroidogenesis at stages prior to 17-hydroxyprogesterone are affected by the organochlorine pesticide, the differences between stimulation and inhibition in such *in vitro* studies may be a reflection of the partial picture obtained when only selected hormones are assayed. Fish exposed *in vivo* may have had impaired pituitary function which if reflected in the availability of endogenous precursors could explain differences between *in vivo* and *in vitro* exposure. The difference between stimulation and inhibition may also reflect the duration of exposure since estradiol was higher in *Anabas testudineus* exposed to 0.00016 mg/l of the organophosphate Metacid-50 or 1.7 mg/l carbaryl than controls up to 15 days exposure, but thereafter levels declined rapidly while in control fish they continued to rise with sexual maturation (Choudhury *et al.*, 1993). The authors suggested that this might have been due to an initial increase in GtH release which could have been confirmed had they been able to measure this hormone. It is interesting to note that had this experiment used an organochlorine known to be estrogenic and vitellogenin (which would have been stimulated by the increased estrogen) measured for only 15 days, the results might well have been attributed to the estrogenic activity of the pesticide. Such partial data clearly must be interpreted with some caution!

In the protogynous *Monopterus albus*, both *in vitro* testosterone production and its aromatisation to estradiol was lower in fish exposed to 9 mg/l malathion for four weeks than in controls and may be the cause for failure of exposed fish to sex-reverse (Singh, 1993). Plasma levels of these steroids were also lower in the exposed fish (Singh, 1989).

While the majority of pesticides fall into the categories of organochlorine, organophosphates or carbamates, there are other classes which have had little attention drawn to their toxicity. The imidazole fungicides, clotrimazole, prochloraz, imazalil and ketoconazole have inhibitory action on mammalian hepatic xenobiotic-metabolising cytochrome P450s (Laignelet *et al.*, 1989; Lewis *et al.*, 1989). Since cytochrome P450 enzymes also play an important role in gonadal steroidogenesis it is possible that they might affect also reproduction. Their action on the xenobiotic transforming enzymes of the liver may also inhibit detoxification of other pollutants. Within the ovary the cytochrome P450 dependent aromatase is essential for conversion of testosterone into estradiol and a recent study has shown that in trout this enzyme is inhibited by imidazole fungicides (Monod *et al.*, 1993). Aromatase activity of the partially purified enzyme, isolated by cell fractionation, was assayed by its conversion of [^{3}H]androstenedione to estradiol in the presence of the fungicides. With all four of the fungicides tested there was a dose dependent decrease in aromatase activity from 10^{-8} to 10^{-4} M concentration (Figure 7.3). Estradiol production by cultured follicles was similarly inhibited by prochloraz over this concentration range. Since other ovarian and testicular steroidal conversions also use cytochrome P450 enzymes these pollutants may

also act at other levels of steroid biosynthesis. This study, using a range of concentrations of a pollutant on a specific enzyme shows clearly how a xenobiotic can directly affect ovarian steroidogenesis and may provide a useful experimental model for the investigation of the mechanism of action of other pollutants. It suggests, furthermore, that any of the xenobiotics which affect P450 enzymes (see Chapter 9) can also affect gonadal steroidogenesis.

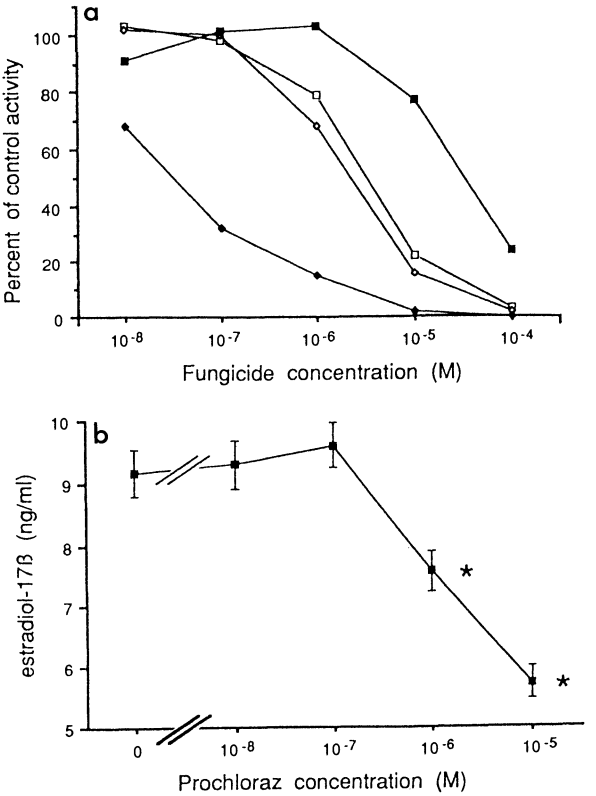


Figure 7.3. a) The effect of imidazole fungicides on ovarian microsomal aromatase in rainbow trout, and b) The effect of prochloraz on estradiol secretion by cultured follicles from a vitellogenic ovary of the rainbow trout. (–□– prochloraz, MW 377; –O– imazalil, MW 297; –■– ketoconazole, MW 531; –♦– clotrimazole;10^{-6}M = 0.38, 0.30, 0.53, and 0.35 mg/l respectively).(Reprinted from *Mar. Environ. Res.*, 35, Monod, G., de Mones, A. and Fostier, A., Inhibition of ovarian microsomal aromatase and follicular oestradiol secretion by imidazole fungicides in rainbow trout., pp 153-157, Copyright 1993 with kind permission of Elsevier Science Ltd, The Boulevard, Langford Lane, Kidlington 0X5 1GB, UK).

7.3.4 Effects of industrial pollutants

Plasma concentrations of androgens, estrogens and corticosteroids in both rainbow trout and carp (of unspecified sex) were decreased after four weeks

intraperitoneal treatment with the PCB Arochlor 1254 (Sivarajah *et al.*, 1978b). Since activity of the hepatic microsomal enzymes was also enhanced and was inversely related to plasma steroid levels it was suggested that the decrease was a result of increased hepatic metabolism. The importance of gonadal glucuronyl transferase was not, however, known at the time of this work so it is also possible that ovarian conjugation could also have been enhanced. It is also not clear why if hepatic cytochrome P450 was stimulated by the pollutants, the ovarian enzymes were not also stimulated, so that the two remained in balance. Information on which specific hepatic and ovarian enzymes were affected would be of considerable interest. The stimulation of cytochrome P450 by PCB is in marked contrast to its inhibition by imidazole fungicides (Monod *et al.*, 1993) (Section 7.3.2). The effect of pollutants on the P450 enzymes will be discussed in detail in Chapter 9. Decreased plasma estradiol and progesterone levels were also found in carp after intraperitoneal administration of PCBs (Yano and Matsuyama, 1986) and again attributed to increased hydroxylation by the hepatopancreas. Exposure of female Atlantic croaker (*Micropogonias undulatus*) to dietary Arochlor 1254 also decreased plasma testosterone but results with estradiol were ambiguous (Thomas, 1988, 1989, 1990a). *In vitro* synthesis of estradiol (per gram tissue) was, however, higher in exposed fish than controls while testosterone production was unaffected. When expressed as ng/ovary, estradiol synthesis did not differ between groups since control fish had larger ovaries. This emphasises one problem with interpreting ovarian synthesis since the proportions of the steroidogenic thecal and granulosa tissue may not increase at the same rate as that of the ovary as a whole, particularly during ovarian recrudescence when the oocyte is undergoing rapid growth by incorporating large amounts of vitellogenin.

Plasma estradiol levels in English sole (*Parophrys vetulus*) collected from a contaminated marine area near Seattle were negatively correlated with hepatic aryl hydrocarbon hydroxylase (AHH) activity and with levels of fluorescent aromatic compounds (FACs) in the bile, but positively related to ovarian PCB concentration (Johnson *et al.*, 1988; Collier *et al.*, 1992). While the AHH and FAC correlation is not unexpected, that of PCB contradicts the other studies cited. It is important to remember that the fish in such studies of polluted waters may well contain a range of other pollutants which might have very different effects and may themselves be correlated with PCB. In such experiments a relationship between one pollutant and an effect does not necessarily imply a cause and effect. The decreased plasma estradiol in sole treated with contaminated sediment extract from this area was attributed to physiological factors such as increased bile flow rather than to changes in estrogen metabolism (Stein *et al.*, 1991), although a later study showed that *in vitro* estrogen synthesis from endogenous precursor was also inhibited in the English sole as well as the rock sole (*Lepidopsetta bilineata*) and the

flathead sole (*Hippoglossoides elassodon*) (Johnson *et al.*, 1993). Since estradiol peaks during vitellogenesis before falling prior to spawning, the time of sampling may be of major importance in determining the relationship between pollution levels and estradiol and vitellogenin concentrations. Low estradiol alone could indicate either retarded development or that fish have progressed further to the prespawning stage. Further correlative evidence such as GSI or gonadal histology is required in such studies. Casillas *et al.* (1991) sampled English sole from polluted and unpolluted areas in Puget Sound during the expected period of vitellogenesis but in addition to measuring estradiol and vitellogenin also measured the spawning success in response to LHRH analogue. This showed that fish containing high hepatic PCB levels and aryl hydrocarbon hydroxylase activity as indicators of pollution had lower estradiol, lower vitellogenin and lower spawning success on LHRH treatment. This clearly indicated that ovarian development had been retarded in the most highly polluted areas. Fish from these areas also suffered from increased incidence of liver lesions. Direct evidence that PAHs, present in such habitats, can directly affect ovarian estrogen synthesis and hence ovarian development is provided by an *in vitro* study with salmon ovarian follicles (Afonso *et al.*, 1997). Both β-naphthoflavone and 20-methylcholanthrene selectively inhibited aromatase activity, but not testosterone synthesis at concentrations of around 0.1 mg/l. If brain aromatase was also affected then feedback regulation could also be affected in both sexes.

In addition to the PCBs, other halogenated hydrocarbons can affect the reproductive system. Polybrominated diphenylethers (PBDE) and polychlorinated naphthalenes (PCN) increased ovarian microsomal progesterone 6β-hydroxylase activity in three-spined sticklebacks but did not affect the 17-hydroxylase (Holm *et al.*, 1994). The relevance of this to reproductive success is not clear since 6β-hydroxylation is not a normal ovarian transformation in teleosts, although it may reflect changes in more vital P450 enzymes which may not be apparent in an artificial cell fraction designed as a bioindicator.

Plasma estradiol and testosterone concentrations were lower in lake whitefish (*Coregonus clupeaformis*) and white sucker (*Catastomus commersoni*) collected below kraft pulp mills than from reference sites (Gagnon *et al.*, 1994a; McMaster *et al.*, 1991; Munkittrick *et al.*, 1991). Testosterone showed a larger response to Ovaprim (GnRH + dopamine receptor antagonist) in exposed than unexposed fish while the response of estradiol did not differ between exposure groups. The lack of effect of bleached kraft mill effluent (BKME) exposure on the response of isolated follicles to GtH suggested that the differences in hormone levels may lie outside the gonad (Gagnon *et al.*, 1994b). In a larger survey throughout Canada, there was a consistent decrease in estradiol but not testosterone levels (Munkittrick *et al.*, 1994). A similar effect was found in males in which 11-

ketotestosterone rather than testosterone was consistently affected. The effect was also apparent in some mills using a non-chlorinated process. Ovarian responsiveness *in vitro* to human chorionic gonadotrophin (hCG) over a range of doses was significantly lower in fish taken from the BKME site than from a reference site (Van der Kraak *et al.*, 1992) (Figure 7.4). 17,20βP production was inhibited to a much greater extent than testosterone. Forskolin, an adenylate cyclase activator and mimic of GtH action which bypasses the GtH receptor, also gave a greater stimulation of steroids in ovarian follicles of fish taken from the reference site but the GtH receptor and adenylate cyclase which represent key elements of the GtH signal transduction cascade was clearly intact in the BKME exposed fish even if functioning at a lower level.

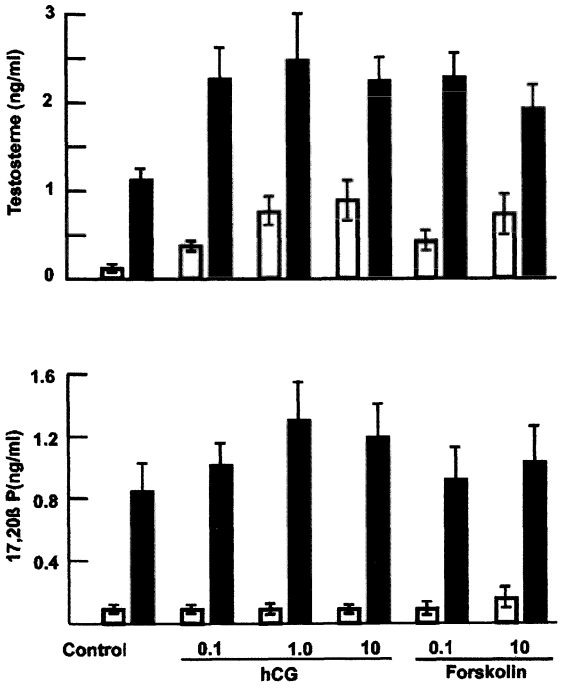


Figure 7.4. In vitro production of testosterone and 17,20βP by white sucker ovarian follicles in response to graded doses of hCG (0.1, 1 and 10 IU/ml) and forskolin (1 and 10μM). Values are mean ± S.E.M. for BKME (open bars) and reference (shaded bars) sites. Reproduced from Van der Kraak, G. J., Munkittrick, K. R., McMaster, M. E., Portt, C. B. and Chang, J. P. (1992). Exposure to bleached kraft pulp mill effluent disrupts the pituitary-gonadal axis of white sucker at multiple sites. *Toxicol. Appl. Pharmacol.* **115**, 224-233, with kind permission of Academic Press, San Diego and the author).

Carp held in two sewage lagoons in southern England did not differ in their estradiol levels from those held in a drinking water reservoir from October until April 1976 (Sivarajah *et al.*, 1979), although the levels

measured (200 pg/ml) indicates that these fish were sampled during the cold winter period of gonadal quiescence before vitellogenesis had begun. Sampling during May-June may have shown a more pronounced effect. This study took place before the widespread use of alkylphenolic detergents which have been implicated as environmental estrogens in such waters, and which might be expected to suppress endogenous estradiol by negative feedback to the pituitary (Purdom *et al.*, 1994). Plasma testosterone levels were significantly lower in female coho salmon in Lake Erie than in Lakes Ontario and Michigan but differences were much smaller than that of 11-ketotestosterone in males (Morrison *et al.*, 1985b). Estradiol did not differ between the lakes. While estradiol concentrations in female flounder (*Pleuronectes americanus*) were not affected by exposure to crude petroleum (Truscott *et al.*, 1983), benzo[*a*]pyrene, a petrochemical derivative decreased circulating estradiol and testosterone in Atlantic croaker (*Micropogonias undulatus*) although it did not affect *in vitro* synthesis of these steroids (Thomas, 1988, 1990a).

Plasma estradiol was decreased by cyanide in rainbow trout (Ruby *et al.*, 1993b). Both phenol and sulphide exposure for 45 days decreased the conversion of injected radiolabelled cholesterol into pregnenolone and progesterone by carp ovaries (Mukherjee *et al.*, 1991). Phenol at the concentration used (8.5 mg/l), however is quite acidic and while seven days at low pH did not affect steroid concentrations in female rainbow trout (Weiner *et al.*, 1986) estradiol was only half the control values in brook trout (*Salvelinus fontinalis*) after 147 days in an acidic environment (Mount *et al.*, 1988a).

7.4 Other biochemical indicators of ovarian activity

While steroidogenesis and egg numbers and size have been used as the major criteria of ovarian function, several other parameters have also been employed as indicators of abnormal functioning of the ovary in response to xenobiotics.

Use of ^{32}P uptake by the ovary as a bioassay for gonadotrophin has already been discussed (Chapter 5). It has also been used to test the response of the ovary itself to challenge by pituitary hormones. Ovaries from *Heteropneustes fossilis* exposed to safe concentrations of cythion (9 mg/l) or hexadrin (0.0006 mg/l) for 4 days had higher incorporation of ^{32}P than those from unexposed fish in response to LH or to homologous pituitary extract from unexposed fish. (Singh and Singh, 1980b). This stimulation might be analogous to the short term stimulation of estradiol observed in *Anabas testudineus* by Choudhury *et al.* (1993) (Section 7.3.2), but stimulation of ^{32}P uptake does not necessarily indicate enhanced steroidogenesis.

Another approach to effects on the biochemical function of the ovary is an examination of lipid metabolism. The ovary is an extremely lipid rich body and lipids play a part in its structural integrity, as an energy source, as constituents of the developing oocytes and as sources of precursor for steroidogenesis. Primary disturbance of lipid balance by xenobiotics could therefore be a cause of some of the other disruptions resulting from exposure to pollutants. Short-term exposure of the Indian catfish for 4 days to safe levels of Cythion (9 mg/l) or hexadrin (0.0006 mg/l) did not affect ovarian lipid content but increased cholesterol content (Singh and Singh, 1980c). As with steroidogenesis, such effects were more pronounced during the period of high ovarian activity in the vitellogenic phase (Singh and Singh, 1980d). Ovarian concentrations of mono-, di- and tri-glycerides and of free and esterified cholesterol in this species also changed in response to exposure to sublethal concentrations of malathion and γ-BHC, the pattern of response varying with stage of the reproductive cycle. These concentrations may, however, have reflected changes in hepatic production and their mobilisation to the ovary (Singh, 1992; Singh and Singh, 1992b). The partial reversal of these changes by simultaneous treatment with the pesticide and *Mystus* GtH lends support to the suggestion that the primary action of the pesticide is at the pituitary-hypothalamic axis (Singh and Singh, 1992c; Singh *et al.*, 1993), but unfortunately no direct comparison was made of lipid patterns in hypophysectomised fish.

Similar perturbations of ovarian lipid content were observed in *Clarias batrachus* with sublethal levels of γ-BHC and malathion (Lal and Singh, 1987), and in *Channa punctatus* with safe levels of the organophosphate fenitrothion and the carbamate carbofuran (Saxena *et al.*, 1986). Effluent from a vegetable oil factory also altered ovarian lipid balance in *Heteropneustes fossilis* with a decrease in total lipid, cholesterol, phospholipid and glyceride levels but an increase in free fatty acid (Kondal *et al.*, 1989).

7.5 Effects on ovulation and spawning

While there is an extensive literature on the effects of pollutants on ovarian recrudescence, the major phase of development of the ovary characterised by rapid increase in GSI as a result of incorporation of vitellogenin into the oocyte, there is relatively little on the final phases of oocyte maturation leading to germinal vesicle breakdown, ovulation and spawning. This is undoubtedly due to the time scale of the two events and the greater ease with which measurements can be taken during the much longer vitellogenic period. Oocyte final maturation can take place within 24 h and sampling an hour later or earlier can make major differences in hormonal

profile. The short duration of this phase of female reproduction makes it difficult to devise an exposure protocol in which the effect is solely due to perturbations in oocyte final maturation, ovulation and spawning. In many cases it must be suspected that the delay in spawning after a long-term exposure can actually be attributed to a delay in ovarian recrudescence as a result of inhibition of pituitary or ovarian function leading to decreased vitellogenesis. In too few cases has the ovary been examined histologically to confirm that the pollutant has blocked only maturation of fully vitellogenic oocytes within a fully developed ovary.

A delay in spawning can have particularly serious effects if sperm release is not similarly delayed. Such a delay may also result in a temporal mismatch between hatching and food availability leading to poor larval survival. A delay between ovulation and spawning may also lead to a deterioration in the quality of eggs held overlong within the body cavity before fertilisation.

7.5.1 Effects of heavy metals

Copper at 162 μg/l completely inhibited spawning in bluegill (*Lepomis macrochirus*) exposed for 22 months (Benoit, 1975) but no examination of the gonads was made to determine whether this was due to inhibited ovarian development or a block at final oocyte maturation. Adult zebrafish (*Danio rerio*) held in 5 mg/l zinc for a 9 day period during which the gametes were maturing showed a delay in spawning (Speranza *et al.*, 1977). Control fish spawned within 1 day of introducing mating pairs into the spawning tank, while treated fish required four and a half days. Since spawning did eventually occur, zinc would appear to act on the final maturational stage rather than on vitellogenesis, but no autopsy was done prior to introducing the mating pairs to confirm this. Zebrafish are known to have a well defined courtship behaviour involving pheromone release and endocrine responses in both sexes (Van den Hurk and Lambert, 1983; Van den Hurk *et al.*, 1987). It is therefore likely that zinc might act by inhibiting oocyte final maturation and release of pheromones by the female, the detection of these pheromones and the response by the male, or the neural and behavioural response to these factors. Zinc also inhibited reproduction in the viviparous guppy, *Poecilia reticulata*. Exposure to 0.17 mg/l for 70 days decreased the numbers of guppies that attained sexual maturity, while 0.61 mg/l caused fewer to give birth and delayed the time until birth of the first brood (Pierson, 1981).

7.5.2 Effects of pesticides

The first phase of the spawning process is migration of the germinal vesicle to the periphery of the oocyte and its subsequent breakdown (GVBD). This is initiated by the maturation inducing steroid, 17,20βP in many species,

released in response to a GtH surge. GtH or LH has often been used to induce ovulation in captive species in aquaculture. This action of LH is inhibited by some pesticides. Malathion (0.01 mg/l), phosdrin (0.000001 mg/l), gardona (0.0001 mg/l) and birlane (0.001 mg/l) decreased the *in vitro* LH induced GVBD of *Mystus vittatus* oocytes to 20-30% of control values (Haider and Upadhyay, 1986). Malathion and endosulfan also decreased the numbers of carp oocytes undergoing GVBD *in vitro* at concentrations of 0.050 and 0.00001 mg/l respectively (Haider and Inbaraj, 1988). At these concentrations the numbers undergoing GVBD were reduced from 41% in control incubations to 18 and 26% with malathion and endosulfan respectively. Unfortunately the authors of these studies did not determine the no-effect dose, but from their dose response curve this is likely to be less than 1/10 of their lowest dose. This suggests that this stage of oocyte development might be one of the most sensitive to pesticides and could provide a relatively simple *in vitro* test of pollutant toxicity. The results could be interpreted to mean that LH induced synthesis of the maturation inducing steroid was inhibited, or that the action of this steroid, in inducing GVBD was inhibited. Incubations with 17,20βP, the presumed maturation inducing steroid, in the presence of the pesticide would clarify this site of action. This is important when determining what may happen *in vivo* where pesticide concentrations in the theca and granulosa cells, in which the maturation inducing steroid is synthesised, may be different to that within the oocyte where GVBD actually occurs. The effective concentrations of the pesticides are considerably lower than those of a number of organochlorines found in eggs (see Chapter 3).

Inhibition of LH induced GVBD as demonstrated in the papers cited above would also be expected to affect LH induced ovulation *in vivo*. γ-BHC at 0.4, but not 0.04 mg/l significantly decreased the numbers of medaka ovulating in response to LH (Hirose, 1975). Natural oviposition in this species ceased after 11 days exposure to 0.001 mg/l, and after 4 days in 0.002 mg/l, of another organochlorine pesticide (chlordecone) (Curtis and Beyers, 1978). Spawning was delayed by about two weeks in bluegill (*Lepomis macrochirus*) exposed to 5 mg/l 2-4D (Cope *et al.*, 1970), but it is not clear whether this is a result of slower oocyte development or a delay in oocyte maturation. A wide range of organochlorine pesticides caused the viviparous mosquitofish (*Gambusia affinis*) to abort (Boyd, 1964) but few experimental details were provided.

7.5.3 Effects of industrial pollutants

Only a few industrial pollutants have been examined for their effects on oocyte maturation and ovulation. Spawning was delayed by one week in minnow *Phoxinus phoxinus* fed 20 mg/kg, and by 3 weeks with 2000 mg/kg of the PCB Clophen A50 (Bengtsson, 1980). White sucker from the effluent

of a bleached kraft mill failed to ovulate in response to intraperitoneal injection with a GnRH analogue, while all fish from a reference site ovulated within 6 h. Those from the BKME site had lower plasma 17,20βP, and unlike fish from the reference site, this hormone failed to respond to the GnRH analogue. Bleached kraft mill effluent also depressed *in vitro* 17,20βP synthesis in white sucker which indicates impaired ovulation. BKME therefore appears to act at multiple sites to inhibit spawning in white sucker (Van der Kraak *et al.*, 1992).

Acid water can also have serious consequences for spawning, eventually leading to extinction of some species, but the mechanism is less clear. In Ontario increased acid fallout has led to the extinction of lake trout (*Salvelinus namaycush*), walleye (*Stizostedion vitreum*), burbot (*Lota lota*) and smallmouth bass (*Micropterus dolomieui*) and the failure of northern pike (*Esox lucius*), rock bass (*Ambloplites rupestris*), pumpkinseed sunfish (*Lepomis gibbosus*), brown bullhead (*Ictalurus nebulosus*) and white suckers (*Catastomus commersoni*) to reproduce successfully (Beamish *et al.*, 1975; Beamish, 1976). The major factor was the inability of 65-75% of the females to release ova for fertilisation which was attributed to alterations in serum calcium resulting from the low pH. Different species had different sensitivities to pH, with reproduction ceasing in smallmouth bass, walleye and burbot at pH 6.0 to 5.5, lake trout, troutperch (*Percopsis omiscomaycus*) at pH 5.5-5.2, brown bullhead, white sucker and rock bass at pH 5.2 to 4.7 while lake herring (*Coregonus artedii*), yellow perch (*Perca flavescens*) and lake chub (*Couesius plumbeus*) spawned down to pH 4.7 to 4.5 (Beamish, 1976). Such data provides valuable information on the susceptibility of natural stocks to increasing acidity and on the best species to use for restocking lakes in which species loss has already occurred.

Ovulation was also delayed in brook trout (*Salvelinus fontinalis*) exposed to pH below 5 (Tam and Payson, 1986) and in perch and whitefish (*Coregonus watmanni*) in lakes with pH 4.75 in which abnormal plasma calcium was again apparent in females (Vuorinen *et al.*, 1990, 1992). The effect of acid water may also be aggravated by high aluminium content since spawning of perch was delayed most in waters in which low pH was associated with high aluminium levels (Rask *et al.*, 1990). Such a delay, together with the slower hatch of perch eggs in acid water (Peterson et al., 1980; Rask, 1983; see also Chapter 8), may result in hatching at a time when water temperature had exceeded the optimal for survival of fry leading to even lower survival.

7.6 Effects on egg numbers and viability

While xenobiotics clearly have effects on gonadal development and spawning, the important factor for the population as a whole is the effect on numbers of normal, healthy and fertile offspring. This comprises a number of inter-related factors. The numbers of eggs produced will clearly give an upper limit to the number of offspring that can be produced and their size, which is related to the amount of nutrient in the form of vitellogenin, will affect the survival of the offspring. There is a balance between egg numbers and their size, which is species dependent, such that salmonids generally produce fewer large eggs, while many cyprinids produce larger numbers of small eggs. Such factors relate to the time from spawning to hatch, so that salmonid eggs can hatch slowly since a large store of nutrient is available in the yolk, while small cyprinid eggs must hatch within a few days after which their nutrients are exhausted. A similar strategy confronts a species in which vitellogenin supply has been limited, either through dietary restriction or by pollution. It can produce the normal number of smaller eggs, or fewer eggs of normal size and such decisions will have been made in response to these factors during vitellogenesis. The mechanism by which such a choice is made remains unclear (Tyler and Sumpter, 1996).

Hatch rate and survival to first feeding will also be decreased by any genetic abnormality that has been caused to the embryo during either egg development, or through absorption of toxicants that have been laid down with the yolk and which will be taken in during embryonic development and incorporated into the larva itself. Once the larvae begin feeding after the yolk-sac stage they will incorporate no further burden of toxicant from maternal sources but will be more vulnerable to uptake from their own environment. Eggs may also take up toxicants via exchange with the surrounding water. Survival of larvae and their growth is not the only factor affecting population. In addition to effects on survival, the potential fertility of the offspring (F1 generation) might also be affected during larval development by pollutants in the yolk derived from their parent or taken up from the water. In this Chapter, only effects derived directly from parental sources are considered, while those arising from pollutant exposure from the external environment after spawning are discussed in detail in Chapter 8. The effects of pollutants on the numbers of eggs produced and their viability to hatch is discussed in this section while post hatch survival is considered in Section 7.7.

7.6.1 Effects of heavy metals

Nine-day exposure of *Danio rerio* to 5 mg/l zinc while the gametes were maturing decreased the numbers of eggs produced to 38% of that of the

unexposed fish and the percentage viability to 21% compared with 90% for controls (Speranza *et al.*, 1977). The survival rate to hatching decreased from 63% in controls to less than 1% in zinc treated fish. There was evidence of embryological abnormalities only in the exposed population. It has already been noted (Section 7.5.1) that the exposed fish delayed spawning for 3-4 days longer than controls and this may have caused the deterioration in egg quality. Information on the ovarian status prior to introducing breeding pairs would have been valuable in confirming this. When the zinc exposed fish were returned to fresh spring water for 9 days, the subsequent mating gave identical survival of eggs and fry to the unexposed fish. Similar effects were found in guppies (*Poecilia reticulata*) which had significantly fewer offspring after exposure for 156 days to 2.6 mg/l zinc (Bieniarz *et al.*, 1994).

There is some evidence that maternal factors may be involved in the increased copper tolerance of larvae from sites contaminated with copper and zinc than those from clean sites (Munkittrick and Dixon, 1988a, 1989). The response was apparent even in white sucker eggs that had not been exposed to high metal concentrations during fertilisation or incubation. The difference in tolerance, however, disappeared shortly after the completion of yolk absorption and was not detectable in older fish indicating that it was not due to the genetic selection of tolerance. Tolerance was highest after the onset of liver function which suggests that maternally transferred metals induce hepatic metallothionein synthesis by the larvae.

Copper at 0.033 g/l decreased the number of viable eggs spawned per female brook trout to approximately half of control values, but lower concentrations were without effect (McKim and Benoit, 1971). Hatchability decreased from 81% in controls to 26% in eggs from exposed fish, and 25% of this mortality occurred as the embryo tried to emerge from the egg. Egg yolk utilisation was greatly decreased and complete resorption of the yolk sac took 4 weeks longer than in controls. At a similar concentration (0.034 g/l) in a copper mining area in Michigan, hatching success of yellow perch eggs was not affected, but the duration of hatching was significantly longer compared to eggs from a nearby unpolluted lake (Ellenberger *et al.*, 1994). Fathead minnows also had fewer spawnings and produced fewer eggs per spawning after exposure to 0.037 mg/l copper during the spawning season (Pickering *et al.*, 1977). Copper exerted its effect only during the spawning period, since fish exposed for 6 months prior to spawning did not differ from those exposed only during spawning season. The disappearance of sauger (*Stizostedion canadense*) and the decline in the walleye (*Stizostedion vitreum*) population in this lake in the 1970s after a century of mining activity was attributed to a similar copper induced disruption of hatch, leading to lack of co-ordination with food availability.

Lead at 1.2 mg/l decreased the numbers of eggs present in the ovaries of spawning *Anabas testudineus* (Tulasi *et al.*, 1989). In guppies fed 500 to

1300 μg/g cadmium in their diet of midge larvae the cumulative numbers of fry produced decreased from 79 to 55% of the control values (Hatakeyama and Yasuno, 1987). Phenylmercury at 0.0002 mg/l also decreased both the numbers of eggs spawned by zebrafish and the percentage of these eggs that hatched (Kihlström *et al.*, 1971).

7.6.2 Effects of pesticides

Compared to the number of studies on the effects of pesticides during gonadal recrudescence, little attention has been paid to the number and viability of the eggs produced.

Mortality was significantly higher for eggs taken from fish in Lake Rerewhakaaitu than in four other New Zealand lakes (Hopkins *et al.*, 1969). Concentrations of DDT and its metabolites in muscle and ovary of fish from this lake and in their fry was over four times higher than in the other lakes. In 156 day feeding experiments with DDT, Macek (1968) showed that exposure of females resulted in lower survival of their offspring. Highest mortalities occurred at the sac-fry stage just before the fry were ready to feed which corresponds to the period at which yolk fat is most rapidly taken up. Surprisingly, exposure of males also affected survival of their fry which may suggest induction of some genetic damage. High mortality, abnormal gastrulation and a high incidence of vertebral deformity were found in winter flounder hatching from eggs which contained 1.1-3.7 mg/kg DDT (Smith and Cole, 1973).

Continuous exposure of sheepshead minnow (*Cyprinodon variegatus*) throughout their lifecycle to Kepone at 0.00078 mg/l significantly reduced the number of eggs spawned per day to 48% of that of controls (Goodman *et al.*, 1982). The fertility of the eggs was decreased from 95 to 85% and embryo survival from 96% to 68%. Lower concentrations of the pesticide were without effect. Temephos at 1 mg/l increased the number of abnormal parturitions while fenitrothion at 0.5 mg/l reduced egg production in the viviparous guppy (*Poecilia reticulata*). The long-term consequence of such exposure is clear since these effects were apparent 2 months after the termination of exposure even though these organophosphate pesticides are rapidly excreted from the body (Yasuno *et al.*, 1980). At 1 mg/l of fenitrothion all females aborted at an early stage of the embryo even two months after cessation of exposure.

While most studies have used a continuous exposure of the pesticide, the real-life situation in many rivers is that of a short pulse of exposure following spraying. Methoxychlor, for example, is used in 7.5 to 15 min pulses to control larval emergence of blackfly in several river systems in Canada. To emulate this, Holdway and Dixon (1986) exposed adult flagfish to a single dose of 1.8 mg/l of the pesticide for 2 h. Compared to unexposed fish, the

time to first guarding behaviour was delayed by 15 days, eggs were laid 10 days later and the total number of eggs laid decreased by 87%. Although the hatch rate was decreased by a third there was no significant increase in numbers of abnormal fry.

7.6.3 Effects of industrial pollutants

White sucker exposed to bleached kraft mill effluent compensated for the lower plasma estradiol levels, and presumably decreased vitellogenin production, by producing the same number of smaller eggs as fish of the same size from an unpolluted site (Munkittrick *et al.*, 1991; McMaster *et al.*, 1992). The exposed fish, however, also grew more slowly and for a given age were smaller than those from the reference site. Since the fecundity of fish usually increases with body size, the age specific fecundity of these fish was also lower than that of the control group. It also suggests that the smaller egg size and lower somatic growth may be due to a decreased ability of the fish to convert energy into somatic and reproductive tissue. Population dynamics of this species may therefore be affected by effluent in two ways. The smaller eggs may have a lower chance of survival, and fewer eggs will be produced during the life-span of the fish.

For eggs fertilised in the laboratory and kept in fresh water, fertilisation rate, time to hatch, hatching success and the percentage of deformities at hatch were not affected by parental exposure to BKME, but the larvae showed a slower growth rate than unexposed fish (McMaster *et al.*, 1992). This may be one of the causes of the smaller fish in exposed effluents. It is also important to note that sucker migrate to clean water to spawn so that although the eggs and larvae will not be directly exposed to effluent, the smaller fry may be more vulnerable to predation than the larger offspring of unexposed parents.

Decreased egg size and lower growth rate of larvae was also observed for brown trout (*Salmo trutta*) exposed to 0.5% BKME in the laboratory (Vuorinen and Vuorinen, 1985), but there was also a lower fertilisation rate of eggs from exposed females. Although less eggs were produced by the exposed fish, this was due predominantly to the smaller size of the fish as found with the Canadian study. The sac fry from these females were smaller and abnormal with a badly curved form (Figure 7.5), had retarded metabolism and could not absorb their yolk, and had a lower heart rate. There were also abnormalities in yolk sac structure with an abnormal network of blood vessels. Almost all fry from the 0.5% BKME exposed group died with symptoms (enlarged yolk sac with greenish-yellow fluid and white spots in the yolk) similar to blue sac disease.

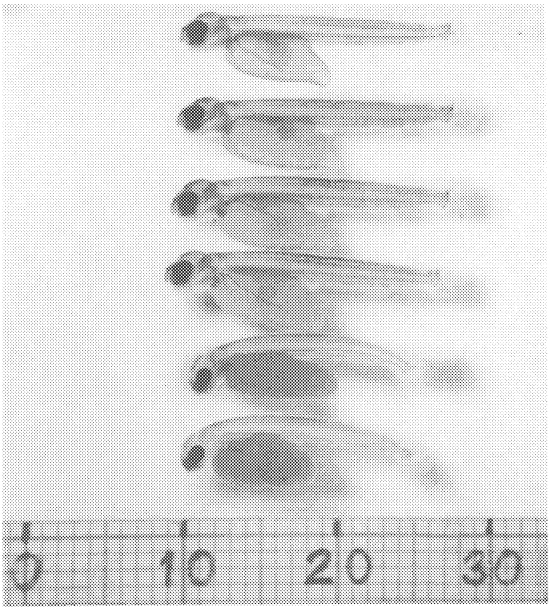


Figure 7.5. Brown trout sac fry of the same age incubated in freshwater. The two sac fry at the top are from a control female, the two in the middle are from 0.2% BKME exposed female and the two at the bottom from a 0.5% BKME exposed female. The scale is in millimetres. (Reproduced from Vuorinen, P. J. and Vuorinen, M. (1985). Effects of bleached kraft mill effluent on reproduction of brown trout (*Salmo trutta* L.) on a restricted diet. *Finnish Fish. Res.* **6**, 92-105, by kind permission of the Finnish Game and Fisheries Research Institute, Helsinki)

While it is often assumed that hatch rate and embryonic survival is attributable to incorporation of maternal pollutants into the yolk, it is also possible that pollutants might directly affect the chromosomes of the germ cells of either sex. Cumulative mortality of embryos and larvae from zebrafish parents, was greatest when both parents had been exposed to BKME, but exposure of the male parent alone caused higher mortality than that of the female alone (Figure 7.6) (Landner *et al.*, 1985).

Only 72% of the eggs from brook trout (*Salvelinus fontinalis*) held in 0.2 mg/l PCB for 21 days hatched compared to 92% of those from unexposed fish (Freeman and Idler, 1975). Although both parents were exposed, the high PCB content of the eggs (78 mg/kg) suggests that maternal transfer of pollutant via the egg was a major contributor to this low hatch rate. In eggs taken from wild-caught lake trout (*Salvelinus namaycush*) in Lake Michigan, hatchability was negatively correlated with total PCB content which ranged from 0.37 to 4.5 mg/kg, but there was a weaker correlation if TCCD (2,3,7,8-tetrachloro-*p*-dioxin) equivalents were used for the calculation, possibly since TCDD equivalents are calculated on activities in mammals and birds and may

not be applicable to fish (Mac and Schwartz, 1992). Spinal deformities and yolk-sac edema (blue-sac disease) were again found as observed above in embryos of BKME exposed parents. No relation, however, was found between levels of PCB (1.9-6.5 μg/g lipid), hexachlorobenzene (0.9-0.16) or DDT and its metabolites (0.71-1.3 μg/g lipid) in eggs of Atlantic salmon (*Salmo salar*) captured in New Brunswick and their hatch rate (Zitko and Saunders, 1979). Concentrations were, however, much lower than the 78 mg/kg of the brook trout eggs of Freeman and Idler cited above.

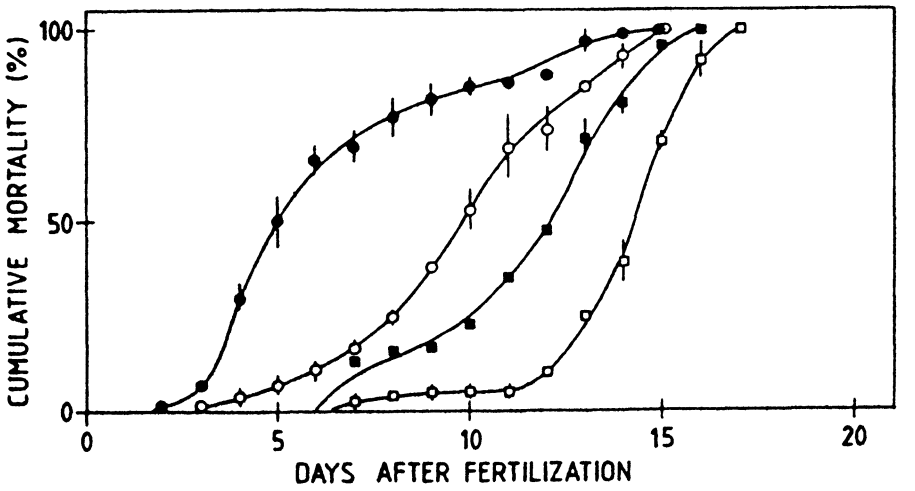


Figure 7.6. Cumulative mortality of embryos and larvae, reared in pure diluent after preexposure of parents for 4 weeks to 2% C+E stage bleach plant effluent. Both parents exposed (●), only male parents (○), only female parents (■) pre-exposed, and control (unexposed parents)(□). (Reproduced from Landner, L., Neilsson, A. H., Sörensen, L., Tärnholm, A. and Viktor, T. (1985). Short-term test for predicting the potential of xenobiotics to impair reproductive success in fish. *Ecotoxicol. Environ. Saf.* **9**, 282-293, with kind permission of Academic Press, San Diego and the author).

Redbreast sunfish (*Lepomis auratus*) sampled at four sites downstream from discharges had decreasing concentrations of PCBs and mercury. Fish taken from the site closest to the discharge had lower fecundity (eggs/g body weight) than those downstream and in the reference site (Adams *et al.*, 1992). This may have been due to the smaller size of the fish, but the decreased amount of functional liver tissue suggests that vitellogenesis might have been impaired. There was a correlation between early embryonic mortality and the concentration of PCB and DDT in eggs of Lake Geneva charr which is probably indicative of the general level of a range of industrial toxicants including heavy metals in the lake (Monod, 1985).

In laboratory studies, injection of the planar PCB 3,3',4,4'-tetrachlorobiphenyl (TCB) at 3 week intervals prior to the spawning season

led to decreased larval survival at doses lower than that which caused decreased ovarian growth, oocyte maturation, circulating reproductive steroids and vitellogenin in white perch (*Morone americana*) (Monosson *et al.*, 1994). TCB did not affect hatch rate, body length of larvae at hatching or larval growth up to 5 days after hatching, but there was a dose dependent decrease in survival at the end of yolk-sac absorption (day 7). Larvae from females treated with 1 or 5 mg/kg did not survive this stage and the survival rate was related to ovarian TCB content with low survival from any parent with more than 0.5 mg/kg ovarian weight TCB. Hatchability was greatly decreased in minnows (*Phoxinus phoxinus*) which had body burdens of 170 mg/kg PCB two months before spawning as a result of feeding with Clophen A50, but was unaffected by concentrations of 15 mg/kg (Bengtsson, 1980) which suggests a greater tolerance than in perch to PCB. Levels of PCBs which do not affect hatch rate may still affect the offspring. Larvae produced by winter flounder taken from areas off Rhode Island polluted by PCBs showed an inverse relationship between PCB content of the eggs and length and weight at hatch (Black *et al.*, 1988). Although in the laboratory the final size of all larvae was not significantly different, such differences in size at hatch may have serious consequences for the wild population since small larvae are more easily predated and have greater difficulty in capturing food.

2,3,7,8-Tetrachloro-*p*-dioxin is extremely potent as a disruptor of reproduction. A single dose of 5 ng (1.7-2 μg/kg body weight) given via the diet to zebrafish (*Danio rerio*) decreased the numbers of eggs and spawnings and after 1-2 spawning cycles no further spawning occurred (Wannemacher *et al.*, 1992). None of the offspring survived and mortality was preceded by severe thoracal edema and malformations of the notochord. Ovarian histology indicated a failure of oocytes to progress past Stage III and undergo vitellogenesis. Although the body weight of exposed fish also decreased this became apparent later than the effect on reproduction. 1 ng had no effect while 20 ng resulted in symptoms of severe toxicity. Similar developmental toxicity and symptoms of blue-sac disease are also observed on exposure of eggs to TCCD (see Chapter 8). A valuable review of reproductive and developmental toxicities of TCCDs in fish and other vertebrates has been provided by Peterson *et al.* (1993).

Survival to hatch of eggs taken from coho salmon in Lake Erie was only 24% compared to 79-86% in eggs from Lake Ontario and Lake Michigan stocks and was attributed to decreased fertilisation success (Morrison *et al.*, 1985b). There was no difference in egg size, embryo size or development time to hatch between the Lakes but post-hatch mortality up to 3 weeks and alevin deformity was significantly higher in the Lake Erie stocks (Figure 7.7). There was no difference in egg or embryo size between groups or of abnormal yolk sac as noted in the studies cited above. Together with the fact that Lake Erie salmon eggs had lower organochlorine and similar heavy metal content to the

other Great Lakes (Morrison *et al.*, 1985a) this suggests that the low survival cannot be ascribed to these pollutants alone.

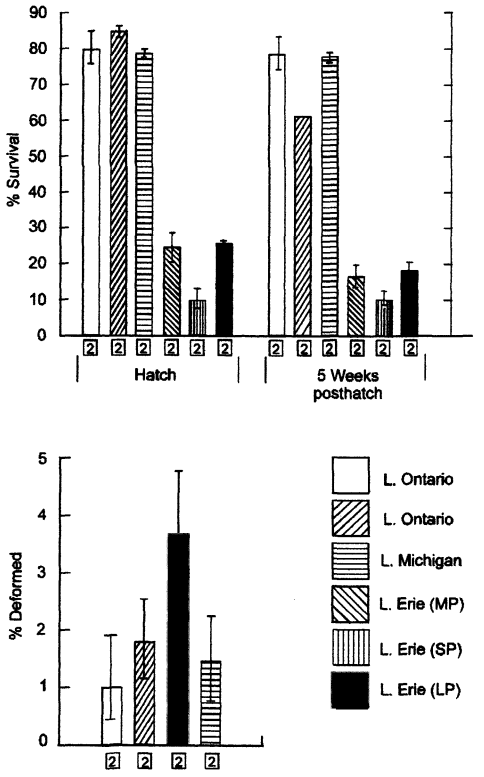


Figure 7.7. A) Survival (% of presumptive fertilised and water-hardened eggs) to hatch and 5 weeks post-hatch of coho salmon eggs collected from Lakes Ontario, Lake Erie and Lake Michigan stocks. Fish were collected from two sites in Lake Ontario. Egg pools of the Lake Erie stock were made from smaller females (<2 kg) (SP), larger females (>2 kg) (LP) and an appropriate sample of mixed size females (MP). B) Incidence of deformed coho salmon alevins (% of successful hatchlings) in groups reared from Lake Ontario, Lake Erie and Lake Michigan stocks. (Redrawn from Morrison, P. F., Leatherland, J. F. and Sonstegard, R. A. (1985). Comparative fecundity and egg survival in coho salmon (*Oncorhynchus kisutch* Walbaum) from Lakes Ontario, Erie and Michigan. *Can. J. Zool.* **63**, 1096-1100 with kind permission of NRC Research Press, Ottawa).

Female white croaker (*Genyonemus lineatus*) taken from a polluted area off Los Angeles which had been used as a dumping ground and the repository of domestic and industrial wastes generated by eight million people, could be induced to spawn only 64% as many eggs per female as those from an uncontaminated reference site and only 80% were fertilised compared to 93% of the control population (Cross and Hose, 1988; Hose *et al.*, 1989). The extremely high ovarian concentrations of DDT (2.1 mg/kg) and PCB (1.7

mg/kg) may well reflect similar toxicant burdens of numerous other xenobiotics and is the most likely explanation for the decline of this and other species in the coastal regions of southern California. In a similar study on the starry flounder, *Platichthys stellatus* captured in San Francisco Bay and induced to spawn in the laboratory there was a significant negative correlation between maternal aryl hydrocarbon hydroxylase (AHH) and percentage of viable eggs, fertilisation rate and embryological success (Spies and Rice, 1988). Embryological success was also negatively correlated with PCB concentration in the eggs. Both AHH and PCB content are probably more indicative of general pollutant levels within the fish, rather than demonstrating a direct causal relationship of PAHs and PCBs. The hatching success of eggs of a number of commercially important species in the Baltic and North Seas was correlated with the levels of PCB residues found in the eggs. Viable hatch was significantly decreased at egg concentrations greater than 120 μg/kg PCB, but organochlorine pesticide residues may also contribute (Von Westernhagen *et al.*, 1981, 1987). In artificially inseminated feral North Sea whiting (*Merlangus merlangus*) the threshold values for ovarian contamination above which hatch viability decreased were set at >0.2 mg/kg for total PCBs, > 0.02 mg/kg for total DDTs and > 0.01 mg/kg for dieldrin (Von Westernhagen *et al.*, 1989).

The limitations of using simplistic measures as bioindicators in a wild population is well illustrated by the poor correlation between ovarian and hepatic PCB concentrations, biliary levels of fluorescent aromatic compounds or hepatic aryl hydrocarbon hydroxylase activities with egg and larval viabilities in English sole (*Parophrys vetulus*) in marine areas off Washington State (Collier *et al.*, 1992). In another study at four sites in this area, production of more smaller eggs was correlated with elevated hepatic and ovarian PCB levels, while elevated biliary FACs were associated with fewer larger eggs (Johnson *et al.*, 1996). Clearly if two pollutants co-existing in the same area have such opposing effects their proportions, as well as the presence of other pollutants of unknown activity, will lead to a very complex situation. Laboratory studies under controlled conditions and field studies in true-life complex environments must be considered together when assessing the relevance of biochemical indicators on fertility and fecundity.

PAHs comprise a number of polycyclic hydrocarbons, of which anthracene and phenanthrene are often used in laboratory models. These compounds are generally believed to become more toxic after exposure to solar ultraviolet radiation, but in fathead minnows anthracene causes reproductive impairment without such exposure (Hall and Oris, 1991). Parents were exposed, in the absence of UV radiation to 0, 0.006 or 0.012 mg/l anthracene for 6 weeks, followed by 3 weeks at 0, 0.012 or 0.02 mg/l. The numbers of eggs produced by anthracene exposed fish was approximately half that of unexposed fish throughout the exposure period. Ultraviolet

radiation, however, acts on the maternally transferred anthracene in the egg to cause decreased hatch rate and larval deformity. Hatching success for eggs from parents initially exposed to 0.012 mg/l and then exposed to UV decreased from control values of 95% to 77% and was zero when the concentration was increased to 0.02 mg/l. After the collected eggs had been placed in clean water, 96 h survivorship of larvae from parents exposed to 0.012 mg/l was unaffected by anthracene in the absence of UV, but decreased from control values of 98% to 85% in eggs which had been exposed to UV. Yolk sac abnormalities, haemorrhage and oedema were present in 8-20% of this latter group. In the high exposure group in which no eggs hatched, the concentration of maternally transferred anthracene was 19 μg/g and the no-effect concentration was estimated to be between 4 and 8 μg/g. Chloroaniline decreased egg release in zebrafish at only 0.04 mg/l (Bresch *et al.*, 1990). This is 1/1000 of the LC_{50} and the authors concluded that the value of 1/100 of the LC_{50} proposed as a safe concentration is too high.

Although there has been some recent concern as to the estrogenic activity of the phthalates used as plasticizers, there have been very few studies of its activity in fish. In the self-fertilising hermaphrodite, *Rivulus marmoratus* 2 mg/l di-n-butylphthalate decreased the numbers of fertile eggs produced and decreased the viability of the embryos (Davis, 1988). 1 mg/l of the phthalate was without significant effect, but there were more skeletal abnormalities in the progeny at both concentrations than in controls. Phthalate appears from this study to cause deformities of the embryo at lower concentrations than that which affects fertility. Although such deformities may arise from chromosomal damage in either male or female gametes, in *Rivulus* both gametes receive identical exposure since they are within the same fish which thus provides an interesting experimental model. Although there is much concern about alkylphenoxy detergents there have been only preliminary studies of their effects on female fertility. Exposure of mature trout to 0.001 or 0.01 mg/l nonylphenol during and before spawning did not affect the fertilisation of eggs, but there was a significantly lower hatching rate (Schwaiger *et al.*, 1997). This may be related to the estrogenic activity of nonylphenol since parental exposure of zebra danios to only 5 ng/l ethynylestradiol for 12 days prior to spawning increased embryo mortality as a result of cytostatic disruption during the early gastrula period (Nash *et al.*, 1997; Kime and Nash, 1998), and estradiol itself has long been known to have anti-mitotic activity in fish eggs (Huffman *et al.*, 1957; Jones *et al.*, 1960). Linear alcoholethoxylates, which unlike the alkylphenolics, presumably have no estrogenic potential since they do not contain a phenolic group also decreased the numbers of clutches laid, the numbers of eggs and the numbers of surviving larvae in fathead minnows at levels as low as 0.08 mg/l (Kline *et al.*, 1996), a level comparable to that of alkylphenolics which results in decreased testicular growth. Although the sample size in this

experiment did not permit statistical analysis, the results suggest that simply substituting linear ethoxylates for the phenolics may not decrease the hazards to fertility. Furthermore, they suggest that not all of the endocrine disrupting effects of the alkylphenolics can be attributed to their action as estrogen mimics. More detailed comparison of the effect of two classes of detergent on reproduction would be of considerable practical importance.

Although asbestos is a common cause of mesothelioma in mammals exposed to the dust, its effects on fish has received little attention which is surprising since it was present in a high proportion of the water supplies of the United States in the late 1970s. Eighteen percent of the 406 water supplies contained chrysotile asbestos at concentrations greater than 10^6 fibres per litre, a concentration which decreased the spawning frequency and number of viable eggs in the Japanese medaka (*Oryzias latipes*) (Belanger *et al.*, 1990). Growth of larval and juvenile fish was also impaired.

Industrialisation of the agricultural process to produce higher crop yields has not been limited to widespread use of pesticides. Increasing use of both natural and artificial mineral fertilisers high in nitrate has led to increased concentrations of these salts in waters in agricultural areas. These are converted by micro-organisms like bacteria and fungi into nitrosamines which are present in high concentration in eutrophied water and can affect fertility. Eggs from carp, for example, held for four seasons in eutrophied culture ponds had a hatch rate in freshwater of only one-quarter of that of eggs from fish held in normal ponds (Bieniarz *et al.*, 1996a, b). This is of considerable practical importance since carp culture ponds are frequently fertilised in the belief that it will increase yield. Eutrophication of natural lakes can also lead to changes in the trophic structure of the whole ecosystem, and in Lake Sjamozero in Karelia this has led to increased mortality of coregonid eggs as a result of increased predation by benthic invertebrates (Sterligova *et al.*, 1988). The possible combination of decreased hatch rate and increased predation could account for major species loss in some areas. Increased maximum total phosphorus levels in Lake Constance was, however, associated with increased fecundity of the whitefish (*Coregonus lavaretus*) after a 2 year time lag and may act via the food chain to alter nutritional status (Hartmann and Quoss, 1993). The effects of eutrophication in wild populations is clearly complex and the exact levels may determine whether they are harmful or beneficial.

Fish in rural areas may also be affected by acid waters resulting from fossil fuel combustion in neighbouring industrial regions. Brook trout, *Salvelinus fontinalis*, in acid water produced fewer eggs but this was due mainly to the smaller size of the fish in acid water (Tam and Payson, 1986; Mount *et al.*, 1988a, b) and the viability of the eggs was high. In another salmonid, *Oncorhynchus mykiss*, however, the progeny of females exposed to pH 4.5 to 5.5 had a lower survival through 7 days of development, hatching

and yolk-sac absorption than control females held at pH 6.5-7.1, but the similarity of offspring survival from both exposed males and exposed females suggested that the viability of gametes from both sexes was affected (Weiner *et al.*, 1986). In flagfish, *Jordanella floridae,* the numbers of eggs spawned in fish held at pH 6.0 was only half that of controls at pH 6.8 and decreased to 13% of control values at pH 5.0 as a result of both fewer spawnings and fewer eggs per spawn. No eggs were produced at pH 4.5. Egg size, and the percentage of fertile eggs were both progressively decreased with decrease in pH. Hatch rate and egg fertility were also decreased, but since fish were spawned, and eggs raised in acid water it is not clear whether this is a maternal effect or due to embryonic exposure. A similar confusion arises in studies of perch, *Perca fluviatilis*, in two lakes of pH 4.4 in Finland in which eggs showed high mortality (Rask, 1992), and in fathead minnows held at pH 5 in which the eggs did not progress past the eyed stage (McCormick *et al.*, 1989).

7.7 Effects of parental exposure on viability of offspring

The majority of the studies on the effects of parental exposure on the offspring have continued only to hatch or to first feeding and very few have examined the effects on later stages of life. Clearly if there are gross deformities in the embryo, there will be high mortality in the very early stages of development. Conversely, relatively small deformities will allow longer survival and may only become apparent at later life stages. These may become apparent in laboratory studies, but in the wild may lead to inability to catch prey, or to increased vulnerability to predation. Mortality rates in the laboratory may therefore be a very conservative estimate of natural survivability.

Heavy mortality of lake trout fry was observed in eggs taken from lakes in the New York area following heavy use of DDT for insect control between 1951 and 1957. The losses were characterised by distended air bladder and air in the intestinal tract, with fry floating upside down and eventually sinking and dying (Burdick *et al.*, 1964). A close relationship was found between mortality and the DDT content of the fry, with all eggs having a DDT content greater than 3 mg/kg showing the syndrome. To confirm this relationship, fry hatched from eggs of brown trout and brook trout fed with a diet of 3.4 mg/kg DDT were compared with those from fish fed DDT free diet. Eggs from DDT exposed fish had significantly greater mortality up to 40 days after first feeding than eggs from unexposed parents (Burdick *et al.*, 1972). Highest mortality occurred after feeding began, but the symptoms associated with this were not compared to those of wild exposed fish. Major deformities were found in this study, but it is not known whether lower doses of DDT, or other

pollutants can cause malfunctions which affect the survival of fry through to sexual maturation. Fry from Baltic salmon which are exposed to a wide range of toxicants, including dioxins and PCBs, suffered from high mortality characterised by discoloured skin, haemorrhages and white precipitates in the yolk sac (Norrgren *et al.*, 1993). This syndrome, designated M74, was also associated with induction of hepatic cytochrome P450 enzymes in both the yolk-sac fry suffering from M74 and in the adult feral females which produced the affected fry. The liver also showed morphological changes associated with induction of these enzymes. This suggested that the fry were affected by xenobiotics passed on from the parent through the yolk, but there were no differences in heavy metals or a range of PCDDs and PCDFs in the roes of females which produced normal and M74 offspring, suggesting that other chemicals might be implicated. Fry mortality from this syndrome was first observed in 1974 and by 1992 accounted for the mortality of 75% of the larvae hatched from feral parents in a fish hatchery.

Of particular concern is whether parental exposure may adversely affect the fertility of the offspring of exposed parents, the F1 generation. Such intergenerational effects on fertility would be particularly difficult to determine in long-lived species, but may be especially relevant to recent mammalian studies which suggest that a decrease in human male fertility and an increase in male genital abnormalities may result from exposure to environmental estrogens during foetal life (Sharpe and Skakkebaek, 1993). Fish may provide a suitable experimental model for similar studies on the effects of such exposure on females since fecundity of fish is so readily measurable.

7.8 Conclusions

Morphological damage is induced in female gonads by a wide range of pollutants, most of which cause atresia of mature oocytes and a failure of immature oocytes to incorporate vitellogenin. In general this is consistent with inhibition of estrogen synthesis and resultant inhibition of hepatic vitellogenesis. While there have been numerous studies on the effects of pollutants, these have covered a wide range of chemicals, exposure levels and times, and species. It is thus almost impossible to draw any conclusion about relative toxicities of different pesticides and make any recommendations on which is the safest. Organochlorines in general are clearly much more toxic (at levels as low as 0.00001 mg/l; 0.01 ppb) than either the majority of organophosphates or carbamates. Such information, however, is of little use unless put into the context of their relative toxicities to the target pest, and the amounts in which they are actually applied to the field. This information is not available in any of the cited papers. There has also been little attempt to

use a range of doses to determine the "no effect" level in many cases, and comments that pesticide A has a greater effect on the ovary than pesticide B are quite meaningless when only a single arbitrary concentration of each has been used and relative toxicities to pests or lethal levels to fish are not given. In too many cases the suspicion remains that the main aim is to get a publishable result rather than to produce really useful information on either the basic science of mechanisms of action or of use in minimising environmental impact and maintaining fish stocks.

Failure of ovarian estrogen synthesis also provides an explanation for many of the examples where exposed fish produce fewer eggs but it is often not clear whether this is due to primary failure of ovarian steroidogenesis, or to a secondary effect originating from inhibition of hypothalamic or pituitary function. Egg numbers are usually dependent on the size of the female, and since exposure to xenobiotics often inhibits growth of the exposed fish, decreased egg production may reflect effects on growth rather than direct effects on the reproductive system. It is therefore important in such studies to compare body weights of fish in the different experimental groups. Egg size and their number are also interdependent. A failure to utilise energy reserves, as reflected in poor growth, or a failure to produce sufficient vitellogenin, resulting from decreased estrogen synthesis, may therefore become manifest in either decreased egg numbers or decreased egg size.

Delayed ovulation and spawning can also often be attributed to the slower ovarian development, but in many of the experiments cited the ovaries have not been examined before the spawning period to determine whether they did in fact differ in developmental stage. *In vitro* oocyte final maturation is extremely sensitive to pollutants and it is therefore possible that this might provide a sensitive assay of pollutant toxicity. Effects of pollution at this level may well contribute to spawning failure. Hatch rates which are frequently affected by pollutants could result from either failure of fertilisation, or from embryonic malfunction. Delayed spawning may result from a temporary inhibition of oocyte final maturation, possibly caused by diminished behavioural stimuli. Egg quality may also deteriorate as a result of a delay in spawning or from perturbations during vitellogenesis which result in poor quality yolk. Symptoms frequently mentioned are those characteristic of blue-sac disease which may be caused by abnormal yolk structure and could reflect errors during vitellogenesis.. High mortality at the hatch stage is frequently observed.

Many of the effects observed are clearly multifactorial, but studies are urgently required to clarify which stages are the most sensitive to disturbance by xenobiotics and to understand the underlying mechanisms of such disturbances. Intergenerational effects whereby fertility of the offspring may be affected by exposure of the parents to pollutants is an area urgently needing investigation.

Chapter 8

Disruption in Eggs, Embryos, Larvae and Juvenile Fish.

8.1 Introduction

The early life stages of fish have for a long time been considered to be the most sensitive stage to pollution and have therefore frequently been used as the basis of toxicity tests. Such tests have, however, usually been carried out on a similar basis to adult toxicity tests such as the LD_{50}. Since the realisation that very low levels of pollutants might disrupt endocrine processes at levels well below those which cause mortality, it has become clear that simple tests on eggs, embryos or larvae which use survival as an endpoint are no longer sufficient. Furthermore, it is no longer so clear whether early life stages are in fact more sensitive to pollutants if all effects are considered, rather than simple toxicity end-points. A major difficulty is in defining the lowest observed adverse effect concentration (LOAEC), since this is dependent upon what end point is observed. The assumption is often made that non-specific cellular toxicity will be similar in both adults and early-life stages, but this does not take into account the fact that endocrine disruption may occur at levels much lower than that which is cytotoxic. If larval growth is taken as an endpoint, then some information is needed as to whether this is a result of decreased feeding, food conversion or oxygen metabolism, or of decreased production of thyroid, interrenal or growth hormones. If malformed embryos are taken as the endpoint then it is important to know whether this is due to general genetic damage, or whether a specific developmental stage, possibly under hormonal control, is affected. Clearly embryo-larval exposure tests, especially in small rapidly maturing species such as zebrafish (*Danio rerio*) and fathead minnows (*Pimephales promelas*), are simple, relatively

inexpensive and well suited to screening large numbers of potentially harmful chemicals (Kristensen, 1994; Meinelt and Staaks, 1994), but their ability to provide data on potential endocrine disruptors has, so far, been largely unexplored.

It is necessary to determine whether exposure at this stage to pollutants, at concentrations below those which cause mortality, affect developmental processes and if so how the concentrations needed to cause an effect relate to those that cause disruption in adults. In particular it is important to determine whether there are effects of early life exposure which only become apparent in adult fish. This includes not only errors in sexual differentiation and function, but changes in key enzymes in the liver and other tissues which would affect circulating hormone levels. The enzymes involved in such metabolism and deactivation are the same as those, which in adults, are affected by a wide range of pollutants (Chapters 6, 7, and 9).

Unfertilised fish eggs contain numerous maternally contributed hormones, but their role in the development of embryonic and juvenile fish is not clear. These steroids are metabolised by the embryo to reduced and conjugated products which are excreted (Yeoh *et al.*, 1996a, b), and any changes in such metabolism could lead to a disturbance of the hormonal milieu under which the embryo develops resulting in abnormalities or changes in sexual differentiation.

In mammals, sexual differentiation occurs in the embryo in response to secretions of hormones from the testis, while during the perinatal period testicular testosterone is aromatized to estradiol by the brain and then imprints behavioural and enzymatic patterns which only become apparent at puberty (Von Saal *et al.*, 1992). Such effects include feedback responsiveness of the pituitary, courtship behaviour and hepatic enzyme activity which in turn can affect interrenal activity. In mammals these pre- and perinatal testosterone secretions only occur in males as a result of the Y-chromosome which causes testicular development, so that only the testis can respond correctly to maternal hCG stimulation. Inhibition of such testicular secretions leads to the default, female, sexual development. Clearly if pollutants inhibit these secretions there will be feminisation of males, or if females are exposed to anthropogenic androgens or estrogens they will become masculinised. In birds the male is the default pattern and hormonal secretion from the heterozygous female are necessary to establish female differentiation. Disruption of normal differentiation and development in mammals and birds as a result of consumption of contaminated fish is discussed in more detail in Chapter 11.

In fish the genetic basis for sexual differentiation is less clear, but the need to produce monosex stocks for aquaculture has shown that sexual determinism can be altered by exposure to androgens or estrogens during early life stages (Yamamoto, 1969). Similarly exposure of natural fish stocks to anthropogenic chemicals with androgenic or estrogenic activity may result

in varying degrees of abnormal sexual differentiation or function. Endocrine disruption in early life stages is not, however, restricted to simple abnormalities of the reproductive system. The sex steroids, thyroid hormones and a range of growth factors are involved in normal development and disruption of the balance of any of these can lead to a low hatch rate or abnormal larvae. The evidence for such effects so far is, however, sparse since most studies have simply recorded the toxic end-points, rather than examining mechanisms. In many cases little is known of the endocrine and paracrine factors which affect development. As a result of the wide use of early life stages for toxicity testing there is a very extensive literature on the effects of pollution on early development. It is neither possible nor desirable to cover this literature in detail, but a selection of the data is given in the following sections so that the general effects of pollution on development can be seen. Those few references in which a clear disruption of endocrine function is shown to be the cause of errors in larval development are discussed in greater detail. It is rather more debatable whether simple developmental abnormalities resulting from exposure of parents, eggs or larvae to anthropogenic pollutants should be classed as endocrine disruption. The definition of Kavlock *et al* (1996) (Chapter 1) comprises " natural hormones in the body responsible for ..... the regulation of developmental processes", but it is not yet clear exactly what role the natural steroid and thyroid hormones play in normal development and what might be the consequences of disruption of their synthesis and action. If, however, the broadest definition of endocrinology is taken, which encompasses a wide array of inter- and intracellular signalling processes, then almost all examples of xenobiotic induced developmental abnormalities will be included within the definitions of endocrine disruptors (Chapter 1) since the correct cellular organisation during larval or embryonic development is highly dependent upon such signals.

This Chapter includes only the effects of exposure of eggs, larvae and juveniles to the pollutants after spawning, since effects resulting from maternal transmission are discussed in Chapter 7. In many cases the effects are the same.

8.2 Exposure of eggs and embryos

8.2.1 Hatchability, abnormalities and survival.

A standard toxicity test will simply measure the number of eggs that hatch, or that survive until adulthood. There is frequently little attempt to understand the cause of mortality, or to detail developmental abnormalities in those that fail to survive to reproductive age. In many respects the effects of

exposure of eggs parallels that of eggs from exposed parents in which the contaminant is passed on in the egg, but frequently the period of exposure, while the egg remains permeable is much shorter than the time during which pollutants can bioaccumulate from the mother. In both cases, large slow hatching eggs, such as those of salmonids, may be particularly vulnerable.

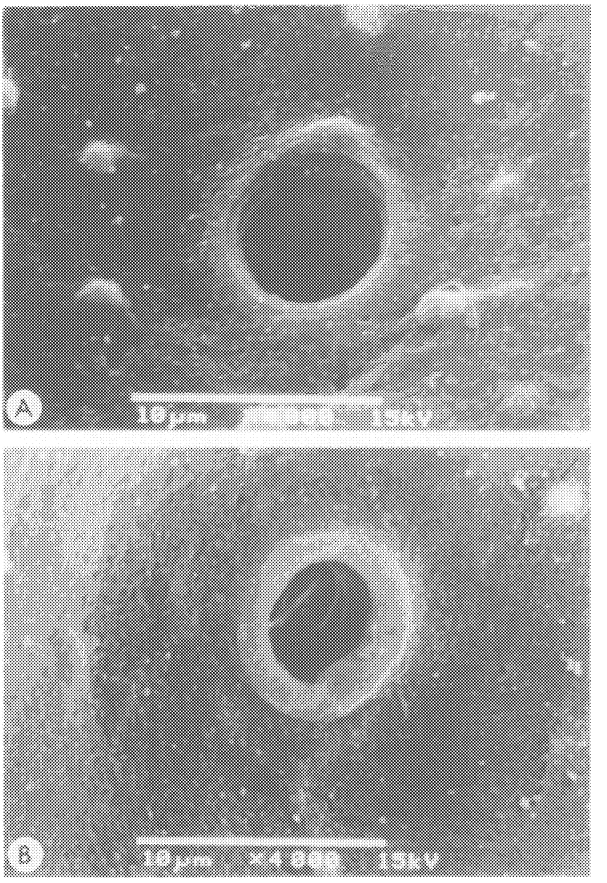


Figure 8.1. Scanning electron micrograph of the micropyle of *Fundulus heteroclitus* egg. A) Control, and B) exposed to 1 mg/l mercuric chloride (Reprinted from Khan, A. T. and Weis, J. S. (1993). Differential effects of organic and inorganic mercury on the micropyle of the eggs of *Fundulus heteroclitus. Environ. Biol. Fish.* **37**, 323-327, Fig. 1 with kind permission of Kluwer Academic Publishers and the author).

Heavy metals, not surprisingly, are toxic to eggs and lead to decreased hatch rate. It is important, however, to differentiate between effects that prevent fertilisation and those that inhibit development of the fertilised egg. In eggs of *Fundulus heteroclitus*, mercury decreased fertilisation by blocking the micropyle and preventing entry of the sperm (Khan and Weis, 1993).

Scanning electron microscopy (Figure 8.1) showed that there were differences in the effects of methyl mercury, which caused blockage by secretion of material through the micropyle, and mercuric chloride which caused a swelling of the walls of the micropyle. The sensitivity to mercury differed between populations taken from polluted and clean habitats, suggesting that some adaptation can occur (Khan and Weis, 1987c, 1993). Methylmercury also increased the percentage of abnormal embryos from eggs exposed to 1 mg/l for 20 min prior to fertilisation (Khan and Weis, 1987c), but the effect was greater in eggs taken from fish in clean water than from a heavily polluted site. At the longer exposure time of 16 days from hatch medaka eggs showed decreased hatch rate and abnormalities with only 15µg/l mercuric chloride (Heisinger and Green, 1975).

Although it might be assumed that eggs become less permeable, and therefore less susceptible, to pollutants after the chorionic hardening which follows fertilisation, exposure of sheepshead minnow eggs to 0.45 mg/l cadmium after hardening delayed hatching and inhibited growth. This was, however, due to binding of cadmium to the chorion rather than direct uptake, and resulted in interference with the calcium uptake which is essential for normal development (Meteyer *et al.*, 1988). A time course study showed that this was more likely to be due to irreversible incapacitation of the calcium pump rather than direct calcium-cadmium competition for binding sites on the chorion. No effect was observed at 0.048 mg/l.

Tributyltin is extremely toxic to minnow eggs, with 90% mortality after exposure to 10 µg/l (Fent, 1992). The hatched larvae had severe skeletal abnormalities with bent tails, were mostly motionless and had opaque eyes. Even at an exposure level of 0.8 µg/l 50 % of the larvae were abnormal. These concentrations compare with 32.5 mg/l which depressed hepatic P450 content and EROD activity in adult eel, rainbow trout, European eel and scup (Fent and Stegeman, 1991; Fent and Bucheli, 1994).

Similar symptoms of skeletal abnormalities were found with carp eggs exposed to the pesticides diazinon, fenitrothion, carbaryl, malathion and phosphamidon, with additional evidence of circulatory failure, and enlargement of the pericardial sac (Kaur and Toor, 1977). Eggs were, in general, 1-2 orders of magnitude more sensitive than fry, but comparison of these levels with those found to cause endocrine disruption (see Appendix) is difficult since there is such a wide variation in literature data between species and authors.

The first 24 h after fertilisation appears to be the most sensitive period for eggs since flagfish eggs exposed to a 2 h pulse of methoxychlor during this period had a decreased hatch rate and were less tolerant to the pesticide when re-exposed as 8-day juveniles (Holdway and Dixon, 1986b), while those exposed 2-3 days after fertilisation were unaffected. Early exposure also resulted in reduced whole body levels of serotonin, indicative of an effect on

indoleamine metabolism. The data suggests that the exposure regimes used for blackfly control may cause long-term neurological damage and the altered neurochemistry in rapidly developing young fish could affect the precisely timed and controlled elaboration of various tissues and permanently alter development. The reproductive and developmental abnormalities which became apparent over the full life cycle (Holdway and Dixon, 1986a) may therefore have been the result of changes in brain serotonin levels. Other organochlorines and PCBs which deplete serotinin and other biogenic amines may act in a similar manner. Some of the effects of organochlorines could be due to their action either as corticoid mimics or as stressors, since a recent study with anuran amphibia has shown that developmental abnormalities caused by exposure of tadpoles to DDT are also caused by exposure to corticosterone (Hayes *et al.*, 1997). Clearly much has yet to be learnt about the role of hormones in early development of non-mammalian vertebrates and how disruption of normal hormonal balances can cause embryonic abnormality.

Using [^{3}H] TCDD Walker *et al.*, (1991) showed that lake trout eggs containing 0.23 μg/kg of the dioxin had a decreased hatch rate, but that TCDD related mortality was greatest at the sac fry stage when they developed yolk sac edema resembling blue-sac disease. TCCD was neither metabolised nor eliminated by the eggs or sac fry, but was rapidly eliminated by fry. The lowest observable adverse effect level for mortality was 0.055 μg/kg egg.

In salmon, acid pH affects the rate of uptake of water into the egg which occurs shortly after fertilisation possibly as a result of interference with the release of colloid from the yolk into the perivitelline space, or to denaturation of this colloid (Peterson and Martin-Robichaud, 1982). The low hatch rates at low pH may in some cases be due to changes in the chorion. The decreased hatchability of perch and salmon eggs at low pH was due to a disturbance in production of the choriolytic enzyme and resulting failure of the inner layer of the chorion to dissolve (Runn *et al.*, 1977; Peterson *et al.*, 1980). Since transfer of perch eggs raised in acid water to a neutral pH at the normal hatching time gave a normal hatch rate of normal fry, the deformities found in the fry hatched at low pH appear to be secondary to the delay in hatch rather than being caused primarily by acidity.

8.2.2 Endocrine disruption

Although many studies have reported decreased hatch rate or deformity, there is little, apart from the possible implication of biogenic amines, that might be classed as endocrine disruption. One of the few such studies to show a clear effect is that of Wester and Canton (1986) in which exposure of medaka eggs to β-hexachlorocyclohexane (an impurity in the insecticide lindane) for one month from fertilisation resulted in formation of a testis-ova

in males (Figure 8.2A). Estrogenic activity of the organochlorine was implicated by the induction of vitellogenin in both sexes. In addition the thyroid gland showed hypertrophy of the epithelial cells and decreased colloid content indicative of hyperactivity which may have been caused by increased numbers of thyrotrophin producing cells in the pituitary. There were also changes in the kidneys and liver. The no-observable effect level of 0.056 mg/l was the same for both histopathological and abnormal behaviour as end points. It would be of some interest to determine whether exposure to this organochlorine for an even shorter period during a critical window could exert a similar effect which disrupted endocrine function at the adult stage without causing long-term behavioural abnormalities. The recent concern about alkylphenolic detergents as a source of environmental estrogens has led to a study of the effects of one degradation product, 4-nonylphenol, on sexual development in medaka. Exposure of medaka to 0.050 or 0.10 mg/l of the phenol from hatch to 3 months of age gave a testis-ova in 50 and 86% of males respectively, but no effect was observed at 0.01 mg/l (Gray and Metcalf, 1997) (Figure 8.2B). There were also increased numbers of female fish in the 0.1 mg/l treated group (67%) compared with controls (33%). The nominal concentration of nonylphenol used in this study (0.05 mg/l) is slightly greater than that found in municipal sewage treatment effluent (0.035 mg/l) (Stephanou and Giger, 1982). The alkylphenoxyethoxylates, from which this compound derives, also degrade to a range of other products some of which are also estrogenic and suggests that the levels pertaining in the regions downstream of these effluents could cause similar effects in wild populations. The choice of the Japanese medaka in both of these studies is particularly apt since, unlike many other teleosts, spontaneous intersex gonads or sex reversal have never been observed in this species. The medaka is unusual in being a differentiated gonochorist in which sexual differentiation of the female gonad begins before hatching while the male differentiates during a critical window around 13 day post-hatch (Yamamoto, 1969). An exposure regime which examined in more detail whether the critical period for exposure to nonylphenol corresponded to this critical window would be of great interest. The effects observed in the medaka suggest that the effects of nonylphenol may be even more pronounced in the majority of teleost species which are undifferentiated gonochorists, where the gonad develops through an ovary like stage before finally differentiating into an ovary or testis. That such effects do occur is suggested by the high proportions of intersex roach found in a number of British rivers which receive sewage effluents containing high levels of estrogenic chemicals, as indicated by the elevated plasma concentrations of vitellogenin in exposed fish (Jobling *et al.*, 1998).

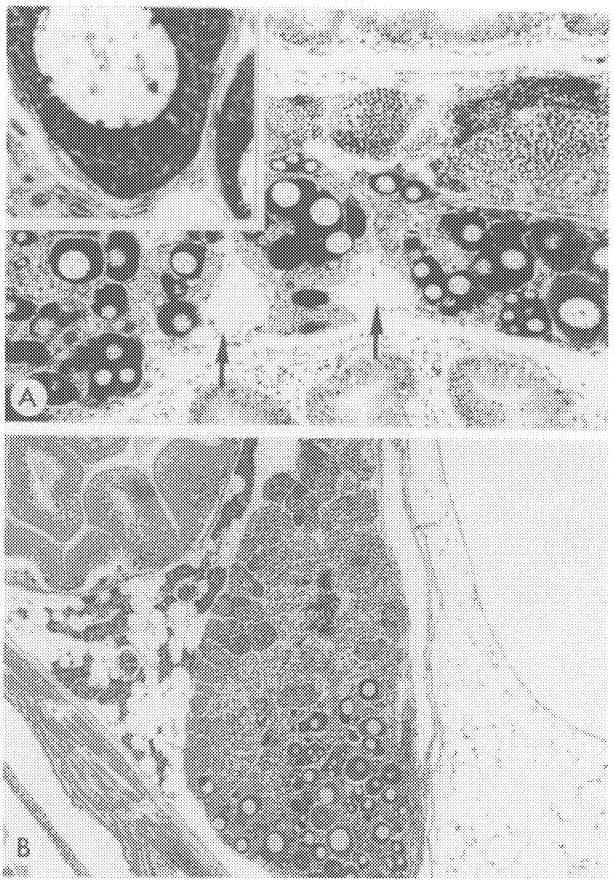


Figure 8.2. Testis-ova from a medaka A) Exposed to 1 mg/l β-HCH for 3 months with evidence of hermaphroditism. Besides spermatogenesis, clusters of oocytes and of adipocytes (arrows) are also present thoughout this organ (Haemotoxylin-eosin x 2000). Inset: detail of an oocyte surrounded by theca cells (H&E stain, x 800). (Reprinted from *Aquat. Toxicol.* **9**, Wester, P. W. and Cantor, J. H., Histopathological study of *Oryzias latipes* (medaka) after long-term β-hexachlorocyclohexane exposure, pp. 21-45, 1986 with kind permisssion of Elsevier Science - NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands). B) Exposed to 100 µg/l nonylphenol from 1 d post-hatch to 3 months. Testis tissue is in the anterior (top) part of the gonad and ovarian tissue in the posterior (bottom) of the gland (H&E stain, x 40) (Reprinted with permission from *Environmental Toxicology and Chemistry*, 1997. Induction of testis-ova in Japanese medaka (*Oryzias latipes*) exposed to *p*-nonylphenol by Gray, M. A. and Metcalf, C. D. Volume. **16**, pp. 1082-1086. Copyright Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL, 1997).

It is of some concern that by replacing persistent and estrogenic organochlorines such as DDT and lindane (containing β-HCH) with "safer" pesticides we have ignored the possibility that the "inert" dispersant with which these chemicals were formulated has often contained an alkylphenolic

detergent which degrades to a persistent and estrogenic alkylphenol. The studies of Wester and Canton (1986), and Gray and Metcalf (1997) show that the effects of both types of chemicals on the development of the medaka gonad are indistinguishable (Figure 8.2). We do indeed seem to have a propensity to grow our food in a "sea of estrogen".

Tributyltin can induce imposex in marine gastropods at concentrations of only 1 ng/l (Gibbs *et al.*, 1987), a concentration far below that which causes larval abnormalities (Section 8.2.1). Since this may be due to its action as an aromatase inhibitor (Fent, 1996), it would be of considerable interest to examine whether it can also affect the sexual differentiation of teleosts at such low concentrations during the critical phases of development. This is particularly relevant since Piferrer *et al.* (1994) have shown that treatment of genetically all-female salmon embryos for only 2 hours during a critical period 3-days after hatching with the aromatase inhibitor 4-(5,6,7,8-tetrahydroimidazo[1,5-α]pyridin-5-yl)benzonitrile monohydrochloride gave 22% males which had testes indistinguishable from testes of normal XY males of similar age.

Fertility can also be affected at concentration levels lower than that which causes apparent morphological abnormalities. Exposure of American flagfish (*Jordanella floridae*) to 0.75 mg/l hydrogen cyanide from fertilisation to hatching reduced hatch rate to 37% of the control group, but also decreased by 30% the egg production of those that did successfully complete hatching and attain sexual maturity (Cheng and Ruby, 1981). Exposure during both embryonic and juvenile stages had similar effects, but in addition decreased the hatching success of the F1 generation. The data also suggested that embryonic exposure to cyanide decreased the spawning period from 12 to 8 days and delayed sexual maturity from 56 to 70 days post-hatch.

While estrogens are well known to cause sex reversal in fish, they may also have a simple anti-mitotic effect (Huffman *et al.*, 1957; Jones *et al.*, 1960) on fish eggs. The 24 h ED_{50} of 2 mg/l for estradiol is, however, extremely high but this value was determined in experiments which were performed to assess use of estrogens in chemotherapy for tumour control. It is therefore probable that embryonic development might be impaired at very much lower estrogen concentrations. The cytostatic disruption of embryonic development of eggs from parents exposed to 5 ng/l ethynylestradiol prior to spawning (Nash *et al.*, 1997; Kime and Nash, 1998), however, suggests that such effects of estrogenic pollutants as a cause of low hatch rate or larval abnormality should not be ignored.

8.3 Exposure of larval and juvenile fish

In addition to exposure of flagfish eggs to methoxyclor, Holdway and Dixon (1986a, b) also examined the effect of a 2 h pulse of this pesticide at either 8 days of age, or at both 8 and 71 days. Mean daily egg production, total egg production and time to spawning of the first generation fish as well as hatchability and incidence of abnormality were adversely affected by a single exposure of 8 day juveniles to methoxychlor at concentrations greater than 0.25 mg/l. It is clear from this study that even a short exposure at juvenile as well as embryonic stages of life may affect the second generation, but the mechanism by which this effect was exerted was not investigated.

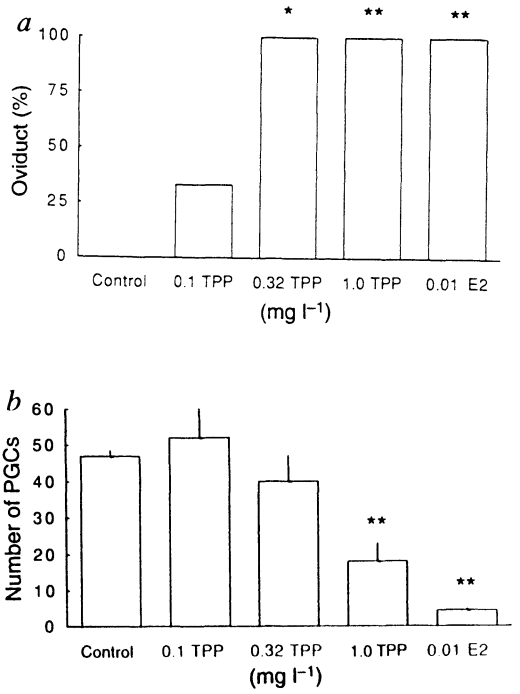


Figure 8.3. The effects of 60 day exposure of XY male carp to 4-tert-pentylphenol (TPP) or estradiol (E2). a) Frequency of occurrence of an oviduct in cross-sections of gonads, b) Total number of primordial germ cells (PGCs) per gonad. (*, $P < 0.01$; **, $P < 0.001$). (Reprinted with kind permission from *Nature* **384**, 221-222. Gimeno, S., Gerritsen, A., Bowmer, T. and Komen, H. (1996). Feminization of male carp. Copyright 1996, Macmillan Magazines Limited).

The medaka is one of the few species of teleost in which intersex fish are unknown, but the presence of such abnormalities in most teleosts does not necessarily indicate endocrine disruption, since a few percent of such

individuals may be found in many populations. Their presence may only be remarked upon if the waters are polluted by suspected endocrine disruptors. Another problem is that if eggs or juvenile fish are exposed before sexual differentiation, the natural population will include both males and females so that small changes in the sex ratio may be difficult to determine. One approach which avoids this difficulty is to use genetically all male carp (XY) obtained by fertilising eggs from females (XX) with sperm from homogametic males (YY) produced by androgenesis (Gimeno *et al.*, 1996). When such 50-day old males were exposed for 90 days to 4-*tert*-pentylphenol (TPP) covering the period of labile sex differentiation almost all fish had developed an oviduct by 60 days exposure, and in some this was apparent after only 30 days exposure to 0.1 mg/l (Figure 8.3). There was also a reduction in the number of primordial germ cells after 40 days. In some fish exposed to TPP the testis was very poorly developed, while at higher doses an ovo-testis was formed (Figure 8.4).

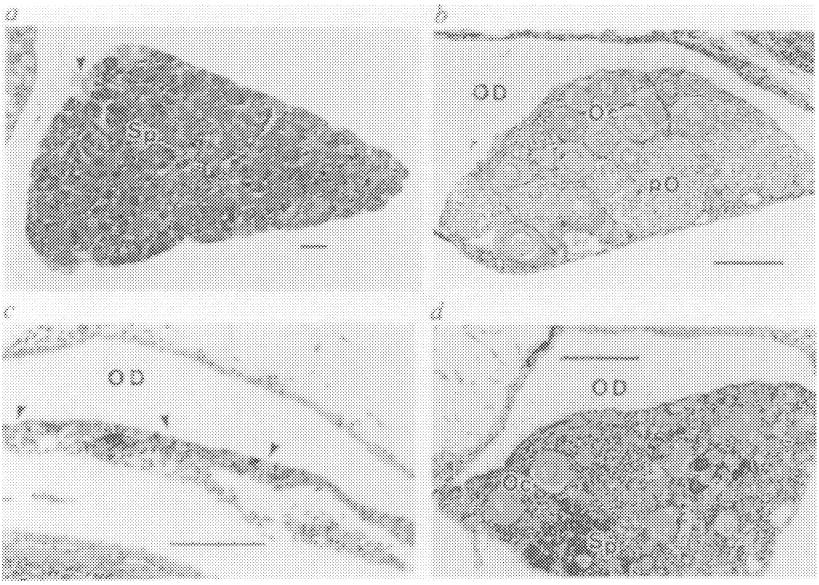


Figure 8.4. a) Testis of normal XY male carp showing spermatogenic stadia (Sp), b) Ovary from normal female (XX) carp showing oviduct (OD), prophase oocytes (pO) and previtellogenic oocytes (Oc), c) Poorly developed testis from an XY male carp exposed to 1 mg/l 4-*tert*-pentylphenol for 60 days showing an oviduct and containing only a few primordial germ cells (arrows), d) Ovo-testis from an XY male carp exposed for 60 days to 4-*tert*-pentylphenol showing the presence of both spermatogenetic stadia, previtellogenic oocytes, an oviduct and primordial germ cells. (Reprinted with kind permission from *Nature* **384**, 221-222. Gimeno, S., Gerritsen, A., Bowmer, T. and Komen, H. (1996). Feminization of male carp. Copyright 1996, Macmillan Magazines Limited).

Further studies (Gimeno *et al.*, 1997) showed that exposure to TPP for 3 days during the embryo-larval period (before 3 day post-hatch) did not affect the process of sexual differentiation, nor the proliferation of primordial germ cells, but longer exposures starting before (24-51 days post hatch) and including sexual differentiation (50-110 day post-hatch) induced formation of an oviduct. The state of development of this duct depended on the exact timing of exposure, and once formed persisted after return to clean water. Exposure before and during sexual differentiation decreased the numbers of primordial germ cells in a dose related manner, but independent of the timing of the exposure. These data show that exposure of cyprinids to estrogens even before the onset of sexual differentiation may result in compromised reproductive function. The exact timing of the critical period sensitive to endocrine disruption may differ between species, since exposure of newly hatched salmonid larvae to estradiol induced feminisation only at 6 months of age (Piferrer and Donaldson, 1992). This demonstration of the effect of a xenoestrogen on sex differentiation of fish exposed at a critical period suggests that all male fish populations might provide a suitable assay for endocrine disruption in fish by suspected estrogenic xenobiotics. The presence of hermaphrodite roach in some English rivers (Jobling *et al.*, 1998) containing sewage effluents is more likely due to exposure at this critical period than to direct effects on adult fish.

There are few studies on the effects of exposure of eggs, embryos or larvae on endocrine systems other than that of reproduction. A recent study (Stephens *et al.*, 1997) has shown that larval and juvenile turbot can exhibit a stress response to the water soluble fraction of crude oil leading to elevated whole body levels of cortisol and thyroxine. The effect was apparent even at 2-days post-hatch, suggesting that turbot have a primitive corticosteroid response at hatch, although older larvae were more responsive as the system matured. Since Barry *et al.* (1995) have suggested that stress during early development may permanently alter the stress response in adults, further studies on the effects of early life exposure on the adult functioning of the hypothalamo-pituitary-interrenal and hypothalamo-pituitary--thyroid axes would be of particular interest.

8.4 Conclusions

While there is a very extensive literature on the use of early life stages to monitor the effects of pollutants, the vast majority of these describe toxicological tests with simple end-points such as hatch rate or survival to a predetermined post-hatch date. A number also record abnormalities in the offspring, but only a fraction describe developmental changes which only become apparent in the adult fish. In part this is due to the need for simple,

rapid and inexpensive toxicity tests, and the consequent need to monitor simple parameters within a short time scale. For the same reason these tests also generally use small, rapidly developing species. The more sophisticated tests may use such species for complete life cycle tests in which exposure begins at hatch and follows changes through to the second generation. Such tests may well pick up endocrine disruption through changes observed in reproductive parameters such as egg numbers or fertilisation rate after exposure throughout one complete life cycle. They provide little information, however, on how such changes may be induced, since decreased numbers of eggs provides little information on whether the effect is exerted at the brain, pituitary, gonad or liver as changes in hormone levels or tissue morphology are rarely monitored. Continuous exposure throughout the life cycle also provides little information as to whether the eggs, embryos, larvae, juveniles or adults are the most sensitive stage for pollution induced disruption. Exposure for short windows during each of these stages would provide such information, but the costs of such procedures for routine monitoring would be prohibitive. It is becoming increasingly recognised that endocrine disruptors do not follow the normal pattern on which standard toxicology testing is based since they may be effective only during a critical window in embryonic development and the consequences only become apparent as the adult fish start their first reproductive season. Perhaps the best starting point for such tests is the complete life cycle exposure so that xenobiotics which affect any of the reproductive parameters can then be examined in more detail to determine the sensitive period and the mechanism of action. It must also be recognised that the small rapidly maturing fish which are used for many tests may not provide a suitable model for those species of greatest commercial importance. A warm water fish which takes only a few months from hatch to first spawning, and which thereafter spawns at regular intervals, may differ greatly in its endocrine control from a large marine species which matures only after several years and thereafter has a single annual spawning event. Fish such as eels and the Pacific salmon in which a single spawning migration is followed by death may be different again.

There has been a general assumption that early life stages are the most sensitive to environmental toxicants. While this may be true for simple mortality tests, it has yet to be validated for the more subtle endocrine and biochemical changes. Except for a very short period after spawning, bioaccumulation is not generally of importance until larval feeding begins after hatching. Long lived carnivorous species in which environmental toxicants can bioaccumulate over several years may therefore be more vulnerable to low levels of pollution during a prolonged adulthood, than to a brief exposure during the relatively short larval period. Laboratory testing of such species is frequently not possible, and perhaps the best method of monitoring is by relating tissue pollutant levels to endocrine and reproductive

parameters. The problem is that in such cases, not all pollutants in the tissue may be analysed and false relationships can result from a partial analysis.

There is now an urgent necessity to compare the levels that are shown to be toxic in current early life cycle tests with those that cause endocrine or reproductive dysfunctions which are only be expressed in adult fish. Although it may appear to be simple to compare literature data on toxicity during early life stages with the data in the Appendix on endocrine disruption, this is severely complicated by the different species used in these two types of tests. It will be necessary to take several species with different reproductive strategies and compare the lowest effective adverse concentrations obtained from a simple standard early life stage toxicity test, a whole life cycle test and an in depth study using exposure during different windows of development using endocrine and developmental dysfunction in adults as the end point (Figure 8.5).

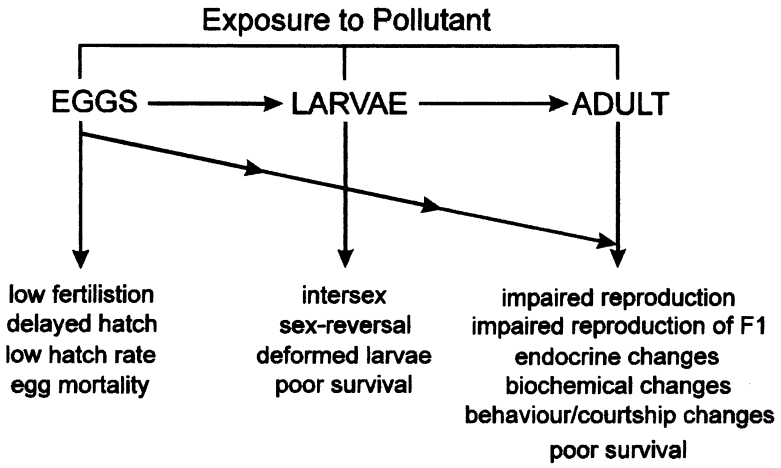


Figure 8.5. Possible effects of exposure to pollution of eggs, larvae and adults

Such studies will not be simple, but they will provide the necessary information on which to base regulatory tests for endocrine disruptors. Clearly endocrine disruption is not of any importance if it only occurs at concentrations at which hatch rate is negligible, while if it occurs at concentrations which current toxicity testing shows to have no adverse effect, then such tests will require substantial revision. It is also important not to extrapolate the data on such tests with small species exposed for a short period to long-lived top predators so that misleading inferences are drawn about the safe levels of exposure for commercially important species such as cod, salmon and eels.

Chapter 9

Disruption of Liver Function

9.1 Introduction

The liver is the major organ of detoxification in all vertebrates and would therefore be expected to play a major role in determining the sensitivity of an organism to a particular pollutant. In fish it is particularly rich in lipids and it is therefore not surprising to find that it is also a major site of bioaccumulation for many organic pollutants (see Chapter 3). Such bioaccumulation increases the risk both of morphological damage to the liver, and of disruption to the activities of detoxifying enzymes. These changes may in turn alter the rate at which further accumulations of the toxin can be deactivated and removed from the organism. This could result in synergisms between different pollutants if the disruption of the deactivating systems caused by one pollutant results in failure to detoxify the second. The hepatic enzymes used for detoxifying xenobiotics are in fact closely related to the enzymes that are used for both synthesis and deactivation of other chemicals naturally present in the body. P450 and conjugating enzymes, for example, play a major role in the deactivation of steroid hormones and if these enzymes are preferentially used to metabolise a pollutant, the hormones may be less rapidly deactivated resulting in a higher plasma concentration. They are also essential for the synthesis of steroid hormones by the interrenal and gonads. The situation is further complicated since the liver is both the target tissue and site of deactivation of the ovarian steroid estradiol. As a target tissue it has estrogen receptors which specifically induce synthesis of the yolk protein vitellogenin. The balance between binding to such receptors and deactivation could be affected by changes both in the deactivating enzymes and in the structural integrity of the liver and its constituent cells.

Because of both its ability to bioconcentrate, and the wide variety of enzymes which are stimulated in the presence of xenobiotics, the liver has been the centre of a large number of toxicological studies. Many of these use the liver simply as a bioindicator of the presence of a pollutant in the water rather than examine what effect the enzyme changes might have on the fish itself. The increased use of hepatic vitellogenin synthesis as a biomarker for exposure to environmental estrogens has, however, led to increased interest on how hepatic function is affected by xenobiotics and how this might affect reproduction.

9.2 Morphological effects

A wide range of pollutants cause changes in liver morphology. In some cases this may be as a result of induction of metabolising enzymes or of vitellogenesis, while in others it is damage resulting from cytotoxic action of the xenobiotic. In other cases the changes may be the preliminary stages of those associated with induction of cancers. Gross morphological changes may be seen in simple histological sections, but the higher resolution of electron microscopy is necessary to resolve the finer details such as changes in rough and smooth endoplasmic reticulum. Structural changes in the liver resulting from chronic exposure of fish to pollutants has potential as a biomarker of exposure (Hinton and Laurén, 1990). Such changes are apparent at the tissue, cell and organelle level, but it is important to distinguish those changes which are induced by anthropogenic toxicants from the natural changes which vary with nutritional status, life stage, sex, season and reproductive phase or by exposure to natural plant toxins, enteric bacterial derived toxins, or viruses. The establishment of archives of histopathological sections from indicator species to provide site-specific historical databases has been suggested by Hinton and Laurén (1990). These authors have classified a range of morphological changes induced by chemical perturbations under the general headings of cellular necrosis, hyperplasia of regeneration, fatty change, high amplitude swelling, nuclear and cytoplasmic inclusions, hepatocyte hypertrophy and hepatocytomegaly. In addition they discuss neoplastic changes, but incidences of hepatocellular carcinoma as exemplified by Cormier and Racine (1990) while clearly indicative of environmental pollution are beyond the scope of this volume.

Some of the morphological changes are indicative of induction of vitellogenesis by xenobiotics and parallel those which occur during ovarian recrudescence. 0.1 mg/l of β-HCH (an impurity in lindane) increased hepatocellular basophilia caused by hypertrophy of the rough endoplasmic reticulum (Wester *et al.*, 1985). The authors attributed this to an excessive production of vitellogenin by the liver resulting from the estrogenic activity of

this organochlorine. They confirmed this by showing that similar changes occurred in young fish, both in the liver and other tissues, after exposure to estradiol and that both estradiol and β-HCH stimulated vitellogenin. This suggested that for β-HCH, the estrogenicity outweighed the hepatotoxic effects of the pesticide. By contrast, the γ-isomer of HCH (lindane) decreased the amount of rough endoplasmic reticulum (RER) in zebrafish and there was a microvesicular fatty vacuolisation (steatosis) characterised by lipid deposition within their cisternae (Braunbeck *et al.*, 1990a) which suggests that this isomer might impair estrogen induced vitellogenesis. Parallel studies on hepatic ultrastructure and vitellogenin production after exposure to this or other pollutants would provide valuable information on estrogenicity versus the inhibition of vitellogenesis resulting from pollutant induced morphological changes. Many pesticides are not estrogenic and may simply inhibit liver function. Following exposure of bluegill (*Lepomis macrochirus*) to 0.1-10 mg/l of the herbicide 2,4-D, morphological abnormalities of the liver included shrinkage and loss of vacuolation of the parenchymal cells and decreased glycogen content (Cope *et al.*, 1970). These abnormalities were maximal 14 days after treatment and then decreased, so that by 28 days they were only apparent in fish exposed to 5-10 mg/l of the herbicide. Even the occasional fish exposed to 0.1 mg/l showed some abnormalities, and although the effects were of short duration this could cause major reproductive dysfunction if high water concentrations coincided with vital stages of the reproductive cycle. The 2 week spawning delay found for fish exposed to 5 mg/l may well have been due to decreased vitellogenin production by damaged livers. Exposure of the catfish, *Heteropneustes fossilis*, to malathion at 1.2 mg/l also showed shrinkage of the hepatocytes after 24 h, and by 48 h there was degeneration of the cell membrane, vacuolation of the cytoplasm and signs of the nuclei becoming pyknotic and eccentric (Dutta *et al.*, 1993). By 96 h there was very extensive damage to the tissue morphology.

Decreased vitellogenin production has also been associated with necrosis caused by the high incidence of liver tumours in winter flounder in Long Island Sound and Boston Harbour (Murchelano and Wolke, 1985; Pereira *et al.*, 1992). High contaminant levels have also been linked to hepatic lesions in English sole both at this location and on the U. S. west coast (Malins *et al.*, 1984; 1985; Krahn *et al.*, 1986; Myers *et al.*, 1991). In Puget Sound a correlation has been shown between idiopathic hepatic lesions, neoplasms and exposure to aquatic pollution in English sole (Myers *et al.*, 1991).

In addition to changes in the gross structure of the liver, pollutants induce ultrastructural changes. Livers from zebrafish exposed to 0.001 mg/l DDT for 24 h, or to 0.0003 mg/l for 72 h had a complete loss of glycogen, decreased cell size and a cytoplasmic compartment filled with rough endoplasmic reticulum (Weis, 1974). In addition to the loss of lipid, glycogenolysis was as extensive as complete fasting for 14 days suggesting that the equilibria in the

rate-limiting steps of glycogenolysis had been upset. The levels used, although substantially less than the maximal found in sprayed areas (0.012 mg/l), only allowed survival of the fish for 7 or 16 days, but accumulation at lower concentrations might well have similar effects over a longer exposure period. The more DDT tolerant guppy, which has a 14 day LD_{50} of 0.018 mg/l, by contrast showed only a gradual accumulation of smooth endoplasmic reticulum in liver cells after 28 day exposure while glycogen, cell size and rough endoplasmic reticulum remained unaffected. The greater tolerance of the guppy was attributed to the higher lipid content of this species compared to the zebrafish since the lipid droplets can sequester the pesticide and protect against toxic effects. Since this would lead to greater bioaccumulation in the guppy after long-term low-level exposure the effects of short and long-term exposure on hepatic ultrastucture may be very different in the two species. Endosulfan is extremely toxic with a No Observed Adverse Effect Concentration for hepatic cytological alterations of 1 ng/l (0.000001 mg/l) in rainbow trout which is 3 orders of magnitude below the LC_{50} for this species and well within the concentrations found in the environment (Arnold *et al.*, 1996a). The changes induced included proliferation of the SER, and circular arrays of RER indicating induction of MFO. Above 50 ng/l there were degenerative effects such as dilation of the intermembranous spaces in the mitochondria, deformation of mitochondria, myelin formation in peroxisomes and cytoplasm, and vesiculation and dilation of RER cisternae. In carp fed for 5 weeks with 0.5 μg endosulfan per kg diet, equivalent to only 15 ng/kg fish per day the livers showed enlarged nucleoli, increased number and size of the Golgi fields and ER lamellae as well as proliferation of peroxisomes and lysosomes (Braunbeck, personal communication). Pathological processes were also apparent from the glycogen and lipid depletion, invasion of phagocytic macrophages and accumulation of myelinated bodies in the endothelial cells of hepatic sinusoids

Rainbow trout exposed for 18-34 days to a combination of 0.00005 mg/l of the organochlorine endosulfan and 0.0005 mg/l of the organophosphate disulfoton showed changes to the ultrastructure of hepatic cells, with irregular nuclei, and alterations to the lysozomes and rough endoplasmic reticulum (Arnold *et al.*, 1995). As with the DDT exposed zebrafish there was a decrease in glycogen content of the hepatocytes (Figure 9.1).

The morphological changes to the liver caused by the chlorinated urea herbicide linuron above 30 μg/l in rainbow trout (Oulmi *et al.*, 1995a) are very similar to those resulting from its potential breakdown products 4-chloroaniline and 3,4-dichloroaniline (Braunbeck *et al.*, 1990b) which suggests that some of the changes may result from these metabolites.

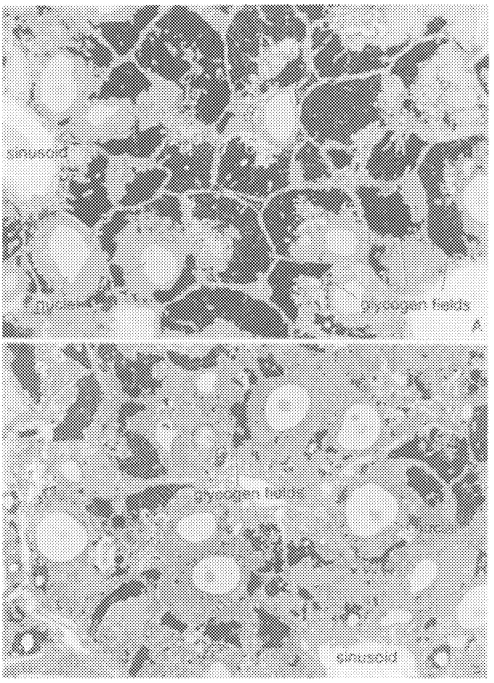


Figure 9.1. The effect of exposure of rainbow trout to 50 ng/l endosulfan and 1, 5, or 10 ng/l disulfoton on liver morphology. A). Control fish showing regular appearance of the liver, with hepatocytes divided into large intensively stained peripheral glycogen fields and perinuclear and peribiliar organelle-containing zones, with few lipid inclusions. B) Hepatocytes from exposed fish are characterised by significant glycogen depletion and an increase in nuclear diameter. (Argentaffian stain, x 1700). (Reprinted from *Aquat. Toxicol.*, **33**, Arnold, H., Pluta, H-J. and Braunbeck, T., Simultaneous exposure of fish to endosulfan and disulfoton *in vivo*: ultrastructural, stereological and biochemical reactions in hepatocytes of male rainbow trout (*Oncorhynchus mykiss*), pp. 17-43, 1995 with kind permisssion of Elsevier Science - NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands).

The liver is particularly vulnerable to organophosphate action since the toxic effects are the result of bioconversions by the monooxygenase system which is very active in this tissue. Although the primary acute toxicity of organophosphates is due to acetylcholinesterase inhibition and would therefore be expected to act predominantly on neural tissue, the organophosphate insecticide disulfoton has effects on rainbow trout liver at levels at which such effects are not apparent macroscopically (Arnold *et al.*, 1996b). Quantitative analysis of the changes was not possible and stereological analysis showed only insignificant effects, but there were clear qualitative changes in liver structures after exposure. At 0.1 μg/l there was dilation, fragmentation and vesiculation of the rough endoplasmic reticulum (RER), but the parallel arrangement of most cisternae was not affected at any

concentration. At low concentrations the RER cisternae formed circular arrays enclosing membrane bound glycogen areas, or organelles such as mitochondria. With increasing disulfoton (>5 μg/l) concentrations these circular arrays were transformed to strongly condensed myelin figures showing the increasing toxic effects of the pesticide. There were also changes in lysosomes, peroxisomes, Golgi fields and mitochondria. The changes were apparent even at the lowest concentration used, 0.1 μg/l which is over four orders of magnitude lower than the 96 h LC_{50} for this species (3 mg/l). In eels, in which the LC_{50} is only 37μg/l, the liver may be even more sensitive to this pesticide, but liver morphology was not examined below 5 μg/l at which it produced complex changes (Arnold and Braunbeck, 1994). The observed changes in both eel and trout liver suggest a biphasic mode indicative of adaptive effects at low concentration, but degenerative effects above 5 μg/l. The disulfoton released into the Rhine during the chemical spillage at Basle in 1986 produced concentrations between 5.3 and 600μg/l which accounts for the high mortality of the Rhine eel population and was sufficient to cause severe liver changes in eel, trout and other species (Braunbeck *et al.*, 1990c; Arnold and Braunbeck, 1994).

Rainbow trout fed dietary levels of 10 or 100 mg/l of the PCB Arochlor 1254 for 229-330 days showed irregular and bizarre nuclear outlines, separation of the nucleolar components and large nuclear pseudoinclusions (Hacking *et al.*, 1977). Increases in smooth endoplasmic reticulum, changes in the rough endoplasmic reticulum, increased lysosomes, reduced glycogen, and increased lipid and hypoxic vacuoles were also frequently seen. There was no difference between control fish and those fed 1 mg/kg of the PCB. The dietary administration of 10 mg/l resulted in a whole fish concentration of 2.3 μg/g which is within the range found in wild populations (see Chapter 3). Weekly injection of carp and rainbow trout with 25 mg/kg body weight Arochlor 1254 for 4 weeks also caused enlargement of the rough endoplasmic reticulum of the hepatocytes (Sivarajah *et al.*, 1978a). Although use of PCBs has ceased in many countries other halogenated organics such as polychlorinated naphthalenes (PCNs) and polybrominated diphenyl ethers (PBDEs) are increasingly used as fire retardants. Their effects on liver morphology, however, differs little from the PCBs. In sticklebacks (*Gasterosteus aculeatus*) they caused intracellular lipid accumulation in the hepatocytes, and the rough endoplasmic reticulum often formed circular structures surrounding the lipid globuli (Holm *et al.*, 1993).

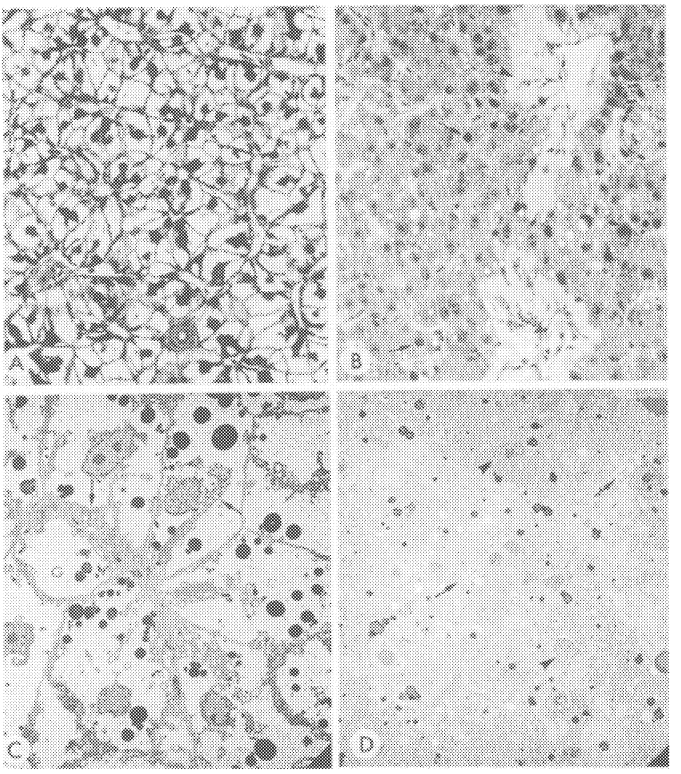


Figure 9.2. The effect of paper mill effluent on morphology of bullhead liver. A) Liver from fish taken above the pulp mill, rich in storage substances (* = glycogen vacuole), B) Liver from fish below the pulp mill, low in glycogen and showing necrosis of single hepatocytes (A, B May-Grünwald/Giemsa, x 485), C) Ultrastructure of liver from fish above the pulp mill with well compartmentalised cytoplasm, large storage areas (G = glycogen, F = fat), and organelle rich zones (arrows), D) Ultrastructure of liver from a fish below the effluent low in glycogen, pycnotic cell nuclei (large arrow heads), widened cisternae of the endoplasmic reticulum (small arrowheads) and giant lysosomes (arrows) (C, D x 1100). (Reproduced from Bucher, F., Hofer, R. and Salvenmoser, W. (1992). Effects of treated paper-mill effluents on hepatic morphology in male bullhead (*Cottus gobio* L.). *Arch. Environ. Contam. Toxicol.* **23**, 410-419, Figs 2, 3, 6, 7 by kind permission of Springer-Verlag, Berlin and the authors).

Effluents from an Austrian pulp mill caused lesions and atrophy in livers of bullhead (*Cottus gobio*), and depletion of glycogen and necrosis of hepatocytes (Figure 9.2 A, B). Nuclei were pycnotic and there was a high incidence of *Zschokkella nova* (Myxosporea) parasitisation (Bucher *et al.*, 1992). Ultrastructural studies showed glycogen depletion, a loss of compartmentalisation of the cytoplasm, reduced and disorganised rough endoplasmic reticulum, and vesicular proliferated smooth endoplasmic reticulum (Figure 9.2 C, D). Giant lysozomes and proliferation of

peroxizomes were common. The increase in smooth endoplasmic reticulum may have been a result of the active detoxification of lipophilic organic pollutants in the effluent since it is the site of action of the mixed function oxidases and conjugating enzymes (see Section 9.3). Fish collected upstream of the mill exhibited normal livers. During the high-water following the spring thaw in the Alps, the incidence of liver damage decreased which suggests that the exposure did not cause irreparable damage to the tissue. Such changes in mixed function oxygenase, however, may well result in alterations to hormone catabolism so that endocrine function could be affected during the period of low water.

The changes induced in liver morphology by a wide range of pollutants are highly complex and comparisons between authors, species and pollutant are not easy. Braunbeck (1994) has compared in detail the ultrastructural changes induced by seven different pollutants (4-chloroaniline, endosulfan, atrazine, diazinon, disulfoton, linuron and ochratoxin) in the liver of rainbow trout and shown that the pattern of changes shows some specificity. Although the author compared the sensitivity of cytological and "conventional" LOAEC, there is a wide variation between the methods in some cases which may partly reflect the unspecified nature of "conventional", but may also reflect the primary site of action of the pollutant. The data presented in the Appendix for endosulfan and diazinon indicates that disruption of liver morphology occurs at similar concentrations to that of reproductive function and suggests that changes in liver morphology may provide a suitable indicator of reproductive dysfunction.

9.3 Effects on hepatic enzymes and proteins

9.3.1 Introduction

The liver contains a large number of enzymes and proteins, some of which are related to its general detoxifying role and can be used as bioindicators of exposure to environmental contamination (Thomas, 1990a). The metallothioneins and the P450 enzymes, which are most commonly used as such indicators, play a natural role within the fish in maintaining a homeostatic equilibrium. While metallothionein has an important role in maintaining constant cellular or tissue levels of essential metals such as zinc, the cytochrome P450 catalysed mixed function oxidases (MFOs) are important in both deactivation and excretion of chemicals such as steroid hormones produced within the fish, as well as their synthesis in other tissues such as the gonads. The activity of both these systems is regulated by substrate availability, so that an increase in metallothionein or MFO indicates increased exposure of the tissue to a heavy metal or an oxidisable substrate. It

does not matter whether this increase originates from natural changes or those induced by exposure to xenobiotics. Since pollutants such as the heavy metals cadmium and mercury, or the organic polyaromatics, PCBs and dioxins also induce increased activity, this provides the basis for their use as a bioindicator.

It is important to remember that fish evolved around 400 million years ago and that since metallothioneins and mixed function oxidases are also present in invertebrates, their lineage is indeed very ancient. Even the most ancient of anthropogenic pollutants, the heavy metals, were first released in significant quantities less than 5000 years ago, while organic pollutants date from only last century. It is only during the last 50-100 years that release of these pollutants into the environment has attained such very high levels. It is therefore clear that the metallothioneins and MFOs did not arise to protect against man-made toxins, and that they evolved primarily to regulate available concentrations of essential metals, and the metabolism of organic chemicals normally present within the organism. Although they may also offer some protection against natural toxins these are relatively rare compared to anthropogenic sources. These systems are far too frequently considered to be present to protect the organism against xenobiotics, a statement that is clearly shown to be false in the case of PAHs and some organophosphates which they transform to more toxic metabolites. Since natural changes within the organism can stimulate production of metallothionein and MFOs to maintain a homeostatic balance, external sources of metals or organic chemicals which mimic those for which they are designed can cause a similar elevation. While such stimulation may provide a suitable bioindicator of pollution exposure, it is important to recognise that this stimulation may disrupt their normal function in maintaining the natural chemical balance. Since zinc is an essential element for normal reproductive function, stimulation of metallothionein production by external sources of other heavy metals may well disrupt reproduction. The changes in metallothionein during the normal reproductive cycle (see Section 9.3.2) suggest that it does indeed provide an important natural balance that may be disrupted by external sources of metal. Similarly MFOs regulate steroid hormone synthesis and metabolism, and stimulation of the enzyme by anthropogenic mimics will upset the hormone balance. It is no co-incidence that MFOs are stimulated primarily by planar ring structures which have steric similarities with the steroids. The concentration by many toxicologists on metallothioneins and MFOs as "indicators of pollution" misses the point that stimulation of these enzymes by pollutants must, almost inevitably, lead to some disruption of the endocrine system.

9.3.2 Metallothioneins

Metallothioneins are low molecular weight (6000-7000 Da) proteins rich in cysteine and present in all living organisms. They are induced as a result of exposure to the essential metals copper and zinc and serve a homeostatic function under normal conditions. Excess of these metals, or the presence of the non-essential heavy metals cadmium and mercury, will induce increased production of the metallothionein. High levels of the protein are therefore indicative of excessive exposure of an animal to heavy metals, and have frequently been used as a bioindicator (Klaverkamp *et al.*, 1984; Hamilton and Mehrle, 1986; Benson *et al.*, 1990; Garvey, 1990). In fish, metallothioneins are present in the kidney, intestine and gills in addition to the liver (Klaverkamp *et al.*, 1984) and are believed to protect against metal toxicity by binding metal ions and reducing their availability to the sulfhydrile groups of other proteins associated with membranes or cytosolic enzymes. The induction of metallothionein by treatment with metal provides protection against subsequent exposure to metals thus increasing the general tolerance of the organism. The toxic effects of the heavy metals only become apparent when the capacity of the metallothionein to sequester them is exceeded such that they may bind to other proteins and cause pathological damage (Hamilton and Mehrle, 1986).

There appear to be chemical differences, not only between mammalian and piscine metallothioneins, but also between teleost species and, more importantly, even between different tissues within the same species. Although an antibody raised against cod hepatic metallothionein cross-reacted with the proteins in liver, brain, anterior and posterior kidney, spleen, intestine and ovaries, the putative metallothionein from cod brain migrated differently from that of the other tissues on gel electrophoresis (Hylland *et al.*, 1994). There were also differences in the response of metallothionein between the different tissues following injection of cadmium or zinc. The metal binding protein from dab ovaries differed from that isolated from the liver in that it was not inducible by cadmium, even though this metal was bound in a dose dependent manner (Kammann *et al.*, 1996).

The binding of metallothionein to heavy metals is readily examined by gel permeation chromatography where the association of the metal to the low molecular weight fraction of the cytosol with high cysteine content can be seen as well as the increase in this fraction in response to metal exposure (see for example Wofford and Thomas, 1984). The binding of metals to metallothionein may not, however, reflect the different proportions of metals in the water. After 2 weeks exposure of fish to a metal contaminated lake, their hepatic metallothionein had bound mainly copper, even though zinc concentrations in the water were 200 times greater than those of copper (Roch *et al.*, 1982; Roch and McCarter, 1984a, b). Clearly the different affinities of

the heavy metals for the protein gives a binding pattern very different from that pertaining in the water and although metallothionein may be used as an indicator of metal exposure, quantitative data must be used with some discretion.

It is also important that appropriate controls are used since metallothionein concentration can vary during the reproductive season (Olsson *et al.*, 1987; Overnell *et al.*, 1987a), probably as a result of changes in plasma estradiol since treatment of fish with this hormone increased metallothionein production (Overnell *et al.*, 1987a; Olsson *et al.*, 1989a). This is reflected in the increased binding of cadmium to the metallothionein fraction in cadmium + estradiol treated flounder compared to those treated with cadmium alone, suggesting stimulated induction of metallothionein synthesis (Povlsen *et al.*, 1990). Olsson *et al.* (1989a), observing the increased concentration of metallothionein bound zinc in association with the increase in metallothionein suggested that the function of this protein was to maintain hepatic zinc homeostasis during exogenous vitellogenesis. In addition to changes in metallothionein during the reproductive season, the levels of the protein also changes in response to stress factors (Overnell *et al.*, 1987b).

The binding of metals to metallothionein is also not as simple as is often implied. There are a number of other proteins present in the liver which can compete for binding of the heavy metal. In rainbow trout co-injected with estradiol and cadmium, the metal did not initially bind to metallothionein, but to a high-molecular-mass cytosolic protein (Hm_r) from which it was gradually redistributed to metallothionein, resulting in induction of metallothionein and binding of most of the metal to this protein. Cadmium inhibition of vitellogenesis diminished during this redistribution (Olsson *et al.*, 1995). In addition there is evidence that cytosolic non-thionein proteins can bind heavy metals and Cope *et al.* (1994) have suggested that this may be a sensitive indicator of cadmium exposure. There are also indications that there are other low molecular weight, heat-stable metal ligands in addition to metallothionein which bind cadmium (Thomas *et al.*, 1983; Stone and Overnell, 1985; Spry and Wood, 1989), although these were not found in other studies on the same species (Olsson *et al.*, 1989b). The induction of cadmium binding thioneins may also cause an imbalance in biologically essential metals, such as copper, as a result of changes in their binding and tissue distribution (Pruell and Engelhardt, 1980).

Amino acid sequencing has shown a close homology between different piscine metallothioneins (Kille *et al.*, 1992). Rainbow trout encodes two distinct metallothioneins while pike, stone loach, flounder and plaice appear to produce only a single isoform. Earlier studies have also suggested the presence of isometallothioneins with two forms in carp liver (Kito *et al.*, 1982) and three in coho salmon and skipjack (McCarter *et al.*, 1982; Takeda

and Shimizu, 1982). Elucidation of the nucleotide sequences encoding these metallothioneins has allowed the development of probes based on their mRNAs for the detection and quantification of metallothionein specific mRNA levels (Kille *et al.*, 1992).

While metallothionein has been widely used as a monitor for heavy metal exposure, there remain problems with its use and results obtained should be used with some discretion. While the induction of metallothionein is indicative of exposure to heavy metals, it gives little information on whether the fish is affected by the heavy metal since it is the excess of metal that is not bound to the protein which is toxic. Since the role of metallothionein in natural fish populations not exposed to anthropogenic sources of heavy metals is to stabilise zinc and copper levels within the tissues, changes in metallothionein concentrations may alter such an equilibrium. As essential zinc plays an important role both in reproduction and in the functioning of many enzymes, any change in its cellular distribution may have effects on the reproductive endocrine system. Displacement of the metal by the chemically similar cadmium and mercury may be responsible for some of the endocrine disrupting effects of these heavy metals (see Appendix). Although most studies have concentrated on hepatic metallothioneins, similar effects are also likely to be found with the gonadal metallothioneins which may directly affect reproductive endocrine function. Induction of hepatic metallothionein may, therefore, provide an indicator of potential endocrine disruption in exposed populations.

9.3.3 Mixed function oxidases (MFO)

The mammalian mixed function oxidase (or monooxygenase) system, which is present predominantly in the smooth endoplasmic reticulum of the liver, catalyses oxidation reactions involving conversion of lipophilic substrates to more hydrophilic compounds which may be later conjugated. The enzymes require molecular oxygen and a carbon monoxide sensitive cytochrome P450. As in mammals the teleost enzyme inducers act through the intracellular Ah-receptor, translocation of the receptor complex to the nucleus and transcriptional activity at the gene (Hahn *et al.*, 1992, 1993). In normal animals the hepatic enzymes are present at relatively low activity when they function as deactivators of endogenous substrates such as steroids. If, however, the animal is exposed to environmental xenobiotics, MFO activity increases so that these chemicals may be rapidly deactivated to hydrophilic products which can be readily excreted. The biotransformation of xenobiotic organic chemicals, which has been extensively reviewed elsewhere (Buhler and Williams, 1989; Foureman, 1989; James, 1989; Sijm and Opperhuizen, 1989; Varanasi *et al.*, 1989), generally involves addition of a hydroxyl group to an aromatic ring, followed by conjugation. Such planar hydroxylated

metabolites of polyaromatic hydrocarbons have a structural similarity to the steroids and are more toxic than their parent compounds. The stimulatory response of the MFO to pollutants can not only provide a bioindicator of pollution exposure (Payne *et al.*, 1987; Melancon *et al.*, 1988; Goksøyr and Förlin, 1992), but may also indicate potential endocrine disruption since a number of chemicals which stimulate MFO activity may also have antiestrogenic or estrogenic potentiating activity as indicated by their modulation of vitellogenin synthesis (Anderson *et al.*, 1996a, b) (see Section 9.4).

The hepatic MFO of fish has many similarities to the mammalian system in its general detoxification of xenobiotics and catabolism of endogenous chemicals such as the steroid hormones. There are, however, some major differences between the mammalian and piscine systems. In particular the increase in smooth endoplasmic reticulum and the accompanying increase in microsomal protein may not be as pronounced as in mammals, while the MFO activity is generally lower (Addison, 1984). While the liver has been the focus of the majority of the studies, it is important to remember that many of the steroidogenic conversions which are essential for gonadal function require these same enzymes in the gonads. Effects which are observed on the liver enzyme could therefore also be manifest within the gonads and affect their function (see Section 9.4). MFO is not a single enzyme, but a complex which is dependent upon a family of cytochrome P450s for their action. Several of these cytochrome P450 isozymes have been isolated and purified (Goksøyr, 1985; Klotz *et al.*, 1983, 1986; Williams and Buhler, 1982, 1984). Different substrates may therefore be expected to have very different effects, and in fish taken from contaminated waters there is always a complex mixture of contaminants which may have acted upon the hepatic enzymes. As with the metallothioneins, MFOs may provide bioindicators of environmental contamination, but care is needed in interpretation and quantitative use is difficult. Table 9.1 shows the effects of a range of pollutants on MFO activity in fish.

Table 9.1. Effect of environmental pollutants on mixed function oxygenase activity. See footnote for abbreviations used. (+, -, 0 indicate increase, decrease or no effect; ip = intraperitoneal injection (per kg body weight); ig = intragastric injection; V= *in vitro*).

Pollutant	Species	Indicator	Exposure		Reference
Metals					
Cadmium	*Oncorhynchus mykiss*	ECOD	0.5 mg/kg ip	0/-	Förlin *et al.*, 1986
Copper	*Dicentrarchus labrax*	EROD	0.0064 mg/l V	-	Viarengo *et al.*, 1997
Mercury	*Dicentrarchus labrax*	EROD	0.004 mg/l V	-	Viarengo *et al.*, 1997
Methyl mercury	*Dicentrarchus labrax*	EROD	0.021 mg/l V	-	Viarengo *et al.*, 1997
Tin (Tributyl/triphenyl)	*Anguilla anguilla*	P450, EROD	>100 mg/l V	-	Fent and Bucheli, 1994
	Cottus gobio	P450, EROD	>36 mg/l V	-	Fent and Bucheli, 1994
	Mullus barbatus	EROD	0.6-1.2 mg/l V	-	Morcillo and Porte, 1997
	Oncorhynchus mykiss	P450, EROD	>22 mg/l V	-	Fent and Bucheli, 1994 Reader *et al.*, 1996
	Stenotomus chrysops	P450, EROD	>6 mg/l V	-	Fent and Stegeman, 1991
Pesticides					
p,p'-DDT	*Oncorhynchus mykiss*		100 mg/kg diet 4 weeks/		
			50 mg/kg ip 14d	0	Buhler and Rasmussen, 1968
	Salvelinus fontinalis		17 mg/kg liver	0	Addison *et al.*, 1977
p,p'-DDE	*Salvelinus fontinalis*		58 mg/kg liver	0	Addison *et al.*, 1977
Chlordane	*Oncorhynchus mykiss*		50 mg/kg ip 4d	0	Buhler and Rasmussen, 1968
	Oncorhynchus kisutch		50 mg/kg ip 4d	0	Buhler and Rasmussen, 1968
Kepone	*Oncorhynchus mykiss*	ECOD, EROD	5mg/kg ip	-	Vodicnik *et al.*, 1981
Mirex	*Oncorhynchus mykiss*	ECOD, EROD	40 mg/kg ip	0	Vodicnik *et al.*, 1981
		ECOD	200 mg/kg diet	0	Law and Addison, 1981
	Salvelinus fontinalis	AHH (BaP)	200 mg/kg diet	0	Law and Addison, 1981

Table 9.1 (continued)

Pollutant	Species	Indicator	Exposure		Reference
Diazinon+ disulfoton	*Oncorhynchus mykiss*	ECOD, EROD	50 mg+ 1µg/l	+	Arnold *et al.*, 1995
Disulfoton	*Oncorhynchus mykiss*	SER	5 µg/l	+	Arnold *et al.*, 1996b
Industrial					
2,3,7,8-TCDD	*Gadus morhua*	EROD	8µg/kg ig	+	Hektoen *et al.*, 1994
	Oncorhynchus mykiss	PROD	2µg/kg ip	+	Linström-Seppa and Oikari, 1989a
		ECOD, EROD	1.2µg/kg ip	+	Vodicnik *et al.*, 1981
		EROD	8µg/kg ig	+	Hektoen *et al.*, 1994
		P450-mRNA	>7.2 ng/l V	+	Zabel and Peterson, 1996
Polychlorinated dibenzodioxin	*Cyprinus carpio*	P450, EROD	64ng/kg liver	+	Van der Weiden *et al.*, 1994
	Oncorhynchus mykiss[a]	P450-mRNA	10-350 µg/l V	+	Zabel *et al.*, 1996
Polychlorinated dibenzofurans	*Cyprinus carpio*	P450, EROD	177 ng/kg liver	+	Van der Weiden *et al.*, 1994
2,3,6,7-Tetrachloro-xanthene	*Oncorhynchus mykiss*[a]	P450-mRNA	>5 ng/l	+	Zabel and Peterson, 1996
Polybromo-diphenylether	*Oncorhynchus mykiss*	EROD	2 µg/kg ip	0	Holm *et al.*, 1994
	Gasterosteus aculeatus	EROD	6 mg/kg ip	0	Holm *et al.*, 1993, 1994
Polybrominated biphenyls	*Oncorhynchus mykiss*	AHH (BaP), ECOD, EROD	150 mg/kg ip	+	Elcomb and Lech, 1978
	Salvelinus fontinalis	AHH (BaP)	200 mg/kg diet	+	Law and Addison, 1981
PCB/PCN	*Gasterosteus aculeatus*	EROD	38/14 mg/kg ip	+	Holm *et al.*, 1993, 1994
	Oncorhynchus mykiss	EROD	2µg/fry	+	Holm *et al.*, 1994
PCBs	*Cyprinus carpio*	P450, APND	25 mg/kg ip	+	Sivarajah *et al.*, 1978b
		AHH (BaP), ECOD, EROD	0.3 mg/kg ip	+	Melancon *et al.*, 1981
		APND	250 mg/kg diet 7d	+	Ito and Murata, 1980

Table 9.1 (continued)

Pollutant	Species	Indicator	Exposure		Reference
	Ictalurus punctatus	AHH (BaP), ECOD, EROD	1 mg/kg ip	+	Ankley *et al.*, 1986
		AH, APND	1 mg/l	+	HillI., 1976
	Oncorhynchus mykiss	P450, APND	25 mg/kg ip	+	Sivarajah *et al.*, 1978b
		AHH (BaP), ECOD, EROD	150 mg/kg ip	+	Franklin *et al.*, 1980
		AHH (BaP), PNAD	500 mg/kg ip	+	Förlin, 1980
		EROD	500 mg/kg ip	+	Andersson *et al.*, 1985
	Pimephalus promelas	CYP1A/EROD	0.1 mg/kg liver	-/+	Lindström-Seppä *et al.*, 1994
	Platichthys flesus	P450/EROD	100 mg/kg ip	0/+	Besselink *et al.*, 1996
	Salvelinus fontinalis	ECOD, EROD	170 mg/kg bw via diet	+	Addison *et al.*, 1981
	Salvelinus namaycush	EROD	6 mg/kg ip	+	Palace *et al.*, 1996
3,3',4,4'-TCB	*Oncorhynchus mykiss*	ECOD, EROD	0.2 mg/kg ip	+	Melancon and Lech, 1983
	Stenotomus chrysops	P450, EROD, mRNA	1 mg/kg ip	+	Gooch *et al.*, 1989
2,3,3',4,4',-PCB	*Stenotomus chrysops*	P450, EROD, mRNA	5 mg/kg ip	0	Gooch *et al.*, 1989
2,3'4,4',5-PCB	*Stenotomus chrysops*	P450, EROD, mRNA	5 mg/kg ip	0	Gooch *et al.*, 1989
2,2',3,,4,4',5'-PCB	*Stenotomus chrysops*	P450, EROD, mRNA	5 mg/kg ip	0	Gooch *et al.*, 1989
2,2'3,3'4,4'-PCB	*Stenotomus chrysops*	P450, EROD, mRNA	5 mg/kg ip	0	Gooch *et al.*, 1989
Crude petroleum	*Pseudopleuronectes platessa*	AHH (BaP)		+	Truscott *et al.*, 1983
	Salmo salar	AHH (BaP)		+	Truscott *et al.*, 1983
	Tautogolabrus adspersus	AHH		+	Walton *et al.*, 1983
Oil spill	*Perca fluviatilis*	AHH, ECOD		+	Lindström-Seppä, 1988
PAHs	*Parophrys vetulus*	AHH	100 g sediment extract/kg bw	+	Collier and Varanasi, 1991
	Salmo trutta	AHH (BaP)		+	Payne and Penrose, 1975

Table 9.1 (continued)

Pollutant	Species	Indicator	Exposure		Reference
3-Methyl-cholanthrene	*Archosargus probatocephalus*	AHH	10 mg/kg ip	+	James and Bond, 1980
		AHH	20 mg/kg ip	+	Bend *et al.*, 1977
	Cyprinodon variegatus	AHH, ECOD, EROD	33 mg/kg ip	+	James *et al.*, 1988
	Fundulus grandis	AHH, ECOD, EROD	20 mg/kg ip	+	James *et al.*, 1988
	Oncorhynchus mykiss	AHH (BaP), PNAD	20 mg/kg ip	+	Förlin, 1980
	Oryzias latipes	AHH, ECOD, EROD	12 mg/kg ip	+	James *et al.*, 1988
	Paralichthys lethostigma	AHH, ECOD	10 mg/kg ip	+	James and Bend, 1980
	Pimephales promelas	ECOD, EROD	38 mg/kg ip	+	James *et al.*, 1988
	Poecilia reticulata	AHH, EROD	9 mg/kg ip	+	James *et al.*, 1988
	Salvelinus fontinalis	ECOD, AH, AE	0.35 mg diet x 9	+	Addison *et al.*, 1978
Benzo[*a*]pyrene	*Anguilla anguilla*	AHH (BaP), EROD	1 mg/kg ip	+	LeMaire-Gony and LeMaire, 1992
	Centropristris striatus	AHH		+	Fair, 1986
	Ictalurus punctatus	AHH	0.1 mg/kg ip	+	Fingerman *et al.*, 1983
	Oncorhynchus mykiss	AHH	30 mg/kg ip	+	Gerhart and Carlson, 1978
α-Naphthoflavone	*Lepomis macrochirus*	AHH	136 mg/l	+	Funari *et al.*, 1987
	Poecilia reticulata	AHH	136 mg/l	+	Funari *et al.*, 1987
β-Naphthoflavone	*Gadus morhua*	AHH, ECOD, EROD	100 mg/kg	+	Goksøyr *et al.*, 1987
	Ictalurus punctatus	EROD	50 mg/kg ip	+	Tate, 1988
	Oryzias latipes	EROD	10% in diet	+	Schell *et al.*, 1987
	Oncorhynchus mykiss	ECOD, EROD	100 mg/kg ip	+	Kleinow *et al.*, 1986
		EROD	100 mg/kg ip	+	Andersson *et al.*, 1985
		AHH, ECOD, EROD	100 mg/kg ip 500 mg/kg diet	+	Eisele *et al.*, 1984
	Oncorhynchus tshawytscha	CYP1A1 mRNA	50 mg/kg ip	+	Campbell and Devlin, 1997
Chrysene	*Oncorhynchus mykiss*	AHH	30 mg/kg ip	+	Gerhart and Carlson, 1978

Table 9.1 (continued)

Pollutant	Species	Indicator	Exposure		Reference
3-Trifluoromethyl -4-nitrophenol	*Oncorhynchus mykiss*	EROD	4.6 mg/l	+	Hewitt *et al.*, 1996
4-Nonylphenol	*Salmo salar*	CYP1A, EROD	125 mg/kg ip	-	Arukwe *et al.*, 1997b
Coal dust	*Oncorhynchus tshawytscha*	CYP1A1 mRNA	60 mg/l	+	Campbell and Devlin, 1997
Municipal wastewater	*Oncorhynchus mykiss*	AHH (BaP)		+	Förlin and Hansson, 1982
		PNAD		+	Förlin and Hansson, 1982
		EMND		-	Förlin and Hansson, 1982
		P450		+	Förlin and Hansson, 1982
Pulp mill	*Catastomus commersoni*	EROD		+	McMaster *et al.*, 1992, Gagnon *et al.*, 1994b Munkittrick *et al.*, 1994
		AHH (PPO/ BaP)		+	McMaster *et al.*, 1991, McMaster *et al.*, 1991
	Coregonus clupeaformis	EROD		+	Munkittrick *et al.*, 1992
	Coregonus lavaretus	EROD		+	Soimasuo *et al.*, 1995
	Lepomis macrochirus x cyanellus	EROD		-	Andersson *et al.*, 1987
	Myoxocephalus quadricornus	EROD		+	Servizi *et al.*, 1993
	Oncorhynchus tshawytscha	EROD		+	Campbell *et al.*, 1996
		CYP1A1 mRNA		+	Andersson *et al.*, 1988b
	Perca fluviatilis	EROD		+	Oikari and Jimenez, 1992
Juvabione/-dehydrojuvabione	*Oncorhynchus mykiss*	EROD	0.5	+	Martel *et al.*, 1997

Table 9.1 (continued)

Pollutant	Species	Indicator	Exposure	Reference
Norwegian fiord polluted	*Gadus morhua*	P4501A1, EROD	+	Goksøyr *et al.*, 1994
Puget Sound	*Parophrys vetulus*	AHH (BaP)	+	Johnson *et al.*, 1988 Collier *et al.*, 1992
	Pleuronectes vetulus	AHH	+	Johnson *et al.*, 1993
River Sava Yugoslav	*Barbus barbus*	AHH (BaP)	+	Kenzic *et al.*, 1983
	Chondrostoma nasus	AHH (BaP)	+	Kenzic *et al.*, 1983
	Cyprinus carpio	AHH	+	Kurelec *et al.*, 1981
	Leuciscus cephalus	AHH (BaP)	+	Kenzic *et al.*, 1983
River Durance, France	*Barbus barbus*	EROD	+	Vindimian *et al.*, 1991
	Chondrostoma nasus	EROD	+	Vindimian *et al.*, 1991
	Leuciscus cephalus	EROD	+	Vindimian *et al.*, 1991
Rotterdam harbour sediment	*Cyprinus carpio*	P450 1A, EROD	+	Van der Weiden *et al.*, 1993
San Francisco Bay/PCB	*Platichthys stellatus*	P450, AHH (BaP), EROD,	+	Spies and Rive, 1988; Spies *et al.*, 1988, 1990
Sewage Lagoon	*Cyprinus carpio*	P450	+	Sivarajah *et al.*, 1979
Tennessee stream/PCB	*Lepomis auritus*	P450, EROD,	+	Adams *et al.*, 1992

AE = aldrin epoxidase; AHH = aryl hydrocarbon hydroxylase; AnH = aniline hydroxylase; APND = aminopyrine N-demethylase; BaP = benzo[a]pyrene; CYP1A = cytochrome P4501A; CYP1A1 mRNA = cytochrome P4501A1 messenger RNA; ECOD = 7-ethoxycoumarin O-deethylase; EMND = ethylmorphine N-demethylase; EROD = ethoxyresorufin-O-deethylase; P450 = cytochrome P450 (used as indicator of P450 dependent enzyme activity); PBDE = polybrominated diphenylethers; PCN = polychlorinated naphthalenes; PNAD = p-nitroanisole-I = injection; O-demethylase; PPO = 2,5-diphenyloxazole; PROD = 7-pentoxyresorufin O-dealkylase activity; SER = increased smooth endoplasmic reticulum in liver cells; TCX = 2,3,6,7-tetrachloroxanthene. a using rainbow trout gonadal cell line (RTG-2)

The commonest measures of MFO activity are ethoxyresorufin-*O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) activity, while P450 content has also frequently been used as an indicator of MFO activity. Spies *et al.* (1990), in fact, found that P450 induction was a more sensitive indicator than EROD or AHH activity for organochlorines in the starry flounder in San Francisco Bay. By contrast in another Pleuronectiformes, the flounder *Platichthys flesus,* there was an increase in EROD activity 10 days after injection of 100 mg/kg body weight of the PCB Clophen A50, while total hepatic cytochrome P450 content remained unchanged. Increased smooth endoplasmic reticulum is also indicative of increased MFO activity, and its increase after exposure to organophosphates shows that these pesticides are active inducers of the enzyme (Arnold *et al.*, 1996a). MFO activity is, in fact, intimately involved in the toxicity of phosphorodithioate pesticides since it converts them into the sulphoxides which are responsible for the inhibition of acetylcholine esterase (Levi *et al.*, 1988).

These MFO enzymes may have significant substrate specificity, and while they have frequently been tested on some common pollutants such as PCBs and PAHs, there are many categories of pollutant which have not been examined at all. Even within the PCBs there are major differences in responsiveness. While intraperitoneal injection of scup (*Stenotomus chrysops*) with 3,3',4,4'-tetrachlorobiphenyl (congener 77) induced MFO as evidenced by increased EROD activity and P450, several penta- and hexachlorinated biphenyls (congeners 105, 118, 128, and 138) failed to do so (Gooch *et al.*, 1989). The lack of MFO inducing activity was correlated with chlorine substitution at any of the *ortho*-positions (2, 2', 6 or 6') which leads to loss of coplanarity of the two rings. The inhibiting effect of mono-*ortho*-chloro substitution was much more pronounced in scup than in mammals which suggests that the Ah receptor may have different ligand binding characteristics to that of mammals. Zabel *et al.* (1996) have suggested that induction of cytochrome P4501A mRNA in fish cell lines may be a better way of determining the relative potencies of polychlorinated aromatic congeners since it may avoid problems associated with the agonistic activity of the congeners which is apparent when EROD activity is used as the indicator. This specificity has obvious relevance to the use of MFO induction as a bioindicator since PCBs always occur in a polluted environment as a mixture of large numbers of congeners which clearly have very different responses in inducing MFO. Failure to induce MFO activity does not necessarily imply a lack in toxicity to other aspects of biological disruption. In fact, it may imply the opposite, since induction of MFO affords some protection by its detoxification.

A number of other aromatic xenobiotics also induce MFO. In addition to TCDD (2,3,6,7-tetrachlorodibenzo-*p*-dioxin), tetrachlorodibenzofuran and tetrachloroxanthene, which are associated with it in pulp mill effluents, are

potent inducers of MFO (Zabel and Peterson, 1996). Although it is often assumed that compounds such as these associated with the chlorinating process are responsible for the MFO activity of pulp mills, the enzyme is also induced by mills that do not use chlorine. This activity may be due to natural products from the wood such as juvabione and dehydrojuvabione (Martel *et al.*, 1997). Unlike PCBs and polychlorinated naphthalenes, however, the polybrominated diphenylether (Bromkal 70-5DE) did not induce EROD activity in the stickleback even though all three compounds were equally effective in decreasing spawning success and causing lipid accumulation in the liver (Holm *et al.*, 1993). Although polybrominated diphenylethers do not induce MFO activity, the polybrominated biphenyls do (Elcombe and Lech, 1978; Law and Addison, 1981) which suggests that it is the ether link rather than the presence of bromine which destroys the inducing activity. Although the bioaccumulation of dibenzothiophenols has been suggested as a biomarker of PAH exposure from certain sources of crude oil products (Krahn *et al.*, 1992) sulphur heterocycles such as dibenzothiophenone are unable to induce MFO activity (Vignier *et al.*, 1985).

Fish monooxygenase systems differ from mammals in having a high responsiveness to the PAHs (3-methylcholanthrene, β-naphthoflavone, benzo[*a*]pyrene etc.) but only a low responsiveness to the phenobarbitals (Goksøyr *et al.*, 1987; Stegeman and Kloepper-Sams, 1987). In mammals there are two groups of MFO; one involves cytochrome P450s that catalyse aniline hydroxylation and aminopyrine N-methylation and are induced by barbiturates or DDT, while the other involves cytochrome P448s and catalyse benzo[*a*]pyrene hydroxylation (aryl hydrocarbon hydroxylase) and ethoxyresorufin *O*-deethylation (EROD). Only this latter type appears to be induced in fish, although the former is present at low level (Addison, 1984). Although the cytochromes differ slightly in the wavelength of their absorbance, they are both generally referred to in the literature as P450s. In both cod and rainbow trout, injection with β-naphthoflavone, but not phenobarbital, induced ethoxycoumarin-*O*-deethylase (ECOD), EROD, biphenyl-4-hydroxylase and oxidised phenanthrene at the 1,2-position (Goksøyr *et al.*, 1987). Ethylmorphine-N-demethylase activity was, however, unaffected in cod and decreased in trout. β-naphthoflavone treatment also shifted hydroxylation of phenanthrene from the 9,10 to 1,2 positions. If such shifts also occur in steroid hydroxylation, this could have major implications for the endocrine system (See Section 9.3.5). Immunochemical studies using antibodies raised against the major P-450 induced in cod (P-450c) showed that while β-naphthoflavone increased the amount of immunoactive protein in liver slices of both species, there were significant species differences (Goksøyr *et al.*, 1987). This may explain the differences in relative toxicity and the extent of 2,3,7,8-TCDD induction of EROD in these two species (Hektoen *et al.*, 1994).

The teleost MFO has little activity against chlorinated hydrocarbon pesticides and, unlike mammals, is not induced by DDT, DDE or chlordane (Buhler and Rasmusson *et al.*, 1968; Addison *et al.*, 1977), although surprisingly it does convert aldrin to dieldrin in some (Addison, 1984) but not all species (Hektoen *et al.*, 1994). There may, however, be significant species differences since, unlike the zebrafish (*Danio rerio*), guppies (*Poecilia reticulata*) showed a proliferation of smooth endoplasmic reticulum (which normally precedes MFO induction) after DDT exposure (Weis, 1974). MFO was not, however, directly measured and even in the guppy the induction was much lower and took longer than in rats. The failure of the teleost liver to act on organochlorine pesticides may well increase the time for which these pollutants are available to fish tissue and hence increase their toxicity in comparison with mammals. Variations in hepatic MFO response to organochlorines may explain the differences in sensitivity of zebrafish and guppies to DDT (Weis, 1974).

The time course for induction of MFO in exposed fish is comparable to that of metallothionein and increases can be detected within a few days of exposure and may remain high for several weeks (Addison, 1984; Collier and Varanasi, 1991).

MFO induction is affected by reproductive status of the fish. This is not surprising since there are major seasonal changes in secretion of the sex steroids, the natural substrates for hepatic MFO. This may be reflected in sex differences in inducibility of MFO in response to xenobiotic exposure of sexually mature but not immature fish (Förlin and Lidman, 1979; Förlin, 1980; Förlin and Andersson, 1984). MFO and cytochrome P-450 is generally higher in mature males than mature females as result of differences in steroid hormone levels (Förlin and Andersson, 1984). Aryl hydrocarbon hydroxylase was lower in females with yolky eggs than in males and less mature female *Platichthys stellatus* and the decrease in AHH activity co-incided with the onset of yolk production (Spies *et al.*, 1988). Estradiol, but not testosterone, administration to juvenile trout prior to treatment with PCB decreased the extent of MFO induction (Stegeman and Kloepper-Sams, 1987). This sex difference was greater in fish than in mammals and, while in mammals the activity was regulated by testosterone, in fish estradiol appeared to the main steroid which affected its induction (Stegeman and Kloepper-Sams, 1987). There is, however, some conflicting evidence that testosterone or 11-ketotestosterone may in some cases be stimulatory, or may act synergistically with estradiol (Hanson, 1982; Pajor *et al.*, 1990). The suppressive role of estradiol in producing sex differences in cytochrome P450 inducible hepatic microsomal enzyme activity was confirmed by Pajor *et al.* (1990), who showed furthermore that different forms of P450 were affected to different extents. The suggestion by these authors that a pituitary factor, possibly growth hormone, also affected hepatic P450, further complicates the use of

MFO activity for pollution monitoring. Further evidence for the suppression of MFO activity by estrogens is provided by a recent study in which both EROD activity and steroid 6β, 16α and 17α-hydroxylases were suppressed by both estradiol and the estrogen mimic 4-nonylphenol (Arukwe *et al.*, 1997b).

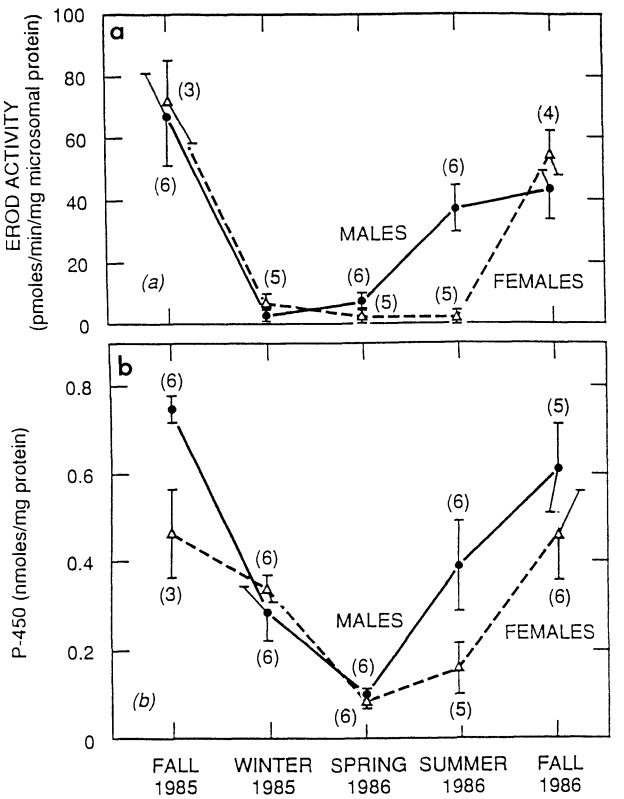


Figure 9.3. Changes in a) EROD activity and b) cytochrome P-450 levels in male (——) and female (- - - -) redbreast sunfish from a Tennessee river (mean ± SEM (n)). (Reprinted with permission from Jimenez, B. D., Oikari, A., Adams, S. M., Hinton, D. E. and McCarthy, J. F. (1990). Hepatic enzymes as biomarkers: Interpreting the effects of environmental, physiological and toxicological variables. In "Biomarkers of Environmental Contamination", (J. F. McCarthy and L.R. Shugart, Eds.), pp.123-142. Copyright CRC Press, Boca Raton FL.).

In some species, aryl hydrocarbon hydroxylase (AHH) shows seasonal changes in activity and declines at the onset of vitellogenesis (Hanson and Gustafsson, 1981; Koivusaari *et al.*, 1981; Walton *et al.*, 1983; Lindström-Seppä, 1985; Luxon *et al.*, 1987). Such seasonal changes in enzyme activity complicate its use as a bioindicator since white suckers showed differences in AHH activity between pulp mill effluent and a control site only during the

summer non-spawning period (Munkittrick *et al.*, 1991), while in English sole (*Parophrys vetulus*) there were differences in activity between regressed and vitellogenic fish taken from polluted, but not clean, sites in Puget Sound (Johnson *et al.*, 1988). The effects of season and sex on MFO activity as indicated by P450 levels and EROD activity is well illustrated by the redbreast sunfish (Figure 9.3) in which levels are lowest during gonadal recrudescence in spring. (Jimenez *et al.*, 1990). MFO activity decreases in fish held in captivity, but this may not be due solely to decreased xenobiotic exposure or changes in feeding status (Collier and Varanasi, 1991). EROD activity is also dependent both upon the temperature at which fish are held and that at which incubation was carried out (Förlin *et al.*, 1984; Anderson and Förlin, 1992). Temperature of acclimation had a more pronounced effect on fed than starved bluegill sunfish and temperature changes may be responsible for much of the effect of seasonality in some species (Figure 9.4) (Jimenez *et al.*, 1990). If MFO activity is to be used as a monitor of xenobiotic exposure it is clearly important that comparisons are made between populations that have had similar exposure to temperature, captivity stress, nutrition etc., and are of comparable sexual and reproductive status. This is frequently difficult in fish caught in the wild for comparison between clean and polluted sites.

While organochlorine pesticides, with the possible exception of aldrin, are unaffected by teleost MFOs, there have been few studies of the organophosphates. A combination of endosulfan (0.00005 mg/l) plus disulfoton (0-0.005 mg/l) increased activities of MFO but higher levels of disulfoton decreased the activity, probably due to lipid peroxidation and destruction of microsomal membranes (Arnold *et al.*, 1995). While endosulfan may be converted by the cytochrome P450 enzymes to the less toxic alcohol metabolites, disulfoton may be converted by oxidative desulphuration to the sulphoxide which is the metabolite responsible for the inhibition of acetylcholine esterase by the phosphorothionates. The effects of the pesticide combination on both the liver and the whole organism will therefore be a balance between their actions on the metabolising enzymes, changes in hepatic morphology and function and the increase or decrease in activities of their metabolites.

Although the liver is generally considered to be the major site of MFO activity, the enzyme is also present in a wide range of other tissues in addition to the gonads discussed above and in Section 9.4. Immunohistochemical staining with a CYP1A-specific antibody has been used to demonstrate enzyme activity in the gastrointestinal tract, gill, kidney, pancreas and spleen of adult fish as well as in the endothelium of post-hatched larvae (Lindström-Seppä *et al.*, 1994). It is also present in embryonic fish where it may be induced by the presence of pollutants in the yolk derived from maternal sources (Binder and Lech, 1984). During late embryonic development and at

the swim-up stage fish which had hatched from eggs spawned in Lake Michigan, and containing high PCB levels (> 2 mg/kg) had higher MFO activity than those from control fish. 7 months after hatching and culture in the laboratory, the MFO levels were the same in both groups of fish as a result of dilution of the maternally derived xenobiotics.

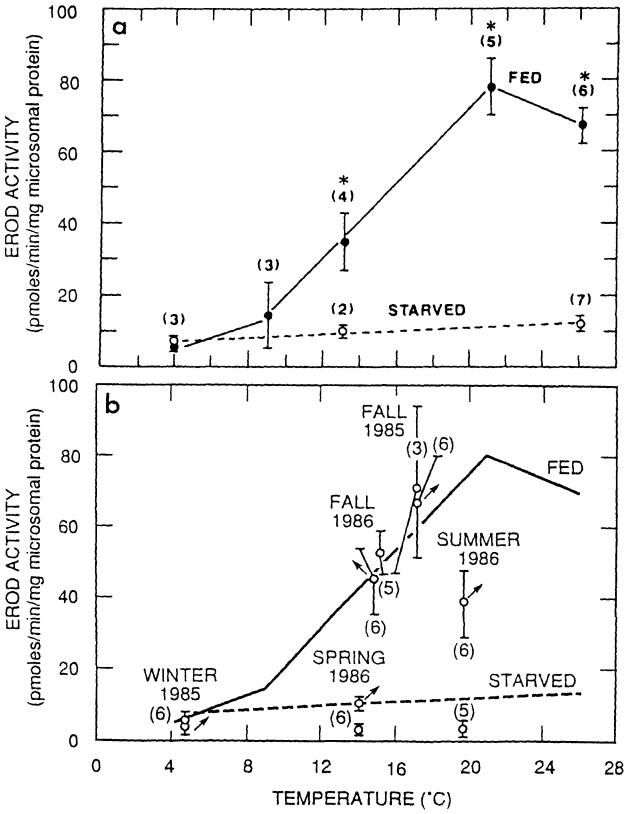


Figure 9.4. a) EROD activity (measured at 30°) of bluegill sunfish acclimated to different temperatures and fed *ad libitum* (——) or denied food for 2 weeks (- - - -), b) EROD activity of redbreast sunfish collected at different seasons in a Tennessee river (see Figure 9.3) plotted as a function of mean water temperature for the month they were collected superimposed on the data from Figure 9.4a . Open circle with arrow indicates a male group (Figure 9.3a is modified with permission from *Environmental Toxicology and Chemistry* 1988, The mixed function oxidase system of bluegill sunfish, *Lepomis macrochirus:* Correlation of activities in experimental and wild fish by Jimenez, B. D., Burtis, L. S., Ezell, G. H., Egan, B. Z., Lee, N. E., Beauchamp, J. J. and McCarthy, J. F. Volume 7, pp. 623-634. Copyright Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL 1988; Fig 3b is reprinted with permission from Jimenez, B. D., Oikari, A., Adams, S. M., Hinton, D. E. and McCarthy, J. F. (1990). Hepatic enzymes as biomarkers: Interpreting the effects of environmental, physiological and toxicological variables. In "Biomarkers of Environmental Contamination", (J. F. McCarthy and L.R. Shugart, Eds.), pp.123-142. Copyright CRC Press, Boca Raton FL.)

Although MFO is induced by a wide range of xenobiotics as part of the normal detoxification response, the response is reliant on the integrity of the detoxifying enzyme system. If the toxicant causes damage to the system before it can be removed, MFO activity may be decreased and may be one reason why the dose response curve becomes linear at high concentrations. In a polluted ecosystem there are a large number of toxicants, not all of which are deactivated by the MFO system, that may cause such disruption. Organotin compounds, for example, decrease EROD activity by inhibiting NADH or NADPH cytochrome c reductases (Fent and Stegeman, 1991; Fent and Buchemi, 1994). EROD activity was noticeably reduced at 0.01 mM (2.9 mg/l) and completely inhibited at 0.3 mM, which compares to the levels of 0.011 mM found in trout exposed to tributyltin (Martin *et al.*, 1989). This inhibition may in turn decrease the hepatic metabolism of the organotin itself to dibutyltin (Reader *et al.*, 1996). Clearly the presence of tributyltin in a polluted ecosystem will decrease the detoxifying activity of the liver towards other xenobiotics and would seriously disrupt the usefulness of MFO as a monitor for PCB and PAH exposure. Other heavy metals also inhibit EROD activity. Cupric, mercuric, and methymercuric ions (100 nM) all inhibited the β-naphthoflavone and benzo[*a*]pyrene induced EROD activity in *Dicentrarchus labrax*, but the effect was drastically reduced if glutathione was added suggesting that this may play a role in the first-line defence against heavy metal toxicity (Viarengo *et al.*, 1997). The interactions between pollutant mixtures of PAHs, PCBs and heavy metals which are normally present in polluted waterways and the P450, metallothionein and glutathione of the liver is clearly very complex and makes predictions of effects extremely difficult.

While pulp mill effluents containing high levels of chlorophenolics can induce MFO activity in caged fish downstream of the mill (Soimasuo *et al.*, 1995) such effluents may also act as hepatotoxicants which kill liver cells and thus reduce enzyme activity (Oikari and Jimenez, 1992). 3,3',4,4'-tetrachlorobiphenyl increased cytochrome P4501A content and EROD activity in fathead minnows (*Pimephales promelas*) at low concentration, but at levels above 1.8 μg/g liver EROD activity was decreased (Lindström Seppä *et al.*, 1994). Such effects clearly limit the quantitative usefulness of MFO as an indicator of pollution exposure.

In many pesticides, the active ingredient is often contaminated with a number of impurities resulting from its synthesis and it is diluted with a carrier that is claimed to be inert. While such impurities and carriers may be inactive against the target organism they be less inert as an endocrine disruptor of other, non-target, life. A lampricide containing 3-trifluoromethyl-4-nitrophenol (TFM), for example, was a potent inducer of mixed function oxygenase activity, but the MFO induction was not associated with the primary formulation since the pure compounds themselves failed to induce

activity (Hewitt *et al.*, 1996). Subfractions, containing a chloro-nitro-trifluoromethyl substituted dibenzo-*p*-dioxin and a range of chloro, and/or nitro, and/or trifluoromethyl substituted phenols, diphenyl ethers and dibenzo-*p*-dioxins had potent MFO inducing activity. Such impurities may be widespread in many pesticides and show that tests on purified ingredients alone are insufficient to detect biologically hazardous activity.

The wide variety of factors affecting MFO induction, particularly reproductive status, together with its lack of inducibility by a large number of classes of pollutants suggest that it is not suitable for routine use as a quantitative measure of either pollutant exposure or of endocrine disruption. Dose response curves often have limited range or lack reliability and require careful calibration (Addison, 1984; Collier and Varanasi, 1991). There may also be differences in responsiveness between different parameters to different pollutants so that fish held at different sites may show a different pattern of EROD activity and P450 level as a result of contributions from different proportions of the components of a mixture of pollutants (Goksøyr *et al.*, 1994). In the channel catfish, for example, there was no increase in P450 after PCB treatment even though EROD activity showed up to a fifteen-fold increase (Ankley *et al.*, 1986). Eggens *et al.* (1995) also found that there was no simple correlation between cytochrome P450 1A content or EROD activity and exposure of plaice and flounder to PCBs or PAHs in the southern North Sea. Consideration must, however, be taken of the large number of modifying factors. These may be accentuated by differences between species. Preliminary studies have shown that roach (*Rutilus rutilus*) held in a lake receiving bleached kraft mill effluent had higher EROD and PROD activity than those from a reference site, while perch (*Perca fluviatilis*) showed no difference between sites (Karels *et al.*, 1997). Perch and roach both showed a lower induction of MFO than bream, rainbow trout and whitefish when caged in the same area polluted by pulp mill effluents (Lindström-Seppä and Oikari, 1989b, 1990a, b). Provided, however, that a suitable baseline is used it may provide a useful indicator that wild fish have been exposed to certain pollutants, or be useful for laboratory testing of the potential harmful effects of a range of chemicals within certain classes.

The specificity of MFO to polyaromatic and chlorinated biphenyl structures makes it particularly useful as an indicator for the presence of such pollutants in aquatic ecosystems. It may also prove useful in screening the constituents of industrial effluents for potential toxicity to fish so that the process may be adjusted to give minimal impact. MFO induction has been used, for example, to determine which of the cooking liquors from kraft pulp mills poses the greatest hazard to downstream fish populations and has shown that the hazardous constituents are most probably natural wood components rather than the bleaching or extraction chemicals (Hodson *et al.*, 1997). While most studies have used either tissue slices or hepatocytes, which have

the disadvantage of variation between fish and laboratories, a more recent method using the PLHC-1 (*Poeciliopsis lucida*) hepatoma cell line have the advantage of standardisation and can be used to compare induction of cytochrome P4501A induction by different samples of polluted waters (Villeneuve *et al.*, 1997).

9.3.4 Other enzymes

In addition to MFOs, the liver also contains a number of other enzymes which are affected by xenobiotics and which may potentially have an effect on the endocrine system. These are illustrated by the limited examples shown in Table 9.2 and discussed below.

Table 9.2. Other hepatic enzymes induced by exposure to pollution

Pollutant	Species	Reference
Glutathione-S-transferase (GST):		
Benzo[*a*]pyrene	*Centropristus striata*	Fair, 1986
Diazinon+disulfoton	*Oncorhynchus mykiss*	Arnold *et al.*, 1995
PCB	*Ictalurus punctatus*	Ankley *et al.*, 1986
PCB/β-naphthoflavone	*Oncorhynchus mykiss*	Andersson *et al.*, 1985
UDP-glucuronyl transferase (UDPGT):		
PCB/β-naphthoflavone	*Oncorhynchus mykiss*	Andersson *et al.*, 1985
β-naphthoflavone	*Pseudopleuronectes americanus*	Foureman *et al.*, 1983
Dibenzanthracene	*Pseudopleuronectes americanus*	Foureman *et al.*, 1983
PCB	*Cyprinus carpio*	Sivarajah *et al.*, 1978b
	Ictalurus punctatus	Ankley *et al.*, 1986
	Oncorhynchus mykiss	Sivarajah *et al.*, 1978b

Glutathione-S-transferase (GST): In mammals these enzymes are usually located in the liver cytosol where they detoxify epoxides and diolepoxides (formed by aryl hydrocarbon hydroxylases) by addition of glutathione to form a thioether conjugate (Jakoby and Habig, 1980; Morganstern *et al.*, 1984; Mannervik, 1985). Conjugation with glutathione may also be important in the detoxification of mercuric ions. (Allen *et al.*, 1988). Most evidence suggests that these enzymes are not generally induced by PAH exposure in fish (Buhler and Williams, 1989; Foureman, 1989; Collier and Varanasi, 1991), although the sea bass (*Centropristis striata*) appears to be an exception (Fair, 1986). 2,3,7,8-TCDD also failed to induce the enzyme in rainbow trout or cod (Hektoen *et al.*, 1994). In rainbow trout, the PCB Clophen A50 or β-naphthoflavone only doubled activity of the enzyme compared to increases of

172- and 49-fold for EROD activity (Andersson *et al.*, 1985). Some of this variability may be accounted for by substrate specificities of different isozymes since Arochlor 1254 increased hepatic GST activity towards 1,2-epoxy-3-(*p*-nitrophenoxy)propane but not four other substrates (Ankley *et al.*, 1986). Even with this substrate, increases were only 20% which is minute compared to those in MFO activity. Cadmium inhibited both glutathione and glucuronide conjugation (Förlin *et al.*, 1986), so the combined effects of inorganic and organic contaminants may be difficult to interpret.

UDP-glucuronyl transferase (UDP-GT): This conjugating enzyme converts alcohols to glucuronides which are water soluble and readily excreted. In fish, in contrast to mammals, these enzymes are present in the gonads and there is evidence that in some species they may act as pheromones (see Chapter 4). Any pollutant that affects hepatic UDP-GT activity may therefore also affect the gonadal enzyme and possibly interfere with chemical signalling between the sexes or balances of free and conjugated steroids within the gonad where the enzyme may provide a protective role. UDP-GT activity was increased in both trout and carp by Arochlor 1254 administered weekly at 25 mg/kg bw for 4 weeks (Sivarajah *et al.*, 1978a). In rainbow trout there was a marked increase in UDP-GT activity 1-4 weeks after injection of β-naphthoflavone or the PCB Clophen A50, but the time course of the induction and its extent was dependent on both the inducer used and the substrate for conjugation (Andersson *et al.*, 1985). By contrast, another study by Castrén and Oikari (1987) suggests that polychlorinated biphenyl (Pyralene 3010; 2 mg/l) does not affect UDP-GT activity. These differences clearly confuse the issue and, since PCBs also induced activity towards *p*-nitrophenol but not to 1-naphthol, suggests that in fish as in the rat, there are multiple forms of UDP-GT (Andersson *et al.*, 1985). While the response of UDP-GT is less marked than monooxygenase activity as determined by EROD, activity remains high for longer (Andersson *et al.*, 1985).

Pulp mill effluent has a variable effect on UDP-GT activity which is probably dependent upon the exposure conditions (Andersson *et al.*, 1987; Soimasuo *et al.*, 1995). 2,4,6-Trichlorophenol (0.2 mg/l), pentachlorophenol (0.02 mg/l) and dehydroabietic acid (1.55 mg/l) which are common toxicants in effluents of the pulp and paper industry all inhibited UDP-GT activity (Castrén and Oikari, 1987). There may also be species differences in inducibility of the enzyme since it was induced in rainbow trout, but not cod microsomal preparations (Goksøyr *et al.*, 1987).

Unlike many enzymes, UDP-GT activity was reported to be unaffected by cadmium (0.75 mg/l) or zinc (1.25 mg/l), but was significantly increased by the slimicide N-methyldithiocarbamate (0.07 mg/l; Vapam) (Castrén and Oikari, 1987).

Epoxide hydroxylase: The microsomal form of the mammalian enzyme has greatest activity towards PAHs and converts epoxides (formed by aryl

hydrocarbon hydroxylases) to diols (Oesch and Golan, 1980). In contrast to mammals, fish appear to be refractory to induction of this enzyme (Andersson *et al.*, 1985; Buhler and Williams, 1989; Foureman, 1989; Collier and Varanasi, 1991).

Miscellaneous other enzymes: A range of other enzymes are affected by exposure of fish to pollutants which may result in either altered detoxification or general cellular metabolism. Mercury inhibited activities of lactate, pyruvate and succinate dehydrogenases in liver and a range of other tissues of *Channa punctatus* (Sastry and Rao, 1981). Lipid peroxidase activity (as indicated by increased malondialdehyde production) in Atlantic croaker liver and ovary was increased by both cadmium (5 mg/l for 6 days + 1 mg/l for 33 days) and by the PCB Arochlor 1254 (4.2 mg/kg body weight/day for 17 days) (Wofford and Thomas, 1988a; Thomas and Wofford, 1993). It is however important to note that age and nutritional status can also affect this enzyme (Wofford and Thomas, 1988b). By contrast, in the same species cadmium decreased, while the PCB increased, glutathione peroxidase activity (Thomas and Wofford, 1993). Since this enzyme protects against oxidative damage, this may be a primary cause of some of the cellular injury caused by pollutants. Liver catalase activity has also been used as an index of cadmium poisoning in the killifish (*Fundulus heteroclitus*) since it was inhibited by both *in vivo* and *in vitro* exposure to the metal at concentrations above 1 mg/l (Pruell and Engelhardt, 1980). Pre-exposure to the metal prevented catalase inhibition at higher subsequent doses, but there was no reliable dose response relationship.

9.3.5 Effects on steroid metabolism

In vertebrates, the liver is the main site of deactivation of steroid hormones which generally involves hydroxylation or reduction of ketonic groups, and subsequent glucuronidation or sulphation of these alcohols. In fish, the situation is more complex since these transformations may also take place in the gonads themselves (Kime, 1993). 5α- and 5β-reduced and, 6- and 7-hydroxylated metabolites together with their glucuronides and sulphates are commonly found in incubations of gonads of a variety of teleost fish and may play a role in rapidly removing hormones from target sites within the ovary and testis. Furthermore some of these products may have pheromonal activity and be involved in prespawning behaviour (see Chapter 4). Gonadal reductases are vital for the synthesis of such essential hormones as estradiol, testosterone, 11-ketotestosterone, 17,20βP and 20βS, while hydroxylation by the P450 mixed function oxidase system is essential for a large part of the biosynthetic sequence. The importance of the mixed function oxidase is clear from reference to Figure 4.3 in which the 20,21-lyase, 17,20-lyase and the 11β, 17α, 21-hydroxylases are all P450 dependent MFOs. It is therefore clear

that any of the pollutants which affect the steroid metabolising enzyme systems of the liver will have similar effects in the gonads and the interrenal gland. Together such perturbations of metabolism of hepatic, interrenal and gonadal enzymes may have a marked effect on the hormonal balance and, by feedback mechanisms, on the functioning of the pituitary and hypothalamus.

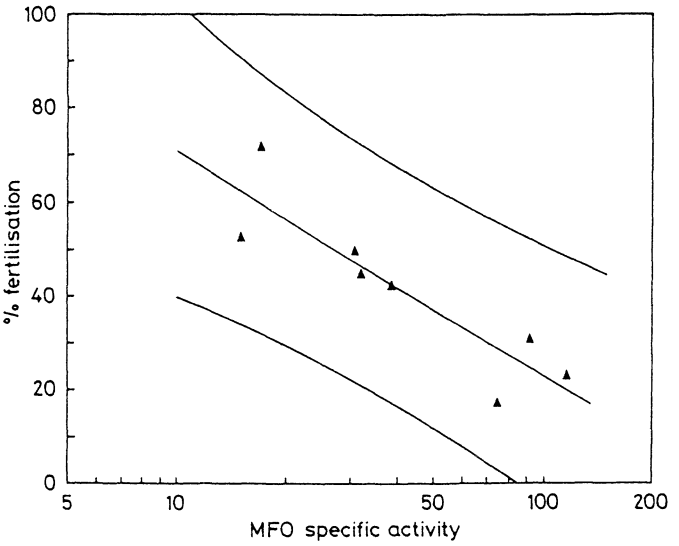


Figure 9.5. Correlation between mixed function oxygenase activity and percentage fertilisation of eggs from starry flounder collected in San Francisco Bay. (Reprinted from *Mar. Environ. Res.* **14**, 426-428. Spies, R. B., Rice, D. W. and Ireland, R. R., Preliminary studies of growth, reproduction and activity of hepatic mixed-function oxidase in *Platichthys stellatus*, Copyright 1984 with kind permission of Elsevier Science Ltd, The Boulevard, Langford Lane, Kidlington 0X5 1GB, UK).

In many of the studies described in this volume, it is not at all clear whether the primary effect of a pollutant leading to inhibition of reproduction is exerted on the pituitary, the gonad or the liver since the outcome is very similar in many cases. Hepatic enzyme activity is more frequently used as a monitor of pollution exposure than of its effects on steroidogenic enzymes. Whether it is the hepatic or gonadal enzyme that is affected by the pollutant, the result will be a perturbation in steroid balance within the fish which may result in disturbance of normal reproductive function. All of the chemicals in Tables 9.1 and 9.2, must therefore be considered to be possible causes of disruption to the reproductive endocrine system, and any xenobiotic which induces MFO activity must be considered to be a potential endocrine disruptor. Evidence of such a relation between MFO induction and reproductive dysfunction was observed in the correlation between MFO activity and percentage fertilisation of eggs of the starry flounder in San

Francisco Bay which is polluted by PCBs and PAHs (Spies *et al.*, 1984, 1985) (Figure 9.5). Imposex in marine gastropods has been linked to organotin inhibition of the aromatase enzyme, which converts testosterone to estradiol, as a result of destruction, or inhibition of the cytochrome P450 enzyme (Fent, 1996). It has yet to be confirmed that organotin can act in a similar fashion in fish, but if it does it would be expected to have equally profound effects on reproductive development and function.

It is not only the activity of the MFO enzymes that may be affected by pollutants. The nature of the metabolite may also change with pollution exposure, but changes may be species dependent. In cod, for example, there was a shift from 17-hydroxysteroid oxidoreductase activity to 16-hydroxylation with androstenedione substrate after β-naphthoflavone exposure, while in rainbow trout the pattern of metabolism was unchanged (Goksøyr *et al.*, 1987). This may be due to changes in relative activities of reducing and hydroxylating enzyme activities since changes in the regiospecific hydroxylation of phenanthrene substrate occurred with both species. By contrast to this, trout exposed to municipal wastewater showed an increase in 17β-HSD activity while that of the 6β-hydroxylase decreased (Förlin and Hansson, 1982). Since there is a sex dependent difference in activities in these enzymes in unexposed fish, such changes may be of physiological significance to the fish. Chlorinated paraffins caused only a marginal shift from 6β-hydroxylase and 5α/β-reductase activity in female flounder (*Platichthys flesus*) which was little different from that induced by the change from seawater to brackish water (Haux *et al.*, 1982). While PCBs increased progesterone hydroxylase activity in the hepatopancreas of carp (Yano and Matsuyama, 1986), the significance of this is not clear since the metabolites were not identified and may equally well have been potentially active metabolites as deactivation products. PCBs, polychlorinated naphthalenes and polybrominated diphenylethers, however, increased ovarian progesterone 6β- but not 17-hydroxylase activity in rainbow trout and stickleback (Holm *et al.*, 1994) indicating a perturbation in the balance of ovarian steroids. It is not clear whether the lack of correlation between hepatic EROD activity and the ovarian hydroxylases reflects tissue or substrate differences, but it does suggest that the steroid deactivating enzymes are stimulated more than those involved in biosynthesis. Although a contaminated-sediment extract had no effect on estradiol metabolism in English sole, it increased the flow of bile which may account for the decreased plasma concentrations of this steroid (Stein *et al.*, 1991).

9.3.6 Effects on xenobiotic metabolism

In the sections above it has been shown that a wide range of pollutants affect the activities of the xenobiotic deactivating enzymes in the liver. PCBs

and dioxins in particular can induce mixed function oxidase activity while heavy metals may induce metallothionein activity. This will increase the rate of metabolism of these compounds and therefore their rate of removal from the organism. In some cases, however, it has been noted that above a certain concentration there is a decrease in activity as a result of either cellular damage or deactivation of the enzyme. At such concentrations the effect on the organism will be disproportionate to the increase in concentration and may result in permanent damage. Many pesticides do not induce increased enzyme activity and damage may result at much lower concentrations. It is in this context that the combinations of xenobiotics found in polluted aquatic environments may be most dangerous. While the ubiquitous PCBs may cause little damage on their own at low concentrations, since they induce MFO activity which will in turn deactivate them, they frequently occur in sediments in association with DDT and its derivatives which cause morphological damage. A cocktail of DDTs, PAHs, PCBs and heavy metals as is found in many areas subjected to long-term pollutant release would be expected to have an effect much greater than the sum of the individual components. There is little data on such interactions, but a combination of cadmium and benzo[*a*]pyrene had a much more harmful effect on liver morphology and showed greater increase in MFO activity than either pollutant alone (LeMaire-Gony and LeMaire, 1992). Although the toxicity of such multicomponent systems is extremely difficult to study, it is important that they be considered when setting legal limits for water quality. The presence of organotin in many aquatic environments further complicates matters since it has an inhibitory, rather than stimulatory, action on MFO activity (Fent, 1996).

9.4 Vitellogenesis

9.4.1 Introduction

A major role of the liver during ovarian recrudescence is the synthesis of the yolk protein, vitellogenin. This is stimulated by estradiol secreted by the ovary in response to pituitary gonadotrophins. Decreased vitellogenesis can therefore result either from a decreased pituitary or ovarian function, or from a primary failure of the hepatic enzymes necessary for synthesis of vitellogenin itself. In a large number of studies (see Appendix) a decreased gonadosomatic index, indicative of low vitellogenin production, is observed in response to exposure to xenobiotics. Such measurements, which are clearly very easy to make and require the minimum of laboratory facilities, provide valuable preliminary information that pollution is having an effect on reproduction (Kime *et al.*, 1998). It does, however, provide little information

on the site of action within the female reproductive endocrine system. If the decreased vitellogenin resulting from pollution exposure can be restored by treatment with estradiol or gonadotrophin, then clearly failure is not at the hepatic level (Chakravorty *et al.*, 1992). Histological study can provide further information by showing whether there is cellular disruption of the liver during vitellogenesis which might be the proximate cause of failure of vitellogenesis. Further studies then become necessary to clarify whether this is due to morphological disruption or to malfunction of specific enzyme systems.

Livers of both male and female fish have receptors for estrogens and are both capable of producing vitellogenin. Since estrogens are the only known stimulus for the hepatic production of vitellogenesis, the presence of vitellogenin in male plasma is indicative of exposure to estrogen. The measurement of vitellogenin in the plasma of male or juvenile fish, therefore, provides a ready means of testing for the presence of environmental estrogens, or the estrogenic activity of specific chemicals (Kime *et al.*, 1998). Although the first evidence of induction of hepatic vitellogenesis in fish by a xenobiotic (β-HCH) was described as long ago as 1985 (Wester *et al.*, 1985) the implications of this were largely ignored until the recent concern about environmental estrogens emerged.

Compounds such as the organochlorine pesticides and PCBs are particularly interesting since they are known to disrupt both ovarian and pituitary function, and some are estrogenic in mammals. Their action within the intact fish may therefore be a combination between toxic effects on the gonad, pituitary and liver, and estrogenic activity resulting in the direct stimulation of hepatic vitellogenesis. The overall result will be determined by the relative toxicities and estrogenicities at these different levels. Estrogenic activity may also cause indirect effects by positive or negative feedback on the hypothalamic-pituitary axis.

9.4.2 Disruption of hepatic vitellogenesis by pollutants

Decreased plasma vitellogenin, like a low gonadosomatic index, does not necessarily demonstrate a disruption of hepatic function and in many cases is more likely to result from low ovarian estrogen secretion. Cadmium, malathion and 3-methylcholanthrene, for example, decreased both estradiol and vitellogenin in *Monopterus albus* (Singh *et al.*, 1989) which suggests that the decreased vitellogenin was a result of inhibited estrogen production. Similar suggestions have been made for the decreased vitellogenin in cyanide exposed rainbow trout (Ruby *et al.*, 1986, 1993b) and cadmium exposed winter flounder (Pereira *et al.*, 1993). The fact that mercury and cadmium caused ovarian lesions as well as decreased plasma vitellogenin in *Lepidocephalichthys thermalis* (Victor *et al.*, 1986) also suggests that the primary disruption is at the ovarian or pituitary level rather than on hepatic

synthesis. In *Clarias batrachus* the endosulfan induced reduction in vitellogenesis was reversed by treatment with estradiol (Chakravorty *et al.*, 1992) indicating that the pesticide acted primarily on the ovary or pituitary rather than directly on the liver. Other examples of decreased vitellogenin can be found in the Appendix.

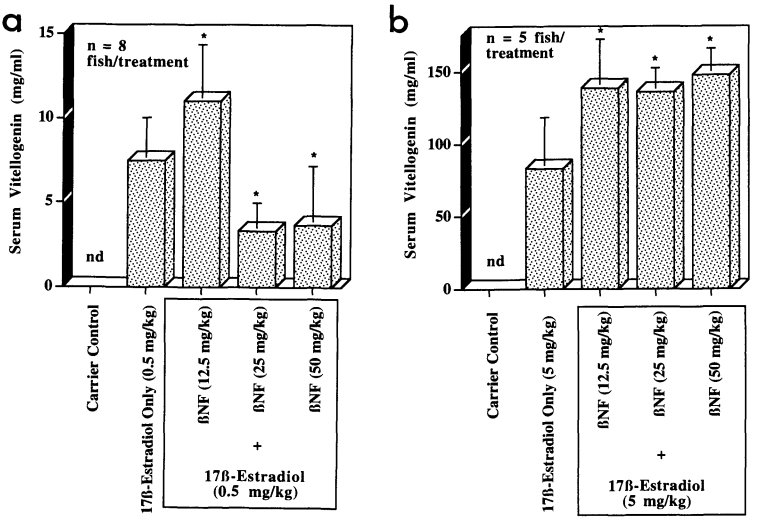


Figure 9.6. Juvenile rainbow trout injected with a) estradiol (0.5 mg/kg) in cod liver oil or estradiol (0.5 mg/kg) + various doses of β-naphthoflavone or b) estradiol (5 mg/kg) in cod liver oil or estradiol (5 mg/kg) + various doses of β-naphthoflavone. Vitellogenin was measured by ELISA 8 days after treatment. * indicates a significant difference from the estradiol positive control. (Reproduced from Anderson, M. J., Olsen, H., Matsumura, F. and Hinton, D. E. (1996). *In vivo* modulation of 17β-estradiol induced vitellogenin synthesis and estrogen receptors in rainbow trout liver cells by β-naphthoflavone. *Toxicol. Appl. Pharmacol.* **137**, 210-218, by kind permission of Academic Press, San Diego and the author).

Of more concern in the present context are the direct effects of pollutants on liver function which cause disruption of vitellogenin synthesis. Such effects may act at the level of the estrogen receptor, for example by antiestrogens blocking the receptor sites, on the expression of the vitellogenin gene, or on the presence of the various elements necessary for the production of vitellogenin. Although there is ample evidence for such disruption, the mechanisms are less clear. An interesting recent study, however, has related the modulation of estradiol-induced vitellogenin synthesis by xenobiotics to their ability to induce cytochrome P4501A1. *In vitro* studies with rainbow trout liver cell cultures showed that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzofuran, 2,3,4,7,8-pentachlorodibenzofuran and β-naphthoflavone were estrogenic, while the PCB 2,3,4,4',5-

pentachlorobiphenyl was estrogen potentiating (Anderson *et al.*, 1996a). In a further study (Anderson *et al.*, 1996b), β-naphthoflavone either inhibited or potentiated the effect of estradiol on vitellogenin synthesis depending on the relative amounts of each used (Figure 9.6). The anti-estrogenic effect of β-naphthoflavone was attributed to action on the Ah-receptor, which is involved in CYP1A1 gene expression (Wang *et al.*, 1993), and may also decrease binding of estradiol to its receptor. The estrogen potentiating effect may be due to alteration of cellular protein phosphorylation via an Ah-receptor mediated mechanism as shown for TCCD in mouse liver (DeVito *et al.*, 1994). The balance between the anti-estrogenic and estrogen potentiating mechanisms will clearly result in varying effects on vitellogenin synthesis and, since estradiol concentration varies seasonally, the effect of MFO inducers may be different at different stages of the reproductive cycle. Since the estrogen receptor is also present in the hypothalamus and pituitary, any of the MFO inducers (Table 9.1) may modulate activity at these sites as well as in the liver. Xenobiotic induction of MFO, or alteration of vitellogenin synthesis may therefore be indicative of more profound disruption of the reproductive endocrine system.

Such direct effects are clearly demonstrated when hepatic tissue or hepatocytes are examined *in vitro*. Both basal and estrogen stimulated production of vitellogenin by hepatocytes of female goldfish was decreased in a dose dependent manner by addition of 0.1-100 nM mercuric chloride (0.02-20 μg/l Hg) or cadmium chloride (0.01-29μg/l Cd) (Yao *et al.*, 1996). Copper and zinc also disrupted vitellogenin synthesis, but no clear dose response pattern was observed. After 10 days of treatment the response to estradiol was restored by removal of copper and zinc, but not cadmium and mercury suggesting a different site of action of the metals. The authors suggest that cadmium and mercury act at the transcriptional level to reduce VTG mRNA production, while zinc and copper act at the receptor level to inhibit VTG gene activation by estradiol. Significantly, perhaps, copper and zinc are essential trace elements in many biological processes, while cadmium and mercury have no natural biological function. Inhibition of both transcription and translation by cadmium as a cause of decreased vitellogenin synthesis in response to estradiol was confirmed by Olsson *et al.* (1995). Furthermore, translation and transcription of metallothionein synthesis was also reduced by this metal when it was injected into rainbow trout with estradiol. After such co-injection cadmium bound to high-molecular-mass cytosolic proteins rather than metallothionein to which it was only gradually redistributed. This redistribution led to an increase in metallothionein and co-incided with induction of vitellogenin mRNA, showing that cadmium inhibits transcription of the vitellogenin gene. The repressed induction of metallothionein by the binding of cadmium to these non-metallothionein proteins results in an increased toxicity of this metal, but preinduction of metallothionein by zinc

injection decreased the sensitivity of the fish to cadmium and decreased the repression of vitellogenin mRNA. Zinc did not affect the estradiol induced synthesis of vitellogenin, which is not surprising since it is an essential element in such synthesis. Since the non-metallothionein cytosolic proteins, but not metallothionein itself, are induced by estradiol, it follows that cadmium will be more toxic to general hepatic function during vitellogenesis than during the spawning and regressed phases of the normal reproductive cycle when estradiol production is low.

A direct effect of cadmium on the liver was also shown by the reduction in the RNA to DNA ratio to half that of the control value after injection of 2 mg cadmium per kg body weight in estrogen injected flounders (*Platichthys flesus*) which co-incided with a significant reduction in plasma vitellogenin concentration (Povlsen *et al.*, 1990). This data again suggests that cadmium interferes with protein synthesis at the transcriptional level.

Calcium is an essential component of vitellogenin so it is not surprising to find that disturbance of normal calcium homeostasis can cause disruption of vitellogenin production. Female rainbow trout exposed to 10μg/l of cadmium for four weeks during vitellogenesis had a complex hypocalcaemic response, resulting from decreased free plasma and protein bound calcium, decreased plasma vitellogenin, and reduced binding of calcium to vitellogenin (Haux *et al.*, 1988). Although these disturbances were believed to result from the effects of cadmium on calcium regulating tissues such as gills, intestine and kidneys, their ultimate effect is to decrease yolk production and incorporation. Exposure of rainbow trout to 0.01 mg/l cyanide during early vitellogenesis also depressed plasma calcium to below the levels required for the production of exogenous yolk by the liver (Da Costa and Ruby, 1984). Atlantic salmon, in contrast, showed increased plasma vitellogenin in response to cyanide exposure but since hepatic levels remained unaffected it was suggested that the toxicant might be decreasing uptake by the oocytes as a result of action on the pituitary (Ruby *et al.*, 1987).

Disturbance of calcium homeostasis may also account for the decreased vitellogenin production of fish exposed to acid waters low in calcium. At pH 5.0 and 0.5 mg/l calcium plasma estradiol, vitellogenin and calcium levels in brook trout were significantly lower than in controls held in neutral water with high calcium (pH 6.5, 7.5 mg/l Ca) (Mount *et al.*, 1988a). Neither low calcium nor low pH alone had any effect. The presence of aluminium also contributed to dysfunction which appeared to depend on the length of exposure or the stage of ovarian development. There was a parallel disturbance in plasma sodium, haematocrit and osmolarity, but it is not clear how much of this was a direct effect on osmoregulation and how much was due to secondary effects resulting from stress. Vitellogenin levels were also lower in rainbow trout at pH 4.5 than in fish held at pH 5.6 or 7.6 (Roy *et al.*, 1990). These authors also suggest that acid conditions may modify the

chemical nature of the yolk so that although eggs are spawned normally the yolk cannot be successfully utilised by the embryo. This suggestion is supported by the finding of Gunn and Keller (1984) and Mills (1984) that although acid stressed populations of lake trout spawned normally, there were no emergent fry as a result of mortality in early life stages. An examination of such changes in the yolk or vitellogenin after pollution exposure would be of considerable interest.

Hepatic damage not surprisingly also leads to decreased synthesis of vitellogenin as found in winter flounder collected from chemically contaminated areas of Boston Harbour which had gross liver lesions (Pereira *et al.*, 1992).

9.4.3 Changes in hepatic lipids

In addition to the changes in vitellogenin production during the female reproductive cycle there are parallel changes in hepatic lipid content reflecting the changes in pituitary secretion. Both the lipid content and the nature of its constituents can be affected by pollutants, although it is unclear what impact this has on the fish as a whole. Since phospholipids are vitellogenin precursors and cholesterol a precursor of the reproductive steroids, perturbations in their balance might have significant effects on reproduction. 0.044 mg/l mercury caused a significant increase in hepatic lipid of *Notopterus notopterus* exposed for 30 days (Verma and Tonk, 1983) together with decreased ovarian content. γ-BHC decreased hepatic concentrations of phospholipids in *Heteropneustes fossilis* as a result of an arrest in synthesis and translocation to the ovary (Singh and Singh, 1992b). Since lipid synthesis is under gonadal steroid control the authors suggested that the dislocation was the result of pesticide action at the pituitary level since the response was abolished by simultaneous administration of gonadotrophin. Cythion and hexadrin also decreased hepatic lipid content in this species during the preparatory, prespawning, and late postspawning phases (Singh and Singh, 1980d) but changes were more ambiguous during the spawning phase (Singh and Singh, 1980a, d). Using ^{14}C-acetic acid, Singh and Kime (1994) showed that 4-week exposure of goldfish to γ-HCH altered the distribution of acetic acid incorporation among the different lipid fractions with increased uptake into total lipid, free fatty acid and cholesterol esters, but decreased uptake into the triglyceride fraction indicating that γ-HCH promoted esterification of free cholesterol, but inhibited esterification of free fatty acids into mono-, di-, and triglycerides. In *Heteropneustes fossilis* the effect of γ-BHC on hepatic lipid balance varied with the stage of the ovarian cycle (Singh, 1992). The pesticides may therefore affect the synthesis and release of lipids required for ovarian recrudescence as well as their transfer from liver to ovary. Changes in hepatic cholesterol were also found after exposure to phenol, ammonia and

sulphide which are present in the effluent of jute and paper mills (Bhattacharya *et al.*, 1984; Kumar and Mukherjee, 1988).

9.4.4 Vitellogenin as a monitor of environmental estrogens

Since estrogens are the only significant stimulant of hepatic vitellogenesis (see Bromage and Cumaranatunga, 1988) the liver provides a suitable system to determine both the estrogenicity of pollutants in the laboratory and whether wild fish have been exposed to anthropogenic estrogens in their natural habitat. In an early study Wester *et al.* (1985) showed that guppies exposed to β-HCH, a minor constituent of the pesticide lindane, had changes in the liver which were attributed to the estrogenic activity of this organochlorine since similar changes were found on exposure to estradiol. In both cases high levels of vitellogenin were identified in the plasma and in sections of hepatic tissue. Changes characteristic of estrogen exposure were also found in the pituitary and testis. These effects were apparent at levels of 0.1 mg/l. Elevated plasma vitellogenin was also found in Atlantic salmon fed a diet of Baltic herring oil containing 60 ng/l dioxin (Von der Decken *et al.*, 1992). The increase was, however, relatively small in the mature females used and unfortunately the effect on males or juveniles in which any vitellogenin production would have been attributable solely to the diet was not examined. The food used was taken from Baltic herring which form a major part of the natural diet of the salmon, and while this clearly shows that wild salmon in the Baltic may suffer endocrine disturbance under wild conditions it is not possible to attribute the effects solely to dioxin since other contaminants would also be present. Baltic fish also contain particularly high levels of both PCBs and DDT which have estrogenic activity (Chapter 3; Colborn and Clement, 1992). Direct evidence that organochlorines are estrogenic in fish was provided by the elevation of vitellogenin after dietary exposure of juvenile rainbow trout (Donohoe and Curtis, 1996). A diet containing 0.4 mg/kg per day of chlordecone elevated vitellogenin to 0.9 μg/l and accumulated the pesticide in the liver at concentrations of 16 μg/g. Injection of trout with 5, 15, or 30 mg/kg *o,p'*-DDT and *o,p'*-DDE but not *p,p'*-DDE resulted in elevated plasma vitellogenin (23-24 μg/l) at sampling 14 days later. In comparison, injection of 5 mg/kg estradiol elevated vitellogenin to 640 μg/l. Further study showed that chlordecone, *o,p'*-DDT and *o,p'*-DDE but not *p,p'*-DDE exhibited a low binding affinity for hepatic cytosolic estradiol binding sites, confirming the structural requirements for estrogenicity found in mammals (Nelson, 1974; Nelson *et al.*, 1978). The hepatic concentrations of the DDTs (14 μg/g) in these experiments, estimated by using [^{14}C]*p,p'*-DDE, were much higher than found in any wild fish (Chapter 3). cDNA probes have recently been introduced to detect induction of vitellogenin by xenobiotics (Ren *et al.*, 1996). In contrast to the findings of Donohoe and Curtis (1996) hepatic

vitellogenin mRNA was induced in rainbow trout injected with 22 or 44 mg/kg *p,p'*-DDE (Ren *et al.*, 1996). The probe was also used to test the estrogenic activity of nonylphenol which induced vitellogenin mRNA at only 0.01 mg/l (Lech *et al.*, 1996). The EC_{50} of 0.014 mg/l was well below the LC_{50} of 0.19 mg/l for this compound, indicating estrogenic activity at concentrations well below those that would cause mortality.

Pulp and paper mill effluent contains a large number of chemicals which disrupt the endocrine system of fish. Some of these, like the organochlorines, may be expected to have estrogenic activity, while other components may have masculinising activity (see Chapters 6 and 7). Effluents from such mills contain, in addition to the many synthetic chemicals, a range of natural compounds which originate from the trees themselves. Bacterial conversion of the plant sterol, β-sitosterol, has been suggested as a cause of the androgenic activity of some effluents (Chapter 6). Recent studies (Tremblay *et al.*, 1995; Mellanen *et al.*, 1996; MacLatchy *et al.*, 1997) have suggested that β-sitosterol itself has endocrine disrupting activity. β-sitosterol induced vitellogenin gene expression in the livers of juvenile rainbow trout, but surprisingly the response of diethylstilbestrol (DES) at the same concentration (45 mg /kg feed) was only 3-fold higher (Mellanen *et al.*, 1996). After intraperitoneal administration of 10 mg of diethylstilbestrol or β-sitosterol, the response of the former was 6 fold greater. Since β-sitosterol differs from cholesterol only by addition of an ethyl group at position 24 on the side chain, its estrogenic activity is surprising. It is important to note that the commercial β-sitosterol used in this study is only >90% pure (with 6.6% kampesterol), and the possibility that the effect results from traces of unrelated impurities of high estrogenic activity cannot be entirely eliminated. Pine and spruce contain a number of stilbenes, which have close structural similarities to the synthetic estrogen diethylstilbestrol which has been implicated in cases of severe disruption of mammalian endocrine function (Colborn and Clement, 1992). In breast cancer cell lines the stilbenes isohapontigen and isohapontin were estrogenic at only 10-15 pM (3 μg/l), while pinosylvin and β-sitosterol, were active at 1 μM (300 μg/l) (Mellanen *et al.*, 1996). The stilbenes piceatannol and resveratrol showed no activity at the concentrations tested, and DES was not tested. The weakness of *in vitro* testing using cells lines was demonstrated by the estrogenicity of β-sitosterol in T-47D, but not in MCF-7 cells and may suggest that metabolism is required to produce activity. Pinosylvin was not active in the juvenile trout test, but the more "estrogenic" isohapontin and isohapontigen were not tested. Further evidence of the estrogenic activity of β-sitosterol comes from receptor studies in which it had a relative potency only slightly less than that of nonylphenol (0.001) in displacing ^{3}H-estradiol from rainbow trout liver receptors (Tremblay *et al.*, 1995).

Elevated plasma vitellogenin may not, however, always be attributable to estrogenic stimulation since although plasma concentrations were higher in

females taken from a polluted area near New York than in a clean area during the spawning season no such differences were observed in spawned females or in small immature fish (Pereira *et al.*, 1992). The high prevalence of liver tumours and low ovarian lipid levels in fish from the polluted area suggested impaired vitellogenin uptake into the oocytes rather than stimulated hepatic vitellogenesis as the cause of elevated plasma levels.

Estrogenic activity does not always originate from anthropogenic wastes discharged into aquatic environment. Commercial fish food diets increased the plasma vitellogenin levels in Siberian sturgeon, indicative of the presence of estrogenic substances (Pelissero *et al.*, 1989, 1991a, b; Pelissero and Sumpter, 1992). The activity was, however, attributed to the presence of natural phytoestrogens such as genistein and coumestrol in the soybean and vegetable material used in commercial fish diets rather than to contamination by a pollutant during manufacture.

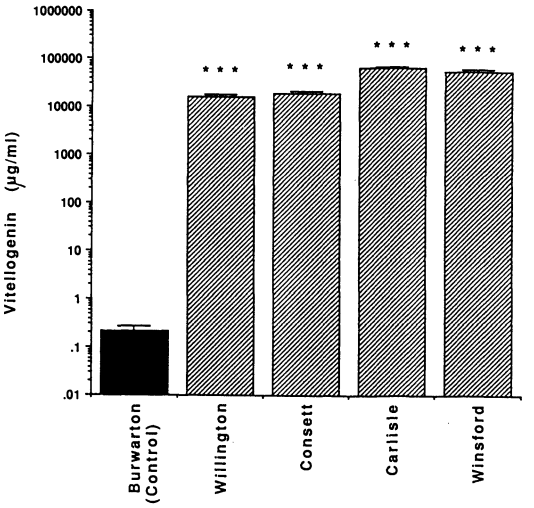


Figure 9.7. The estrogenic response of male rainbow trout (n=20) placed in the effluent channels of sewage treatment works near the towns of Willington, Consett, Carlisle and Winsford in northern England or on a fish farm (Burwarton) supplied with well water. Plasma vitellogenin levels (± SEM) are shown after 3 weeks exposure. (Reproduced from Sumpter, J. P., Jobling, S. and Tyler, C. R., 1996. Oestrogenic substances in the aquatic environment and their potential impact on animals, particularly fish. *In* "Toxicology of Aquatic Pollution. Physiological, Molecular and Cellular Approaches" (E. W. Taylor, Ed), pp. 206-224. *Soc. Exp. Biol. Seminar Ser.* **57**, by kind permission of Cambridge University Press).

Reports of the presence of hermaphrodite fish in the lagoons of sewage treatment works led Purdom *et al.* (1994) to examine the sewage effluent for the presence of estrogenic substances. They placed male rainbow trout in

cages below the sewage outflow and measured plasma vitellogenin concentrations over the following weeks. After 67 days mean vitellogenin levels in the exposed males was 174 μg/ml compared to 0.03 μg/ml in control fish. Extended studies at further sites showed that in some areas male vitellogenin levels were increased to 147,000 μg/ml and that high levels were present in fish held in effluent channels of sewage treatment works near four English towns (Purdom *et al.*, 1994; Sumpter *et al.*, 1996) (Figure 9.7). There were seasonal changes in the extent of vitellogenin induction, probably as a result of changes in water flow and the extent of effluent dilution (Harries *et al.*, 1996).

In a further study, the effects of holding caged male fish at different distances downstream from the effluent on plasma vitellogenin was monitored (Harries *et al.*, 1997). Extremely high plasma levels of vitellogenin (52 mg/ml) were found even 5 km below the effluent source in the River Aire in northern England. This river receives effluents from textile mills and nonylphenol, one of the breakdown product of the detergents used to wash sheep fleece, was present at all sites at concentrations greater than 25 μg/l. In addition, lipophilic estrogenic pesticides such as lindane, toxaphene and dieldrin used to control external parasites in sheep associate with the grease in wool and may have been washed off during the wool scouring process. In addition to the high vitellogenin levels, the caged males also had significantly lower GSI than laboratory controls from the same stock. Elevated plasma vitellogenin levels have also been found in immature European eels in the river Seine below the Paris sewage outfall (Burzawa-Gerard and Billard, Personal communication). Sexually immature eels may spend up to 20 years in European rivers before their spawning migration to the Sargasso sea, so they may have high cumulative exposure to environmental estrogens in the effluents and sediments before sexual differentiation. Since sexual differentiation in the eel is very labile (Tesch, 1977; Beullens *et al.*, 1997), this exposure may be related to the lack of males found in many European rivers and have severe repercussions on future populations (see Fig. 1.2). Of the marine species, those that inhabit or spawn in estuaries or areas close to coastal sewage works are most likely to show indications of induced vitellogenesis resulting from anthropogenic sources. In a study of flounder off the coast of northern England Lye *et al.* (1997) found elevated levels of vitellogenin in male flounder, together with increased incidence of testicular abnormalities.

The nature of the environmental estrogens in sewage effluent has been the focus of intensive recent studies (Sumpter *et al.*, 1996). A large number of chemicals, including the organochlorine pesticides, PCBs, PAHs and polychlorinated dibenzodioxins which have estrogenic properties in mammals (see Colborn and Clement, 1992) and are widespread in the aquatic environment were initially suspect. More recently interest has centred on the

nonyl- and octylphenol polyethoxylates (APEs) which are used extensively as surfactants and are biodegraded in sewage treatment works to the persistent hydrophobic octyl- and nonylphenols which accumulate in sediment and sludge. 30μg/l of octylphenol had a similar vitellogenin inducing potency to 2 ng/l ethynylestradiol, and significant but lower activity was found for nonylphenol, nonylphenoldiethoxylate and nonylphenoxycarboxylic acid (Jobling *et al.*, 1996) (see Fig. 6.5). The minimum effective level of octylphenol was 4.8 μg/l. These concentrations are well within the levels reported from a range of sources including sewage effluents, and very high levels are found downstream of pulp and textile mills. A recent study suggests that the estrogenic components of some sewage effluent which stimulate vitellogenin in fish may, in fact, be the natural estrogens estrone and estradiol, together with some of the synthetic estrogen ethynylestradiol, and that such activity is not attributable to other components of the effluent (Desbrow *et al.*, 1998; Routledge *et al.*, 1998). *In vivo* data showing elevated plasma vitellogenin must, however, be interpreted with some caution as shown by the data of Janssen *et al.* (1995, 1997) in which Rhine sediment induced premature vitellogenesis (Chapter 7). Although vitellogenin was abnormally elevated in females, it was not detectable in similarly exposed males which indicated that external estrogen was not the stimulus. This suggests that male or juvenile fish would provide the most reliable monitors for exposure to environmental estrogens. If, however, females had not been tested in these studies abnormalities of the female reproductive system would not have been detected.

The capacity of the teleost liver to respond specifically to estrogens in the production of vitellogenin provides a very useful screening assay for compounds with potential estrogenic activity. A sensitive bioassay using cultured hepatocytes has been applied to a wide range of such compounds (Jobling and Sumpter, 1994; Pelissero *et al.*, 1993; Sumpter and Jobling, 1996).

Production of vitellogenin, requires the binding of estrogen to hepatic cytosolic receptors, followed by their translocation to the nucleus where they stimulate transcriptional activity. Use of the hepatic estrogen receptor provides a useful screening assay for the potential estrogenic effects of xenobiotics (Thomas and Smith, 1993). The estrogen receptor of the spotted sea trout (*Cynoscion nebulosus*), however, differs from that of mammals and mammalian studies are not necessarily good predictors of estrogenic activity in fish. While the synthetic antiestrogens clomiphene, tamoxifen and nafoxidine displaced ^{3}H-estradiol from its cytosolic receptor, Kepone (chlordecone) had an affinity only one tenth of that with the mammalian receptor and was only 1/10,000 as effective as estradiol. Several DDT derivatives and PCB mixtures failed to displace estradiol from the seatrout receptor even at concentrations as high as 10^{-3}M although they bind to the

mammalian receptor. Copper, zinc and cadmium were actually more effective than these chlorinated hydrocarbons causing 50% displacement of the estradiol at 10^{-3}M (112, 201, and 64 mg/l respectively). Teleosts are an extremely diverse group and it is not yet clear how much difference there is in the estrogen receptors between species. Jobling *et al.* (1995) has used displacement of estradiol from rainbow trout receptors to test the binding of a range plasticizers, petrochemicals, agrochemicals and food additives. Butylbenzylphthalate (BBP) showed significant binding to the receptor at 10^{-6} M (0.23 mg/l), but none of the other chemicals tested bound at less than 10^{-3} M. Estrogenic activity of the compounds was also tested by measuring their direct stimulation of the transcriptional activity of the estrogen receptor on transiently transfected MCF7 cells using the reporter plasmids pTKLUC and pERE-TKLUC (Jobling *et al.*, 1995). Octylphenol, BBP and di-n-butylphthalate all stimulated transcriptional activity of the estrogen receptor at 10^{-6} M concentrations.

A simple colourimetric screen for estrogenic activity has also been developed by integrating the human estrogen receptor into the yeast genome which also contained expression plasmids carrying estrogen-responsive sequences controlling the reporter gene lac-Z which encodes the enzyme β-galactoside (Routledge and Sumpter, 1996). This has been applied to demonstrate estrogenic activity of alkylphenolics and some organochlorine pesticides. This assay, however, used a mammalian receptor which may differ from that of fish, although a similar procedure using the trout estrogen receptor has now been developed (Petit *et al.*, 1996). This demonstrated estrogenic activity for the pesticides methyldiclofop, biphenyl, chlordecone and lindane. Some caution must be exercised with the use of some *in vitro* screens since they will only detect estrogenic activity where this is due to the primary pollutant and not when it is due to metabolites which may be formed *in vivo*. This is exemplified in the use of rainbow trout hepatocyte primary cultures in which the effects of xenobiotics were tested for expression of the estrogen receptor and vitellogenin genes (Flouriot *et al.*, 1995). Chlordecone, nonylphenol, the PCB mixture Arochlor 1245 and lindane induced accumulation of mRNA for both the estrogen receptor and vitellogenin. The antiestrogens, 4-hydroxytamoxifen and ICI 164,384 prevented these effects of the xenobiotics, showing that the effects were mediated by the estrogen receptor. Of the four xenobiotics, however, only chlordecone and nonylphenol displaced binding of [^{3}H]estradiol to estrogen receptor enriched extracts or activated the estrogen-dependent reporter gene cotransfected with an expression vector ER cDNA. This suggests that only these two chemicals are direct inducers of the estrogen receptor and vitellogenin, and that PCBs and lindane act through their hepatic metabolites. By contrast pentachlorophenol acts as an antagonist of estradiol induced expression of the estrogen receptor and vitellogenin genes.

In addition to vitellogenin, estrogen also stimulates hepatic synthesis of the zona radiata (eggshell) proteins. A recent study (Arukwe *et al.*, 1997) suggests that measurement of these proteins may be an even more sensitive indicator of the presence of estrogens than that of vitellogenin. A single injection of 1 mg/kg body weight 4-nonylphenol significantly increased plasma zona radiata protein (Zrp) levels in the plasma of Atlantic salmon, while vitellogenin increased only when the dose was increased to 125 mg/kg body weight. Of the three zona radiata proteins Zrp-β was more responsive that either Zrp-α or Zrp-γ. When tested with oil refinery effluent containing alkylphenolic detergents, a 4 week exposure of salmon to 10% effluent increased Zrp levels, while vitellogenin only increased in 50% effluent.

9.5 Conclusions

Due to its high lipid content and the consequent bioaccumulation of organic pollutants, together with its role in the catabolism of steroid hormones and in the synthesis of vitellogenin the teleost liver provides a good marker for the toxic effects of pollutants. There is clear evidence that some pollutants may cause morphological damage to the liver which may lead to formation of neoplasms or through cellular disorganisation may affect the activity of key enzymes which in turn may alter reproductive and other endocrine function. Some of the morphological changes, such as in smooth endoplasmic reticulum may be associated with induction of specific xenobiotic metabolising enzymes such as mixed function oxidases. Although there is now an extensive literature on the morphological changes induced in the liver by a wide range of pollutants there has been little attempt to relate this to how the fish, and in particular, its reproductive system might be affected. The suggestion that primary cultures of hepatocytes might be used for ultrastructural investigations (Zahn *et al.*, 1995), might be profitably be extended to include effects on MFO induction and vitellogenesis (with and without addition of estrogen) within the same investigation.

Certain pollutants may induce specific protein synthesis or enzyme activity. Heavy metals (zinc, cadmium, copper, mercury) induce metallothionein production while PCBs, PAHs and dioxins induce MFO activity. This induction has been used as a bioindicator of exposure to these pollutants, but only rarely is there a dose-response relationship and the response is affected by a wide range of external influences. Furthermore the previous exposure history affects the induction and permits acclimation to exposed waters. The use of these enzymes and proteins as biomonitors of exposure is not quantitative and even qualitative assessment requires careful validation. MFO activity is also of limited usefulness since many pollutants, especially the pesticides, have little effect on the enzyme system. Although

other enzymes are affected by pollutants, the change is small compared to that of metallothioneins and MFO. Although quantitative use of these biomarkers is of limited use, they have much greater potential as indicators of disruption of the normal chemical equilibrium of the organism, particularly of the essential trace metals and of steroid metabolising enzymes. Since these are intimately involved in the normal functioning of the endocrine system, any xenobiotic which affects metallothionein or MFO activity can be considered a potential endocrine disruptor and warrants further investigation into its effects on the whole animal.

Since vitellogenin synthesis is estrogen dependent, induction of this protein, especially in male and juvenile fish, is a clear sign of exposure to environmental estrogens and may provide a valuable biomonitor of such pollutants. Vitellogenesis by hepatocyte culture can also be used as a screening procedure for estrogenic activity of suspected pollutants. The use of transfected yeast and similar screens must, however, be used with some caution since they will not pick up effects caused by estrogenic activity of hepatic metabolites. Although elevated plasma vitellogenin provides a valuable biomarker for the exposure of fish to chemicals with estrogenic activity, it should be used only in the first screening. It is then important to clarify what effect this exposure has had on the fish itself. Exposure to an estrogen would suggest that the gonads and plasma steroid levels in the affected fish should be examined for signs of reproductive impairment or abnormalities in sexual differentiation. Although estrogen exposure will elevate vitellogenin, in the absence of gonadotrophin-I, it will not be incorporated into the oocytes of females, and as in males and juveniles will remain at elevated levels in the plasma. This may cause secondary problems since high doses of estradiol administered to juvenile trout in attempts to produce all-female stock caused kidney damage and eventual failure as a result of vitellogenin accumulation (Herman and Kincaid, 1988). There is also some evidence that vitellogenin can feedback to the ovary and inhibit estrogen production (Reis-Henriques *et al.*, 1997), so that an excess of vitellogenin stimulated by exogenous estrogen might disturb gonadal steroidogenic activity in both males and females.

By contrast, decreased vitellogenesis may have multiple causes and in most cases is due to disruption of the endocrine system at the hypothalamus, pituitary or ovary. Any change in catabolising enzymes may have a major effect on hormonal balance and result in endocrine disruption which may be particularly important during gonadal recrudescence and spawning. There are few instances in which combinations of pollutants have been examined, but such combinations may be particularly important since damage caused by one pollutant may alter the effect of others. Studies of MFO or metallothionein induction, for example, often do not consider the possible interaction of other components of the polluted ecosystem.

Chapter 10

Disruption of Thyroid and Interrenal Function.

10.1 The endocrinology of the thyroid and interrenal glands

Compared with the studies on reproductive function, relatively little is known about the effects of pollutants on the functions of the thyroid and interrenal tissues. This is undoubtedly due to the fact that in fish, unlike mammals, these tissues do not form distinct glands, but are scattered in small clumps which makes experimental isolation and examination more difficult. There is considerable overlap in function between the thyroid and interrenal with regard to control of growth and metabolism. It is therefore appropriate to consider these tissues together with related endocrine functions such as the stress response, which also involves the chromaffin tissue analogue of the mammalian adrenal medulla, osmoregulation and factors affecting growth.

The thyroid gland in fish, as in mammals, is primarily concerned with regulating metabolic rate which may in turn affect both activity and growth of the fish. Growth may, for example, be stimulated by thyroxine treatment, although the results are closely dependent upon the conditions under which fish are maintained (Barrington *et al.*, 1961). Conversely, growth is inhibited by irradiation which decreases or abolishes activity of the thyroid gland (Norris, 1969). The thyroid is also involved in a less specific manner with larval development, behaviour and reproduction and there are interactions between growth hormone, the gonadal hormones and thyroid function (Matty, 1985). There is also evidence that the thyroid may be involved in the parr-smolt transformation, the spawning migration and osmoregulation (Grau, 1987).

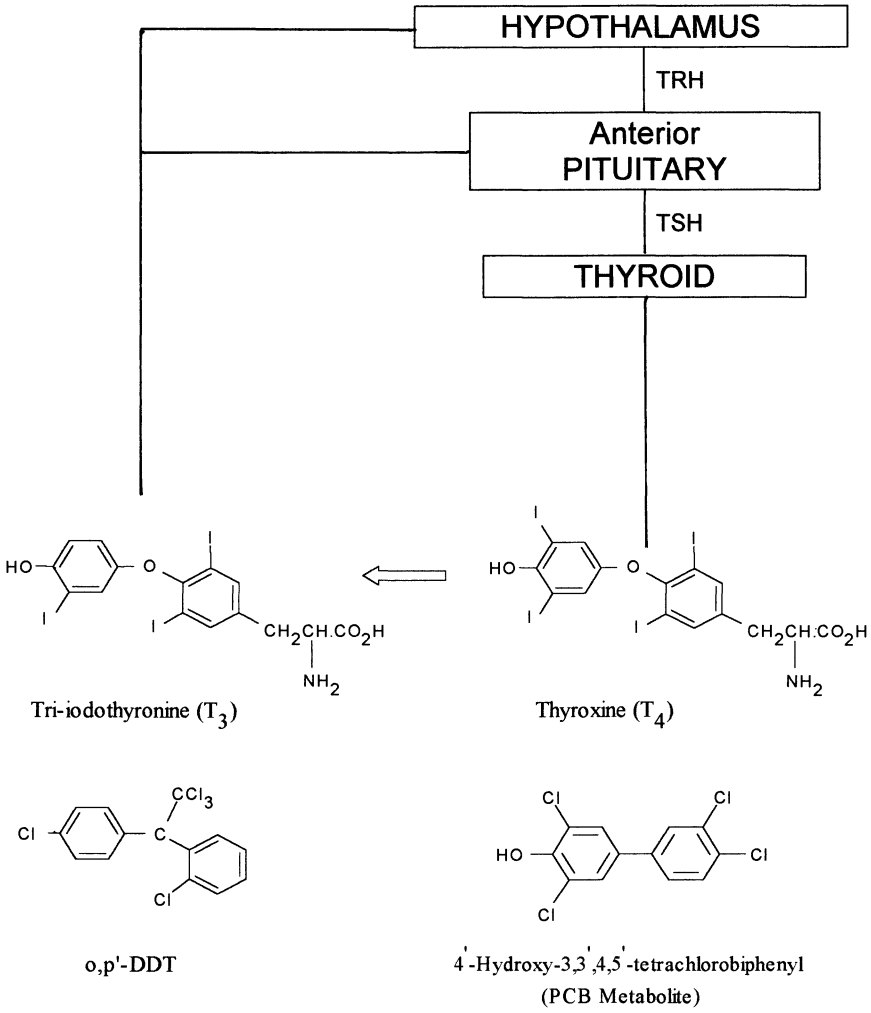
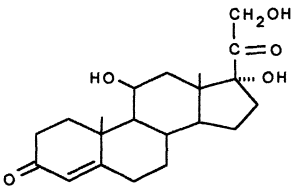


Figure 10.1. Control of thyroid hormone secretion.

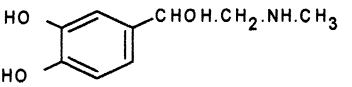
Regulation of thyroid activity is effected in a very similar manner to that of the gonadal steroids (Figure 10.1). Metabolic or neural stimuli act via the hypothalamus which secretes thyrotrophin-releasing hormone (TRH) which in turn acts on the thyrotrophs of the pituitary to release the thyroid-stimulating hormone (TSH). This in it's turn stimulates the thyroid to secrete the hormones thyroxine (T_4) and triiodothyronine (T_3). T_3 is the active hormone and is derived from T_4 by removal of one iodine by the 5'-monodeiodinase enzyme which is present in both the thyroid and the liver. This conversion could therefore be affected by xenobiotics which act on hepatic enzymes. The thyroid hormones are iodinated biphenyl ethers and have close structural

similarities to the DDTs, PCBs, dioxins and their metabolites (Figures 2.6 and 10.1). It is therefore not surprising to find that in mammals the PCB metabolite 4'-hydroxy-3,3',4,5'-tetrachlorobiphenyl competes very strongly with thyroxine for its binding site on the carrier binding protein (Brouwer *et al.*, 1990). Iodine is essential for synthesis of the thyroid hormones T_3 and T_4, and in the absence of their production there can be no negative feedback to the pituitary which will continue to secrete TSH to stimulate the thyroid, resulting in hypertrophy or goitre. Unlike mammals in which the thyroid is a discrete gland situated around the trachea, that of teleosts forms diffuse follicles scattered around the ventral aorta and its branches.

Growth hormone (GH) is secreted by the anterior pituitary, but unlike mammals, growth in fish continues throughout life and does not cease with sexual maturation. The size of a fish reflects both food availability and age. Pollutants can therefore affect growth, both by action at the level of pituitary GH secretion and at the behavioural level by affecting foraging. They can also act indirectly by affecting availability of plant or animal food resources. Conversion of these resources into somatic growth will in turn be affected by general metabolic activity, under thyroid and interrenal control, and by liver function which can all be influenced by pollution. In temperate species there is a distinct seasonal cycle of growth, apparent in rings on scales and otoliths, which can be used in assessing age. This reflects both the temperature dependence of feeding and metabolism, and the distribution of resources between gonadal and somatic growth. In the female, the partition of calcium and protein resources between somatic growth and vitellogenin production for the developing oocytes will show a distinct seasonal cycle. Differences in growth between clean and polluted habitats can therefore have a multiplicity of causes and this Chapter will be concerned predominantly with the few studies which have examined endocrine causes.



Cortisol



Adrenalin (Epinephrine)

Figure 10.2. Hormones of the interrenal gland (cortisol) and chromaffin tissue (adrenaline) in fish.

The teleost interrenal gland differs from its mammalian homologue, the adrenal gland, in that it does not constitute a distinct structure comprising a cortex and medulla, but the steroid secreting interrenal and adrenaline producing chromaffin tissues are dispersed within the head kidney (Chester Jones and Mosley, 1980). As with mammals, corticosteroid production is stimulated by pituitary adrenocorticotrophin (ACTH) in response to stress, and the hormone cortisol (Figure 10.2) plays a key role both in carbohydrate metabolism and in the stress response. There is no convincing evidence that the teleost interrenal secretes the mammalian mineralocorticoid, aldosterone, in any significant amount. Corticosteroids are synthesised by very similar pathways to that of the gonadal steroids involving P450 and mixed function oxidases (Henderson and Kime, 1987). Their synthesis is, therefore, like the gonadal steroids likely to be affected by any xenobiotic which affects mono-oxygenase activity (Chapter 9). Conversely, activity of hepatic cytochrome P450 may be modulated by cortisol (Devaux *et al.*, 1992) and, in a further link with the liver, cortisol may also modulate metallothionein activity (Hyllner *et al.*, 1989). The interplay between these activities is clearly complex even in a normal fish, but the interrelationships between interrenal P450, cortisol secretion and hepatic metallothioneins and cytochromes in fish exposed to pollutants targeting multiple interacting sites is indeed complicated.

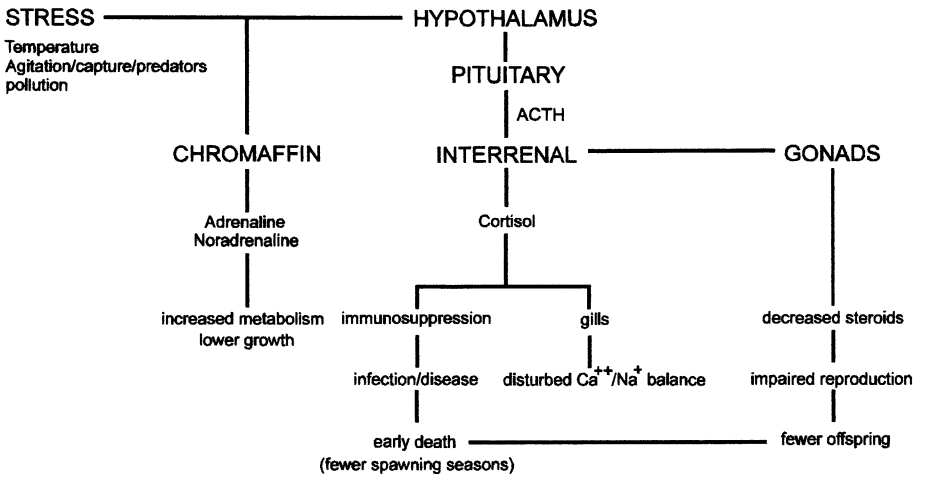


Figure 10.3. Schematic diagram of the stress response in fish

Unlike mammals it is not possible to isolate pure adrenal cortex or medullary tissue in fish, or to assess the effects of experimental treatment with toxicants on adrenal weight or morphology. As with mammals there is a complex interplay between the interrenal gland and the gonads, such that

stress may affect the reproductive potential while the reproductive status affects the stress response (Kime *et al.*, 1980). High levels of cortisol, indicative of stress, in both fish and mammals may also lead to suppression of the immune system and susceptibility to disease (Figure 10.3). The most extreme example is, of course, the Pacific salmon (*Oncorhynchus* spp.), in which the gonads mature only during the spawning migration which is associated with fasting and extremely plasma high cortisol levels (Hane *et al.*, 1966), possibly resulting from impaired hormone clearance (Idler and Truscott, 1963). The mass post-spawning mortality shows all the symptoms of hypercortisolism, with deterioration of skin integrity and infection (Robertson *et al.*, 1963). The role of the interrenal in osmoregulation is not as clear as in mammals since mineral balance is also in part regulated by both the gills and the urophysis.

Pollutants may affect interrenal function via two distinct mechanisms. As a general external stressor, pollution can cause a range of malfunctions in the animal ranging from general discomfort to actual tissue damage. These effects, like elevated temperature, agitation of the water, handling, presence of predators etc. will lead to a stress situation and hence, via pituitary ACTH release, to increased cortisol secretion. This may then act as an immunosuppressant leading to infection, disease and early death (Pickering, 1981; Pickering and Duston, 1983; Pickering and Pottinger, 1989). Clearly such effects will affect fish populations since they will have early mortality and in most species have fewer spawning seasons. They may also produce fewer eggs or sperm as a result of stress and indirect inhibition of gonadal steroidogenesis. Although such stressors clearly alter the endocrine status, they cannot be classed as endocrine disruptors since the endocrine system is not the *primary* site of action as defined in Chapter 1. The endocrine system is disrupted as a secondary effect to action elsewhere in the organism, and for this reason it is essential that any study of the effects of endocrine disruptors uses fish which are as stress-free as is practicable. Stress effects may, however, indirectly affect normal endocrine function since exposure of fish to the stress of low levels of pollutants may affect the response of the hypothalamo-pituitary-interrenal axis to natural stresses. Most studies of the interrenal have involved exposure of fish to pollutant levels which cause a significant increase in plasma cortisol but there is as yet little experimental data to show whether exposure to pollutants at even lower levels causes an increased stress response to natural environmental and behavioural stressors. Such studies should show whether a fish exposed to levels of a pollutant that does not increase cortisol has a stress response to predators or handling which does not occur in unexposed fish. Such pollutant induced hypersensitivity to stressors would qualify as endocrine disruption by the definitions given in Chapter 1.

The second mechanism by which pollutants may affect interrenal function, and that which will be a major concern in this Chapter, is by direct disruption of the hypothalamo-pituitary-interrenal axis, which may result in secondary symptoms such as changes in osmoregulatory function, susceptibility to infection and disease, and altered growth. Direct effects of pollutants on pituitary function, discussed in Chapter 5, include not only modulation of the corticotrophs which affect ACTH secretion and hence interrenal activity, but also arginine vasotocin (AVT) which interacts with cortisol in maintaining osmoregulatory homeostasis.

10.2 Effects on the thyroid

The most extensive field study of thyroid dysfunction has been that of Leatherland and co-workers on salmon in the heavily polluted Great Lakes. Salmonid species in these lakes suffer a high incidence of goitres. Although early studies had suggested that these may have been due to the low iodine content of the lakes they are now attributed to environmental factors (Leatherland, 1992, 1993). The thyroid gland of salmonids, unlike that of mammals, is in the form of diffuse follicles scattered around the ventral aorta. It cannot be readily weighed and can generally be detected only by histological examination. Estimates by Leatherland (1992, 1993) showed that the degree of thyroid enlargement in coho salmon (*Oncorhynchus kisutch*) was between 1×10^3 and 1×10^{12} and that there had been a consistent 100% prevalence of such thyroid hyperplasia for the previous 18 years regardless of salmon species, lake of origin or gender. Grossly visible thyroid lesions in the Lake Ontario coho salmon stock have, however, declined from 20-30% in the 1970s to 1% at present. Such lesions were not apparent in native Pacific stocks, or in young salmon taken from the Great Lakes and reared on clean spring water, so a genetic basis could be discounted. Iodine deficiency was also discounted since it was not supported by flesh iodine levels, the access of salmon as top predator to an adequate dietary supply of iodine, the lack of gender differences despite the fact that the ovary competes with the thyroid for available iodine, and the ability of Great Lakes salmon to secrete thyroid hormones at extremely high levels. The presence of high levels of aromatic organochlorines in the salmon, together with their known antithyroidal effects in mammals (Bastomsky, 1997a, b; McKinney *et al.*, 1985a, b) suggested that these chemicals might be the causative agents. Lesions were not found, however, in either rainbow trout or coho salmon that had been fed PCB contaminated diets or in hatchery reared trout or salmon fed a diet of Great Lakes salmon (Leatherland and Sonstegard, 1979, 1980b, 1982), and in the Great Lakes salmon themselves there was no correlation between tissue organochlorine content and degree of thyroid enlargement. Rodents fed a diet

of Great Lakes salmon for 2 months, however, showed a marked thyroid hyperplasia and hypertrophy, and the degree of thyroid enlargement was dependent upon the dose of PCB administered (Sonstegard and Leatherland, 1979; Leatherland and Sonstegard, 1980c; Chu *et al.*, 1984; Cleland *et al.*, 1988). The differential response of mammals and salmon may be due to differences in the extent of binding of thyroid hormones to protein between mammals and fish since McKinney *et al.* (1985a, b, c, 1987) have suggested that halogenated aromatic hydrocarbons may bind to thyroid binding sites. Although direct causal links between body burdens of PCBs in salmon and thyroid dysfunction were not established it is important to remember that assay methods are limited in what they measure and that some organochlorine metabolites may not be detected. Some may be present in the forage base of the fish and although they may not be bioaccumulated, they may still have a biological activity.

In addition to chlorinated hydrocarbons, Leatherland (1992) also considered the possible impact of other factors and found that the degree of eutrophication of the lake of origin of the salmon was closely correlated with the size of the thyroid lesions. Some products of bacterial metabolism have been shown to cause goitres in humans (Gaitán, 1973; Gaitán *et al.*, 1973) and Lake Erie water inhibited *in vitro* thyroid activity in mammalian tissue (Leatherland, 1992). Although salmon in Lake Erie had decreased fecundity (see Chapters 6 and 7) this was not directly related to the presence of thyroid lesions (Leatherland and Sonstegard, 1987). PCBs and dioxins have a close structural similarity to the thyroid hormones but there have been no studies in fish on their competition for binding sites. The action of such pollutants at the level of feedback on the pituitary would be of particular interest in the context of goitre formation.

Although the causative agent for the thyroid dysfunction in Great Lakes salmon is not yet established, pollution clearly plays an important role. The Great Lakes have been the repository for anthropogenic wastes for over a century, and may harbour many complex chemicals which may be responsible both for these effects, and the others described elsewhere in this volume. The immediate relevance of these studies to human health is clear from the studies of Beierwaltes (1987a, b) documenting the high incidence of goitre in the State of Michigan that could not be attributed to an iodine deficiency.

There have also been extensive laboratory studies into the effects of anthropogenic pollutants on thyroid activity in fish, predominantly for pesticides in India, while European studies have concentrated more on the effects of industrial pollutants and increased acidity. Relatively few studies have examined morphological effects. Both the organophosphate fenitrothion (1.5 mg/l), and the carbamate carbofuran (5 mg/l) caused a decrease in the diameters of the thyroid follicles and the amount of colloid, but an increase in the thickness of the epithelium after 120 days exposure (Saxena and Mani,

1988). In addition, the thyroids of fenitrothion treated fish showed invasion of the follicular lumen by blood corpuscles and breakdown of the epithelium. The changes in thyroid structure with both pesticides were progressive and apparent by 30 days exposure. It is not clear whether this was a real difference between the two types of pesticide, or whether it is simply due to the use of a more toxic quantity of the organophosphate. Studies using several doses would be useful in resolving whether there are qualitative differences between different types of pollutants.

Monopterus albus exposed to cadmium, malathion or 3-methylcholanthrene showed similar disintegration of the thyroid structure with thicker epithelia and decreased colloid (Singh, 1989). Mercury, in the form of the inorganic chloride, organic methylmercury or the commercial Emisan-6 (6% methoxyethylmercuric chloride) also decreased the colloid levels in the thyroid follicles of *Clarias batrachus* and *Liza parsia* which had hypertrophied epithelial cells (Kirubagaran and Joy, 1989; Pandey *et al.*, 1993). Uptake of radioiodine was inhibited and levels of protein bound iodine decreased. These changes led to decreased plasma levels of the hormones T_3 and T_4 after two weeks exposure to organic mercury (Kirubagaran and Joy, 1994). The levels of mercury used (0.05 mg/l), however, were 50 times higher than that which caused an instant decrease in motility of sperm from a closely related catfish (Rurangwa *et al.*, 1998; Chapter 6) suggesting that fish in which thyroid malfunction occurs are unlikely to be part of a viable reproducing population. Mercury is accumulated in fish as methylmercury and T_4 levels were doubled in juvenile rainbow trout exposed to only 0.006 mg/l methylmercuric chloride for 4 h, although concentrations did not differ from controls after 72 h exposure (Bleau *et al.*, 1996). Similar elevations were found with higher levels (0.028 mg/l) of mercuric chloride. Exposure of the catfish *Clarias batrachus* to 5 mg/l lead nitrate for 150 days gave very similar symptoms to that found with mercury (Katti and Sathyanesan, 1987b). Histological observations showed hypertrophy of the thyroid with a doubling of the epithelial cell diameters, and uptake of radioiodine was decreased to only one tenth of the controls. The authors suggest that lead might act by inhibiting the ATPase implicated in TSH action, or may interfere with the sulphyl-iodide transport system by binding to the sulphur and preventing it from combining with iodine. The hypertrophy of the pituitary thyrotrophs would appear to be a secondary effect resulting from the thyroxine deficiency.

DDT treatment of tilapia also gave goitrous, hypertrophied but inactive thyroid follicles (Shukla and Pandey, 1986b). Follicular size and nuclear diameters were greatly increased, but the colloid was vacuolated and mostly exhausted. The changes were reversible and a normal thyroid was restored after 20 days in clean water. The goitrous changes have more in common with the Great Lakes salmonids, and the greater structural similarity between DDT

and thyroid hormones compared to other pesticides is suggestive of interference with T_3/T_4 feedback at the pituitary.

Not surprisingly, pollutants also affect thyroid hormone secretion, but unfortunately there has been no systematic comparison between plasma hormone levels, TSH levels and thyroid structure. Although Singh (1989) found that both plasma T_3 and T_4 increased after 7 day exposure of tilapia to 3 mg/l cadmium, Hontela *et al.* (1996) have shown that the effects of this metal are both time and dose dependent. After 2-4 h exposure of rainbow trout to 0.4 to 2.4 mg/l cadmium there was a significant increase in T_4, but not of T_3, but after one week exposure to 0.4 or 0.8 mg/l of this metal T_4 was decreased, while T_3 was again unaltered. These changes may not, therefore, have any adverse effects on the fish since it is T_3 rather than T_4 that is the biologically active hormone. It is not clear from these studies whether cadmium affects the enzymes involved in iodine oxidation, conversion of T_4 to T_3, or the breakdown and excretion of the hormones. Since cadmium induced changes in T_4 parallel the changes in interrenal cortisol secretion, it is also possible that this steroid may affect the rate of metabolism and clearance of the thyroid hormones as suggested by Brown *et al.* (1991). The generally stimulatory effect of cadmium on thyroid hormones is in marked contrast to the clear inhibitory effects of mercury (Kirubagaran and Joy, 1989, 1994) and reflects the somewhat anomalous effects of this metal on reproduction (Chapters 5-7).

Malathion, γ-BHC, endosulfan and carbaryl affect the plasma concentrations of T_3 and T_4 in a number of Indian freshwater species, but the effects vary unpredictably between species and pesticides (Yadav and Singh, 1986, 1987a, b; Singh, 1989; Sinha *et al.*, 1991a, b, 1992a, b). Such variations may result from changes in response with time (Hontela *et al.*, 1996) or with the stage of the reproductive cycle (Yadav and Singh, 1987a). Some of these effects may also be exerted by the action of pesticides on thyroid peroxidase which is involved in the enzymatic iodination of tyrosine (Yadav and Singh, 1987b, c; Sinha *et al.*, 1991b, 1992a). There was, however, no clear dose or time dependent alteration in plasma T_3 and T_4 in flounder treated for 2-10 days with the PCB Clophen A50 (Besselink *et al.*, 1996), while T_3, but not T_4 was significantly reduced in vitellogenic rainbow trout treated for 12 days with 0.01 mg/l HCN (Ruby *et al.*, 1993b) suggesting that HCN acts on the monodeiodination of T_4. Although the decreased circulating levels of the active hormone, T_3, during vitellogenesis might have implications for its availability to the developing oocytes after spawning, hatch rate and larval development were not recorded.

In addition to pesticide runoffs, water in agricultural areas is also likely to have high ammonia content from fertilisers. Ram and Sathyanesan (1987c) found that "safe" levels (100 mg/l) of ammonium sulphate caused hypertrophy of the thyroid follicles after 6 months exposure. There was,

however, also degeneration of the liver and preliminary observations by this group had also shown hypertrophy of the cortical and medullary cells of the interrenal. Since ammonia can cause damage to the gill tissue, it is not clear whether these effects are primarily due to action on the thyroid, liver and interrenal, or are merely the side effects of stress resulting from irritation and degeneration of gill function.

Although there was little or no decrease in plasma thyroid hormones in rainbow trout exposed to low pH (Brown *et al.*, 1984, 1986), exposure to a combination of low pH and aluminium supported the hypothesis that extrathyroidal conversion of T_4 to T_3 was impaired by aluminium (Brown *et al.*, 1990). Aluminium reduced both the affinity of hepatic T_4 5'-monodeiodinase for T_4 and the amount of functional enzyme which is a major site for plasma T_3 generation. Low pH and aluminium together impaired peripheral thyroid function, but it is not clear whether they act directly on the enzymes or whether they are mediated via cortisol which showed a much greater increase in the presence of aluminium than with low pH alone. The growth of trout was also suppressed by low pH in the presence of aluminium but not at low pH alone (Sadler and Lynam, 1987; Mount *et al.*, 1988b; Reader *et al.*, 1988). Since exogenous treatment of fish with thyroid hormones leads to increased somatic growth (Higgs *et al.*, 1982; Hilton *et al.*, 1987) it is probable that the effect of aluminium on fish growth acts via impairment of the hepatic monodeiodination enzyme. There may be considerable species variation in both the response and tolerance to aluminium under acid conditions. Brown trout (*Salmo trutta*) showed an elevation of both T_3 and T_4 after exposure to pH 5 and 12.5 or 25 μg/l aluminium, but fish did not survive past 24 h at 50 μg/l (Waring *et al.*, 1996a). By contrast the rainbow trout (*Oncorhynchus mykiss*) used by Brown *et al.* (1990) were exposed to pH 4.7 and an aluminium concentration of 540 μg/l for 7 days without mortality.

Plasma concentrations of the thyroid hormones T_3 and T_4 are also affected by stress since cortisol promotes the conversion of T_4 to T_3 and increases the plasma clearance of T_3 (Vijayan *et al.*, 1988; Brown *et al.*, 1991). It is therefore important to determine whether the fish concerned are stressed and whether stress-induced elevation of cortisol might be the cause of altered thyroid hormone levels rather than direct effects of the xenobiotic on the thyroid gland, or on the hepatic conversion of T_4 into T_3. Measurement of both cortisol and thyroid hormones would in many cases resolve such actions. Such parallel measurements showed, for example, that perch from a habitat contaminated with PAHs, PCBs and heavy metals had lower T_4 than control fish but T_3 did not differ, while the capture-induced cortisol levels were lower in the fish exposed to pollutant (Hontela *et al.*, 1995). This suggests that in this case the xenobiotics acted directly on both the interrenal and thyroid function.

In salmonids the thyroid is involved in smoltification and seawater adaptation (Dickhoff *et al.*, 1978; Folmar and Dickhoff, 1980; Grau *et al.*, 1981), and there is some evidence that PCBs and fuel oil can disrupt the normal pre-migratory pattern of thyroid hormones and survival of fish on transfer to saltwater (Folmar *et al.*, 1982). Heavy metals such as arsenic (Nichols *et al.*, 1984), copper and zinc (Lorz *et al.*, 1976) can also affect the seawater adaptation by delaying the normal increase in plasma thyroxine associated with the parr-smolt transformation. The presence of such metals in mining waters in salmon rivers could, therefore, have a significant effect on fish populations.

10.3 Growth

Growth can be affected by pollutants via a very wide range of mechanisms, only some of which involve the endocrine system. Juvenile bluegill (*Lepomis macrochirus*), for example, show reduced growth in the presence of only 37 μg/l cadmium, but this was a result of decreased foraging rather than lower utilisation of consumed material (Bryan *et al.*, 1995), presumably resulting from the neurotoxic action of cadmium. Feeding rates of mummichogs were also affected by exposure to 0.01 mg/l mercury or methylmercury for two weeks (Weis and Khan, 1990). In wild fish, it is particularly important to determine whether the pollutant acts directly on the endocrine system of the fish, or whether growth is inhibited by secondary effects such as changes in feeding behaviour or depletion of the food base of the fish resulting from high pollutant levels in the sediment (Munkittrick, 1988b).

There was a negative correlation between growth and body burden of mercury in juvenile walleye (*Stizostedion vitreum*) fed dietary methylmercury (0.1 and 1 μg/g) for 6 months (Friedmann *et al.*, 1996). All fish consumed the full amount of diet so this was not due to loss of appetite or foraging behaviour but, since plasma cortisol was suppressed, mercury probably acts via the interrenal or thyroid and hence on utilisation of the diet. The lower growth rate of Atlantic salmon parr exposed to crude oil was also due to decreased utilisation rather than decreased feeding efficiency, and since the timing of smoltification is length dependent this may result in delay of the seaward migration of juvenile salmon (Vignier *et al.*, 1992). Such decreases in utilisation of food resources is particularly hazardous to species which are at the limits of their natural habitats. Bluegill sunfish (*Lepomis macrochirus*) in northern latitudes, for example, are dependent on growth and lipid deposition during the short summer months for survival throughout the winter, and the decreased food conversion efficiency after exposure to the

wood preservative pentachlorophenol may lead to increased overwintering mortality (Samis *et al.*, 1991).

While decreased growth might be expected to be an obvious consequence of exposure to pollutants, there are a number of cases in which exposed fish have more rapid growth than unexposed controls. Perch and roach nearest to the effluent of a pulp mill on the Baltic coast had higher growth rates than fish further away (Sandström *et al.*, 1988) and in minnows exposed to the PCB Clophen A50 growth was highest in fish with the highest body burdens (Bengtsson, 1980). In both studies reproductive function was impaired. Since in normal fish there must be a partition between somatic and gonadal growth during the reproductive season, the results suggest that this energy partition is impaired by the pollutant. It is not clear whether this is the cause or the result of inhibited reproductive function, or whether it is induced by stimulation of the thyroid hormones.

10.4 The stress response

10.4.1 Introduction

The General Adaptation Syndrome of Selye (1950) divides the response of an animal to external stressors into three stages - alarm, resistance and exhaustion. The initial phase, the "fight or flight" response, involves release of adrenaline (epinephrine) from the chromaffin tissue that in fish lines the walls of the posterior cardinal veins, and is homologous with the mammalian adrenal medulla, followed by release of cortisol from the interrenal tissue (Figure 10.2). Together these responses result in mobilisation of energy reserves with increased plasma glucose, stimulation of the heart etc. If the stressor persists then continued release of these hormones will lead to physical deterioration, decreased resistance to disease and eventually death. The stress response system may respond to pollutants in two distinct ways. The pollutants may act as stressors and, through physical, physiological or sensory mechanisms, induce the normal stress response, or they may disrupt the normal endocrine response to stress by direct action on the hypothalamo-pituitary-interrenal axis. While the former mechanism will increase plasma cortisol and adrenaline as a normal stress response, the latter may suppress (or stimulate) the normal stress response to external stressors such as capture or agitation. Although only the latter is a true endocrine disruptor, both will be discussed since it is important to be able to differentiate the two mechanisms and stress may have secondary effects on a range of other endocrine disrupting processes.

10.4.2 Pollution induced stress

The two possible effects of pollutants on the stress response have opposing responses in plasma cortisol which enables them to be distinguished. The action of a pollutant which causes general discomfort to the exposed animal is perceived as a stressor and consequently will lead to increased adrenaline and cortisol secretion. This in turn will result in metabolic and osmoregulatory disturbances which become manifest in elevated blood glucose concentrations or changes in ionic composition (Pickering, 1981; Giesy, 1988; Folmar, 1993). The high level of stress induced may make it impossible for the fish to respond to other stressors such as predators and if prolonged will inevitably lead to early mortality. Elevated cortisol, together with elevated plasma glucose has frequently been used as an indicator of stress induced by pollutants, but the rapidity with which cortisol is elevated may make it difficult to differentiate between pollutant induced and capture stress. Plasma glucose, because it reflects the stress experienced several hours earlier may, in some cases, be the more reliable parameter. Changes in interrenal nuclear diameter have also been used as indicators of stress caused by pollution. The use of these as indicators of exposure to a variety of pollution stressors including fuel oil, polyaromatic hydrocarbons, pulp mill effluent, copper, landfill leachate, municipal sewage, copper, ammonia and nitrate has been reviewed (Donaldson *et al.*, 1984; Thomas, 1990b). Concern with the effects of acid rain on commercially important salmonids in temperate areas of the northern hemisphere has led to a large number of studies on the effects of high pH on the interrenal system. In most of these, acidity is perceived as a general stressor leading to increased plasma cortisol levels. In the majority of cases there are associated disturbances in thyroid function, osmoregulatory balance and gas exchange, but it is not clear whether the elevated cortisol is the cause of such disturbances or, perhaps more likely, the result of primary action at the gills which in turn leads to a secondary stress response. The combination of acid water with aluminium has a much greater impact on plasma cortisol levels and mortality than acid alone (Goss and Wood, 1988; Brown *et al.*, 1990), but it is clear that this was the result of chronic stress resulting from hypoxia and osmoregulatory imbalance caused by action at the gills. Wild brown trout in natural acid waters also had higher cortisol than fish in neutral waters and this was increased by artificial exposure to aluminium (Whitehead and Brown, 1989). Low calcium content also increased the response of cortisol to low pH (Brown *et al.*, 1989). Prior exposure to acid may, however, also increase the interrenal responsiveness to other stressors such as handling (Barton *et al.*, 1985).

Cortisol levels increase rapidly during the first few hours after administration of the pollutant, but then can often return to baseline values. This initial increase probably relates to the alarm phase of Selye's General

Adaptation Syndrome (GAS) (Selye, 1950). During this phase of steep increase in plasma concentrations, cortisol can often display a linear dose response curve from which the no effect concentration can be calculated (Donaldson and Dye, 1975; Schreck and Lorz, 1978; Swift, 1981). Such indicators may, however, not be universally applicable since plasma cortisol did not increase in coho salmon exposed to acutely lethal concentrations of cadmium (Schreck and Lorz, 1978). These authors have suggested that cortisol increases only reflect the awareness of the fish to the stressor, such as its irritant effects on the eyes, skin, gills, or on buccal or nasal chemoreceptors. As such, the stress response only occurs in response to fright, discomfort or pain. Osmoregulatory disturbance can presumably also cause discomfort. Cortisol measurements thus have limited value in detecting the presence of pollutants which are not sensed as "harmful" by the fish, but which nevertheless may cause long-term malfunction. Cadmium appears to vary considerable in the stress response evoked in different species. In contrast to the closely related coho salmon, cortisol increased rapidly in rainbow trout exposed to 0.1 mg/l for 24 h (James and Wigham, 1986) or 0.4 mg/l cadmium for a week and remained elevated for a week after exposure (Hontela *et al.*, 1996). There may well be differences between acutely exposed laboratory fish and feral fish in which long-term chronic exposure may lead to interrenal dysfunction (Hontela *et al.*, 1992, 1995, 1996, 1997). Cadmium also elevated cortisol in tilapia, probably as a result of damage to the gills leading to homeostatic imbalance (Pratap and Wendelaar Bonga, 1990). Long-lived fish, such as eels, which feed in the sediments are likely to be exposed to heavy metal pollutants over a long time span. American eels (*Anguilla rostrata*) exposed to environmentally realistic levels of cadmium (150 μg/l) for 16 weeks had chronically elevated cortisol even after 8 weeks, although glucose levels had returned to normal and fish were able to respond to additional stress (Gill *et al.*, 1993). This elevated cortisol may well affect the eels metabolism, immune system, gonadal maturation and ability to migrate to its spawning grounds. Since eels may spend up to 20 years in freshwater before their spawning migration, it is probable that bioaccumulation may cause such disruption in waters of much lower metal concentrations. Since cadmium accumulates in the gills where it can cause damage to oxygen uptake it is, perhaps, not surprising that dietary uptake in bass did not elevate cortisol (Weber *et al.*, 1992). Mercury exposure also increased plasma cortisol levels in juvenile rainbow trout 24 h after exposure to very low concentrations (28 μg/l mercuric chloride and 6 μg/l for methylmercury), but levels had returned to normal after one week of exposure (Bleau *et al.*, 1996).

Respiratory malfunction is probably one of the most common inducers of the stress response since many xenobiotics are absorbed through the gills. Exposure of flounder to the water soluble fraction of crude oil, for example,

resulted in a dramatic decline in blood oxygen content within 48 h leading to the classic stress response of increased cortisol and noradrenaline which was probably responsible for the increased blood haematocrit and haemaglobin within the first few hours (Alkindi *et al.*, 1996). A similar effect was observed in striped mullet (*Mugil cephalus*) exposed to crude oil which irritated olfactory and gill epithelia (Thomas *et al.*, 1980). That crude oil may have such effects in wild populations was shown by the elevated cortisol levels in eels collected near the site of the Amoco Cadiz wreck 8 months after the oil spillage (Leloup-Hatey and Hardy, 1985). Ammonia and nitrite are also perceived as stressors only when they enter the fish and cause methaemoglobinaemia which reduces the oxygen carrying capacity of the blood (Tomasso *et al.*, 1981). Laboratory studies showed that striped bass responded to the noxious response of benzene by elevation of cortisol and other responses typical of the alarm phase of the General Alarm Syndrome, but adaptation occurred (MacFarlane and Benville, 1986). Populations of wild bass in San Francisco Bay, however, have declined by 75% since the early 1960s, and fish captured with levels of 4 mg/l benzene in the liver were in poor physiological and reproductive condition and heavily parasitised suggesting that long-term benzene exposure may have led to the exhaustion phase of the GAS and immunosuppression. The Californian bass, however, are also exposed to high levels of agrochemicals, petrochemicals and industrial and urban wastes and fish captured during the summer "die off" had many symptoms of liver disease. Liver, kidney, intestine and thyroid follicles of moribund fish showed a range of histopathological changes, and it was difficult to find interrenal tissue in the head kidney since the amount of tissue was drastically reduced even though the high plasma cortisol was indicative of chronic stress (Young *et al.*, 1994). Plasma cortisol concentrations in carp were elevated after exposure to sub-lethal concentrations of aldrin, atrazine, DDT, dieldrin, endrin, HCB, lindane, methanol and toluene (Gluth and Hanke, 1985), and although the time course of the response varied between the chemicals, comparisons are not possible since only a single dose of each was used.

Since plasma cortisol increases in response to handling stress, considerable care has to be taken in sampling if an effect of the pollutant alone is to be monitored. The effects of capture and confinement stress affect not only cortisol and glucose, but also the sex steroids and it is essential that capture methods be standardised for any experiment in which pollution effects are examined (Jardine *et al.*, 1996). The role of temperature must also not be neglected since it may affect both the primary action of the pollutant and the stress response of the pituitary-interrenal axis as well as the rate of hepatic catabolism of both pollutant and cortisol. The matter is clearly complex since the temperature effects on both the plasma cortisol and glucose responses varies widely between different pollutants (Gluth and Hanke, 1984).

There have been suggestions that other, secondary effects of stress indicative of increased cortisol might provide a good model for monitoring pollutant induced stress. Increased nuclear diameter of interrenal cells is indicative of elevated cortisol secretion, and the cortisol induced increase in glucose can be used as a stress indicator (Brown *et al.*, 1984, 1986). Changes in the structure of fish skin, including increased secretory activity and cell death by apoptosis can be induced by injection of ACTH (Iger, 1992) and similar changes occurred in trout exposed to acidified water (Iger *et al.*, 1994). These effects were apparent 7 days after exposure to acid by which time the plasma cortisol had returned to control values. Salmonids exposed to acid stress were also hyperactive, but suffered loss of appetite, possibly as a result of the elevated glucose levels in the plasma (Jones *et al.*, 1987). In many studies, fish are exposed to rapid changes in pH, but Balm and Pottinger (1993) found that a gradual decrease in pH allowed fish to adapt without involvement of the pituitary-interrenal axis. Baseline plasma ACTH and cortisol did not differ between controls and exposed fish, nor was there any sensitisation to additional stress in the acid exposed group. *In vitro*, the baseline and ACTH stimulated cortisol secretions were the same in both groups. Ultrastructural changes in the gills suggested a locally regulated adaptive mechanism possibly involving paracrine factors. Further studies (Balm *et al.*, 1995) showed that neither prolactin nor α-MSH were involved in adaptation of skin or gills to acid pH. These data suggest that the primary action of an acid stressor is at the gills, and only when they are unable to maintain homeostasis is the pituitary interrenal axis invoked as a stress response.

10.4.3 Endocrine disruption of the stress response

True endocrine disruption of interrenal function involves a direct action of the pollutant either on the hypothalamic-pituitary-interrenal axis or on the liver such that hepatic catabolism of cortisol is affected. Unlike the secondary effects, mediated via the General Adaptation Syndrome, endocrine disruption generally suppresses interrenal function and consequently may lead to a lowered stress response, and hence decreased ability of the fish to respond appropriately to external threats from predators or altered environmental conditions. Although endocrine disruption could also theoretically lead to hypersensitivity of the pituitary-interrenal system there are no examples of such action in the literature.

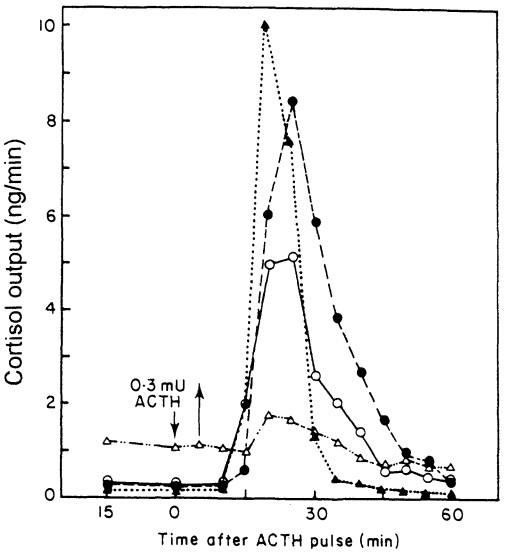


Figure 10.4. The response to ACTH of superfused interrenal tissue taken from tilapia 1 day (△–··–△), 130 days (○——○) and 225 days (● - - - ●) after a single injection of 50 mg/kg bw o,p'-DDD compared to control fish (♦·····♦). (Reproduced from Ilan, Z. and Yaron, Z., 1980. Suppression by organochlorines of the response to adrenocorticotrophin of the interrenal tissue in *Sarotherodon aureaus* (Teleostei). *J. Endocrinol.* 87, 185-193 by kind permission of the Journal of Endocrinology Ltd).

Although tilapia had higher resting levels of cortisol after injection with *o,p'*-DDD (50 mg/kg), there was no further increase in the steroid level after stress induced by confinement in a net and this lack of response persisted for 120 days (Ilan and Yaron, 1983). This was not because the interrenal was maximally stimulated already by the pesticide, since the response of superfused interrenal tissue to ACTH was much lower in DDD injected than in control fish and only 225 days after injection was the response the same as in controls (Figure 10.4). Pre-treatment of tissue with *o,p'*-DDD or addition of the pesticide to the superfusion medium also suppressed the ACTH response (Ilan and Yaron, 1980). Since *o,p'*-DDD did not affect cortisol production if N^6,$O^{2'}$-dibutyryl cyclic AMP was used instead of ACTH, it is likely that *o,p'*-DDD acts by inhibiting the generation of cyclic AMP. Disappearance of injected ^{3}H-cortisol from plasma was much slower in the DDE treated group than in controls indicating that the elevated levels of endogenous hormone in this group was probably due to decreased clearance as a result of hepatic malfunction. This was confirmed by the lower rate of *in vitro* catabolism of ^{3}H-cortisol. The long delay before recovery from a single injection of DDD correlated well with the persistence of the xenobiotic in plasma of the treated fish. Initial levels of 8.2 mg/l had declined to 0.4 mg/l 100 days after treatment, but had decreased little more by 213 days. Even this

concentration of DDD in the superfusion medium partially suppressed the ACTH response (Ilan and Yaron, 1980). This inhibitory effect of the DDT metabolites DDE and DDD on cortisol secretion is in marked contrast to the stimulatory effect of DDT (Gluth and Hanke, 1984, 1985) which is probably a simple stress response. It is not clear whether this is a real difference or reflects differences in experimental protocol such as dose, method of administration or species - a direct comparison would be of considerable interest since these organochlorines usually occur together in polluted sediments and in fish tissue. Although there are a number of other papers which report the suppression of cortisol levels by pollutants, few other authors have attempted to define the mechanism so clearly.

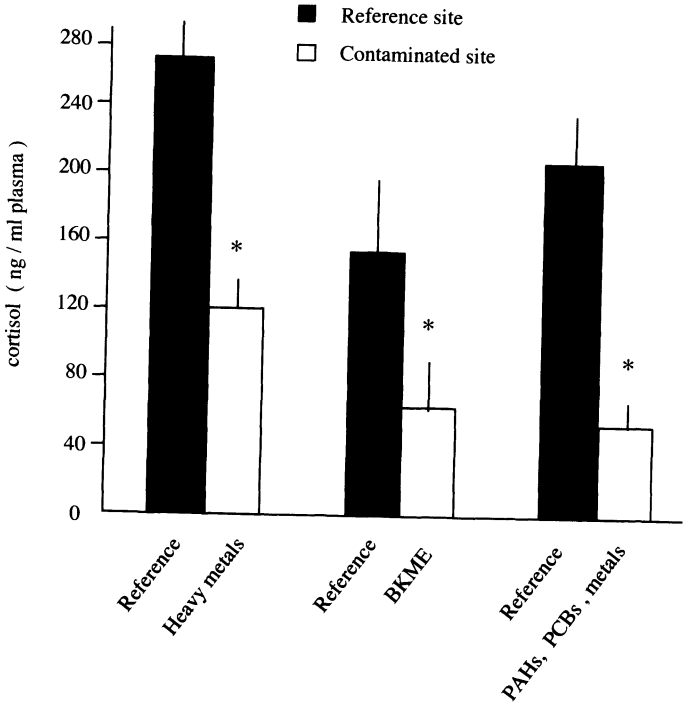


Figure 10.5. The impaired stress response of yellow perch (*Perca flavescens*) to capture at three sites contaminated by heavy metals, bleached kraft mill effluent or by mixtures of organics and heavy metals in Quebec (Reproduced from Hontela, A. (1997). Endocrine and physiological responses of fish to xenobiotics. *Rev. Toxicol.* 1, 159-206 by kind permission of IOS Press, Amsterdam. The Fig is modified from Hontela *et al.*, *Ecotoxicol.* 5, 1-12 (1996), *Arch. Environ. Contam. Toxicol.* 22, 278-283 (1992), *Environ. Toxicol. Chem.* 14, 725-731 (1995) and Brodeur *et al.*, *Can. J. Fish. Aquat. Sci.* **54**, 2752-2758.

A methylmercury diet (0.1mg/kg) which gave a mean body burden of 0.25 mg/kg suppressed cortisol in juvenile walleye (*Stizostedion vitreum*) which

may have contributed to the decreased growth of these fish (Friedman *et al.*, 1996). Decreased plasma cortisol was also found in northern pike (*Esox lucius*) taken from a lake and which contained a very high concentration (6-16 mg/kg) of mercury in their muscle tissue (Lockhart *et al.*, 1972). Mercuric chloride, methylmercuric chloride and methoxyethylmercury (Emisan-6) also decreased plasma cortisol levels in *Clarias batrachus* (Kirubagaran and Joy, 1991). The cytological changes of the pituitary ACTH cells and hypertrophy of the interrenal suggested that enzymes involved in the biosynthesis of cortisol were the primary site of action.

Pike and perch taken from sites containing high sediment levels of PAHs, PCBs and mercury both had a significantly decreased response to capture stress, the extent of the decrease relating to the extent of contamination (Figure 10.5) (Hontela *et al.*, 1992). The atrophied appearance of the pituitary corticotrophs suggested that the fish may have attained the exhaustion phase of the GAS after prolonged exposure to pollution and were no longer able to respond to stressful stimuli, but a direct effect on interrenal steroidogenesis might also have contributed. A similar suppression of the cortisol response to stress together with disruption of pituitary morphology was found at sites where these species had been exposed to bleached kraft mill effluent (Hontela *et al.*, 1997) or to sediments containing PCBs, PAHs and heavy metals (Hontela *et al.*, 1992, 1995).

This impaired response to stress in polluted environments forms the basis of the ACTH challenge test (Hontela, 1997; Girard *et al.*, 1997). Fish are quickly captured by seine net to avoid injury and held for 24 h to allow capture induced cortisol levels to return to basal values. Fish are then injected with saline or ACTH and sampled 2 h later. In yellow perch taken from a polluted site this test showed that the fish had impaired ability to respond to a standard ACTH dose (Figure 10.6). The failure to reverse the low cortisol level by ACTH was indicative of impaired interrenal rather than pituitary/hypothalamic function. An *in vitro* test, based on the same principles, uses head kidney pieces perifused for 10 min with ACTH and provides similar information by comparing tissues from fish captured in clean and contaminated sites. The test has been used in field studies at heavy metal contaminated sites in northern Quebec and organic and heavy metal contaminated sites in the St Lawrence River (Hontela, 1997). This is a similar procedure to that used by Ilan and Yaron (1980) for *o,p'*-DDD (see above). Such standardised tests may provide the basis for regulatory testing protocols for interrenal dysfunction in wildlife.

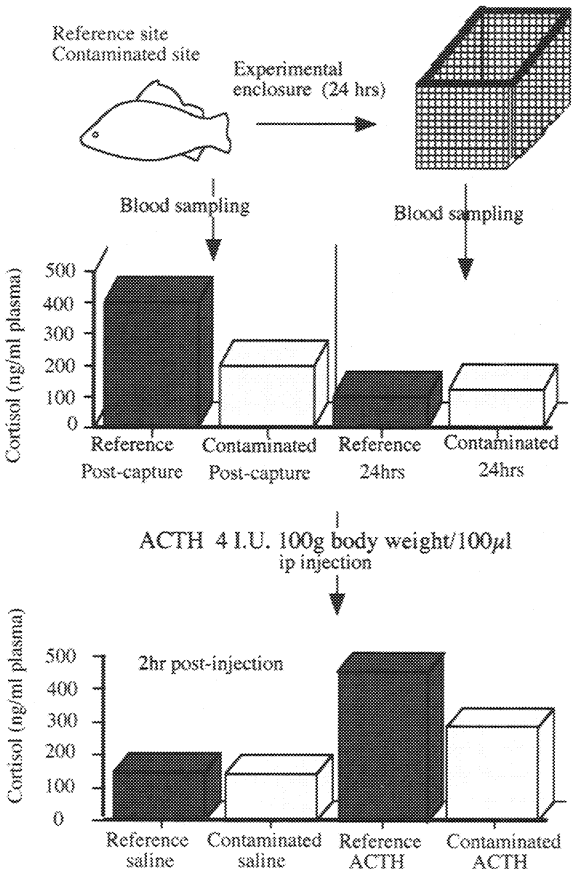


Figure 10.6. Field use of the *in vivo* ACTH challenge test to assess the functional integrity of interrenal tissue in yellow perch. (Reproduced from Hontela, A. (1997). Endocrine and physiological responses of fish to xenobiotics. *Rev. Toxicol.* **1**, 159-206 by kind permission of IOS Press, Amsterdam. The Figure is modified from Girard, C., Brodeur, J. and Hontela, A. (1997). Responsiveness of the interrenal tissue of yellow perch (*Perca flavescens*) from contaminated sites to an ACTH challenge test *in vivo*. *Can J. Fish Aquat. Sci.* **55**, 438-450).

Cortisol biosynthesis involves very similar enzymatic sequences to those involved in gonadal steroidogenesis. Since the plasma levels of the reproductive steroids can be affected by pollutants (Chapters 5 and 6), and the activities of many of the mixed function oxygenases involved in their biosynthesis and metabolism can be modulated by pollution (Chapter 9) it would not be surprising if similar effects were observed in interrenal tissue of exposed fish. *o,p'*-DDD is, for example, known from mammalian work to inhibit the mitochondrial 11β-hydroxylase which converts 11-deoxycortisol into cortisol (Hart *et al.*, 1971, 1973), but there have been no such studies in

fish. Bleached kraft pulp mill effluent (BKME) has been implicated in such changes of reproductive function, and has more recently also been shown to suppress plasma cortisol levels in exposed white suckers (McMaster *et al.*, 1994). This suggests that many of the pollutants which cause reproductive dysfunction through disruption of gonadal steroidogenesis, or which affect hepatic MFO activity, might also have similar effects on the stress response through disruption of interrenal steroidogenesis. Such studies are few, most probably a reflection on the greater difficulty in isolating interrenal tissue, but there are certainly good indications of suppressed interrenal steroidogenesis after exposure to pollutants. Spawning coho salmon taken from Lake Erie showed parallel decreases in plasma 11-ketotestosterone and cortisol compared with fish taken from Lakes Ontario and Michigan at comparable stages of sexual development (Morrison *et al.*, 1985b). The Lake Erie fish also had smaller interrenal cells and nuclear area than fish from other sites, indicative of reduced steroidogenic activity. Direct evidence for the disruptive effects of Arochlor 1254 on interrenal steroidogenesis was obtained by incubating [^{14}C]progesterone with head kidneys of cod fed a diet containing 1 to 50 μg/g of the PCB for 5½ months (Freeman *et al.*, 1982), but the limited number of incubations and failure to fully identify products, together with lack of any obvious pattern with increasing PCB concentration makes it difficult to interpret the data. Further evidence of direct action of pollutants on interrenal steroidogenesis is provided by the decreased responsiveness of superfusions of tilapia interrenal tissue to ACTH stimulation after *in vivo* exposure to cadmium or copper (Pelgrom *et al.*, 1994).

In contrast to the inhibitory action of DDT metabolites on hepatic clearance of cortisol, PCBs can enhance the rate of clearance of the steroid by hepatocytes (Vijayan *et al.*, 1997). In rainbow trout treated with 3,3',4,4'-tetrachlorobiphenyl for six weeks, plasma cortisol was elevated, uptake of [^{3}H]cortisol by hepatocytes was increased and its catabolism enhanced. The increase in hepatic EROD activity suggested that these changes were a result of increased mono-oxygenase activity. Treated fish had both a lower condition factor and lower plasma glucose concentrations indicative of increased carbohydrate metabolism resulting from elevated cortisol. Since data has already been cited to show that PCB induced changes in MFO can affect interrenal biosynthesis, there is clearly a balance between changes in steroid metabolism by the liver and the interrenal, and the relative effects of any pollutant at these two sites will determine whether plasma cortisol is increased or decreased.

Endocrine disruption encompasses not only direct effects on the endocrine glands, but also effects on the receptors for their hormones. There are some preliminary indications that acidity may affect the numbers of specific cortisol receptors in gills of Atlantic salmon (Weisbart *et al.*, 1989). Such studies are few and data on how other pollutants affect the receptors for hormones of

both the interrenal and other endocrine tissues would be of considerable interest.

In contrast to the extensive studies on the effects of pollutants on cortisol secretion, those which examine the effects on the catecholamines are very sparse. Cadmium at the environmentally realistic levels of 150 μg/l decreased the baseline plasma concentrations of dopamine, adrenaline and noradrenaline in American eels (*Anguilla rostrata*) below that of controls after 16 weeks exposure (Gill and Epple, 1992). Although the initial response of dopamine to mild short-term stress was impaired, there was no clear impact on the stress response of adrenaline or noradrenaline. The results were less consistent at a lower exposure of 75 μg/l cadmium. Since organochlorine and organophosphate pesticides, PCBs and PAHs affected brain levels of catecholamines in fish (McDonald and Fingerman, 1979; Fingerman and Russell, 1980; Thomas *et al.*, 1981; Holdway and Dixon, 1986b), their action on the chromaffin tissue and the consequent impact on the stress response would be of some considerable interest. Plasma cortisol levels were increased by exposure to only one tenth the concentration of PAH that caused observable behavioural disturbance resulting from depleted levels of brain catecholamine (Thomas *et al.*, 1981). It would be important to determine whether such affects on catecholamine production by chromaffin tissue occurred at lower levels than those which caused general behavioural or locomotor changes as a result of action on the central nervous system.

Fish in the wild are exposed to a mixture containing many different pollutants. In the preceding sections it has been shown that while some xenobiotics elicit a simple stress response, others disrupt the pituitary-interrenal axis and suppress the response to external stressors. Combinations of different pollutants may therefore result in unpredictable actions on the stress response of fish. In some cases the stress response caused by one pollutant may be negated by inhibition of the interrenal activity by another. This is exemplified by the fact that copper induced a normal stress response in tilapia, while fish exposed to a combination of copper and cadmium did not differ in plasma cortisol levels from control fish (Pelgrom *et al.*, 1995a).

10.5 Osmoregulation

Although many of the classical experiments of endocrinology, involving interrenalectomy and cortisol replacement have clearly shown that the hypothalamo-pituitary-interrenal axis plays a role in normal osmoregulation (Eddy, 1981) by action at the gills, intestine and kidney, most of the osmoregulatory disturbances resulting from pollutants reported in the literature appear to be the cause rather than the result of changes in interrenal function. Since the primary tissue contact of water-borne pollutants is with

the gills, and the gills are a major site of osmoregulation this is perhaps not surprising. The heavy metals arsenic and mercury for example appear to have their primary action on gill Na^+-K^+-ATPase resulting in hydromineral disturbance (Stagg *et al.*, 1992; Hwang and Tsai, 1993). Copper and zinc, however, reduced the influx of chloride but did not alter Na^+-K^+-activated ATPase in the stenohaline bluegill (*Lepomis macrochirus*) (Heath, 1987). Copper also increased chloride cell proliferation and an increase in their diameter and decreased plasma sodium (Pelgrom *et al.*, 1995a, b), while cadmium increased the number of lysozomes and apoptic chloride cells as well as inhibiting active branchial Ca^{2+}-transport activity (Pelgrom *et al.*, 1997). The cadmium and zinc inhibition of transepithelial calcium influx in rainbow trout gills may be due to displacement of Ca^{2+} ions from the protein carriers which facilitate the diffusion (Verbost *et al.*, 1987; Sauer and Watanabe, 1988). In tilapia, however, neither copper nor cadmium affected calcium influx, although a combination of the two metals did, underlining the importance of looking at the effects of combinations as well as single metals (Pelgrom *et al.*, 1997).

While the gills are the primary point of contact for pollutants in the water, uptake of dietary pollutants may affect other organs such as the liver and kidneys. Damage to the kidneys or liver by pollutants such as mercury, atrazine, or organochlorine pesticides (Hilmy *et al.*, 1983b; Kirubagaran and Joy, 1988c; Oulmi *et al.*, 1995a, b) may also result in osmoregulatory disturbances. The urophysis, a caudal neurosecretory system in teleosts (Larson and Bern, 1987) and the corpuscles of Stannius also play a role in osmoregulation of teleost fish but the direct actions of xenobiotics at these sites has not yet been examined. The correlation between urotensin I and pH, and between urotensin II and total water aluminium (Hontela *et al.*, 1989) was more likely to be due to action of pollutants at the gills and attempts by the urophysis to restore homeostasis. Similarly, the elevated brain levels of arginine vasotocin (AVT) detected seven days after exposure of trout to acid water containing aluminium (Hontel *et* al., 1991, 1993) was more likely due to primary action at the gills rather than the pituitary. Although there were changes in the morphology of pituitary prolactin secreting cells after exposure of tilapia to cadmium, this was due to increased activity in an attempt to restore calcium homeostasis due to action of the metal on the gills (Fu *et al.*, 1989). Since catecholamines, prolactin, calcitonin, the neurohypophysial hormones arginine vasotocin (AVT) and isotocin (IT), the urotensins, as well as cortisol and the thyroid hormones all affect gill transport (Mayer-Gostan *et al.*, 1987) it is clear that endocrine disruption at a number of loci can affect osmoregulatory balance. To date many of these have not been examined. In such investigations it is important to determine whether the endocrine system is directly affected by the pollutant or whether endocrine activity is perturbed

in an effort to restore homeostasis as a result of a pollution induced disequilibrium at other sites.

It has yet to be shown whether the direct endocrine disrupting effects of pollutants on the hypothalamo-pituitary-interrenal axis causes changes in osmoregulatory function at concentrations which do not have direct effects on ion transport in the gills and kidney.

10.6 The immune system and disease

Although there is clear evidence of a high incidence of disease in fish living in polluted waters (Klontz, 1984; Bucke and Waterman, 1988; Dethlefsen, 1989; MAFF, 1991) cause and effect is not clearly established. Nor is it clear whether, if pollution is the cause, it is due to increased numbers of pathogenic organisms or to increased susceptibility of fish to these organisms as a result of suppression of their immune system.

There is an extensive literature on disruption of the immune system in fish (Anderson *et al.*, 1996). Immunosuppression resulted from exposure to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, tributyltin, zinc, PCBs, TCCD and PAHs, endrin, malathion, trichlorphon, DDT, and bayluscide. The parameters measured included phagocytosis, lymphocyte numbers, macrophage activity, serum antibody levels, and susceptibility to pathogens. The mechanism by which these pollutants exert their effects is not generally clear and, although direct disruption of the immune system itself can undoubtedly occur, many of the cases of diseased fish could be attributed to action on non-specific tissues which is manifest as a stress reaction leading to elevated cortisol and hence immunosuppression (Brown *et al.*, 1979).

There is ample evidence that cortisol can suppress the immune system (Ellis, 1981; Anderson *et al.*, 1982; Stave and Roberson, 1985; Trripp *et al.*, 1987) based on experiments in which high plasma cortisol levels, elevated by injections of this steroid, caused disrupted immune function. At high levels of toxicant exposure, the increased level of stress induced cortisol may therefore increase susceptibility to infection and disease and lead to increased mortality. Such effects may occur at levels below that which causes mortality by a direct toxic action, but above that at which true endocrine disruption occurs. The stress response involves increased secretion of both cortisol from the interrenal and catecholamines (adrenaline and noradrenaline) from the chromaffin tissue. These in turn act, via the peripheral circulation, on the anterior kidney, spleen and thymus to affect the lymphocytes, while macrophages and lymphocytes can be directly affected by both cortisol and catecholamines (Figure 10.7) (Schreck, 1996). There is evidence that pollutants can affect the immune system by causing atrophy of the thymus

since in guppies treated with tributyltin oxide (TBTO) for a month this gland had almost disappeared, and the no-observed effect concentration was estimated as only 0.01 μg/l (Wester and Canton, 1987; Wester and Roghair, 1994). Cortisol appears to act by altering the number and affinity of specific receptors on the leukocytes, but little is known of the mechanism by which catecholamines exert their action.

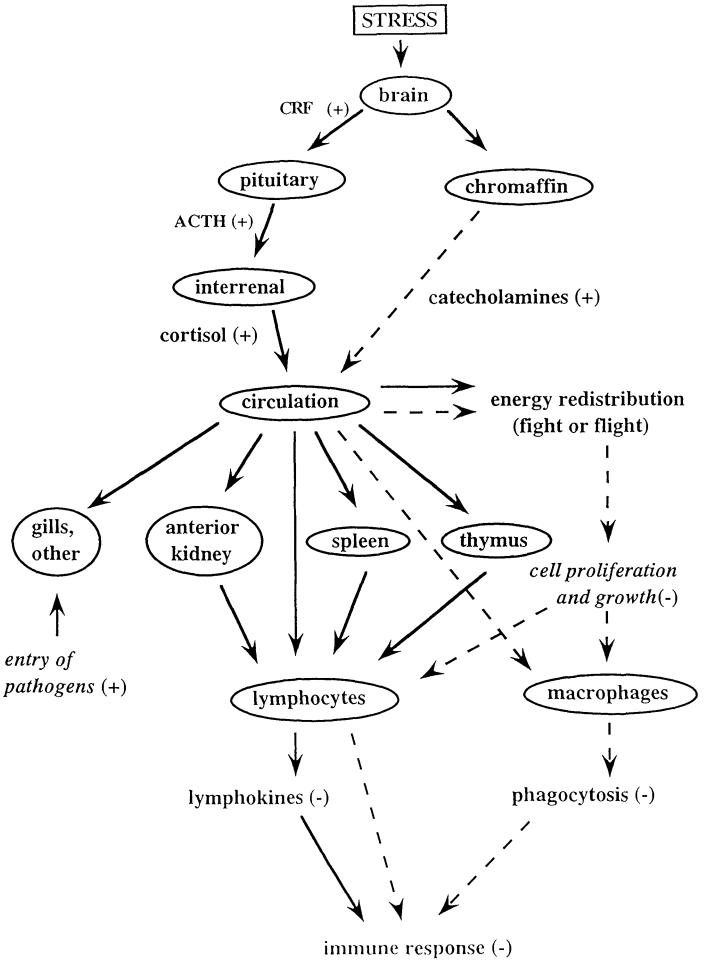


Figure 10.7. The physiological stress response and the mechanisms whereby the immune system is affected. Solid arrows denote pathways mediated by the hypothalamic-hypophyseal-interenal axis, while dashed arrows denote those mediated by catecholamines. Italicized words denote modes of action that are inferred, but not based on direct data. (+) and (-) indicate the direction of the response. (Reproduced from Schreck, C. B. (1996). Immunomodulation:Endogenous factors. In "The Fish Immune System. Organism, Pathogen and Environment", (G. Iwama and T. Nakanishi, Eds.), pp. 311-337 by kind permisssion of Academic Press, San Diego)

Although exposure of rainbow trout to 0.12-0.15 μg/l endrin for 60 days resulted in a four-fold increase in plasma cortisol throughout the exposure period, together with a diminished immune response this does not on its own demonstrate that the pesticide acts solely via cortisol (Bennett and Wolke, 1987). For such a demonstration it would be necessary to show that the same immunosuppression occurred in both endrin treated and non-specifically stressed fish which had the same high plasma cortisol levels over the same time period.

The data summarised above concerns only the effect of stress, mediated via cortisol and catecholamines, on the immune response. Perhaps even more interesting is how the immune system responds to pollution induced suppression of interrenal function. Does exposure to endocrine disrupting chemicals which inhibit the normal stress response lead to a decreased susceptibility to disease? What action does a long-term decrease in basal cortisol secretion have on the immune system? How is it affected by combinations of pollutants which both induce the stress response, and inhibit the extent of the response. How is it affected by endocrine modulators which affect the interrenal, but not the chromaffin tissue?

The immune system involves interactions between the cells of the immune system which are mediated by both direct cell to cell contact and via release of cytokines, which are soluble factors that play a regulatory or enhancing role within the immune system (Manning and Nakanishi, 1996). These factors, which include the interleukins, tumour necrosis factors, interferons and transforming growth factors act as intercellular messengers which mediate activities of the cells of the immune system. As such they are included in the broader definitions of endocrinology, and direct effects of pollutants on the synthesis and action of these factors can be considered as endocrine disruption. Data on such disruption as a cause of immune dysfunction in fish, however, is not yet available.

10.7 Conclusions

Although changes in thyroid morphology or levels of the hormones T_3 and T_4 show changes after exposure to a wide range of pesticides and industrial pollutants, there is rarely either a clear dose response, or a consistent and predictable effect. Frequently the changes are transient and can be affected by the stage of the reproductive cycle. Disruption of thyroid function can therefore be taken as indicative of the presence of anthropogenic chemicals, but does not in itself generally provide a sufficiently reliable indicator to be used as a biomonitor, although clearly the goitrous fish found in the Great Lakes are an exception. Of particular concern must be the relation between thyroid function and plasma cortisol, such that thyroid activity can be altered

by non-specific stressors. The function of thyroid hormones as metabolic regulators and the decreased growth found in fish with low plasma T_3 suggests that in any study of pollutants growth should be compared between exposed and control fish and, if there are any differences, that thyroid hormones might be worth measuring. If the aim is to determine whether the test pollutant is an endocrine disruptor it is important to note whether food consumption differs between the control and treated fish, since lack of feeding is indicative of ill health resulting in loss of appetite, while failure to convert the food into somatic growth may be due to endocrine disruption or to a generalised stress effect acting via the interrenal. Many of the effects observed for reproductive dysfunction occur at levels at which there is "no apparent ill-health or visible difference between control and exposed groups". Such differences clearly must include weights of fish in both groups, but this is often not clearly stated. The absence of weight differences in such studies would clearly indicate that the reproductive system is the most sensitive and has not been subjected to general non-specific stress acting via the interrenal and thyroid glands.

The importance of avoiding uncontrolled stress is, of course, obvious if endocrine disruption of the hypothalamic-pituitary-interrenal axis is under investigation. In many cases, however, it may be necessary to induce stress in a controlled manner so that the response to external stressors can be examined. It is apparent that in many of the studies of pollution the use of sub-lethal levels of the xenobiotic must be more carefully defined than hitherto. It is no longer sufficient to simply use a level just below that which kills a fish, but which quite clearly is causing it some discomfort and altering the interrenal function through a normal stress response. In far too many cases only a single dose of the pollutant is used, and comparisons are often made between the action of two or more chemicals each used at a single "sub-lethal" dose. Such experiments have little value in either real terms or in providing scientific evidence of endocrine disruption, although they have been included in this volume for the sake of completeness. To provide comprehensive data it is necessary to use a wide range of concentrations and to carefully monitor the status of the fish. This will enable determination of the levels at which the fish gives obvious signs of stress, their feeding behaviour is affected, they feed normally but fail to grow, they fail to respond normally to external stressors, interrenal and/or pituitary corticotrophs are affected, liver catabolism is affected, and finally the level at which the exposed fish do not differ in any respect from the controls (the no observable adverse effect concentration).

In terms of the fish as a whole it is important to determine how the levels of a pollutant which affect the reproductive system compare with those which either cause endocrine disruption of the hypothalamic-pituitary-interrenal axis or which elicit a stress response. Clearly concentrations which either cause

such a high stress response that they lead to immunosuppression and disease, or which so inhibit the ability of the fish to respond by natural "fight or flight" mechanisms to predators, will not survive long enough for endocrine disruption of the reproductive system to have any real meaning.

Chapter 11

The Fish-Wildlife-Human Connection.

11.1 Introduction

The data presented in this volume clearly show that the endocrine system of fish can be compromised by a wide range of anthropogenic pollutants that have been introduced into the aquatic ecosystem. Fish do not, however, live in isolation from other animal communities since they provide a food resource on which many other aquatic and terrestrial animals are dependent. These animals include the marine mammals such as seals and whales, fish eating birds, and terrestrial mammals such as mink, otters and of course humans. The effects of pollution on the more visible species, such as fish eating birds and marine mammals, is both more apparent to us than that which may be occurring unseen in the aquatic ecosystem and serves as a warning that we are threatening our own food resources, our health and ultimately our species.

11.2 Health hazards from dietary fish

11.2.1 Communities at risk

Many of the early studies cited in Chapter 3 measured the concentrations of specific pollutants in tissues of fish to determine whether they were fit for human consumption. For this reason the studies usually concentrated on edible muscle tissue and viscera, including liver and gonads, were generally ignored. They aimed to determine the minimum level which might affect human health in urban societies where fish is consumed in relatively small quantities, but such levels may not be valid in societies in which fish constitutes a major source of protein and in which the liver and gonads (fish

roe) may be also be consumed. Such communities, which comprise both indigenous North American populations around the Great Lakes and Arctic coasts and numerous small fishing communities throughout the world, are generally widely scattered and information on the effects of such high levels of consumption is often not readily available to the scientific community. The communities themselves may have little idea that the diet which has sustained them for generations may be the cause of their ill health or decreased fertility. The threat to such communities is often first seen in the ill health, or reproductive failure, of local birds and mammals which consume fish of similar origin to their own diet. Scientific examination of such wildlife for early signs of developmental and functional abnormalities, together with determination of tissue pollutant levels can link cause and effect. If the symptoms found in such wildlife are also apparent in local fish-eating communities, there is also a warning to other human communities which may consume fish in lower quantities. If, for example, clinical infertility is found in a community which consumes a large amount of fish, decreased fertility might be expected in urban communities where fish forms only part of the diet, or where it forms the diet of other domesticated animals which provide protein sources. By the nature of its proximity to major sources of pollutants and to urban populations, and to its historic role in providing protein sources to large urban areas, the Great Lakes area has been the subject of the most intensive such investigation. There have been no comparable studies on populations around the highly polluted North and Baltic seas, probably as a result of the difficulty in identifying such high-risk communities in the more populous areas of Europe in which dietary intake is generally more varied. The fish-eating species most used for such investigation are the marine mammals, mink and birds, particularly herring gulls and the American bald eagle - which may have more than symbolic significance for the population of North America!

While the effects of pollutants on fish may serve as a warning of their potential effects on humans and terrestrial wildlife, effects that are apparent in fish eating mammals and birds may conversely serve as a warning that similar effects are occurring in the fish stocks from which they derive their diet. Dolphins, whales, seals, otters and mink and fish eating birds such as gulls and sea eagles are relatively long-lived top-predators and effects on their health would be expected to be similar to the less visible piscine top predators which consume a very similar diet. Such species as eels, cod and salmon are not only the most likely to suffer first from the effects of pollution, they are also the species of greatest commercial importance.

11.2.2 Effects on fish eating birds

In "Silent Spring" Carson (1962) demonstrated the effects of indiscriminate pesticide spraying on bird populations which resulted in

mortality, reproductive failure, egg-shell thinning and hatching failures. While such problems are now much diminished in the general avian population, they remain prevalent in a number of species which take fish from polluted habitats as their main dietary source. Herring gull populations in the Great Lakes region declined rapidly in the 1960s and 1970s, and the symptoms were similar to those described by Carson (1962) as resulting from pesticide spraying. Similar evidence of reproductive and developmental failure was found in other gulls, terns, and cormorants and included symptoms of congenital abnormality, low hatchability and fledging rates, undersized chicks and unresorbed yolk sacs (Gilbertson, 1988). In one gull colony the eggs contained 30 mg/kg DDE and 100 mg/kg PCB, as well as dieldrin, heptachlor epoxide and hexachlorobenzene. The small size of the chicks that pipped the shell but failed to hatch was similar to that caused by antithyroid compounds and it was suggested that it might be due to the presence of antithyroid compounds in the yolk derived from maternal diet (Gilbertson and Fox, 1977), while the chick oedema was attributed to the presence of polychlorinated dibenzofurans amongst the PCBs. The presence of thyroid active contaminants in Great Lakes fish is also discussed in Chapter 10. To determine whether the hatching failure was due to contaminants within the egg, or to contaminant induced changes in parental behaviour, egg exchange experiments were conducted between clean maritime and "dirty" colonies (Peakall *et al.*, 1980). Parents in the contaminated area were less attentive to their nests and applied less heat to the eggs than those in a clean area suggesting that diet affected parental behaviour. The time for which the parents left the eggs unattended was correlated with the organochlorine residues in the eggs. It is not clear whether these behavioural changes were the result of pollutant induced neurological damage or mediated through changes in the hormonal profile which regulates reproductive behaviour. Chicks from eggs laid in a contaminated area showed lower hatch rate, were smaller, had more oedema and unresorbed yolk sacs than controls (Gilbertson and Fox, 1977).

The great blue heron (*Ardea herodias*) on the coast of British Columbia, which forages in estuaries on which are situated Canada's largest concentration of forest product industries including pulp, paper and lumber mills and wood treatment operations, provides an excellent biomonitor of the effects on predators of fish exposed to such effluents (Elliott *et al.*, 1989). Eggs from these herons contained unusually high levels of polychlorinated dibenzodioxins and dibenzofurans (PCDDs and PCDFs). The similar high levels of PCDDs found in the related grey heron (*Ardea cinerea*) in the Netherlands (Van den Berg *et al.*, 1987) show that such effects are not limited to pulp mill areas and can also be caused by general industrial effluents. Levels of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) were higher than those which gave embryotoxic effects in chicken egg injection studies. In the

Canadian study PCDD levels were threefold higher in 1987 than in 1986 and no young were produced. This was attributed to predation by bald eagles rather than direct embryotoxic effects of the organochlorines but, as with the gulls around the Great Lakes, parental guarding behaviour could have been affected. It is perhaps not surprising that bald eagles in British Columbia had extremely high levels of PCDDs (Elliot *et al.*, 1996). One bird had liver concentrations of over 6 μg/kg, and the ratio of TCDD to TCDF was taken to indicate induction of hepatic cytochrome P4501A. Many birds also had very high levels of DDT and DDD, with levels greater than 50 mg/kg in livers of some birds. Potentially many of these birds would appear to be susceptible to endocrine dysfunction, but this was not examined in this study. The concentrations of PCBs and TCDDs in fish below the dams of three Michigan rivers, however, were sufficiently high to be considered to pose a hazard to the reproductive productivity of bald eagles in the region (Giesy *et al.*, 1995).

In addition to the organochlorines, mercury levels are high in eggs of many fish-eating birds. Although levels in herring gulls in the Great Lakes area declined from 1972 to 1992 after restrictions were placed on some uses of the metal, concentrations now appear to have stabilised and will probably not decline further unless additional restrictions are imposed (Koster *et al.*, 1996). In the Great Lakes, the mercury concentrations have now fallen below the level at which reproductive impairment is likely to occur from exposure to this metal alone, but it could contribute to the cumulative effects of other pollutants. Levels of mercury in eggs of gulls in areas bordering the North Sea are, however, much higher (6-7 μg/g) and have been associated with hatching failure (Becker *et al.*, 1993).

Many of the reproductive defects resulting from the widespread application of pesticides were summarised by Carson (1962) and more recent evidence that pollutant burdens can result in endocrine disruption has been reviewed by Fox (1992, 1993) and Fry (1995). These effects include the feminization of gull embryos, alterations of sex ratios, production of supernormal clutches and abnormal mating behaviour such as female-female pairing. Such effects predominate in fish eating birds and are usually associated with high tissue concentrations of organochlorines, such as DDT, methoxychlor and mirex, which have been shown to be estrogenic in birds (Eroschenko and Palmiter, 1980; Fry and Toone, 1981). *o,p'*-DDT at 2-5 mg/kg, a level much lower than found in many gull colonies, can for example induce feminization in male gull embryos (Fry and Toone, 1981). The biochemical markers, such as induction of cytochrome P4501A1 as indicated by elevated EROD or AHH activity, found in exposed fish are also apparent in many fish eating birds. The lack of parental attentiveness, which may have contributed to loss of the clutches of the Canadian herons (see above), and has also been observed in gulls and terns, is suggestive of thyroid mediated toxicity (Fox, 1993). Such inattentiveness has been induced in doves fed a

PCB contaminated diet which altered thyroxine levels, decreased metabolic rate and the ability to thermoregulate at low temperatures. Studies into the parental care of appropriate fish species would be of considerable interest in this context, but to date such studies have been few and the results inconclusive (Jones and Reynolds, 1997). Great Lakes herring gulls collected from 1974-1983 had a high incidence of goitre which reflects similar abnormalities in salmon from these lakes (see Chapter 10). Similar goitres and abnormalities have been induced by feeding DDT, DDE, dieldrin and PCBs to birds. The small size of herring gull chicks around the Great Lakes that pipped the shell but failed to hatch may have been due to the presence of anti-thyroid compounds, since such compounds can produce similar symptoms (Gilbertson, 1988). A more recent study has shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin can affect plasma thyroid and sex steroid concentrations as well as estrogen receptor levels in adult great blue herons (Janz and Bellward, 1997). The presence of the symptoms of chick oedema disease in eggs collected from Great Lakes colonies of gulls has a remarkable similarity to the blue-sac disease found in fish eggs exposed to maternal xenobiotics (Chapters 7 and 8). A range of other developmental abnormalities have been described in other fish eating birds (see Fox, 1993; Fry, 1995 for references).

11.2.3 Effects on marine mammals

Both marine and terrestrial piscivorous mammals, such as seals, whales, mink and otters, are threatened by the concentration of aquatic pollutants into fish tissues. In recent years there have been a number of incidences of high mortality of seals, whales and dolphins in the North Sea. Although the primary cause of death has been disease, possibly due to the introduction of new strains of viruses, the very high levels of PCBs in the affected fish suggests that pollutant induced disruption of the immune system may have been a major contributory factor. Bergman and Olsson (1985) related the widespread incidence of disease in seals to adrenocortical hyperplasia induced by high tissue PCB levels leading to immunosuppression. There were also reproductive disorders and hormonal osteoporosis which indicates general endocrine disruption. Many of the symptoms of skin disease were similar to those found in rice oil disease (Yusho) in Japan in 1967 where up to 67 μg PCB per kg body weight per day was ingested over 3 months from contaminated rice oil. Intake of PCBs by Baltic seals from their diet of fish is estimated to exceed this and to have continued for several years. Since the carcasses are frequently washed up onto populous shores, and the animals have a significant emotional appeal, such incidents have been rather more newsworthy than similar mortalities of other less visible or emotive species. Mortality is, of course, only the extreme case of pollution induced

malfunction, and suggests that many more of the species may be suffering from sub-lethal effects.

The collapse of the common seal (*Phoca vitulina*) population in the western Wadden Sea off the Netherlands, from 3000 animals in 1950 to less than 500 in 1975 was associated with a sharp decline in pup production (Reijners, 1986). Tissue pollutant concentrations in these mammals differed from those in northern areas of the Wadden Sea, which were unaffected by the decline, only in their content of PCB which emanates from the River Rhine. Groups of seals fed either fish from the polluted western part of the Wadden Sea or fish from the north-east Atlantic did not differ in their plasma progesterone or estradiol within either the pregnant or non-pregnant groups, but the reproductive success of those fed the diet from the Wadden Sea was significantly lower. The hormonal profile showed that the pollutant exerted its effect at the stage of the reproductive process around implantation and that the follicular, luteal and post-implantation processes were unaffected.

There have been numerous studies of the concentrations of pollutants in cetaceans (see Muir *et al.*, 1996a, b). Since these animals are long lived, consume fish and have a large amount of lipophilic blubber it would be expected that organic pollutant levels would be high. In beluga whales in the St. Lawrence river estuary, for example, concentrations of PCBs, DDT and mirex in blubber were 8-412, 3-389 and 0.2-6.8 mg/kg respectively (Muir *et al.*, 1996a). These levels are far higher than found in fish liver, but whether comparable levels are found in hepatic or gonadal tissue is not clear. For obvious reasons, there has been little direct study of the relation between pollutant concentration and endocrine function in cetaceans, but there is evidence that P450 enzymes are stimulated indicative of the presence of potential endocrine disrupting factors (Watanabe *et al.*, 1989; Norstrom *et al.*, 1992; White *et al.*, 1994). Many of the symptoms, such as increased prevalence of tumours and a high incidence of lesions of the digestive system, together with evidence of immunosuppression found in beluga whales of the St Lawrence (Beland *et al.*, 1993) have similarities to those of the Baltic seals. The beluga may be particularly susceptible since it accumulates much higher levels of organochlorines in its blubber than do the other cetaceans and seals in the St. Lawrence which showed no such lesions. The temporal trends in blubber concentrations in beluga whales paralleled that in fish and seabirds in the St. Lawrence estuary during the 1980s (Muir *et al.*, 1996b).

11.2.4 Effects on terrestrial mammals

Mink are of major commercial importance in the Great Lakes area, and until recently derived their food from fisheries in the lakes. The effects of such diet on ranch mink has been well documented (Aulerich and Ringer, 1977; Gilbertson, 1988). The first evidence of reproductive dysfunction in stocks of

mink on ranches became apparent in the late 1960s and was attributed to agents in their diets of fish from Lakes Michigan and Erie. Although DDT was at first suspected, the levels present in the lake fish diet when added to an uncontaminated diet did not cause any decrease in fertility. Lake Michigan salmon, however, also contained 10-15 mg/kg of PCBs, and when this concentration was added to a control diet, the clinical symptoms were similar to those of mink fed the lake fish. The levels of PCBs in mink that died as a result of either diet were very similar. Cessation of use of Great Lakes fish in mink diet led to an end of the reproductive problems faced by ranchers. The high levels of PCBs and TCDD equivalents (TCCD-EQ) in fish below hydroelectric dams also poses a threat to reproductive function in mink feeding in these areas (Giesy *et al.*, 1994b).

In the United Kingdom, the Eurasian otter (*Lutra lutra*) disappeared from large areas of England in the 1960s when organochlorine pesticides were in widespread use. Similar population decreases were recorded over much of western Europe. Although populations have begun to recover in Wales, south-west England and the peripheral areas of mainland Europe since use of these chemicals was restricted they have continued to decline in the more central industrial areas. It is not clear whether this is simply due to loss of habitats or to the wide range of industrial pollutants to which they are now exposed in these areas (Chanin, 1985). PCB levels were much higher in otters in Shetland and England (2 mg/kg body weight) than in Scotland and although there was a strong negative correlation between body condition and PCB level the population in Shetland was increasing (Kruuk and Conroy, 1996). The lack of correlation of PCB with age suggests that otters may bioaccumulate less than mink, and this may account for a thriving population in areas which contain levels of PCBs which would cause reproductive failure in mink. The increasing use of pesticides to control sea lice in salmon ranches around the northern European coasts may, however, pose a particular threat to local populations of otters and seals feeding on fish and crustaceans in the vicinity of the sea cages if application is not sufficiently regulated.

Some effects of pollutants are, however, less readily detectable. Rats fed for 20 days with a diet of 30% Lake Ontario salmon showed a behavioural difference to control animals (Daly *et al.*, 1989; Daly, 1993). Differences were recorded only for negative experiences and not for positive ones, so may not be detectable in simple reward experiments. Furthermore, the offspring of parents who had consumed Lake Ontario salmon, but were fed an uncontaminated diet from weaning to adulthood also showed these behavioural changes as adults. Such behavioural problems may account for the lack of parental care found in some birds, but second generational effects in such populations would be particularly difficult to detect. It would also be extremely difficult to determine whether parental care in otters or mink might be similarly affected.

11.2.5 Effects on fish eating human communities

Although the majority of human populations consume a varied diet, there are some isolated communities where consumption of fish is the major protein source and such communities are at similar risk to that of fish-eating birds and mammals. Although game fisherman in the Great Lakes are warned of the dangers of eating their catch, they usually do not use this as their sole food resource. The Inuit communities in Arctic regions have little choice, and although many areas now have access to air-freighted food supplies from further afield, their traditional way of life is dependent upon hunting. Although such areas are some thousands of kilometres from the sources of pollution, their food resources of whale, seal and fish are top predators which forage further afield. The marine mammals are protected against the cold Arctic waters by a thick layer of blubber which forms a major part of the traditional inuit diet (muktuk). Not surprisingly this contains very high levels of anthropogenic pollutants (up to 400 mg/kg DDT and PCB) (Muir *et al.*, 1996a), and only 10 g muktuk can contain more than the maximum weekly intake of PCBs as recommended by the Canadian health authorities. Livers of ringed seals contain 20-200 times the recommended levels of mercury and high amounts of cadmium (Pearce, 1997). Although fish and fish-eating mammals might be expected to contain high levels of pollutants, arising from foraging in lower latitudes in closer proximity to industrial effluents, the finding of high levels in caribou is indicative of high local fallout of pollutant. Such pollution arises from condensation of atmospheric pollutants emitted in warmer, more southerly industrial areas, and may produce a flush of toxins in the aquatic ecosystem during the early summer snow melt (see Pierce, 1997). The exposure of such remote, traditional and independent communities to the toxins produced in the affluent industrial communities some thousands of kilometres to the south is but one price that is paid for the high living standards of western "civilisation" and illustrates the clear global dimension to aquatic and atmospheric pollution.

It is in such highly exposed populations that the first signs of human health hazards are likely to become apparent, ironically because it is often such remote populations that are chosen as controls living in a pristine environment. The remoteness of such communities, their understandable resentment against intrusion by outsiders, and limited local medical facilities have restricted the amount of information available about endocrine disruption. The very high concentrations of xenobiotics found in their blood and tissue would, however, suggest that endocrine disruption would be similar to that observed in fish and other fish eating mammals and described elsewhere in this volume.

Studies on children born to women who ate contaminated fish from the Great Lakes during pregnancy suggest that the effects of pollutants such as

PCB can affect the behaviour pattern in a similar manner to that described for rats and birds (Sections 11.2.2, 11.2.4). Newborns had more jerky, unbalanced movements and startles and a greater number of abnormally weak reflexes, while at 5-7 months they performed less well in a visual recognition memory test (Jacobson and Jacobson, 1988; Jacobson *et al.*, 1990; Lonkey *et al.*, 1996). In follow up studies (Jacobson and Jacobson, 1993, 1996) the children who had the highest *in utero* exposure were three times more likely to have low average IQ and twice as likely to be at least two years behind in reading comprehension as normal children by age 11. The greatest effects were related to memory and attention, which closely reflects that observed in PCB exposed wildlife populations. Although larger amounts of PCBs were transferred in breast milk than *in utero*, the behavioural effects were associated only with transplacental transfer suggesting that the period during which the fetal brain develops is the most sensitive. In the context of recent concern about reproductive abnormalities and decreased sperm counts, evidence in this population of effects due to fetal and neonatal exposure on neural development of the pituitary, imprinting of sexual behaviour patterns which only become apparent at puberty, and the fertility of both sexes, would be of great interest, but the population studied is small and it will be at least another decade before any such effects become apparent.

Although these studies, and those on rats and birds, deal with behavioural changes rather than direct endocrine disruption they show that many of the effects of exposure to anthropogenic pollutants are very subtle and may only be expressed in the offspring of exposed parents. Such xenobiotic induced changes in behaviour are clearly induced at an early stage of embryonic development. Parallel abnormalities might also be expected in endocrine development, possibly as a result of neural abnormalities in the pathways controlling the function of hypothalamus. The close parallelism between the effects of PCBs on attentiveness in humans and wildlife suggests that other developmental effects observed in fish and birds which receive pollutants via the egg may also occur in humans through placental transfer. As with the behavioural studies, many of these relatively minor disruptions may escape detection in routine clinical examination. Humphrey (1988) found no significant differences between medical complaints *reported* by fish-eating and control groups in a cohort study, but suggested that further studies should test multigenerational effects on their children, possible metabolic and immunological impacts, and onset of diseases such as hypertension, arthritis, and cancer. To these should also be added endocrine disruption. The prevalence of a high incidence of unexplained goitres in human populations in the Great Lakes area and its parallel with that in fish populations has already been discussed (Chapter 10).

11.3 Hazards to human populations.

The majority of this volume has been devoted to the effects of anthropogenic pollutants on fish which inhabit polluted aquatic environments. Clearly humans who have a high dietary imput of such fish will also accumulate these same pollutants in their tissues. This applies equally well to the Inuit, communities around the Great Lakes and to peasant farmers who consume fish from paddy fields treated with pesticides. The relevance to the majority populations of the world who consume a mixed diet in which meat and vegetables predominate is less clear.

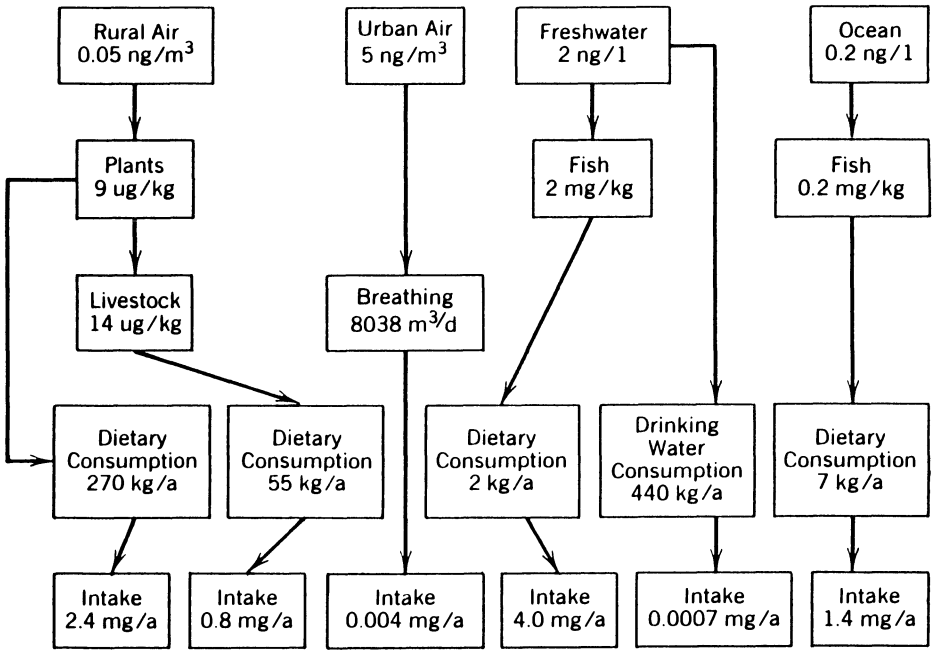


Figure 11.1. Pathways for human intake of PCBs in the Great Lakes area. (Reprinted with kind permission of John Wiley and Sons Inc. from Hallett, D. J. Ecosystem management of persistent toxic chemicals - back to basics. *In* "Toxic Contaminants and Ecosystem Health: A Great Lakes Focus", (M. S. Evans, Ed.), pp. 491-509. Copyright John Wiley & Sons, 1988. Data is from Bennett, B. G., *Sci. Total Environ.* 29, 101-111).

Modelling studies for PCBs and TCCD in the Great Lakes area suggest that only around half the human intake of these chemicals was attributable to consumption of freshwater fish and that the rest was from atmospheric pollution incorporated into vegetables and meat (Hallett, 1988) (Figure 11.1). Even consumption of "pesticide-free" organic produce will not completely prevent dietary intake of potential endocrine disruptors.

The resultant PCB concentrations in human adipose tissue in Ontario was 2-2.5 mg/kg between 1976 and 1981, while in Canada as a whole levels were 0.9 mg/kg. At the same time DDT levels throughout Canada were over 2 mg/kg (see Hallett, 1988). These levels are comparable to those found in many fish (Chapter 3). Since these studies were done the manufacture of PCBs and DDT has ceased in Canada, but as with fish these values indicate a general level of toxic contamination and similar distribution patterns probably pertain for many of the chemicals in current agricultural, industrial and domestic use. As shown in the Inuit studies, atmospheric fallout can be a very significant means of global distribution. In the two decades since these studies were carried out, there has been a great increase in combustion of petroleum products for transport and provision of energy and PAH intake, through the routes described for PCBs, is probably much more significant. As shown earlier, both PCBs and PAHs can be considered potential endocrine disruptors. In addition to these sources human dietary intake in Western Europe and North America has moved from simple intake of fresh meat and vegetables to a predominance of processed food. These contain large numbers of food additives, colourings, preservatives and flavourings, few of which have been tested for endocrine disrupting activity. While few of these chemicals bioaccumulate in human tissue, the constant intake will provide a continuous burden of exposure. The incidences of food sensitivities to these additives is difficult to gauge except in a few cases where clinical symptoms are presented. The hyperactivity induced in some children by tartrazine food colourings may be obvious but, at a lower level, a slight increase in activity may not be noticeable. Reported incidences of food allergies and sensitivities appear to be increasing, but this may be due to greater awareness, and some of these cases refer to natural products such as nuts and wheat. Other allergies, such as asthma have also shown dramatic increases during the last few decades. Whether the increased exposure to anthropogenic chemicals in general is contributing to this apparent increased sensitivity, acting through disturbance of the endocrine and immune systems is, at present unclear. In addition to food there is also greatly increased exposure to a wide range of cosmetic products, exhaust fumes of urban traffic, and vapours from chemicals such as preservatives and fire-retardants in materials in both the home and workplace. Recent concerns about the estrogenic effects of alkylphenolics and phthalates has shown just how widespread can be the use of apparently inert materials which have subsequently been shown to have endocrine disrupting activity. The majority of substances to which we are now exposed have never been tested except by simple toxicity tests which examine lethal, rather than endocrine disrupting activity.

While Western Europe and North America have suitable regulatory bodes for testing potentially harmful chemicals and issuing regulatory guidelines, such organisation is totally lacking in many developing economies in which

pollution control takes second place to competition and cheap production processes. In the former Soviet Union, large areas can be considered nothing less than an environmental disaster zone, the effects of which may be yet to come on their populations. The infrastructure to examine the effects of 80 years of neglect of the environment no longer exists. Even in the West, the political will is lacking to take many of the unpalatable, and electorally harmful, steps to protect the environment.

The long-term health hazards of these chemicals are extremely difficult to assess. In addition to the many chemicals shown to disrupt the endocrine system (see Appendix), induction of P450 by a wide range of xenobiotics can be considered to indicate the presence of potential endocrine disruptors. At one level ingestion of these chemicals will result in clear clinical symptoms such as infertility but, at a sub-clinical level, slight alterations to thyroid, adrenal, gonadal or immune function may be manifest only by some minor discomforts, which nevertheless can over time affect health. Similarly, major behavioural abnormalities require psychiatric help while mild learning and behavioural difficulties, which are not noticeable in individuals, may affect the general cohesion of society. The possibility that the chemicals to which we are exposed may cause slight behavioural changes and learning difficulties in our children which is only detectable by sophisticated testing is particularly insidious. The evidence of such behavioural changes from rats, birds and humans exposed to polluted fish is a warning that other sources of chemicals, or cocktails of small amounts of many chemicals might have similar activity.

A number of recent illnesses, such as those related to use of organophosphates in sheep dipping, Gulf War Syndrome, Sick Building Syndrome, Chronic Fatigue Syndrome etc. present symptoms which are often not well defined and do not fit with a clear diagnosis of clinical illness. In some cases, incidences of the symptoms do not appear to differ greatly from those of comparable unexposed groups and on this basis compensation for the claimants is denied. The effects are frequently attributed to stress, but xenobiotics themselves can be stressors and the resultant endocrine and physiological responses are not distinguishable from that of work or domestic-related mental stress. Stress, from whatever cause, may in turn lead to immunosuppression and increased susceptibility to bacterial and viral infection. Whatever may be the cause of these particular syndromes, the effects of true endocrine disruption will most probably be at this borderline level of statistical significance, and it is at this level, rather than in overt clinical symptoms, that endocrine disruption is likely to be most apparent in the majority of human populations. Both sheep dipping and service in the Gulf War involved exposure to organophosphate pesticides which can, from evidence obtained in experiments with fish and other vertebrates, be classed as endocrine disruptors, but whether this is the cause of the illness or whether it is related more to the stress of farming and battle, to other quite unrelated

factors or a to combination of all three is almost impossible to determine. Fox (1993) has discussed the theoretical background to use of biomarkers as indicators of impairment of function, and the continuum from early physiological disturbance to incurable disease (Figure 11.2). Such biomarkers include induction of a stress response, induction of detoxification systems, inhibition of specific enzymes, metabolic impairment, impaired growth, impairment of the immune and reproductive systems and impaired organ or tissue function based on functional tests or histopathological alterations. These biomarkers cover all of the disruptions caused to fish by exposure to the endocrine disrupting xenobiotics as described in Chapters 5-10, as well as many of the symptoms of non-specific ill-health found humans.

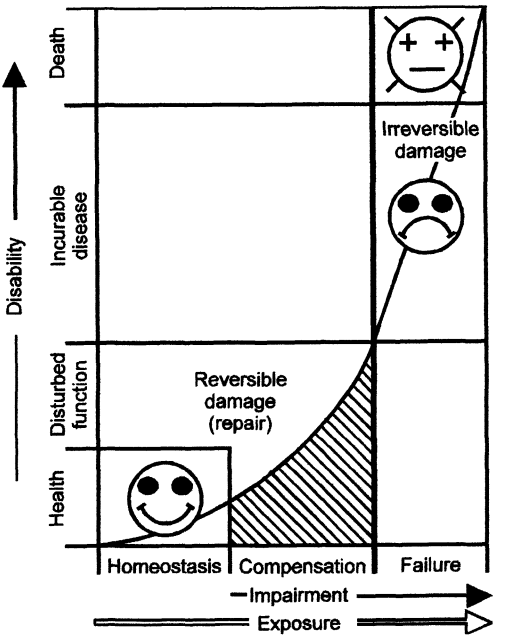


Figure 11.2. The exposure-response curve. The shaded area indicates the region of effects due to endocrine disruption. (Modified from Fox, G. A. (1993). What have biomarkers told us about the effects of contaminants on the health of fish-eating birds in the Great Lakes? The theory and a literature review. *J. Great Lakes Res.* 19(4), 722-736, by kind permission of the International Association of Great Lakes Research, Ann Arbor, MI).

The recent debates on whether the reported decreases in human sperm count (Sharpe and Skakkebaek, 1993) are real or a quirk of data collection and statistics again clearly illustrates the level at which endocrine disruptors manifest themselves in the short term and the difficulties in collecting comparable data over a long period during which trends would become apparent. A decrease in sperm counts from say 1950 to 1990 could be

attributable to changes in laboratory and clinical methodology, the presence of environmental estrogens, or to the many changes in life-style over this period which include increased use of motor vehicles, central heating, plastic packaging, cosmetics, preservatives, consumption of processed foods, exposure to pesticides etc. These are, of course, not mutually exclusive since many of these changes have involved increased exposure to xenobiotics. Similar problems arise with many of the other possible symptoms of endocrine disruption.

11.4 Testing for endocrine modulators

The data presented in this volume shows that a very wide range of anthropogenic chemicals can cause endocrine disruption in fish. The evidence, although patchy, suggests that levels of these chemicals found in wild populations of fish may be affecting their development, reproduction, activity, behaviour and ability to cope with stress. A great deal of the data refers to the persistent organochlorines, such as DDT and PCBs, which have not been in widespread production in many countries for several decades, but which nevertheless will maintain their effects for some decades to come through their persistence in the aquatic environment. Many thousands of other chemicals, many of which are considered "essential" to our present way of life are increasingly making their way into the environment and few have received more than the legal minimum of toxicity testing. Regulatory tests for the endocrine disrupting activities of these chemicals have yet to be designed that can show the full range of possible effects including transgenerational effects. The activity of chemicals, such as phthalates and alkylphenolics, that have recently been shown to be estrogenic has been discovered by chance rather than by any regulatory tests, and most of the studies describing endocrine disrupting effects have been carried out in the laboratories of either universities or environmental protection agencies rather than those concerned with toxicology testing. Chemicals already licensed for use on the basis of standard toxicity tests are currently not required to undergo any further testing for endocrine disrupting activity. This includes the majority of pesticides currently in use, and which on the basis of the data available from fish and other animal studies can be considered as potential endocrine disruptors. The numbers of such chemicals already released into the environment make such complete testing impossible and priority must be given to chemicals shown in animal studies to have endocrine disrupting activity, or which on the basis of structure activity relationships, may be considered to be potential endocrine disruptors. A strategy for such testing of the endocrine modulating effects on wildlife has recently been presented, which comprises analysis of structure activity relationships, *in vitro* and *in*

vivo tests, field studies and risk assessment strategies (Tattersfield *et al.*, 1997).

Scientific studies have so far concentrated too much on showing that chemicals have an effect, frequently using only a single dose. There is now a need to find the minimum dose, administered in a manner appropriate to the species, which will cause an endocrine modulating effect and to obtain dose response relationships since the type of effects may differ at different concentrations. Most importantly, an integrated approach is required to show which stage of the life cycle is most sensitive to the xenobiotic and the mechanism by which it exerts its effects. This involves examining not only a single tissue or hormone, but the full range of endocrine glands and their secretions. There is also an urgent need to examine transgenerational effects so that the effects of parental exposure on their offspring, and that of their offspring, throughout their life cycle can be determined. Much of the data obtained for commercial pesticide formulations need a re-evaluation since many of these, such as lindane (Wester and Canton, 1985, 1986; Wester, 1991) and some lampricides (Hewitt *et al.*, 1996.) contain impurities which have different effects to the primary pesticide, while others may contain "inert" dispersants such as alkyl phenolics which are now shown to be endocrine modulators. Due to commercial secrecy the nature of the products in many formulations is very difficult to ascertain, but if the hazards of these formulations is to be assessed it is absolutely essential that such information is available to the scientific community and to regulatory authorities.

The majority of studies so far have been concerned with "classical" endocrine effects. Endocrinology now comprises a wide range of signalling mechanisms and factors which regulate the relationships between cells, and includes cellular messengers implicit in both immune function and embryonic development. The basic mechanisms underlying such inter- and intra-cellular signalling are still far from understood, but these too are potential sites of action of endocrine modulators in their widest definition. Information in this area is totally lacking, but is potentially of even greater importance than that which is already available.

It is important to realise that many of the very diverse chemicals to which we are exposed can have very similar effects at the endocrine level. Current "safe" levels of many pesticides are derived from toxicity tests of single chemicals and neglect the additional contribution which exposure to other chemicals, such as PAHs from car exhausts, detergents, food additives etc., may make to sub-clinical endocrine dysfunction. This may be a valid assumption at the level of clinical toxicity, but does not hold true at the level of endocrine disruption. Toxicity tests are generally short-term but continuous exposure over a human lifespan, even to chemicals which do not bioaccumulate, may cause cumulative damage to the endocrine, nervous and immune systems. It is too simplistic to expect to find a clear correlation

between any of the dysfunctions of the endocrine, nervous and immune systems and a single factor in our society. The evidence of changes in behaviour induced by *in utero* exposure to PCBs emphasises both the dangers of exposure to anthropogenic chemicals and the difficulties in providing sufficient evidence of the cause of relatively small effects which nevertheless decrease the human potential of both the exposed individuals and society as a whole. Investigation of the behavioural changes induced by a known single factor, such as PCB contaminated fish, is very simple compared to the vastly more complex situation of exposure to the wide range of chemicals to which the majority of the human population is now exposed. The chemicals listed in the Appendix are but a minute fraction of those that may be affecting both present and future generations.

While concern inevitably centres on hazards to human health, studies on other vertebrates such as fish can provide data not readily available from human studies. Fish provide an excellent model with which to examine fertility effects since both sexes produce large numbers of gametes which are released into the external environment and are readily quantified. This also facilitates studies on embryonic development and transgenerational effects. The effects of endocrine disruptors on the fish themselves are, of course, also of major concern and may make a significant contribution to the recent decline in fisheries stocks or of the ability of fish populations to compensate for overfishing.

11.5 Conclusion

There is now clear evidence for the deterioration of many wildlife habitats, and warnings that the effects found in wildlife may impact upon human health and affect future generations (Colborn and Clement, 1992; Colborn *et al.*, 1993, 1996; Colborn, 1994). The aquatic and terrestrial environment contains, and will contain for decades to come, the remains of our past industrial wastes. Daily we are both increasing the quantity of such waste and adding new chemicals with unknown potential for endocrine disruption. The full effects of exposure of human communities to such toxins may not become apparent for another generation, but the data from fish and other wildlife suggests that such effects will not be immediately obvious, that many will be subtle and may be at the limits of statistical significance, yet alone show clear clinical signs of abnormality. Together with the evidence of ozone depletion and global warming there are clear signs that we may be bequeathing to future generations a global environment which is less fit for habitation. There are, in fact, many parallels between the cases of global warming and endocrine disruption. Both involve the recent rapid increase in emission of anthropogenic chemicals and while in both cases there is a strong scientific

basis for predicting threats to human society, the evidence that it is occurring at the present moment does not attain a sufficient level of certainty or statistical significance. Clear evidence that the predictions have been fulfilled will, in both cases, not be available for several decades, but if action is not taken now to curb the emissions it will then be too late to prevent catastrophic changes both to our civilisation and to our planet. The slowness of the democratic political process to initiate remedial measures and to extend public awareness of the problems in the case of fossil fuel consumption and climate change, and its reluctance to take electorally and commercially unpopular action, does not bode well for the much more complex measures necessary to combat the pollution of the global environment by chemicals which threaten the very basic processes of life itself. The scientific community is perhaps better equipped to initiate such awareness and to press for action to bring in sustainable environmental policies which will safeguard the future both of our species and of others that share our planet.

Appendix

Table 1. Effects of heavy metal pollution on the fish endocrine system (V = mg/l *in vitro*; D = mg/kg diet; bwD =dietary administration/kg body weight of fish; T = tissue concentration; I = mg/kg fish injected ; all other concentrations refer to holding water; mg/l. or mg/kg body weight = ppm)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Arsenic	*Colisa fasciatus*	2-14	15-30	Degeneration of testis and ovary	Shukla and Pandey, 1984b, d, e
	Oncorhynchus kisutch	0.3	180	Delayed increase in T_4, decreasd saltwater survival	Nichols *et al.*, 1984
Cadmium	*Clarias batrachus*	0.005 I	x8	Testicular degeneration, inhibited spermatogenesis	Ahsan and Ahsan, 1974
	Clarias gariepinus	50	1	Exposed sperm had decreased motility	Kime *et al.*, 1996
	Lebistes reticularis	225	30	Histophysiological abnormality of testis, arrested spermatogenesis	Sehgal and Pandey, 1984; Sehgal *et al.*, 1984
	Lepidocephalichthys thermalis	0.2	20	Degenerative lesion in ovary impaired vitellogenesis	Victor *et al.*, 1986
	Monopterus albus	3	7	Reduced testosterone, estradiol and vitellogenin, reduced GSI, histological change in gonads and thyroid	Singh, 1989
	Micropogonias undulatus	1	30	Increased ovarian growth, steroido-genesis, vitellogenesis and GtH secretion	Thomas, 1990, 1993
		1	40	Increased ovarian growth, plasma estradiol and in vitro GtH	Thomas, 1989
		1 V	0.1	Increased in vitro GtH and in response to LHRH	Thomas, 1993
	Oncorhynchus mykiss	0.1	28	Decreased calcium and plasma vitellogenin	Haux *et al.*, 1988
	Oncorhynchus mykiss	10-1000V	0.08	Increased *in vitro* synthesis of T & 11βT by testis	Kime, 1984

Table 1 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
		0.4-2.4	0.1-7	Changes in plasma thyroxine	Hontela *et al.*, 1996
	Oreochromis mossambicus	0.02	6	Decreased response to ACTH in interrenal superfusions	Pelgrom *et al.*, 1994
		0.02-0.07	6	Inhibits the stress response to copper	Pelgrom *et al.*, 1995
	Pleuronectes americanus	25-50	71	Decreased plasma vitellogenin, decreased HSI and GSI	Pereira *et al.*, 1993
	Platichthys flesus	2 I	10	Depressed plasma vitellogenin	Povlsen *et al.*, 1990
	Poecilia reticulata	270 D	30-210	Decreased number fry	Hatakeyama and Yasuno, 1987
		0.01-0.02	182	Decreased number fry	Hatakeyama and Yasuno, 1987
	Puntius sarana	20	10	Testicular and pituitary damage	Kumari and Dutt, 1991
	Puntius ticto	26	4	Decreased testis size and abnormalities	Pundir and Saxena, 1990
		8	30	Damage to pituitary cells, reversed by 30 days in clean water	Pundir and Saxena, 1992
	Salvelinus fontinalis	0.010-0.025	1	Testicular damage, inhibition androgen synthesis	Sangalang and O'Halloran, 1973
		0.010-1 V	0.2	Inhibition KT synthesis from pregnenolone	Sangalang and O'Halloran, 1973; Freeman and Sangalang, 1977a
		0.001	10-90	Higher plasma T and KT	Sangalang and Freeman, 1974
Chromium	*Oncorhynchus mykiss*	0.005-1 V	0.03	Decreased fertilisation rate of exposed gametes	Billard and Roubaud, 1985
Copper	*Atherinops affinis*	0.018-0.1 V	2	Exposed egg & sperm gave reduced fertilisation and larval abnormalities	Anderson *et al.*, 1991
	Catastomus commersoni	0.012-0.015	life	Decreased egg size, increased larval deformity	Munkittrick and Dixon, 1989
	Lebistes reticularis	0.5	30	Histophysiological abnormality of testis, arrested spermatogenesis	Sehgal *et al.*, 1984

Table 1 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
	Oncorhynchus kisutch	0.005	172	Impaired parr-smolt transformation, migration & seawater survival	Lorz and McPherson, 1976
	Oreochromis mossambicus	0.05	6	Decreased response to ACTH in interrenal superfusions	Pelgrom *et al.*, 1994
	Perca flavescens	0.034	life	Delayed egg hatch	Ellenberger *et al.*, 1994
	Puntius conchonius	0.2	60-120	Decreased. spermatogenesis, ovarian atresia	Kumar and Pant, 1984
	Pimephales promelas	0.037	120	Fewer eggs, fewer spawnings	Pickering *et al.*, 1977
	Salvelinus fontinalis	0.002-0.032	240	Reduced number viable eggs and hatchability	McKim and Benoit, 1971
Iron	*Oncorhynchus mykiss*	0.005-1 V	0.03	Decreased fertilisation rate of exposed gametes	Billard and Roubaud, 1985
Lead	*Anabas testudineus*	1-20	30	Reduced GSI, reduced number of eggs, increased spawning	Tulasi *et al.*, 1989
	Clarias batrachus	5	275	Retarded ovarian growth, induction nuclear inclusions	Katti and Sathyanesan, 1987a
		5	150	Accumulation of neurosecretory material in neurohypophysis, degeneration nucleus preopticus & nucleus lateralis tuberis	Katti and Sathyanesan, 1986
		5	150	Hypertrophy thyroid and thyrotrophs, decreased thyroid I uptake	Katti and Sathyanesan, 1987b
	Colisa fasciatus	15	4	Reduced spermatogenic activity, haemorrhage in testis	Srivastava, 1987
	Micropogonias undulatus	0.2 bwD	30	Impaired ovarian growth, steroidogenesis and GtH secretion	Thomas, 1990a

Table 1 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
		68 bwD	30	Decreased GSI, plasma testosterone & oestradiol	Thomas, 1988
	Oncorhynchus mykiss	0.01	12	Decreased transformation spermatogonia to spermatocytes	Ruby *et al.*, 1993a
	Puntius conchonius	0.12	60-120	Decreased spermatogenesis, ovarian atresia	Kumar and Pant, 1984
Mercuric chloride	*Channa punctatus*	0.017	7-91	Decreased GSI, oocyte diameter & oocyte maturity	Dey and Bhattacharya, 1989
		0.01	182	Reduced GSI, inhibited development of ovary and testis, smaller gonadotrophs	Ram and Sathyanesan, 1983
		0.01	180	Protein & lipid decreased in liver & ovary, increased hepatic & decreased ovarian cholesterol	Ram and Sathyanesan, 1984
		0.01	180	Degeneration of neurones of nucleus preopticus	Ram and Joy, 1988
	Clarias batrachus	0.05	90-180	Decreased GSI, impaired vitellogenesis, oocyte degeneration	Kirubagaran and Joy, 1988b
		0.05	90-180	Impaired testicular 3βHSD, GSI & spermatogenesis	Kirubagaran and Joy, 1988a, 1992
		0.05	90-180	Hypertrophied thyroid follicles, colloid depletion, inhibited I uptake	Kirubagaran and Joy, 1989
		0.05	90-180	Decreased plasma cortisol, hypertrophied interrenal and pituitary	Kirubagaran and Joy, 1991
		0.05	180	Inactive gonadotrophs, stimulated thyrotrophs	Joy and Kirubagaran, 1989

Table 1 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
	Clarias gariepinus	0.001-1.0	0.0006	Exposed sperm had decreased motility and fertilisation rate	Rurangwa *et al.*, 1998
	Fundulus heteroclitus	0.01-0.05 V	0.001-0.017	Exposure of sperm reduced fertilisation rate	Khan and Weis, 1987a
	Lepidocephalichthys thermalis	0.1	20	Degenerative lesion in ovary, lytic changes in oolemma	Victor *et al.*, 1986
	Liza parsia	0.5	10	Vacuolisation of thyroid colloid follicles	Pandey *et al.*, 1993
	Notopterus notopterus	0.4-0.9	30	Hepatic lipid decreased, ovarian lipid increased	Verma and Tonk, 1983
	Oncorhynchus mykiss	0.001-1 V	0.028	Exposed gametes had lower fertilisation rate	Billard and Roubaud, 1985
	Oryzias latipes eggs	0.0015	16	Decreased hatching success	Heisinger and Green, 1975
Methyl mercuric chloride	*Clarias batrachus*	0.04	90-180	Decreased GSI, impaired vitellogenesis, oocyte degeneration	Kirubagaran and Joy, 1988b
				Impaired testicular 3βHSD, GSI & spermatogenesis	Kirubagaran and Joy, 1988b, 1992
		0.04	90-180	Hypertrphied thyroid follicles, colloid depletion, inhibited I uptake	Kirubagaran and Joy, 1989
		0.05	90-180	Decreased plasma cortisol, hypertrophied interrenal and pituitary	Kirubagaran and Joy, 1991
		0.125	14	Decreased plasma T_3 and T_4	Kirubagaran and Joy, 1994
	Esox lucius	lake	life	Decreased plasma cortisol	Lockhart *et al.*, 1972
	Fundulus heteroclitus	0.01-0.05V	0.003	Exposed sperm had decreased motility and fertilisation rate	Khan and Weis, 1987b
		0.5-5 V	0.014	Exposed eggs gave malformed embryos	Khan and Weis, 1987c

Table 1 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
	Oncorhynchus mykiss	1-10 V	0.02	Exposed sperm had decreased viability	McIntyre, 1973
	Oryzias latipes	0.001-0.01	30-90	Induced vitellogenesis and hermaphroditism	Wester, 1991
		1 V	0.014	Blockage of the micropyle of eggs	Khan and Weis, 1993
	Poecilia reticulata	0.001-0.01	30-90	Induced vitellogenesis and hermaphroditism	Wester, 1991
		0.002	90	Necrosis of sperm, no mature sperm, interrenal hyperplasia	Wester and Canton, 1992
	Stizostedion vitreum (juv)	0.1 D	180	Decreased plasma cortisol	Friedmann *et al.*, 1996
Phenyl-mercuric acetate	*Danio rerio*	0.0002-0.02	50	Fewer eggs laid, lower spawning frequency	Kihlström *et al.*, 1971
Emisan (methoxy ethyl	*Channa punctatus*	0.2	182	Reduced GSI, gonadal degeneration, reduced pituitary gonadotrophs	Ram and Sathyanesan, 1987a
mercuric chloride)		0.2	182	Degeneration of neurones of nucleus preopticus	Ram and Joy, 1988
	Clarias batrachus	0.5	90-180	Decreased GSI, impaired vitellogenesis, oocyte degeneration	Kirubagaran and Joy, 1988b
				Impaired testicular 3βHSD, GSI & spermatogenesis	Kirubagaran and Joy, 1988a, 1992
		0.5	90-180	Hypertrophied thyroid follicles, colloid depletion, inhibited I uptake	Kirubagaran and Joy, 1988b
		0.05	90-180	Decreased plasma cortisol, hyper-trophied interrenal and pituitary	Kirubagaran and Joy, 1991

Table 1 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Tin (dibutyl)	*Poecilia reticulata*	0.32	30	Atrophy of thymus gland	Wester and Canton, 1987
		>0.00001	30	Atrophy of thymus gland	Wester and Canton, 1987
Tin (tributyl)	*Phoxinus phoxinus*	0.004	10	Incomplete hatching, malformation	Fent and Meier, 1992
Tin (triphenyl)	*Phoxinus phoxinus*	0.016	10	Reduced hatch rate, delayed hatching time, malformations	Fent and Meier, 1994
Zinc	*Danio rerio*	5		Delayed spawning, decreased egg viability	Speranza *et al.*, 1977
	Catastomus commersoni	0.2-0.3	life	Decreased egg size, increased larval deformity	Munkittrick and Dixon, 1989
	Poecilia reticulata	0.2-0.6	70	Delayed sexual maturity, fewer offspring, delayed birth first brood	Pierson, 1981
		2.6	156	Fewer offspring	Bieniarz *et al.*, 1994
	Puntius conchonius	11	60-120	Decreased spermatogenesis, ovarian atresia	Kumar and Pant, 1984

Table 2. Effects of organochlorine pesticides on the fish endocrine system. For structures and chemical names of pesticides see Table 2.1 and Figure 2.2.

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Aldrin	*Heteropneustes fossilis*	0.85	28	Reduced pituitary activity	Singh and Singh, 1981, 1982a
		0.85	28	Reduced level of GnRH-like factor in hypothalamus	Singh and Singh, 1982a
	Puntius conchonius	0.00005	60-120	Increased oocyte atresia	Kumar and Pant, 1988
Chlordecone (Kepone)	*Cyprinodon variegatus*	0.00007-0.0001	141	Reduced egg production, decreased hatch success and shorter progeny	Goodman *et al.*, 1982
	Heteropneustes fossilis	0.024	60	Ovarian and testicular degeneration	Srivastava and Srivastava, 1994
	Oncorhynchus mykiss (juv)	0.4/day D	231	Increased plasma vitellogenin	Donohoe and Curtis, 1996
	Oryzias latipes	0.001-0.002	4-9	Inhibition of oviposition	Curtis and Beyers, 1978
2,4-D	*Lepomis macrochirus*	5	150	Delayed spawning, hepatic lesions	Cope *et al.*, 1970
DDD	*Oreochromis aureus*	50 I	1x	Decreased *in vitro* response of interrenal to ACTH,	Ilan and Yaron, 1980, 1983
				Decreased catabolism and clearance of cortisol, no stress response	Ilan and Yaron, 1983
DDE	*Clupea harengus*	0.018 ovary T	life	Reduction in viable hatch	Hansen *et al.*, 1985
	Oncorhynchus mykiss (juv)	22-44 I	1x	Induction of hepatic vitellogenin mRNA	Ren *et al.*, 1996
DDT	*Merlangus merlangus*	0.02	life	Hatch success decreased at egg levels >	Von Westernhagen *et al.*, 1989
	Pleuronectes americanus	0.001-0.002	varied	Decreased fertilisation, deformed embryos	Smith and Cole, 1973
DDTs[a]	*Danio rerio*	0.003	3	Ultrastructural changes in liver	Weis, 1974

Table 2 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
	Oncorhynchus mykiss	polluted lake	life	High egg mortality	Hopkins *et al.*, 1969
	Oncorhynchus mykiss (juv)	15 I	x 3	Increased plasma vitellogenin and hepatic estrogen binding sites	Donohoe and Curtis, 1996
	Oreochromis mossambicus	0.001	20	Inhibited testis 3βHSD and 17βHSD	Bhattacharya and Pandey, 1989
		0.001	20	Ovarian damage, inhibited HSD-reversed in clean water	Shukla and Pandey, 1985
		4	20	Damage to pituitary cells	Shukla and Pandey, 1984a
		0.001	20	Goitrous, non-functional thyroid	Shukla and Pandey, 1986b
	Poecilia reticulata	0.001	28	Minor untrastructural changes to liver	Weis, 1974
	Salmo trutta	1.1-3.4/kg	98-308	Decreased fry survival	Burdick *et al.*, 1972
	Salvelinus fontinalis	0.5-2 /week D	156	Mortality of fry from treated male and female fish	Macek, 1968
	Salvelinus namaycush	polluted lake	life	Fry mortality	Burdick *et al.*, 1964
Dieldrin	*Merlangus merlangus*		life	Hatch success decreased at egg levels > 0.02	Von Westernhagen *et al.*, 1989
Endosulfan	*Channa striata*	0.00075-0.001	2-30	Inhibition oocyte development, reduced GSI	Kulshrestha and Arora, 1984
		0.00075-0.001	2-30	Testicular damage, failure of spermatogenesis	Arora and Kulshrestha, 1984
	Clarias batrachus	0.0015	16	Decreased plasma vitellogenin	Chakravorty *et al.*, 1992
		0.0015-0.008	4-16	Changes in plasma thyroid hormone levels	Sinha *et al.*, 1991a, b
	Cyprinus carpio	0.050-1 V	1.5	Inhibition of LH induced GVBD	Haider and Inbaraj, 1988

Table 2 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
		0.0005 D	28	Morphological changes to liver	Braunbeck, personal communication
	Oncorhynchus mykiss	0.00001	28	Morphological changes to liver	Arnold *et al.*, 1996a
	Oreochromis mossambicus	0.0002-0.0015	28-63	Inhibited male reproductive behaviour	Matthiessen and Logan, 1984
		0.001	20	Damage to pituitary and hypothalamus, decreased HSD	Shukla and Pandey, 1986a
		0.001	20	Decreased thyroid activity, goiterogenesis and hyperplasia	Bhattacharya, 1995
	Rasbora daniconius	0.001	5-75	Decreased oocyte diameter, oocyte rupture, reduced GSI	Rastogi and Kulshrestha, 1990
Endrin (Hexadrin)	*Heteropneustes fossilis*	0.0006-0.008	28	Decreased liver lipid, no effect liver cholesterol, no effect ovarian lipid, increased ovarian cholesterol	Singh and Singh, 1980d
		0.0006	4	Increased ovarian cholesterol, no effect on liver & ovarian lipid	Singh and Singh, 1980c
		0.0006-0.008	28	Reduced ^{32}P uptake by testis, lower pituitary and serum GtH, increased testis lipid	Singh and Singh, 1980a
		0.008	28	Reduced pituitary activity and ovarian ^{32}P uptake	Singh and Singh, 1980b, e, 1982a
		0.008	28	Reduced GnRH-like factor in hypothalamus	Singh and Singh, 1982a
β-HCH	*Oryzias latipes (eggs)*	0.003-1	30-90	Induced vitellogenesis and hermaphroditism	Wester, 1991

Table 2 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
	Poecilia reticulata	0.003-1	30-90	Excessive vitellogenin production, GtH stimulation	Wester *et al.*, 1985
		0.003-1	30-90	Induced vitellogenesis and hermaphroditism	Wester, 1991
γ-HCH	*Carassius auratus*	0.01-0.1	28	Changes in hepatic lipid synthesis	Singh and Kime, 1994
		0.01-0.1	28	Decreased GSI and GtH, altered *in vitro* steroid synthesis	Singh *et al.*, 1994
		1-20 V	0.12	Inhibited endogenous steroidogenesis	Kime and Singh, 1996
	Clarias batrachus	0.002-0.008	28	Changes in hepatic and ovarian lipids	Lal and Singh, 1987
		2-8	4-28	Decreased plasma sex steroids	Singh and Singh, 1987a, b
	Danio rerio	0.04	life	Morphological changes to liver	Braunbeck *et al.*, 1990
	Heteropneustes fossilis	0.004-0.016	28	Reduced hepatic, ovarian and plasma lipids	Singh, 1992; Singh and Singh, 1992
		0.005-0.02	28	Reduced plasma steroid levels	Singh and Singh, 1992a
		16	28	Suppressed GnRH, GtH & plasma steroids and inhibited ovarian growth	Singh and Singh, 1991, 1992c; Singh *et al.*, 1993
		8-16	28	Changes in plasma thyroid hormones	Yadav and Singh, 1986, 1987a, b
	Oreochromis mossambicus	2-4	10	Testicular damage, lowered GSI	Pandey and Shukla, 1980
		4	20	Damage to pituitary cells	Shukla and Pandey, 1984a
	Oryzias latipes	0.005-0.020	28	Oocyte atresia, inhibited LH-induced *in vitro* ovulation, retarded embryonic development	Hirose, 1975
	Rutilus rutilus	1-20 V	0.12	Changes in *in vitro* steroid production	Singh and Kime, 1995
Linuron	*Oncorhynchus mykiss*	0.03	35	Changes to hepatic and renal ultrastructure	Oulmi *et al.*, 1995a

Table 2 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Methoxy chlor	*Ictalurus punctatus*	127-380 I	1 x	Induction of vitellogenesis in immature fish	Nimrod and Benson, 1996
	Jordanella floridae	1.4	0.08	Delayed spawning, decreased egg production and hatch rate	Holdway and Dixon, 1986a
	Puntius conchonius	0.000007	60-120	Increased oocyte atresia	Kumar and Pant, 1988
Mirex	*Oncorhynchus mykiss*	3-300 D	180	Inhibited estradiol induced plasma vitellogenin	Chen *et al.*, 1986
Aldrin, DDD, DDT, dieldrin, endrin, heptachlor, lindane, methoxy-chlor, toxaphene	*Gambusia affinis*	sublethal	?	Caused abortion	Boyd, 1964

[a] DDT and metabolites, different isomers of DDD, DDE, DDTare not distinguished in this Table

Table 3. Effects of organophosphorus pesticides on the fish endocrine system. For structures and chemical names of pesticides see Table 2.1 and Figure 2.3

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Chlorfenvin - phos	*Mystus vittatus*	0.003	84	Inhibition vitellogenesis, and loss 3β HSD	Haider and Upadhyaya, 1985
		0.00001-0.001V	1	Inhibition LH-induced GVBD	Haider and Upadhyaya, 1986
Cythion	*Channa punctatus*	2	182	Inhibited gonadal development & GSI, reduced pituitary gonadotrophs, no effect on hepatic lipid or cholesterol.	Ram and Sathyanesan, 1987b
	Clarias batrachus	1-4	4-28	Decreased plasma sex steroids	Singh and Singh, 1987a
	Heteropneustes fossilis	9-35	28	Decreased liver lipid, no effect liver cholesterol no effect ovarian lipid, increased ovarian cholesterol	Singh and Singh, 1980d
		9	4	Increased ovarian cholesterol, no effect on liver & ovarian lipid	Singh and Singh, 1980c
		9-35	28	Reduced ^{32}P uptake by testis, lower pituitary, and serum GtH increased testis lipid	Singh and Singh, 1980a
		35	28	Reduced pituitary activity and ovarian ^{32}P uptake	Singh and Singh, 1980a, b, e
		35	28	Reduced GnRH-like factor in hypothalamus	Singh and Singh, 1982a
Diazinon	*Oncorhynchus mykiss*	0.04	30	Cytological changes to liver	Braunbeck, 1994
	Salmo salar	0.001	5	Decreased olfactory response to $PGF_{2\alpha}$ and female urine	Moore and Waring, 1996a

Table 3 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
	Oncorhynchus mykiss	0.0001	28	Changes in liver ultrastructure	Braunbeck, 1994; Arnold *et al.*, 1996b
Elsan	*Channa punctatus*	0.017	7-91	Decreased GSI, oocyte diameter	Dey and Bhattacharya, 1989
Fenitro-thion	*Channa punctatus*	1.5	120	Decreased GSI, inhibition oocyte growth, oocyte atresia	Mani and Saxena, 1985
		1.5	150	Decreased GSI, inhibition oocyte growth, oocyte atresia	Saxena and Garg, 1978
		1.5	120	Decreased ovarian RNA, protein & total lipid	Saxena *et al.*, 1986
		1.5	120	Inhibited spermatogenesis, decreased testis weight necrosis of spermatids	Saxena and Mani, 1985, 1987
		1.5	120	Histological changes to thyroid	Saxena and Mani, 1988
	Cyprinus carpio	0.3-1.5	180	Reduced gonadal 3βHSD	Kapur *et al.*, 1978
	Garra mullya	1	30	Decreased ovarian weight, oocyte atresia	Pawar and Katdare, 1983
	Poecilia reticulata	0.1-1.5	60	Abortion, reduced egg production	Yasuno *et al.*, 1980
Fenthion (Lebaycid)	*Tilapia leucosticta*	7	14	Ovarian atresia	Kling, 1981
Malathion	*Danio rerio*	0.5-1.1	7	Decreased ovarian DNA, RNA increased amino acids, enzyme changes	Ansari and Kumar, 1987
	Clarias batrachus	0.002-0.008	28	Changes in hepatic and ovarian lipids	Lal and Singh, 1987
		1-4	28	Decreased plasma sex steroids	Singh and Singh, 1987b
		0.5	30	No effect on testis morphology	Sadhu and Mukhopadhyay, 1985
		0.003-0.007	4-16	Changes in plasma thyroid hormones	Sinha et al., 1991a, 1992a
	Cyprinus carpio	0.05-1 V	1.5	Inhibition of LH induced GVBD	Haider and Inbaraj, 1988
	Heteropneustes fossilis	0.005-0.02	28	Reduced hepatic, ovarian and plasma lipids	Singh, 1992

Table 3 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
		0.005-0.02	28	Reduced plasma steroid levels	Singh and Singh, 1992a
		1.2	1	Morphological damage to liver	Dutta *et al.*, 1993
		10-20	28	Changes in thyroid hormones	Yadav and Singh, 1986, 1987a, b
	Monopterus albus	9	7	Reduced testosterone, estradiol and vitellogenin, reduced GSI, histological change in gonads and thyroid	Singh, 1989, 1993
	Mystus vittatus	2.5	84	Decreased ovarian GSI, & vitellogenesis, loss 3βHSD	Haider and Upadhyaya, 1985
		0.01-1 V	1	Inhibition LH-induced GVBD	Haider and Upadhyaya, 1986
	Oreochromis mossambicus	2-4	20	Reduced ovarian activity, oocyte diameter, GSI, increased atresia	Shukla *et al.*, 1984
		2-4	10	Histophysiological abnormalities of testis, decreased GSI	Pandey and Shukla, 1982
		4	20	Damage to pituitary cells	Shukla and Pandey, 1984a
Methyl-parathion (Metacid-50, Paramar M50)	*Anabas testudineus*	0.0001	90	Decreased oestrogen and GSI after 15 days	Choudhury *et al.*, 1993
	Channa punctatus	0.0001	2-30	Decreased serum and pituitary GtH	Ghosh *et al.*, 1990
	Heteropneustes fossilis	32	28	Reduced pituitary activity	Singh and Singh, 1981, 1982a
		32	28	Reduced GnRH-like factor in hypothalamus	Singh and Singh, 1982a
	Rasbora daniconius	0.1	5-75	Decreased oocyte diameter, oocyte rupture,reduced GSI	Rastogi and Kulshrestha, 1990

Table 3 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Mevinphos (Phosdrin)	*Mystus vittatus*	0.0001	84	Inhibition vitellogenesis, and loss 3β HSD	Haider and Upadhyaya, 1985
		0.0001-0.000001V	1	Inhibition LH-induced GVBD	Haider and Upadhyaya, 1986
Monocroto-phos	*Puntius conchonius*	0.053	60-120	Increased oocyte atresia	Kumar and Pant, 1988
Parathion	*Poecilia reticulata*	0.01-1	40	Degeneration of all germinal cells of testis	Billard and De Kinkeln, 1970
Quinalphos	*Clarias batrachus*	0.025	15	Decreased testis 3βHSD, 17βHSD and seminiferous tubule diameter, increased testis cholesterol	Bagchi *et al.*, 1990
Temephos	*Poecilia reticulata*	0.25-1.5	60	Reduced normal birth	Yasuno *et al.*, 1980
TEPA	*Poecilia reticulata*	1-25	6	Testicular atrophy, decreased male fertility	Stock and Cope, 1969
Tetrachlor-vinphos (Gardona)	*Mystus vittatus*	0.146	84	Inhibition vitellogenesis, and loss 3β HSD	Haider and Upadhyaya, 1985
		0.001-0.1V	1	Inhibition LH-induced GVBD	Haider and Upadhyaya, 1986

Table 4. Effects of carbamate and other pesticides on the fish endocrine system.

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Atrazine	*Cyprinus carpio*	1.0	30	Changes to liver ultrastructure	Braunbeck, 1994
	Danio rerio	0.04	30	Changes to liver ultrastructure	Braunbeck, 1994
Carbofuran	*Channa punctatus*	5	120	Decreased GSI, inhibition oocyte growth, oocyte atresia	Mani and Saxena, 1985
		5	120	Inhibited spermatogenesis, decreased testis weight	Saxena and Mani, 1985
		5	120	Decreased ovarian RNA, protein & total lipid	Saxena *et al.*, 1986
		5	120	Decreased testis weight, delayed sperm formation	Saxena and Mani, 1987
		5	120	Histological changes to thyroid	Saxena and Mani, 1988
	Clarias batrachus	0.5	30	Morphological damage to testes	Sadhu and Mukhopadhyay, 1985
	Colisa lalia	0.7	20	Inhibited ovarian development, increased atresia	Sukumar and Karpagaganapathy, 1992
	Rasbora daniconius	0.1	5-75	Decreased oocyte diameter, oocyte rupture, reduced GSI	Rastogi and Kulshrestha, 1990
Carbaryl	*Anabas testudineus*	1.66	90	Decreased estrogen and GSI after 15 d	Choudhury *et al.*, 1993
	Channa punctatus	2	150	Decreased GSI, inhibition oocyte growth, oocyte atresia	Saxena and Garg, 1978
		1.7	2-30	Decreased serum and pituitary GtH, higher larval mortality	Ghosh *et al.*, 1990
	Channa striata	10-20	2-30	Inhibition oocyte development, reduced GSI	Kulshrestha and Arora, 1984
		10-20	2-30	Testicular damage, failure of spermatogenesis	Arora and Kulshrestha, 1984

Table 4 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
	Clarias batrachus	5-12	4-16	Changes in plasma thyroid hormones	Sinha *et al.*, 1991a, 1992b
	Pimephales promelas	0.008-0.68	270	Decreased hatchability and number eggs,	Carlson, 1971
Esfen-valerate	*Lepomis macrochirus*	0.001	2 x	Spawning delayed 15 days, low larval survival	Tanner and Knuth, 1996
		0.00008	2x	Reduced larval growth	Tanner and Knuth, 1996

Table 5. Effects of industrial pollutants on the fish endocrine system. For structures of chemicals see Figs. 2.5 - 2.8

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Alkyl-phenols	*Oncorhynchus mykiss*	0.02-22	2-4	Estrogenic, stimulates vitellogenin from male hepatocytes	Jobling and Sumpter, 1994
Ammonia	*Channa punctatus*	0.017	7-91	Decreased GSI, oocyte diameter & oocyte maturity	Dey and Bhattacharya, 1989
		36	1-90	Changes in hepatic and plasma cholesterol	Bhattacharya *et al.*, 1984
Ammonium sulphate	*Channa punctatus*	100	180	Thyroid hypertrophy, liver degeneration	Ram and Sathyanesan, 1987c
Anthracene	*Pimephales promelas*	0.006-0.020	42-63	Decreased egg number, survival eggs and fry, deformed fry	Hall and Oris, 1991
Asbestos	*Oryzias latipes*	10^4 fibres/l	56	Decreased spawning frequency, fewer viable eggs	Belanger *et al.*, 1990
		10^6 fibres/l	56	Decreased larval & juvenile growth	Belanger *et al.*, 1990
Benzo[*a*] pyrene	*Micropogonias undulatus*	4 bwD	30	Impaired ovarian growth and steroidogenesis	Thomas, 1990a
		979 bwD	30	Decreased GSI, plasma testosterone & estradiol	Thomas, 1988
Chlorinated paraffins[a]	*Platichthys flesus*	1000 bwD	2 x	Changes in steroid catabolism	Haux *et al.*, 1982
4-Chloro-aniline	*Danio rerio*	0.04-1	119	Decreased fertilisation rate and egg release	Bresch *et al.*, 1990
		0.04	life	Changes to liver ultrastructure	Braunbeck *et al.*, 1990b
	Oncorhynchus mykiss	1 V	1	Cytological changes to hepatocytes	Braunbeck, 1993
		0.04	60	Changes to liver ultrastructure	Braunbeck *et al.*, 1990c

Table 5 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Cyanide	*Jordanella floridae eggs*	0.07	to hatch	Decreased pituitary size, delayed sexual maturity with fewer eggs	Cheng and Ruby, 1981
	Oncorhynchus mykiss	0.01-0.03	7	Reduced serum calcium	Da Costa and Ruby, 1984
		0.001	12	Reduced plasma vitellogenin and GSI	Ruby *et al.*, 1986
		0.01	12	Loss pituitary basophils, inhibited development spermatogonia	Ruby *et al.*, 1993a
		0.01	12	Decreased vitellogenin, estradiol & thyroid hormones	Ruby *et al.*, 1993b
		0.01	12	Elevated brain dopamine, inhibited gonadal development	Szabo *et al.*, 1991
		0.005-1 V	0.03	Decreased fertilisation rate of exposed gametes	Billard and Roubaud, 1985
		0.01	20	Abnormal oocytes, delayed yolk deposition, atresia	Lesniak and Ruby, 1982
	Salmo salar	0.005	12	Inhibition ovarian uptake of vitellogenin	Ruby *et al.*, 1987
Detergent	*Oncorhynchus mykiss*	5-100	-	Decreased motility and fertilising ability of exposed sperm	Mann and Schmid, 1961
Di-n-butyl-phthalate	*Rivulus marmoratus*	1-2	147	Decreased number eggs, decreased hatchability deformed offspring	Davis, 1988
Eutrophied pond	*Cyprinus carpio*	-	1400	Fewer eggs, decreased sperm volume & % GVBD	Bieniarz *et al.*, 1996
3-Methyl-cholanthr-ene	*Monopterus albus*	4	7	Reduced testosterone, estradiol and vitellogenin, reduced GSI, histological change in gonads & thyroid	Singh, 1989
	Oncorhynchus kisutch	0.13 V	0.75	Aromatase inhibitor	Afonso *et al.*, 1997

Table 5 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
β-Naphtho-flavone	*Oncorhynchus kisutch*	0.13 V	0.75	Aromatase inhibitor	Afonso *et al.*, 1997
Nitrate	*Cyprinus carpio*	15 V	1	Decreased hatch rate	Bieniarz *et al.*, 1996
Nitrite	*Cyprinus carpio*	1 V	1	Decreased hatch rate	Bieniarz *et al.*, 1996
Petroleum (crude oil)	*Pleuronectes americanus*	oil slick	12-34	Decreased plasma androgens	Truscott *et al.*, 1983
	Pleuronectes americanus (m)	oil sediment	120	Decreased plasma androgen glucuronides, increased HSI	Truscott *et al.*, 1992
	Salmo salar	oil slick	7-28	Decreased plasma androgens	Truscott *et al.*, 1983
Phenol	*Channa punctatus*	10	1-90	Changes in hepatic and plasma cholesterol	Bhattacharya *et al.*, 1984
	Cyprinus carpio	12	7-30	Increased HSI, decreased GSI, increased ovarian and hepatic cholesterol content	Kumar and Mukherjee, 1988; Mukherjee *et al.*, 1991
Pentachloro phenol	*Oncorhynchus mykiss*	0.022-0.049	18	Oocyte atresia	Nagler *et al.*, 1986
4-Nitro-phenol	*Danio rerio*	1	life	Changes to liver ultrastructure	Braunbeck *et al.*, 1989
4-Nonyl-phenol	*Ictalurus punctatus*	79-237 I	1 x	Induction of vitellogenesis in immature fish	Nimrod and Benson, 1996
	Oncorhynchus mykiss (juv)	0.01	3	Induction of hepatic vitellogenin mDNA	Lech *et al.*, 1996
	Oncorhynchus mykiss			Competition with ^{3}H estradiol for hepatic receptor	Tremblay *et al.*, 1995
	Oryzias latipes (eggs)	0.05	90	Testis-ova in males	Gray and Metcalf, 1997
	Salmo salar	1-125 I	1x	Induced vitellogenin and zona radiata protein	Arukwe *et al.*, 1997a

Table 5 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
		1-5 I	1x	Decreased plasma estradiol	Arukwe *et al.*, 1997b
4-t-Pentyl-phenol	*Cyprinus carpio*	0.1	60	Feminization of males exposed during sexual differentiation	Gimeno *et al.*, 1996, 1997, 1998
	Cyprinus carpio	0.032-1.0	90	Inhibited spermatogenesis, decreased GSI, vitellogenin production	Gimeno *et al.*, 1998
Dinitro-*o*-cresol	*Anguilla anguilla*	0.05	28	Ultrastructural changes to hepatocytes	Braunbeck and Völkl, 1991
	Leuciscus idus	0.05	28	Ultrastructural changes to hepatocytes	Braunbeck and Völkl, 1991
TCB	*Morone americana*	0.2-5 I	3x	Decreased GSI, decreased larval survival	Monosson *et al.*, 1994
	Oncorhynchus mykiss	5mg/kg implant	42	Increased plasma cortisol, increased hepatic clearance	Vijayan *et al.*, 1997
PCB	*Clupea harengus*	-	-	Viable hatch decreased with egg pollution	Von Westernhagen *et al.*, 1987
		0.12 ovary T	life	Reduction in viable hatch	Hansen *et al.*, 1985
	Cyprinus carpio	250 I	1	Lower plasma progesterone testosterone & oestradiol, increased steroid catabolism	Yano and Matsuyama, 1986
		25 I	28	Damage to liver, oocytes and spermatozoa, reduced androgens & estrogens	Sivarajah *et al.*, 1978a, b
	Gadus morhua	1-50 D	165	Testicular abnormalities, inhibited spermatogenesis	Sangalang *et al.*, 1981
		1-50 D	92	Changes in in vitro testicular and interrenal steroidogenesis	Freeman *et al.*, 1982

Table 5 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
				Viable hatch decreased with egg pollution	Von Westernhagen *et al.*, 1987
	Ictalurus punctatus	1-100 I	1x	Changes in hepatic enzymes	Ankley *et al.*, 1986
	Merlangus merlangus			Viable hatch decreased with egg content > 0.2	Von Westernhagen *et al.*, 1987, 1989
	Micropogonias undulatus	0.05 bwD	30	Impaired ovarian growth, vitellogenesis, steroidogenesis & GtH	Thomas, 1990a
		5 bwD	17	Impaired ovarian growth, decreased plasma estradiol and *in vitro* GtH	Thomas, 1989
		3 bwD	30	Decreased GSI and plasma testosterone	Thomas, 1988
	Oncorhynchus mykiss	3-300 D	180	Inhibited estradiol induced plasma vitellogenin	Chen *et al.*, 1986
		25 I	28	Damage to liver, oocytes and spermatozoa, reduced androgens, estrogens. Increased P450	Sivarajah *et al.*, 1978a, b
		10 D	229	Ultrastructural changes to liver	Hacking *et al.*, 1977
		3-300 D	180	Inhibited oestradiol induced plasma vitellogenin	Chen *et al.*, 1986
	Oncorhynchus mykiss larvae	2.5	0.125	Altered sex ratio and abnormal gonads	Matta *et al.*, 1998
	Salvelinus fontinalis	0.2	21	Increased *in vitro* 11β-hydroxylation of T, decreased hatch rate	Freeman and Idler, 1975
	Phoxinus phoxinus	20-2000 D	45	Reduced hatching time, low hatchability	Bengtsson, 1980
	Platichthys flesus			Viable hatch decreased with egg pollution	Von Westernhagen *et al.*, 1987

Table 5 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
	Platichthys stellatus			Embryological success decreased by egg PCB content	Spies and Rice, 1988
PCB, PCN, PBDE	*Gasterosteus aculeatus*	diet	100	Hepatic lipid accumulation	Holm *et al.*, 1993
PCB, PAH, Hg	*Esox lucius*	sediment	life	Decreased stress response, degenerative pituitary corticotrophs	Hontela *et al.*, 1992
	Perca flavescens	sediment	life	Decreased stress response, degenerative pituitary corticotrophs	Hontela *et al.*, 1992
PCB/DDT[b]	*Genyonemus lineatus*	4 ovary	life	Failure to induce spawning, decreased fecundity,	Hose *et al.*, 1989
				Fertility & early oocyte loss	Cross and Hose, 1988
PCB/DDT	*Salvelinus alpinus*	Lake Geneva	life	Embryo mortality	Monod, 1985
TCCD	*Danio rerio*	0.002 D	1x	Decreased egg numbers & spawnings, larval mortality/deformity	Wannemacher *et al.*, 1992
β-Sitosterol	*Carassius auratus*	0.3	12	Decreased T, KT due to decreased cholesterol availability	MacLatchy and Van der Kraak, 1995; MacLatchy *et al.*, 1997
	Oncorhynchus mykiss	45 D	28	Expression of hepatic vitellogenin gene	Mellanen *et al.*, 1996
				Competition with ^{3}H estradiol for hepatic receptor	Tremblay *et al.*, 1995
Sulphide	*Cyprinus carpio*	9	7-30	Increased HSI, decreased GSI, increased ovarian and hepatic cholesterol content	Kumar and Mukherjee, 1988; Mukherjee *et al.*, 1991
Thiourea	*Mystus vittatus*	300	28	Decreased spermatogenesis, regression of interstitial cells	Misra and Pandey, 1985
Urea	*Colisa fasciatus*	2500	15-30	Morphological damage to testis	Shukla and Pandey, 1984d
Acid pH	*Ambloplites rupestris*	pH 5.2-4.7	life	Spawning failure	Beamish, 1976

Table 5 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
	Catastomus commersoni	pH 3-7 V	sperm	Slight decrease in motility	Mohr and Chalanchuk, 1985
	Catastomus commersoni	pH 5.2-4.7	life	Spawning failure	Beamish, 1976
	Coregonus artedii	pH 4.7-4.5	life	Spawning failure	Beamish, 1976
	Couesius plumbeus	pH 4.7-4.5	life	Spawning failure	Beamish, 1976
	Cyprinodon n. nevadensis	pH 5-8.3	42	Decreased egg production, spawning and egg viability	Lee and Gerking, 1980
	Esox lucius, E. niger	pH 3.9-7.9V	sperm	Decreased sperm motility	Duplinsky, 1982
	Ictalurus nebulosus	pH 5.2-4.7	life	Spawning failure	Beamish, 1976
	Jordanella floridae	pH 4.5-6.0	20	Impaired egg production and fertility	Craig and Baksi, 1977
				Decreased yolk deposition, retarded oocyte growth	Ruby *et al.*, 1977
		pH 4.5-6.0	20	Less mature sperm, smaller cysts in testis	Ruby *et al.*, 1978
	Lota lota	pH 6.0-5.5	life	Spawning failure	Beamish, 1976
	Micropterus dolomieui	pH 6.0-5.5	life	Spawning failure	Beamish, 1976
	Oncorhynchus mykiss	pH 4.5-7.6	20	Inhibition vitellogenin synthesis	Roy *et al.*, 1990
		pH 4.5-5.5	42	Reduced survival of progeny in first 7 d	Weiner *et al.*, 1986
	Perca flavescens	pH 4.7-4.5	life	Spawning failure	Beamish, 1976
	Perca fluviatilis	pH 4.4	life	Eggs died before hatching	Rask, 1992
	Percopsis omiscomaycus	pH 5.2-5.5	life	Spawning failure	Beamish, 1976
	Pimephales promelas	pH 8-6	60-150	Oocyte atresia, decreased egg numbers and survival	McCormick *et al.*, 1989
	Salmo salar	pH 4.7-5.6	life	Decreased T and KT	Freeman *et al.*, 1983

Table 5 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
		pH 4.0-4.5	1	Decreased water uptake by ova	Peterson and Martin-Robichaud, 1982
	Salvelinus fontinalis	pH 4.48	21	Inhibition vitellogenin synthesis	Tam *et al.*, 1987
		pH 4.5-7.3	300	Reduced number eggs, delayed ovulation	Tam and Payson, 1986
		pH 4.5	30-73	Increased atresia vitellogenic oocyte, suppression of gonadotropes	Tam *et al.*, 1990
		pH 4.4-5.0	147	Decreased growth, egg numbers, decreased estradiol and vitellogenin	Mount *et al.*, 1988a
		pH 5.6	7	Increased brain and pituitary AVT	Hontela *et al.*, 1991
	Salvelinus namaycush	pH 5.5-5.2	life	Spawning failure	Beamish, 1976
	Stizostedion vitreum	pH 6.0-5.5	life	Spawning failure	Beamish, 1976
Coal dust (PAH)	*Oncorhynchus tshawytscha*	60	8	Induction of hepatic CYP1A1 mRNA	Campbell and Devlin, 1997
Oil refinery effluent	*Salmo salar*	20%	28	Induction of vitellogenin and zona radiata protein	Arukwe *et al.*, 1997a
Textile-mill effluent	*Heteropneustes fossilis*	3-7%	7-120	Oocyte atresia	Murugesan and Haniffa, 1992
Power station effluent	*Abramis brama*	warm effluent	life	Changes in gonadal maturation and spawning time	Luksienė, 1978
	Esox lucius	warm effluent	life	Changes in gonadal maturation and spawning time	Luksienė, 1982
	Perca fluviatilis	warm effluent	life	Changes in gonadal maturation and spawning time	Luksienė, 1982
	Rutilus rutilus	warm effluent	life	Changes in gonadal maturation and spawning time	Luksienė, 1981; Luksienė, and Sandström, 1994

Table 5 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Pulp mill[d]	*Catastomus commersoni*	effluent	life	Reduced plasma sex steroid, decreased egg size and GSI, reduced sperm motility	McMaster *et al.*, 1992
		effluent	life	Lowered response to GtH and GnRH, Decreased steroid production, increased liver size and mixed function oxygenase activity	Van der Kraak *et al.*, 1992 Gagnon *et al.*, 1994a; McMaster *et al.*, 1991
				Decreased male 11KT, increased female T in resonse to GnRH	Gagnon *et al.*, 1994b
		effluent	life	Decreased plasma cortisol	McMaster *et al.*, 1994
	Coregonus clupeaformis	effluent	life	Decreased steroid levels, increased liver size and mixed function oxygenase activity	Munkittrick *et al.*, 1992
	Cottus gobio	effluent	life	Changes in hepatic ultrastructure	Bucher *et al.*, 1992
	Danio rerio	0.03-0.09	7-28	Decreased hatchability and survival of larvae	Landner *et al.*, 1985
	Esox lucius	sediment	life	Decreased stress response, degenerative pituitary corticotrophs	Hontela *et al.*, 1997; Hontela, 1998
	Gambusia affinis	15 km downstream	life	Masculinization of females, precocious development of males	Howell *et al.*, 1980
		effluent from 4 km below	153	Masculinization of females	Drysdale and Bortone, 1989
	Heterandia formosa	effluent	life	Masculinization of females	Bortone and Davis, 1994
	Perca fluviatilis	3% effluent	life	Reduced gonad growth	Sandström *et al.*, 1988
	Perca flavescens	sediment	life	Decreased stress response, degenerative pituitary corticotrophs	Hontela *et al.*, 1997

Table 5 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
	Rutilus rutilus	3% effluent	life	Reduced gonad growth	Sandström *et al.*, 1988
	Salmo trutta	0.2-0.5% effluent	90	Decreased egg numbers, lower egg fertilisation. Fry mortality	Vuorinen and Vuorinen, 1985
Vegetable oil factory effluent	*Heteropneustes fossilis*	5-15%	150	Decreased gonadal lipid, increased fatty acid	Kondal *et al.*, 1989
	Channa punctatus	5%	120	Retarded ovarian growth, atresia	Saxena and Bhatia, 1983
Industrial pollutant[c]	*Channa punctatus*		30	Changes in hepatic and plasma cholesterol	Bhattacharya *et al.*, 1984
Contaminated sediment[e]	*Parophrys vetulus*	sediment extract I	1x	Decreased plasma estradiol	Stein *et al.*, 1991
		polluted site	life	Decreased plasma estradiol and ovarian growth	Johnson *et al.*, 1988
Sewage lagoon[f]	*Cyprinus carpio*		180	No effect on plasma steroids or cytochrome P450	Sivarajah *et al.*, 1979
Sewage effluent	*Cyprinus carpio*	effluent	21	Estrogenic, stimulated vitellogenesis	Purdom *et al.*, 1994
		effluent	life	Elevated vitellogenin, decreased plasma testosterone	Folmar *et al.*, 1996
	Oncorhynchus mykiss	effluent	21	Estrogenic, stimulated vitellogenesis	Purdom *et al.*, 1994; Harries *et al.*, 1997
	Platichthys flesus	effluent	life	Testicular abnormalities & vitellogenin and in males	Lye *et al.*, 1997
Sewage sludge	*Pomatoschistus minutus*	0.1%	30-150	Fewer eggs and larvae at the population level	Waring *et al.*, 1996

Table 5 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Municipal wastewater	*Oncorhynchus mykiss*	1:1-1:5	14	Altered hepatic steroid catabolism	Förlin and Hansson, 1982
Rhine sediment	*Pleuronectes flesus*	polluted	1095	Advanced oocyte maturation	Janssen *et al.*, 1995, 1997
St. Lawrence River	*Perca flavescens*	PAH/PCB /metal	life	Decreased cortisol and thyroxine response to stress	Hontela *et al.*, 1995; Brodeur *et al.*, 1997; Hontela, 1998
Tennessee River	*Lepomis auritus*	PCB+Hg	life	Decreased fecundity and functional liver, increased EROD, P-450 & NADPH, increased liver parasites	Adams *et al.*, 1992
Lake Erie	*Oncorhynchus kisutch*	polluted	life	Over-ripe eggs, fry deformity, low fertilisation rate	Flett *et al.*, 1991
		polluted	life	Lower KT & female T, decreased cortisol & interrenal activity	Morrison *et al.*, 1985b
		polluted	life	Pituitary and serum GtH & plasma KT suppressed	Leatherland *et al.*, 1982
		polluted	life	Fewer gonadotrophs	Leatherland and Sonstegard, 1980c, 1981
Lake Ontario	*Carassius auratus*	polluted	life	No seasonal changes in ovary, high pituitary activity	Munkittrick and Leatherland, 1984
Lake Michigan	*Salvelinus namaycush*	PCB polluted	life	Decreased hatch, abnormalities	Mac and Schwartz, 1992
Puget Sound WA	*Parophrys vetulus*	PCB+PAH	life	low estradiol and vitellogenin, poor reproductive success	Casillas *et al.*, 1991
Rhode Is. Coast	*Pleuronectes americanus*	PCB	life	Decreased length and weight at hatch	Black *et al.*, 1988

Table 5 (continued)

Pollutant	Species	Dose (mg/l; mg/kg)	Time (days)	Effect	Reference
Baltic Sea	*Lota lota*		life	Decreased gonadal maturation, sterility	Pulliainen *et al.*, 1992
	Salmo salar		life	High larval mortality from exposed parents, induced hepatic P450	Norrgren *et al.*, 1993
North Sea	*Gadus morhua* *Limanda limanda* *Merlangus merlangus* *Platichthys flesus* *Pleuronectes platessa*		life	Decreased hatch, malformed embryos	Dethlefsen, 1989

[a] Witachlor 149 (n-paraffin, 12 carbons, 40% chlorine w/w) and Hülz 70C (n-paraffin, 12 carbons, 70% chlorine w/w); [b] California coastal waters containing chlorinated hydrocarbons, notably DDT and PCBs; [c] Industrial pollutant = 0.033 mg/l mercuric chloride + 33.3 mg/l cadmium chloride + 3.3 mg/l phenol + 9 mg/lammonia or factory effluent; [d] Kraft pulp mill effluent = 0.03mg/l 2,4-dichlorophenol + 0.05 mg/l 2,4,6-trichlorophenol + 0.09 mg/l 3,4,5-trichloroguaiacol + 0.04 mg/l 4,5,6-trichloroguaiacol + 0.050 mg/l tetrachloroguaiacol; [e] Contaminated sediment extract containing aromatic hydrocarbons and PCBs; f Containing 1-2 x 10-6 mg/l PCB; Carbamide = urea; PCB = Chlophen =Arochlor 1254 = polychlorinated biphenyls; TCB = 3,3',4,4'-tetrachlorobiphenyl (move to industrial)

References

Adams, S. M., Crumby, W. D., Greeley, M. S. Jr., Ryon, M. G. and Schilling, E. M. (1992). Relationship between physiological and fish population responses in a contaminated stream. *Environ. Toxicol. Chem.* **11**, 1549-1557.

Addison, R. F. (1984). Hepatic mixed function oxidase (MFO) induction in fish as a possible biological montoring system. *In* "Contaminant Effects on Fisheries", (V. W. Cairns, P. V. Hodson, and J. O. Nriagu, Eds.), pp. 52-60. Wiley, New York.

Addison, R. F., Zinck, M. E. and Willis, D. E. (1977). Mixed function oxidase enzymes in trout (*Salvelinus fontinalis*) liver: absence of induction following feeding of *p,p'*-DDT or *p,p'*-DDE. *Comp. Biochem. Physiol.* **57C**, 39-43.

Addison, R. F., Zinck, M. E. and Willis, D. E. (1978). Induction of hepatic mixed function oxidase (MFO) enzymes in trout (*Salvelinus fontinalis*) by feeding Arochlor 1254 or 3-methylcholanthrene. *Comp. Biochem. Physiol.* **61C**, 323-325.

Addison, R. F., Zinck, M. E. and Willis, D. E. (1981). Time- and dose-dependence of hepatic mixed function oxidase activity in brook trout *Salvelinus fontinalis* on polychlorinated biphenyl residues: implications for "biological effects monitoring". *Environ. Poll.* **25A**, 211-218.

Afonso, L. O. B., Campbell, P. M., Iwama, G. K., Devlin, R. H. and Donaldson, E. M. (1997). The effect of the aromatase inhibitor fadrazole and two polynuclear aromatic hydrocarbons on sex steroid secretion by ovarian follicles of coho salmon. *Gen. Comp. Endocrinol.* **106**, 169-174.

Ahel, M., McEvoy, J. and Giger, W. (1993). Bioaccumulation of the lipophilic metabolites of nonionic surfactants in freshwater organisms. *Environ. Poll.* **79**, 243-248.

Ahsan, S. N. and Ahsan, J. (1974). Degenerative changes in the testis of *Clarias batrachus* (Linn.) caused by cadmium chloride. *Ind. J. Zool.* **15**, 39-43.

Al-Mohanna, M. M. (1994). Residues of some heavy metals in fishes collected from (Red-Sea coast) Jizan, Saudi-Arabia. *J. Environ. Biol.* **15**, 149-157.

Alkindi, A. Y. A., Brown, J. A., Waring, C. P. and Collins, J. E. (1996). Endocrine, osmoregulatory, respiratory and haematological parameters in flounder exposed to the water soluble fraction of crude oil. *J. Fish Biol.* **49**, 1291-1305.

Allen, P. (1995). Accumulation of lead and cadmium in the edible tissues of *Oreochromis aureus* during acute exposure. *J. Fish Biol.* **47**, 559-748.

Allen, P., Min, S. Y. and Keong, W. M. (1988). Acute effects of mercuric chloride on intracellular GSH levels and mercury distribution in the fish *Oreochromis aureus*. *Bull. Environ. Contam. Toxicol.* **40**, 178-184.

Anderson, D. P. (1996). Environmental factors in fish health: immunological aspects. In "The Fish Immune System. Organism, Pathogen and Environment", (G. Iwama and T. Nakanishi, Eds.), pp. 289-310. Academic Press, San Diego.

Anderson, D. P., Roberson, B. S. and Dixon, O. W. (1982). Immunosuppression induced by a corticosteroid or an alkylating agent in rainbow trout (*Salmo gairdneri*) administered a *Yersinia ruckeri* bacterin. *Dev. Comp. Immunol.* **Suppl. 2**, 197-204.

Anderson, B. S., Middaugh, D. P., Hunt, J. W. and Turpen, S. L. (1991). Copper toxicity to sperm, embryos and larvae of topsmelt *Atherinops affinis*, with notes on induced spawning. *Mar. Environ. Res.* **31**, 17-35.

Anderson, M. J., Miller, M. R. and Hinton, D. E. (1996a). *In vitro* modulation of 17β-estradiol-induced vitellogenin synthesis: Effects of cytochrome P4501A1 inducing compounds on rainbow trout (*Oncorhynchus mykiss*) liver cells. *Aquat. Toxicol.* 34, 327-350.

Anderson, M. J., Olsen, H., Matsumura, F. and Hinton, D. E. (1996b). *In vivo* modulation of 17β-estradiol induced vitellogenin synthesis and estrogen receptors in rainbow trout liver cells by β-napthoflavone. *Toxicol. Appl. Pharmacol.* **137**, 210-218.

Andersson, T. and Förlin, L. (1992). Regulation of the cytochrome P450 enzyme system in fish. *Aquat. Toxicol.* **24**, 1-20.

Andersson, T., Pesonen, M. and Johansson, C. (1985). Differential induction of cytochrome P450 dependent monooxygenase, epoxide hydrolase, glutathione transferase and UDP glucuronosyltransferase activities in the liver of the rainbow trout by beta Napthoflavone or Clophen A50. *Biochem. Pharmacol.* **34**, 3309-3314.

Andersson, T., Bengtsson, B. E., Forlin, L., Hardig, J. and Larsson, A. (1987). Long-term effects of bleached kraft mill effluents on carbohydrate metabolism and hepatic xenobiotic biotransformation enzymes in fish. *Ecotoxicol. Environ. Saf.* **13**, 53-60.

Andersson, E., Borg, B. and Lambert, J. G. D. (1988a). Aromatase activity in brain and pituitary of immature and mature Atlantic salmon (*Salmo salar* L.) parr. *Gen. Comp. Endocrinol.* **72**, 394-401.

Andersson, T., Förlin, L., Härding, J. and Larsson, A. (1988b). Biochemical and physiological disturbances in fish inhabiting coastal waters polluted with bleached kraft mill effluent. *Mar. Environ. Res.* **24**, 233-236.

Andersson, T., Forlin, L., Olsen, S., Fostier, A. and Breton, B. (1993). Pituitary as a target organ for toxic effects of P4501A1 inducing chemicals. *Mol. Cell. Endocrinol.* **91**, 99-105.

Ankley, G. T., Blazer, V. S., Reinert, R. E. and Agosin, M. (1986). Effects of Arochlor 1254 on cytochrome P-450-dependent monoxygenase, glutathione-S-transferase, and UDP-glucuronyltransferase activities in channel catfish liver. *Aquat. Toxicol.* **9**, 91-103.

Ansari, B. A. and Kumar, K. (1987). Malathion toxicity: Effect on the ovary of the zebra fish *Brachydanio rerio* (Cyprinidae). *Int. Rev. ges. Hydrobiol.* **72**, 517-528.

Ansari, B. A. and Kumar, K. (1988). Diazinon toxicity: Effect on protein and nucleic acid metabolism in the liver of zebrafish, *Brachydanio rerio* (Cyprinidae). *Science Total Environ.* **76**, 63-68.

Arnold, H. and Braunbeck, T. (1994). Disulfoton as a major toxicant in the chemical spill at Basel in November 1986: Acute and chronic studies with eel and rainbow trout. *In* "Sublethal and Chronic Effects of Pollutants on Freshwater Fish" (R. Muller and R. Lloyds, Eds.), pp. 79-86, Blackwell, Oxford.

Arnold, H., Pluta, H-J. and Braunbeck, T. (1995). Simultaneous exposure of fish to endosulfan and disulfoton *in vivo*: ultrastructural, stereological and biochemical reactions in hepatocytes of male rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* **33**, 17-43.

Arnold, H., Pluta, H-J. and Braunbeck, T. (1996a). Cytological alterations in the liver of rainbow trout *Oncorhynchus mykiss* after prolonged exposure to low concentrations of waterborne endosulfan. *Dis. Aquat. Org.* **25**, 39-52.

Arnold, H., Pluta, H-J. and Braunbeck, T. (1996b). Sublethal effects of prolonged exposure to disulfoton in rainbow trout (*Oncorhynchus mykiss*): Cytological alterations in the liver by a potent acetylcholine esterase inhibitor. *Ecotox. Environ. Safety* **34**, 43-55.

Arora, N. and Kulshrestha, S. K. (1984). Comparison of the toxic effects of two pesticides on the testes of a fresh water teleost *Channa striatus* Bl. *Acta Hydrochim. Hydrobiol.* **12**, 435-441.

Arukwe, A., Knudsen, F. R. and Goksøyr, A. (1997a). Fish zona radiata (eggshell) protein: a sensitive biomarker for environmental estrogens. *Environ. Health. Persp.* **105**, 418-422.

Arukwe, A., Förlin, L. and Goksøyr, A. (1997b). Xenobiotic and steroid transfornation enzymes in Atlantic salmon (*Salmo salar*) liver treated with an estrogenic compound, 4-nonylphenol. *Environ. Toxicol. Chem.* **16**, 2576-2583.

Aulerich, R. J. and Ringer, R. K. (1977). Current status of PCB toxicity to mink, and effect on their reproduction. *Arch. Environ. Toxicol. Chem.* **6**, 279-292.

Backe, G. A., Gutenmann, W. H. and Lisk, D. J. (1971). Residue of total mercury and methyl mercuric salts in lake trout as a function of age. *Science* **172**, 951-952.

Badsha, K. S. and Goldspink, C. R. (1982). Preliminary observations on the heavy metal content of four species of freshwater fish in NW England. *J. Fish. Biol.* **21**, 251-267.

Bagchi, P., Chatterjee, S., Ray, A. and Deb, C. (1990). Effect of quinalphos, organophosphorus insecticide, on testicular steroidogenesis in fish, *Clarias batrachus*. *Bull. Environ. Contam. Toxicol.* **44**, 871-875.

Balm, P. H. M. and Pottinger, T. G. (1993). Acclimation of rainbow trout (*Oncorhynchus mykiss*) to low environmental pH does not involve an activation of the pituitary-interrenal axis, but evokes adjustments in branchial ultrastructure. *Can. J. Fish. Aquat. Sci.* **50**, 2532-2541.

Balm, P. H. M, Iger, Y., Prunet, P, Pottinger, T. G. and Wendelaar Bonga, S. E. (1995). Skin ultrastructure in relation to prolactin and MSH function in rainbow trout (*Oncorhynchus mykiss*) exposed to environmental acidification. *Cell Tiss. Res.* **279**, 351-358.

Barrington, E. J. W., Barron, N. and Piggins, D. J. (1961). The influence of thyroid powder and thyroxine upon the growth of rainbow trout (*Salmo gairdneri*). *Gen. Comp. Endocrinol.* **1**, 170-178.

Barry, T. P., Ochai, M. and Malisan, J. A. (1995). *In vitro* effects of ACTH on interrenal corticosteroidogenesis during early larval development in rainbow trout. *Gen. Comp. Endocrinol.* **99**, 382-387.

Barton, B. A., Weiner, G. S. and Schreck, C. B. (1985). Effect of prior acid exposure on physiological responses of juvenile rainbow trout (*Salmo gairdneri*) to acute handling stress. *Can. J. Fish. Aquat. Sci.* **42**, 710-717.

Bastomsky, C. H. (1977a). Goiters in rats fed polychlorinated biphenyls. *J. Physiol. Pharmacol.* **55**, 288-292.

Bastomsky, C. H. (1977b). Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Endocrinol.* **101**, 292-296.

Batten, T. F. C. and Ingleton, P. M. (1987). The structure and function of the hypothalamus and pituitary gland. *In* "Fundamentals of Comparative Vertebrate Endocrinology", (I.

Chester Jones, P. M. Ingleton and J. G. Phillips, Eds.), pp. 285-412. Plenum Press, New York and London.

Beamish, R. J. (1976). Acidification of lakes in Canada by acid precipitation and the resulting effects on fishes. *Water Air Soil Poll.* **6**, 501-514.

Beamish, R. J., Lockhart, W. L., van Loon, J. C. and Harvey, H. H. (1975). Long-term acidification of a lake and resulting effects on fishes. *Ambio* **4**, 98-102.

Beattie, J. H. and Pascoe, D. (1978). Cadmium uptake by rainbow trout, *Salmo gairdneri*, eggs and alevins. *J. Fish. Biol.* **13**, 631-637.

Becker, P. H., Schuhmann, S. and Koepff, C. (1993). Hatching failure in common terns (*Sterna hirundo*) in relation to environmental chemicals. *Environ. Poll.* **70**, 207-213.

Beierwaltes, W. H. (1987a). The most common thyroid disease in the State of Michigan is endemic goitre not due to iodine deficiency. *Washington City Med. Soc. Bull.* **Sept. 1987**, 3-10.

Beierwaltes, W. H. (1987b). The diagnosis and treatment of endemic goitre, the most common thyroid disease in the United States: a challenge to physicians.. *Washington City Med. Soc. Bull.* **Oct. 1987**, 2-10.

Béland, P., DeGuise, S., Girard, C., Lagacé, A., Martineau, D., Michaud, R., Muir, D. C. G., Norstrom, R. J., Pelletier, É., Ray, S. and Shugart, L. R. (1993). Toxic compounds and health and reproductive effects in St. Lawrence beluga whales. *J. Great Lakes Res.* **19**, 766-775.

Belanger, S. E., Cherry, D. S. and Cairns, J. Jr. (1990). Functional and pathological impairment of Japanese medaka (*Oryzias latipes*) by long-term asbestos exposure. *Aquat. Toxicol.* **17**, 133-154.

Bellepaire, C. and Ollevier, F. (1987). L'anguille: son histoire et son élevage. *Aquacult. et Dévelop. Cahiers Ethologie appliquée*, **7**, 85-106.

Bend, J. R., James, M. O. and Dansette, P. M. (1977). *In vitro* metabolism of xenobiotics in some marine mammals. *Ann. N. Y. Acad. Sci.* **298**, 505-521.

Bengtsson, B-E. (1980). Long-term effects of PCB (Clophen A50) on growth, reproduction and swimming performance in the minnow, *Phoxinus phoxinus*. *Water Res.* **14**, 681-687.

Bennett, R. O. and Wolke, R. E. (1987). The effect of sublethal endrin exposure on rainbow trout, *Salmo gairdneri* Richardson. I. Evaluation of serum cortisol concentrations and immune responsiveness. *J. Fish. Biol.* **31**, 375-385.

Benoit, D. A. (1975). Chronic effects of Cu on survival, growth and reproduction of blue gills (*Lepomis macrochire*). *Trans. Am. Fish. Soc.* **104**, 353-358.

Benoit, D. A., Leonard, E. N., Christensen, G. M. and Fiandt, J. T. (1976). Toxic effects of cadmium on three generations of brook trout (*Salvelinus fontinalis*). *Trans. Am. Fish. Soc.* **105**, 550-560.

Benson, W. H., Baer, K. N. and Watson, C. F. (1990). Metallothionein as a biomarker of environmental metal contamination: Species-dependent effects. *In* "Biomarkers of Environmental Contamination", (J. F. McCarthy and L. R. Shugart, Eds.), pp. 267-287. Lewis, Boca Raton.

Bentley, P. J. (1982). "Comparative Vertebrate Endocrinology." 2nd Edition, Cambridge University Press, Cambridge.

Bergman, A. and Olsson, M. (1985). Pathology of Baltic grey seal and ringed seal females with special reference to adrenocortical hyperplasia: Is environmental pollution the cause of widely distributed disease syndrome? *Finn. Game Res.* **44**, 47-62.

Berqvist, P. A., Bergek, S., Hallback, H., Rappe, C. and Slorach, S. A. (1989). Dioxins in cod and herring from seas around Sweden. *Chemosphere*, **19**, 551-556.

Besselink, H. T., van Beusekom, S., Roex, E., Vethaak, A. D., Koeman, J. H. and Brouwer, A. (1996). Low hepatic 7-ethoxyresorufin-*o*-deethylase (EROD) activity and minor

alterations in retinoid and thyroid hormone levels in flounder (*Platichthys flesus*) exposed to the polychlorinated biphenyl (PCB) mixture, Clophen A50. *Environ. Poll.* **92**, 267-274.

Beullens, K., Eding, E. H., Gilson, P., Ollevier, F., Komen, H. and Richter, C. J. J. (1997). Gonadal differentiation, intersexuality and sex ratios of European eel (*Anguilla anguilla* L.) maintained in captivity. *Aquaculture* **153**, 135-150.

Bhattacharya, L. (1995). Histological and histochemical alterations in the thyroid activity of endosulphan treated *Oreochromis mossambicus*. *J. Environ. Biol.* **16**, 347-351.

Bhattacharya, L. and Pandey, A. K. (1989). Inhibition of steroidogenesis and pattern of recovery in the testes of DDT exposed cichlid - *Oreochromis mossambicus*. *Bangladesh. J. Zool.* **17**, 1-14.

Bhattacharya, T., Ray, A. K. and Bhattacharya, S. (1984). Response of *Channa punctatus* (Bloch) under short and long term exposures to industrial pollutants: hepatic and blood cholesterol changes. *Comp. Physiol. Ecol.* **9 Suppl.** 435-438.

Bieniarz, K., Epler, P. and Sokolowska-Mikolajczyk, M. (1994). Effect of zinc on guppy (*Poecilia reticulata*) reproduction. *Pol. Arch. Hydrobiol.* **41**, 489-493.

Bieniarz, K., Epler, P., Kime, D., Sokolowska-Mikolajczyk, M., Popek, W. and Mikolajczyk, T. (1996a). Effects of N,N-dimethylnitrosamine (DMNA) on *in vitro* oocyte maturation and embryonic development of fertilized eggs of carp (*Cyprinus carpio* L.) kept in eutrophied ponds. *J. Appl. Toxicol.* **16**, 153-156.

Bieniarz, K., Epler, P., Sokolowska-Mikolajczyk, M., Chyb, J. and Popek, W. (1996b). Carp reproduction in highly eutrophic pond conditions. *Bull. Environ. Contam. Toxicol.* **57**, 842-848.

Billard, R. and Cosson, M. P. (1992). Some problems related to the assessment of sperm motility in freshwater fish. *J. Exp. Zool.* **261**, 122-131.

Billard, R. and de Kinkeln, P. (1970). Stérilisation des testicules de guppies par des doses non létales de parathion. *Ann. Hydrobiol.* **1**, 91-99.

Billard, R. and Roubaud, P. (1985). The effect of metals and cyanide on fertilisation in rainbow trout (*Salmo gairdneri*). *Water Res.* **19**, 209-214.

Binder, R. L. and Lech, J. J. (1984). Xenobiotics in gametes of Lake Michigan lake trout (*Salvelinus namaycush*) induce hepatic monooxygenase activity in their offspring. *Fund. Appl. Toxicol.* **4**, 1042-1054.

Black, D. E., Phelps, D. K. and Lyon, R. L. (1988). The effect of inherited contamination on egg and larval winter flounder, *Pseudopleuronectes americanus*. *Mar. Environ. Res.* **25**, 45-62.

Bleau, H., Daniel, C., Chevalier, G., van Tra, H. and Hontela, A. (1996). Effects of acute exposure to mercury chloride and methylmercury on plasma cortisol, T_3, T_4, glucose and liver glycogen in rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* **34**, 221-235.

Bletchly, J. D. (1984). Polychlorinated biphenyls. Production, current use and possible rates of further disposal in OECD member countries. I "PCB Seminar", (M. C. Barres, H. Koeman and R. Visser Eds.), pp. 343-372. Ministry of Housing, Physical Planning and Environment, Amsterdam.

Bortone, S. A. and Davis, W. P. (1994). Fish intersexuality as indicator of environmental stress. Monitoring fish reproductive systems can serve to alert humans to potential harm. *Bioscience* **44**, 165-172.

Bortone, S. A. and Drysdale, D. T. (1981). Additional evidence for environmentally induced intersexuality in poeciliid fishes. *Assoc. Southeastern Biol. Bull.* **28**, 67.

Bortone, S. A., Davis, W. P. and Bundrick, C. M. (1989). Morphological and behavioural characters in mosquitofish as potential bioindication of exposure to kraft mill effluent. *Bull. Environ. Contam. Toxicol.* **43**, 370-377.

Boyd, C. E. (1964). Insecticides cause mosquitofish to abort. *Prog. Fish. Cult.* **26**, 138.

Braunbeck, T. (1993). Cytological alterations in isolated hepatocytes from rainbow trout (*Oncorhynchus mykiss*) exposed to 4-chloroaniline. *Aquat. Toxicol.* **25**, 83-110.

Braunbeck, T. (1994). Detection of environmentally relevant pesticide concentrations using cytological parameters: Pesticide specificity in the reaction of rainbow trout liver? *In* "Sublethal and Chronic Effects of Pollutants on Freshwater Fish", (R. Muller and R. Lloyd, Eds.), pp. 15-29. Blackwell, Oxford.

Braunbeck, T. and Volkl, A. (1991). Induction of biotransformation in the liver of eel (*Anguilla anguilla* L.) by sublethal exposure to dinitro-*o*-cresol: An ultrastructural and biochemical study. *Ecotox. Environ. Saf.* **21**, 109-127.

Braunbeck, T. and Volkl, A. (1993). Toxicant-induced cytological alterations in fish liver as biomarkers of environmental pollution? A case-study on hepatocellular effects of dintro-*o*-cresol in golden ide *(Leuciscus idus melanotus)*. In "Fish in Ecotoxicology and Ecophysiology", (T. Braunbeck, W. Hanke and H. Segner, Eds.), pp. 55-80. VCH, Weinheim and New York.

Braunbeck, T., Storch, V. and Nagel, R. (1989). Sex-specific reaction of liver ultrastructure in zebrafish (*Brachydanio rerio*) after prolonged sublethal exposure to 4-nitrophenol. *Aquat. Toxicol.* **14**, 185-202.

Braunbeck, T., Gorge, G., Storch, V. and Nagel, R. (1990a). Hepatic steatosis in zebra fish (*Brachydanio rerio*) induced by long-term exposure to gamma-BHC. *Ecotoxicol. Environ. Saf.* **19**, 355-374.

Braunbeck, T., Storch, V. and Bresch, H. (1990b). Species-specific reaction of liver ultrastructure of zebra fish (*Brachdanio rerio*) and trout (*Salmo gairdneri*) after prolonged exposure to 4-chloroaniline. *Arch. Environ. Contam. Toxicol.* **19**, 405-418.

Braunbeck, T., Burkhardt-Holm, P. and Storch, V. (1990c). Liver pathology in eels (*Anguilla anguilla* L.) from the Rhine river exposed to the chemical spill at Basle in November 1986. *Limnol. aktuell* **1**, 371-392.

Bresch, H., Beck, H., Ehlermann, D., Schlaszus, H. and Urbanek, M. (1990). A long-term toxicity test comprising reproduction and growth of zebrafish with 4-chloroaniline. *Arch. Environ. Contam. Toxicol.* **19**, 419-427.

Brodeur, J. C., Girard, C. and Hontela, A. (1997). Use of perifusion to assess *in vitro* the functional integrity of interrenal tissue in teleost fish from polluted sites. *Environ. Toxicol. Chem.*, **16**, 2171-2178.

Bromage, N. R. and Cumaranatunga, R. (1988). Egg production in the rainbow trout. *In* "Recent Advances in Aquaculture", (J. F. Muir and R. J. Robert, Eds.), Vol. III, pp. 65-138. Croom Helm, London and Sydney.

Bromage, N., Duston, J., Randall, C., Brook, A., Thrush, M., Carrillo, M. and Zanuy, S. (1990). Photoperiodic control of teleost reproduction. *In* "Progress in Comparative Endocrinology", (A. Epple, C. G. Scanes and M. H. Stetson, Eds.), pp. 620-626. Wiley-Liss, New York.

Bromwich, P, Cohen, J., Stewart, I. and Walker, A. (1994). Decline in sperm counts: An artefact of changed reference range of "normal"? *Brit. Med. J.* **309**, 19-22.

Brouwer, A., Murk, A. J. and Koeman, J. H. (1990). Biochemical and physiological approaches in ecotoxicology. *Functional Ecol.* **4**, 275-281.

Brown, E. R., Koch, E., Sinclair, T. F., Spitzer, R. and Callaghan, O. (1979). Water pollution and diseases in fish (An epizootiological survey). *J. Environ. Path. Toxicol.* **2**, 917-925.

Brown, S. B., Eales, J. G., Evans, R. E. and Hara, T. J. (1984). Interrenal, thyroidal, and carbohydrate responses of rainbow trout (*Salmo gairdneri*) to environmental acidification. *Can. J. Fish. Aquat. Sci.* **41**, 36-45.

Brown, S. B., Evans, R. E. and Hara, T. J. (1986). Interrenal, thyroidal, carbohydrate and electrolyte responses in rainbow trout (*Salmo gairdneri*) during recovery from the effects of acidification. *Can. J. Fish. Aquat. Sci.* **43**, 714-718.

Brown, J. A., Edwards, D. and Whitehead, C. (1989). Cortisol and thyroid hormone responses to acid stress in the brown trout, *Salmo trutta* L. *J. Fish. Biol.* **35**, 73-84.

Brown, S. B., MacLatchy, D. L., Hara, T. J. and Eales, J. G. (1990). Effects of low ambient pH and aluminium on plasma kinetics of cortisol, T_3 and T_4 in rainbow trout (*Oncorhynchus mykiss*). *Can. J. Zool.* **68**, 1537-1543.

Brown, S. B., MacLatchy, D. L., Hara, T. J. and Eales, J. G. (1991). Effects of cortisol on aspects of 3,5,3'-triiodo-L-thyronine metabolism in rainbow trout (*Oncorhynchus mykiss*). *Gen. Comp. Endocrinol.* **81**, 207-216.

Brungs, W. A., Leonard, E. N. and McKim, J. M. (1973). Acute and long-term accumulation of copper by the brown bullhead, *Ictalurus nebulosus*. *J. Fish. Res. Board. Can.* **30**, 583-586.

Bryan, M. D., Atchison, G. J. and Sandheinrich, M. B. (1995). Effects of cadmium on the foraging behavior and growth of juvenile bluegill, *Lepomis macrochirus*. *Can. J. Fish. Aquat. Sci.* **52**, 1630-1638.

Bucher, F., Hofer, R. and Salvenmoser, W. (1992). Effects of treated paper-mill effluents on hepatic morphology in male bullhead (*Cottus gobio* L.). *Arch. Environ. Contam. Toxicol.* **23**, 410-419.

Bucke, D. and Watermann, B. (1988). Effects of pollutants on fish. *In* "Pollution of the North Sea, an Assessment", (W. Salomons, B. L. Bayne, E. K. Duursma and U. Forstner, Eds.), pp. 612-623. Springer Verlag, Berlin.

Buhler, D. R. and Rasmussson, M. (1968). The oxidation of drugs by fishes. *Comp. Biochem. Physiol.* **25**, 223-239.

Buhler, D. R. and Williams, D. E. (1989). Enzymes involved in metabolism of PAHs by fish and other aquatic animals: Part I, oxidative enzymes (or phase I enzymes). In " Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment", (U. Varanasi, Ed.), pp. 151-184. CRC Press, Boca Raton.

Burdick, G. E., Harris, E. J., Dean, H. J., Walker, T. M., Skea, J. and Colby, D. (1964). The accumulation of DDT in lake trout and the effect on reproduction. *Trans. Am. Fish. Soc.* **93**, 127-136.

Burdick, G. E., Dean, H. J., Harris, E. J., Skea, J., Karcher, R. and Frisa, C. (1972). Effect of rate and duration of feeding DDT on the reproduction of salmonid fishes reared and held under controlled conditions. *NY Fish Game J.* **19**, 97-115.

Bye, V. J. (1984) The role of environmental factors in the timing of reproductive cycles. *In* "Fish Reproduction: Strategies and Tactics", (G. W. Potts and R. J. Wootton, Eds.), pp. 187-205. Academic Press, London.

Callard, G. V., Petro, Z. and Ryan, K. J. (1981). Biochemical evidence for aromatization of androgen to estrogen in the pituitary. *Gen. Comp. Endocrinol.* **44**, 350-364.

Campbell, K. R. (1994). Concentrations of heavy metals associated with urban runoff in fish living in stormwater treatment ponds. *Arch. Environ. Contam. Toxicol.* **27**, 352-356.

Campbell, P. M. and Devlin, R. H. (1997). Increased CYP1A1 and ribosomal protein L5 gene expression in a teleost: The response of juvenile chinook salmon to coal dust exposure. *Aquat. Toxicol.* **38**, 1-15.

Campbell, P. M., Pottinger, T. G. and Sumpter, J. P. (1992). Stress reduces the quality of gametes produced by rainbow trout. *Biol. Reprod.* **47**, 1140-1151.

Campbell, P. M., Pottinger, T. G. and Sumpter, J. P. (1994). Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. *Aquaculture* **120**, 151-169.

Campbell, P. M., Kruzynski, G. M., Birtwell, I. K. and Devlin, R. H. (1996). Quantitation of dose-dependent increases in CYP1A1 messenger RNA levels in juvenile chinook salmon exposed to treated bleached-kraft mill effluent using two field sampling techniques. *Environ. Toxicol. Chem.* **15**, 1119-1123.

Canario, A. V. M. and Power, D. M. (Eds.). (1995). "Fish Pheromones; Origins and Modes of Action." University of Algarve, Faro, Portugal.

Carlson, A. R. (1971). Effects of long-term exposure to carbaryl (Sevin) on survival, growth, and reproduction of the fathead minnow (*Pimephales promelas*). *J. Fish. Res. Bd. Can.* **29**, 583-587.

Carpene, E., Gumiero, B., Fedrizzi, G., Serra, R. (1994). Trace elements (Zn, Cu, Cd) in fish from rearing ponds of Emilia romagna region (Italy). *Sci. Total Environ.* **141**, 139-146.

Carson, R. (1962). "The Silent Spring", Houghton Mifflin, USA (1965 paperback edition, Penguin, Middlesex, U.K.).

Casillas, E., Misitano, D., Johnson, L. L., Rhodes, L. D., Collier, T. K., Stein, J. E., McCain, B. B. and Varanasi, U. (1991). Inducibility of spawning and reproductive success of female English sole (*Parophrys vetulus*) from urban and nonurban areas of Puget Sound, Washington. *Mar. Environ. Res.* **31**, 99-122.

Castrén, M and Oikari, A. (1987). Changes of the liver UDP-glucuronosyltransferase activity in trout (*Salmo gairdneri* Rich.) acutely exposed to selected aquatic toxicants. *Comp. Biochem. Physiol.* **86C**, 357-360.

Cerniglia, C. A. and Heitkamp, M. A. (1989). Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment. *In* "Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment", (U. Varanasi, Ed.), pp. 41-68. CRC Press, Boca Raton.

Chakravorty, S., Lal, B., and Singh, T. P. (1992). Effect of endosulfan (Thiodan) on vitellogenesis and its modulation by different hormones in the vitellogenic catfish *Clarias batrachus. Toxicol.* **75**, 191-198.

Chang, J. P. and Peter, R. E. (1983). Effects of dopamine on gonadotropin release in female goldfish, *Carassius auratus. Neuroendocrinol.* **36**, 351, 357.

Chang, J. P., Peter, R. E., Nahorniak, C. S. and Sokolowska, M. (1984). Effects of catecholaminergic agonists and antagonists on serum gonadotropin concentrations and ovulation in goldfish: evidence for specificity of dopamine inhibition of gonadotropin secretion. *Gen. Comp. Endocrinol.* **55**, 351-360.

Chanin, P. (1985). "The Natural History of Otters". Christopher Helm, London.

Chen, T. T., Reid, P. C., Van Beneden, R. and Sonstegard, R. A. (1986). Effect of Arochlor 1254 and Mirex on estradiol-induced vitellogenin production in juvenile rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci.* **43**, 169-173.

Cheng, S. K. and Ruby, S. M. (1981). Effects of pulse exposure to sublethal levels of hydrogen cyanide on reproduction of American flagfish. *Arch. Environ. Contam. Toxicol.* **10**, 105-116.

Chester Jones, I. and Mosley, W. (1980). The interrenal gland in Pisces. Part 1. Structure. In " General, Comparative and Clinical Endocrinology of the Adrenal Cortex", (I. Chester Jones and I. W. Henderson, Eds.), Vol. 3, pp. 395-472. Academic Press, London.

Chester Jones, I., Ingleton, P. M. and Phillips, J. G. (Eds.) (1987). "Fundamentals of Comparative Vertebrate Endocrinology". Plenum Press, New York and London.

Choudhury, C., Ray, A. K., Bhattacharya, S. and Bhattacharya, S. (1993). Non-lethal concentrations of pesticide impair ovarian function in the freshwater perch, *Anabas testudineus. Environ. Biol. Fish.* **36**, 319-324.

Chu, I., Villeneuve, D. C., Valli, V. E., Ritter, L., Norstrom, R. J., Ryan J. J. and Becking, G. C. (1984). Toxicological response and its reversibility in rats fed Lake Ontario or Pacific coho salmon for 13 weeks. *J. Env. Sci. Health* **B19**, 713-731.

Cleland, G., Leatherland, J. F. and Sonstegard, R. (1988). Toxic effects in CS7B1/6 and DBA/2 mice following consumption of halogenated aromatic hydrocarbon contaminated Great Lakes coho salmon (*Oncorhynchus kisutch* Walbaum). *Environ. Health Perspect.* **75**, 153-157.

Coccini, T., Candura, S. M., Manzo, L., Costa, L. G. and Tonini, M. (1993). Interaction of the neurotoxic pesticides ivermectin and lindane with enteric GABA-A receptor-ionophore complex in the guinea pig. *Eur. J. Pharmacol. Environ. Toxicol. Pharmacol.* **2**, 1-6.

Colborn, T. (1994). The wildlife/human connection: Modernizing rsk decisions. *Environ. Health. Perspect.* **102**, 55-59.

Colborn, T. and Clement C. (Eds) (1992). "Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection". Princeton Scientific Publishing, Princeton.

Colborn, T., vom Saal, F. S. and Soto, A. M. (1993). Developmental effects of endocrine disrupting chemicals in wildlife and humans. *Environ. Health Persp.* **101**, 378-384.

Colborn, T., Dumanoski, D. and Myers, J. P. (1996). "Our Stolen Future". Little Brown & Co., London.

Cole, L. M., Nicholson, R. A. and Casida, J. E. (1993). Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pesticide Biochem. Physiol.* **46**, 47-54.

Collier, T. K. and Varanasi, U. (1991). Hepatic activities of xenobiotic metabolizing enzymes and biliary levels of xenobiotics in English sole (*Parophrys vetulus*) exposed to environmental contaminants. *Arch. Environ. Contam. Toxicol.* **20**, 462-473.

Collier, T. K., Stein, J. E., Sanborn, H. R., Hom, T., Myers, M. S. and Varanasi, U. (1992). Field studies of reproductive success and bioindicators of maternal contaminant exposure in English sole (*Parophrys vetulus*). *Sci. Total Environ.* **116**, 169-185.

Contreras-Sanchez, W. M., Schreck, C. B. and Fitzpatrick, M. S. (1995). Effect of stress on the reproductive physiology of rainbow trout, *Oncorhynchus mykiss. In* "Proceedings of the 5th International Symposium on Reproductive Physiology of Fish, Austin 1995", (F. W. Goetz and P. Thomas, Eds.), p. 183 , Fish Symposium 95, Austin.

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

Cope, O. B., Wood, E. M. and Wallen, G. H. (1970). Some chronic effects of 2,4-D on the bluegill (*Lepomis macrochirus*). *Trans. Am. Fish. Soc.* **99**, 1-12.

Cope, W. G., Wiener, J. G., Atchison, G. J. (1994). Hepatic cadmium, metal-binding proteins and bioaccumulation in bluegills exposed to aqueous cadmium. *Environ. Toxicol. Chem.* **13**, 553-562.

Cormier, S. M. and Racine, R. N. (1990). Histopathology of Atlantic Tomcod: A possible monitor of xenobiotics in northeast tidal rivers and estuaries. *In* "Biomarkers of Environmental Contamination", (J. F. McCarthy and L. R. Shugart, Eds.), pp. 59-71. Lewis Publishers, Boca Raton FL.

Craig, G. R. and Bakshi, W. F. (1977). The effects of depressed pH on flagfish reproduction, growth and survival. *Water Res.* **11**, 621-626.

Crim, L. W., Peter, R. E. and Billard, R. (1981). Onset of gonadotropic hormone accumulation in the immature trout pituitary gland in response to estrogen or aromatizable androgen steroid hormones. *Gen. Comp. Endocrinol.* **44**, 374-381.

Cross, J. N. and Hose, J. E. (1988). Evidence for impaired reproduction in white croaker (*Genyonemus lineatus*) from contaminated areas off southern California. *Mar. Environ. Res.* **24**, 185-188.

Curtis, L. R. and Beyers, R. J. (1978). Inhibition of oviposition in the teleost *Oryzias latipes*, induced by subacute kepone exposure. *Comp. Biochem. Physiol.* **61C**, 15-16.

Cuvin-Aralar, M. L. A. (1994). Survival and heavy metal accumulation of 2 *Oreochromis niloticus* (L.) strains exposed to mixtures of zinc, cadmium and mercury. *Sci. Total Environ.* **148**, 31-38.

Da Costa, H. and Ruby, S. M. (1984). The effect of sublethal cyanide on vitellogenic parameters in rainbow trout *Salmo gairdneri*. *Arch. Environ. Contam. Toxicol.* **13**, 101-104.

Daly, H. B. (1993). Laboratory rat experiments show consumption of Lake Ontario salmon causes behavioral changes - support for wildlife and human research results. *J. Great Lakes Res.* **19**, 784-788.

Daly, H. B., Hertzler, D. R. and Sargent, D. M. (1989). Ingestion of environmentally contaminated Lake Ontario salmon by laboratory rats increases avoidance of unpredictable aversive nonreward and mild electric shock. *Behav. Neurosci.* **6**, 1356-1365.

Davis, W. P. (1988). Reproductive and developmental responses in the self-fertilising fish, *Rivulus marmoratus*, induced by the plasticizer, di-n-butylphthalate. *Environ. Biol. Fishes* **21**, 81-90.

Davis, W. P. and Bortone, S. A. (1992). Effects of kraft mill effluent on the sexuality of fishes: an environmental early warning? *In* "Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection", (T. Colborn and C. Clement, Eds.), pp. 113-127. Princeton Scientific Publishing, Princeton.

Daye, P. G. and Glebe, B. D. (1984). Fertilization success and sperm motility of Atlantic salmon (*Salmo salar* L.) in acidified water. *Aquaculture* **43**, 307-312.

De Boer, J. (1989). Organochlorine compounds and bromodiphenyl ethers in livers of Atlantic cod (*Gadus morhua*) from the North Sea, 1979-1987. *Chemosphere* **18**, 2131-2140.

Denayer, B. (1994). "Glasaalbemonstering te nieuwpoort tijdens het voorjaar 1994." Ministry of the Flemish Community, Belgium. 1BR. Wb.V.R.94.26 pp. 1-7.

Denton, T. E., Howell, W. M., Allison, J. J., McCollum, J. and Marks, B. (1985). Masculinization of female mosquitofish by exposure to plant sterols and *Mycobacterium smegmatis*. *Bull. Environ. Contam. Toxicol.* **35**, 627-632.

Desaiah, D. (1982). Biochemical mechanism of chlordecone neurotoxicity: A review. *Neurotoxicology* **3**, 103-110.

Desbrow, C., Routledge, E. J., Brighty, G. C., Sumpter, J. P. and Waldock, M. (1998). Identification of Estrogenic Chemicals in STW effluent. 1. Chemical fractionation and *in vitro* biological screening. *Environ. Sci. Technol.* 32, 1549-1558

Dethlefsen, V. (1989). Fish in the polluted North Sea. *Dana* **8**, 109-129.

Dethlefsen, V. and Tiews, K. (1985). Review on the effects of pollution on marine fish life and fisheries in the North Sea. *J. Appl. Ichthyol.* **1**, 97-118.

Devaux, A., Pesonen, M. Monod, G. and Andersson, T. (1992). Glucocorticoid-mediated potentiation of P-450 induction in primary culture of rainbow trout hepatocytes. *Biochem. Pharmacol.* **43**, 898-901.

DeVito, M. J., Xinfrang, M., Babish, J. G., Menache, M. and Birnbaum, L. S. (1994). Dose-response relationships in mice following subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: CYP1A1, CYP1A2, estrogen receptor, and protein tyrosine phosphorylation. *Toxicol. Appl. Pharmacol.* **124**, 82-90.

Dey, S. and Bhattacharya, S. (1989). Ovarian damage to *Channa punctatus* after chronic exposure to low concentrations of elsan, mercury and ammonia. *Ecotoxicol. Environ. Saf.* **17**, 247-257.

Dickhoff, W. W., Folmar, L. C. and Gorbman, A. (1978). Changes in plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* **36**, 229-232.

Donaldson, E. M. and Dye, H. M. (1975). Corticosteroid concentrations in sockeye salmon (*Oncorhynchus nerka*) exposed to low concentrations of copper. *J. Fish. Res. Bd. Can.* **32**, 522-539.

Donaldson, E. M., Fagerland, U. H. M. and McBride, J. R. (1984). Aspects of the endocrine response to pollutants in salmonids. *In* "Contamination Effects on Fisheries", (V. W. Cairns, P. V. Hodson and J. O. Nriagu, Eds.), pp. 213-221. Wiley, New York.

Donohoe, R.M. and Curtis, L.R. (1996). Estrogenic activity of chlordecone, *o,p'*-DDT and *o,p'*-DDE in juvenile rainbow trout: induction of vitellogenesis and interaction with hepatic estrogen binding sites. *Aquatic Toxicology* **36**, 31-52.

Drysdale, D. T. and Bortone, S. A. (1989). Laboratory induction of intersexuality in the mosquitofish, *Gambusia affinis*, using paper mill effluent. *Bull. Environ. Contam. Toxicol.* **43**, 611-617.

Duplinsky, P. D. (1982). Sperm motility of northern pike and chain pickerel at various pH values. *Trans. Am. Fish. Soc.* **111**, 768-771.

Dutta, H. M., Adhikari, S., Singh, N. K., Roy, P. K. and Munshi, J. S. D. (1993). Histopathological changes induced by malathion in the liver of a freshwater catfish, *Heteropneustes fossilis* (Bloch). *Bull. Environ. Contam. Toxicol.* **51**, 895-900,

Ebrahimi, M., Nysten, K., Roelants, I., Ollevier, F. and Kime, D. E. (1995). Use of computer assisted sperm analysis (CASA) for monitoring sperm quality: application for determining effects of heavy metal pollutants. *In* "Larvi95 - Fish & Shellfish Larviculture Symposium", (P. Lavens, E. Jaspers and I. Roelants, Eds.), *European Aquaculture Soc., Special Publication No. 24, Gent, Belgium*. 47-49.

Eddy, F. B. (1981). Effects of stress on osmotic and ionic regulation in fish. In "Stress and Fish", (A. D. Pickering, Ed.), pp. 77-102. Academic Press, New York.

Eggens, M., Bergman, A., Vethaak, D., Van der Weiden, M., Celander, M. and Boon, J. P. (1995). Cytochrome P4501A indexes as biomarkers of contaminant exposure - results of a field-study with plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) from the southern North Sea. *Aquat. Toxicol.* **32**, 211-225.

Eisele, T. A., Coulombe, R. A., Pawlowski, N. E. and Nixon, J. E. (1984). The effects of route of exposure and combined exposure of mixed function oxidase inducers and suppressors on hepatic parameters in rainbow trout (*Salmo gairdneri*). *Aquat. Toxicol.* **5**, 211-226.

Eisenreich, S. J., Looney, B. B. and Thorton, J. D. (1981). Airborne organic contaminants in the Great Lakes ecosystem. *Environ. Sci. Tech.* **15**, 30-38.

Elcombe, C. R. and Lech, J. J. (1978). Induction of monooxygenation in rainbow trout by polychlorinated biphenyls: a comparative study. *Environ. Health Perspect.* **23**, 309-314.

Ellenberger, S. A., Baumann, P. C. and May, T. W. (1994). Evaluation of effects caused by high copper concentrations in Torch Lake, Michigan, on reproduction of yellow perch. *J. Great Lakes Res.* **20**, 531-536.

Elliott, J. E., Butler, R. W., Nostrom, R. J. and Whitehead, P. E. (1989). Environmental contaminants and reproductive success of Great Herons, *Ardea herodias*, in British Columbia. *Environ. Poll.* **59**, 91-114.

Elliott, J. E., Wilson, L. K., Langelier, K. W. and Norstrom, R. J. (1996). Bald eagle mortality and chlorinated hydrocarbon contaminants in livers from British Columbia, Canada 1989-94. *Environ. Poll.* **94**, 9-18.

Ellis, A. E. (1981). Stress and the modulation of defense mechanisms in fish. *In* "Stress and Fish" (A. D. Pickering, Ed.), pp. 147-169, Academic Press, New York.

Eroschenko, V. P. and Palmiter, R. D. (1980). Estrogenicity of kepone in birds and mammals. *In* "Estrogens in the Environment", (J. A. McLachlan, Ed.), pp. 305-325. Elsevier/North Holland.

European Commission. (1996). European workshop on the impact of endocrine disruptors on human health and wildlife. Weybridge, 2-4 Dec. 1996. *Report EUR 17549, Environment and Climate Research Programme, DG XII, European Commission.*

Fair, P. H. (1986). Interaction of benzo(a)pyrene and cadmium on GSH-S-transferase and benzo(a)pyrene hydroxylase in the black sea bass *Centropristis striata. Arch. Environ. Contam. Toxicol.* **15**, 257-263.

Falandysz, J. (1984). Organochlorine pesticides and polychlorinated biphenyls in livers of cod from the Southern Baltic, 1981. *Z. Lebensm. Unters. Forsh.* **179**, 311-314.

Falandysz, J. (1986). Organochlorine pesticides and polychlorinated biphenyls in cod from the Southern Baltic, 1983. *Z. Lebensm. Unters. Forsh.* **182**, 136-139.

Falandysz, J. (1994). Polychlorinated biphenyl concentrations in cod-liver oil: Evidence of a steady-state condition of these compounds in the Baltic area oils and levels noted in Atlantic oils. *Arch. Environ. Contam. Toxicol.* **27**, 266-271.

FAO (1994). "Review of nominal statistics for capture and culture fisheries of Europe." European Inland Fisheries Advisory Commission, 18th Session, Rome 1994. EIFAC/XVII/94/Inf.19

Felton, S. P., Grace, R. and Landolt, M. (1994). Significantly higher levels of zinc and copper found in wild compared to hatchery-reared coho salmon smolts *Oncorhynchus kisutch. Dis. Aquat. Org.* **18**, 233-236.

Fent, K. (1992). Embryonic effects of tributyl tin on the minnow *Phoxinus phoxinus. Environ. Poll.* **76**, 187-194.

Fent, K. (1996). Ecotoxicology of organotin compounds. *Crit. Rev. Toxicol.* **26**, 1-117.

Fent, K. and Bucheli, T. D. (1994). Inhibition of hepatic microsomal monooxygenase system by organotins *in vitro* in freshwater fish. *Aquat. Toxicol.* **28**, 107-126.

Fent, K. and Meier, W. (1992). Tributyltin-induced effects on early life stages of minnows *Phoxinus phoxinus. Arch. Environ. Contam. Toxicol.* **22**, 428-438.

Fent, K. and Meier, W. (1994). Effects of triphenyltin on fish early life stages *Arch. Environ. Contam. Toxicol.* **27**, 224-231.

Fent, K. and Stegeman, J. J. (1991). Effects of tributyltin chloride *in vitro* on the hepatic microsomal monoxygenase system in the fish *Stenotomus chrysops. Aquat. Toxicol.* **20**, 159-168.

Ferrando, M. D., Sancho, E. and Andreu, E. (1992). Persistence of some pesticides in the aquatic environment. *Bull. Env. Contam. Toxicol.* **48**, 747-755.

Fingerman, S. W. and Russell, L. C. (1980). Effects of polychlorinated biphenyl, Arochlor 1242, on locomotor activity and on the neurotransmitters dopamine and norepinephrine in the brain of the Gulf killifish, *Fundulus grandis. Bull. Environ. Contam. Toxicol.* **25**, 682-687.

Fingerman, S. W., Brown, L. A., Lynn, M. and Short, E. C. Jr. (1983). Responses of channel catfish to xenobiotics. Induction and partial characterization of a mixed function oxygenase. *Arch. Environ. Contam. Toxicol.* **12**, 195-202.

Flett, P. A., Munkittrick, K. R. and Leatherland, J. F. (1991). Reproductive problems in Lake Erie coho salmon. *In* "Proceedings 4th International Symposium on Reproductive

Physiology of Fish", (A. P. Scott, J. P. Sumpter, D. E. Kime and M. S. Rolfe, Eds.), pp. 151-153. FishSymp91, Sheffield.

Flik, G., van der Velden, J. A., Seegers, H. C. M., Kolar, Z. and Wendelaar Bonga, S. (1989). Prolactin cell activity and sodium fluxes in tilapia (*Oreochromis mossambicus*) after long-term acclimation to acid water. *Gen. Comp. Endocrinol.* **75**, 39-45.

Flouriot, G., Pakdel, F., DuCouret, B. and Valotaire, Y. (1995). Influence of xenobiotics on rainbow trout liver estrogen receptor and vitellogenin gene expression. *J. Mol. Endocrinol.* **15**, 143-151.

Folmar, L. C. (1993). Effects of chemical contaminants on blood chemistry of teleost fish: a bibliography and synopsis of selected effects. *Environ. Toxicol. Chem.* **12**, 337-375.

Folmar, L. C. and Dickhoff, W. W. (1980). The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. *Aquaculture* **21**, 1-37.

Folmar, L. C., Dickhoff, W. W., Zaugg, W. S. and Hodgins, H. O. (1982). The effects of Arochlor 1254 and No. 2 fuel oil on smoltification and seawater adaptation of coho salmon (*Oncorhynchus kisutch*). *Aquat. Toxicol.* **2**, 291-299.

Folmar, L. C., Denslow, N. D., Rao, V., Chow, M., Crain, A. D., Marcino, J. and Guillette Jr. L. J. (1996). Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (*Cyprinus carpio*) captured near a major sewage treatment plant. *Environ. Health Perspect.* **104**, 1096-1101.

Förlin, L. (1980). Effects of Clophen A50, 3-methylcholanthrene, pregnenolone-16α-carbonitrile and phenobarbital on the hepatic microsomal cytochrome P-450 dependent monooxygenase system in rainbow trout, *Salmo gairdneri*, of different age and sex. *Toxicol. Appl. Pharmacol.* **54**, 420-430.

Förlin, L. and Andersson, T. (1984). Influence of biological and environmental factors on hepatic steroid and xenobiotic metabolism in fish: Interaction with PCB and β-naphthoflavone. *Mar. Environ. Res.* **14**, 47-58.

Förlin, L. and Hansson, T. (1982). Effects of treated municipal wastewater on the hepatic, xenobiotic, and steroid metabolism in trout. *Ecotoxicol. Environ. Saf.*. **6**, 41-48.

Förlin, L. and Lidman, N. (1979). *O*-Demethylation of *p*-nitroanisole by mixed function oxidase system of the rainbow trout (*Salmo gairdneri*) liver. *Gen. Pharmacol.* **10**, 411-415.

Förlin, L., Andersson, T., Koivusaari, U. and Hansson, T. (1984). Influence of biological and environmental factors on hepatic steroid and xenobiotic metabolism in fish: interaction with PCB and β-naphthoflavone. *Mar. Environ. Res.* **14**, 47-58.

Förlin, L., Haux, C, Karlsson-Norrgren, L., Runn, P. and Larsson, A. (1986) Biotransformation enzyme activities and histopathology in rainbow trout, *Salmo gairdneri*, treated with cadmium. *Aquat. Toxicol.* **8**, 51-64.

Foureman, G. L. (1989). Enzymes involved in the metabolism of PAH by fishes and other aquatic animals: Hydrolysis and conjugation enzymes (or Phase II enzymes). *In* "Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment", (U. Varanasi, Ed.), pp. 185-202. CRC Press, Boca Raton.

Foureman, G. L., White, N. B. Jr. and Bend, J. R. (1983) Biochemical evidence that winter flounder, *Pseudopleuronectes americanus*, have induced hepatic cytochrome P-450-dependent monooxygenase activities. *Can. J. Fish. Aquat. Sci.* **40**, 854 - 865.

Fox, G. A. (1992). Epidemiological and pathobiological evidence of contaminant-induced alterations in sexual development in free-living wildlife. *In* "Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection" (T. Colborn and C. Clement, Eds.) pp. 147-158. Princeton Scientific Publishing, Princeton NJ.

Fox, G. A. (1993). What have biomarkers told us about the effects of contaminants on the health of fish-eating birds in the Great Lakes? The theory and a literature review. *J. Great Lakes Res.* **19**, 722-736.

Franklin, R. B., Elcombe, C. R., Vodicnik, M. J. and Lech, J. J. (1980). Comparative aspects of the disposition and metabolism of xenobiotics in fish and mammals. *Fed. Proc.* **39**, 3144-3149.

Freeman, H. C. and Idler, D. R. (1975). The effect of polychlorinated biphenyl on steroidogenesis and reproduction in the brook trout (*Salvelinus fontinalis*). *Can. J. Biochem.* **53**, 666-670.

Freeman, H. C. and Sangalang, G. (1977). Changes in steroid hormone metabolism as a sensitive method of monitoring pollutants and contaminants. *Proc. 3rd. Aquat. Toxicity Workshop, Halifax, NS, Nov. 1976. Environ. Prot. Service Tech. Rep. No. EPS-5-77-1, Halifax, Canada. pp. 123-132.*

Freeman, H. C., Uthe, J. F. and Sangalang, G. (1980). The use of steroid hormone metabolism studies in assessing the sublethal effects of marine pollution. *Rapp. P.-v. Réun. Cons. int. Explor. Mer*, **179**, 16-22.

Freeman, H. C., Sangalang, G. and Flemming, B. (1982). The sublethal effects of a polychlorinated biphenyl (Arochlor 1254) diet on the Atlantic cod (*Gadus morhua*). *Sci. Total. Environ.* **24**, 1-11.

Freeman, H. C., Sangalang, G. B., Burns, G. and McMenemy, M. (1983). The blood sex hormone levels in sexually mature male Atlantic salmon (*Salmo salar*) in the Westfield River (pH 4.7) and the Medway River (pH 5.6) Nova Scotia. *Sci. Total. Environ.* **32**, 87-91.

Freeman, H. C., Uthe, J. F. and Silk, P. J. (1984a). Polychlorinated biphenyls, organochlorine pesticides and chlorobenzenes content of livers from Atlantic cod (*Gadus morhua*) caught off Halifax, Nova Scotia. *Environ. Monitoring Assess.* **4**, 389-394.

Freeman, H. C., Sangalang, G. B. and Uthe, J. F. (1984b). The effects of pollutants and contaminants on steroidogenesis in fish and marine mammals. *In* "Contamination Effects on Fisheries", (V. W. Cairns, P. V. Hodson and J. R. Nriagu, Eds.), pp. 197-211. Wiley, New York.

Friedmann, A.S., Watzin, M.C., Brinck-Johnsen, T. and Leiter, J.C. (1996). Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). *Aquat. Toxicol.* **35**, 265-278.

Fry, D. M. (1995). Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environ. Health Perspect.* **103, Suppl. 7**, 165-171.

Fry, D. M. and Toone, C. K. (1981). DDT-induced feminization of gull embryos. *Science* **213**, 922-924.

Fryer, J. N., Tam, W. H., Valentine, B. and Tikkala, R. E. (1988). Prolactin cell cytology, plasma electrolytes, and whole body sodium efflux in acid-stressed brook trout (*Salvelinus fontinalis*). *Can. J. Fish. Aquat. Sci.* **45**, 1212-1221.

Fu, H., Lock, R. A. C. and Wendelaar Bonga, S. E. (1989). Effect of cadmium on prolactin cell activity and plasma electrolytes in the freshwater teleost *Oreochromis mossambicus*. *Aquat. Toxicol.* **14**, 295-306.

Funari, F., Zoppini, A., Verdino, A., De Angelis, G. and Vittozi, L. (1987). Xenobiotic-metabolizing enzyme systems in test fish. I. Comparative studies of liver microsomal monooxygenases. *Ecotox. Environ. Saf.* **13**, 24-31.

Gagnon, M. M., Dodson, J. J., Hodson, P.V., van de Kraak, G. and Carey, J. H. (1994a). Seasonal effects of bleached kraft mill effluent on reproductive parameters of white sucker (*Catostomus commersoni*) populations of the St. Maurice River, Quebec, Canada. *Can. J. Fish. Aquat. Sci.* **51**, 337-347.

Gagnon, M. M., Dodson, J. J. and Hodson, P. V. (1994b). Ability of BKME (bleached kraft mill effluent) exposed white suckers (*Catostomus commersoni*) to synthesize steroid hormones. *Comp. Biochem. Physiol.* **107C**, 265-273.

Gaitán, E. (1973). Water-borne goitrogens and their role in the etiology of endemic goiter. *World Rev. Nutr. Dietetics* **17**, 53-90.

Gaitán, E., Medina, P., DeRouen, T. A. and Zia, M. S. (1973). Goitre prevalence and bacterial contamination of water supplies. *J. Clin. Endocrinol. Metab.* **51**, 957-961.

Garvey, J. S. (1990). Metallothionein: A potential biomonitor of exposure to environmental toxins. *In* "Biomarkers of Environmental Contamination", (J. F. McCarthy and L. R. Shugart, Eds.), pp. 267-287. Lewis, Boca Raton.

Gerhart, E. H. and Carlson, R. M. (1978). Hepatic mixed-function oxidase activity in rainbow trout exposed to several polycyclic aromatic compounds. *Environ. Res.* **17**, 284-295.

Ghiasuddin, S. M. and Matsumura, F. (1982). Inhibition of gamma-aminobutyric acid (GABA)-induced chloride uptake by gamma-BHC and heptachor epoxide. *Comp. Biochem. Physiol.* **73C**, 141-144.

Ghosh, P., Bhattacharya, S. and Bhattacharya, S. (1990). Impairment of the regulation of gonadal function in *Channa punctatus* by Metacid-50 and Carbaryl under laboratory and field conditions. *Biomed. Environ. Sci.* **3**, 106-112.

Ghosh, P., Bose, S., Ghosh, S., Bhattacharya, S. and Bhattacharya, S. (1991). Fish oocyte membrane binds inorganic mercury in an *in vitro* system. *In* "Current Themes in Comparative Endocrinology", (R. N. Saxena, K. Muralidhar, L. Bhagat, N. Sehgal, T. Saxena and P. Kaushal, Eds.), pp. 102-103. University Press, Delhi.

Gibbs, P. E., Bryan, G. W., Pascoe, P. L. and Burt, G. R. (1987). The use of the dog-whelk, *Nucella lapillus*, as an indicator of tributyltin (TBT) contamination. *J. Mar. Biol. Assoc. U. K.* **67**, 507-523.

Giesy, J. P., Versteeg, D. J. and Graney, R. L. (1988). A review of selected clinical indicators of stress-induced changes in aquatic organisms. In "Toxic Contaminants and Ecosystem Health: A Great Lakes Focus" (M. S. Evans, Ed.), pp. 169-200, Wiley, New York.

Giesy, J. P., Verbrugge, D. A., Othout, R. A., Bowerman, W. W., Mora, M. A., Jones, P. D., Newsted, J. L., Vandervoort, C., Heaton, S. N., Aulerich, R. J., Bursian, S. J., Ludwig, J. P., Ludwig, M., Dawson, G. A., Kubiak, T. J., Best, D. A. and Tillitt, D. E. (1994a). Contaminants in fishes from Great-Lakes-influenced sections and above dams of three Michigan rivers. I. Concentrations of organo-chlorine insecticides, polychlorinated-biphenyls, dioxin equivalents, and mercury. *Arch. Environ. Contam. Toxicol.* **27**, 202-212.

Giesy, J. P., Verbrugge, D. A., Othout, R. A., Bowerman, W. W., Mora, M. A., Jones, P. D., Newsted, J. L., Vandervoort, C., Heaton, S. N., Aulerich, R. J., Bursian, S. J., Luwig, J. P., Dawson, G. A., Kubiak, T. J., Best, D. A. and Tillitt, D. E. (1994b). Contaminants in fishes from Great Lakes-influenced sections and above dams of three Michigan rivers. II: Implications for health of mink. *Arch. Environ. Contam. Toxicol.* **27**, 213-223.

Giesy, J. P., Bowerman, W. W., Mora, M. A., Verbrugge, D. A., Othoudt, R. A., Newsted, J. L., Summer, C. L., Aulerich, R. J., Bursian, S. J., Ludwig, J. P., Dawson, G. A., Kubiak, T. J., Best, D. A. and Tillitt, D. E. (1995). Contaminants in fishes from Great Lakes-influenced sections and above dams of three Michigan Rivers: III. Implications for health of bald eagles. *Arch. Environ. Contam. Toxicol.* **29**, 309-321.

Gilbertson, M. (1988). Epidemics in birds and mammals caused by chemicals in the Great Lakes. In "Toxic Contaminants and Ecosystem Health: A Great Lakes Focus", (M. S. Evans, Ed.), pp. 133-165. Wiley, New York.

Gilbertson, M. and Fox, G. A. (1977). Pollutant-associated embryonic mortality of Great Lakes herring gulls. *Environ. Poll.* **12**, 211-216.

Gill, T. S. and Epple, A. (1992). Effects of cadmium on plasma catecholamines in the American eel, *Anguilla rostrata. Aquat. Toxicol.* **23**, 107-117.

Gill, T. S., Leitner, G., Porta, S. and Epple, A. (1993). Response of plasma cortisol to environmental cadmium in the eel, *Anguilla rostrata* Lesueur. *Comp. Biochem Physiol.* **104C**, 489-495.

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

Gimeno, S., Komen, H., Venderbosch, P. and Bowmer, T. (1997). Disruption of sexual differentiation in genetic male common carp (*Cyprinus carpio*) exposed to an alkylphenol during different life stages. *Environ. Sci. Technol.* **31**, 2884-2890.

Gimeno, S., Komen, H., Jobling, S., Sumpter. J. and Bowmer, T. (1998). Demasculinisation of mature male common carp, *Cyprinus carpio*, exposed to 4-tert-pentylphenol during spermatogenesis. *Aquat. Toxicol.* (In Press)

Girard, C., Brodeur, J. and Hontela, A. (1997). Responsiveness of the interrenal tissue of yellow perch (*Perca flavescens*) from contaminated sites to an ACTH challenge test *in vivo. Can. J. Fish. Aquat. Sci.* **55**, 438-450

Gluth, G. and Hanke, W. (1984). The comparison of physiological changes in carp, *Cyprinus carpio*, induced by several pollutants at sublethal concentrations. II. The dependency on the temperature. *Comp. Biochem. Physiol.* **79C**, 39-45.

Gluth, G. and Hanke, W. (1985). The comparison of physiological changes in carp, *Cyprinus carpio*, induced by several pollutants at sublethal concentrations. I. The dependency on exposure time. *Ecotoxicol. Environ. Saf.* **9**, 179-188.

Goetz, F. W. and Thomas, P. (Eds.) (1995). "Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, University of Texas at Austin, Austin, Texas, 2-8 July, 1995". Fish Symposium 95, Austin.

Goetz, F. W., Ranjan, M., Berndtson, A. K. and Duman, P. (1987). The mechanism and hormonal regulation of ovulation: The role of prostaglandins in teleosts ovulation. *In* "Proceedings of the Third International Symposium on Reproductive Physiology of Fish, St. Johns, Newfoundland, Canada, August 2-7, 1987", (D. R. Idler, L. W. Crim and J. M. Walsh, Eds.), pp. 235-238. Memorial University, St. Johns.

Goksøyr, A. (1985). Purification of hepatic microsomal cytochromes P-450 from β-naphthoflavone-treated Atlantic cod (*Gadus morhua*), a marine teleost fish. *Biochim. Biophys. Acta.* **840**, 409-417.

Goksøyr, A. and Förlin, L. (1992). The cytochrome P450 system in fish, aquatic toxicology, and environmental monitoring. *Aquat. Toxicol.* **22**, 287-312.

Goksøyr, A., Andersson, T., Hansson, T., Klunsøyr, J., Zhang, Y. and Forlin, L. (1987). Species characteristics of the hepatic xenobiotic and steroid biotransformation systems of two teleost fish, Atlantic cod (*Gadus morhua*) and rainbow trout (*Salmo gairdneri*). *Toxicol. Appl. Pharmacol.* **89**, 347-360.

Goksøyr, A., Beyer, J., Husoy, A. M., Larsen, H. E., Westrheim, K., Wilhelmsen, S. and Klungsoyr, J. (1994). Accumulation and effects of aromatic and chlorinated hydrocarbons in juvenile Atlantic cod (*Gadus morhua*) caged in a polluted fjord (Sorfjorden, Norway). *Aquat. Toxicol.* **29**, 21-35.

Gooch, J. W., Elskus, A. A., Kloepper-Sams, P. J., Hahn, M. E. and Stegeman, J. J. (1989). Effects of ortho- and non-ortho substituted polychlorinated biphenyl congeners on the hepatic monooxygenase system in scup (*Stenotomus chrysops*). *Toxicol. Appl. Pharmacol.* **98**, 422-433.

Goodman, L. R., Hansen, D. J., Manning, C. S. and Faas, L. F. (1982). Effects of Kepone on the sheepshead minnow in an entire life-cycle toxicity test. *Arch. Environ. Contam. Toxicol.* **11**, 335-342.

Goss, G. G. and Wood, C. M. (1988). The effects of acid and acid/aluminium exposure on circulating plasma cortisol levels and other blood parameters in the rainbow trout, *Salmo gairdneri*. *J. Fish Biol.* **32**, 63-76.

Grau, E. G. (1987). Thyroid hormones. *In* "Vertebrate Endocrinology: Fundamentals and Biomedical Implications", (P. K. Pang and M. P. Schreibman, Eds.), Vol. 2, pp. 85-102. Academic Press, San Diego.

Grau, E. G., Dickhoff, W. W., Nishioka, R., Bern, H. A. and Folmar. L. C. (1981). Lunar phasing of the thyroxine surge preparatory to seaward migration of salmonid fishes. *Science*, **211**, 607-609.

Gray, M. A. and Metcalf, C. D. (1997). Induction of testis-ova in Japanese medaka (*Oryzias latipes*) exposed to *p*-nonylphenol. *Environ. Toxicol. Chem.* **16**, 1082-1086.

Green, M. B., Hartley, G. S. and West, T. F. (1977). "Chemicals for Crop Protection and Pest Control". Pergamon, Oxford.

Green, M. B., Hartley, G. S. and West, T. F. (1987). "Chemicals for Crop Improvement and Pest Management". Pergamon, Oxford.

Gümgüm, B., Unlu, E., Tez, Z. and Gulsun, Z. (1994). Heavy metal pollution in water, sediment and fish from the Tigris River in Turkey. *Chemosphere* **29**, 111-116,

Gundersen, D. T. and Pearson, W. D. (1992). Partitioning of PCBs in the muscle and reproductive tissues of paddlefish, *Polyodon spathula*, at the falls of the Ohio River. *Bull. Environ. Contam. Toxicol.* **49**, 455-462.

Gunn, J. M. and Keller, W. (1984). Behaviour and ecology of salmonid fishes exposed to episodic pH depressions. *Environ. Biol. Fish* **4**, 241-252.

Haati, H. and Pertilla, M. (1988). Levels and trends of organochlorines in cod and herring in the Northern Baltic. *Mar. Poll. Bull.* **19**, 29-32.

Hacking, M. A., Budd, J. and Hodson, K. (1977). The ultrastructure of the liver of the rainbow trout: normal structure and modifications after chronic administration of a polychlorinated biphenyl Arochlor 1254. *Can. J. Zool.* **56**, 477-491.

Hahn, M. E., Poland, A., Glover, E. and Stegeman, J. J. (1992). The Ah receptor in marine animals: Phylogenetic distribution and relationship to P4501A inducibility. *Mar. Environ. Res.* **34**, 87-92.

Hahn, M. E., Lamb, T. M., Schultz, M. E., Smolowitz, R. M. and Stegeman, J. J. (1993). Cytochrome P4501A induction and inhibition by 3,3',4,4'-tetrachlorobiphenyl in an Ah receptor-containing fish hepatoma cell line (PLHC-1). *Aquat. Toxicol.* **26**, 185-208.

Haider, S. and Inbaraj, R. M. (1988). *In vitro* effect of malathion and endosulfan on the LH-induced oocyte maturation in the common carp, *Cyprinus carpio* (L.). *Water, Air Soil Poll.* **39**, 27-31.

Haider, S. and Upadhyaya, N. (1985). Effect of commercial formulation of four organophosphorus insecticides on the ovaries of a freshwater teleost, *Mystus vittatus* (Bloch) - A histological and histochemical study. *J. Environ. Sci. Health.* **B20**, 321-340.

Haider, S. and Upadhyaya, N. (1986). Effect of commercial formulation of four organophosphorus insecticides on the LH-induced germinal vesicle breakdown in the oocytes of a freshwater teleost, *Mystus vittatus* (Bloch) - a preliminary *in vitro* study. *Ecotoxicol. Environ. Saf.* **12**, 161-165.

Hall, A. T. and Oris, J. T. (1991). Anthracene reduces reproductive potential and is maternally transferred during long-term exposure in fathead minnows. *Aquat. Toxicol.* **19**, 249-264.

Hallett, D. J. (1988). Ecosystem management of persistent toxic chemicals - back to basics. *In* "Toxic Contaminants and Ecosystem Health: A Great Lakes Focus", (M. S. Evans, Ed.), pp. 491-509. Wiley, New York.

Hamilton, S. J. and Mehrle, P. M. (1986). Metallothionein in fish: review of its importance in assessing stress from metal contaminants. *Trans. Am. Fish. Soc.* **115**, 596-609.

Handy, R. D. (1993). The effect of acute exposure to dietary Cd and Cu on organ toxicant concentrations in rainbow trout, *Oncorhynchus mykiss. Aquat. Toxicol.* **27**, 1-14.

Hane, S., Robertson, O. H., Wexler, B. C. and Krupp, M. A. (1966). Adrenocortical responses to stress and ACTH in Pacific salmon (*Oncorhynchus tschawytscha*) and steelhead trout (*Salmo gairdnerii*) at successive stages in the sexual cycle. *Endocrinol.* **78**, 791-800.

Hansen, P. D., von Westernhagen, H., and Rosenthal, H. (1985). Chlorinated hydrocarbons and hatching success in Baltic herring spring spawners. *Mar. Environ. Res.* **15**, 59-76.

Hanson, T. (1982). Androgenic regulation of hepatic metabolism of 4-androstene-3,17-dione in rainbow trout, *Salmo gairdneri. J. Endocrinol.* **92**, 409-417.

Hanson, T. and Gustafsson, J.-Å. (1981). *In vitro* metabolism of 4-androstene-3,17-diones by hepatic microsomes from rainbow trout (*Salmo gairdneri*): Effects of hypophysectomy and estradiol 17β. *Gen. Comp. Endocrinol.* **44**, 181-188.

Harms, U. and Kerkhoff, M. A. T. (1988). Accumulation by fish. *In* "Pollution of the North Sea. An Assessment", (W. Salomons, B. L. Bayne, E. K. Duursma and U. Förstner, Eds.), pp. 567-578. Springer-Verlag, Berlin.

Harries, J. E., Sheahan, D. A., Jobling, S., Matthiessen, P., Neall, P., Routledge, E. J., Rycroft, R., Sumpter, J. P. and Tylor, T. (1996). A survey of estrogenic activity in United Kingdom inland waters. *Environ. Toxicol. Chem.* **15**, 1993-2002.

Harries. J. E., Sheahan, D. A., Jobling, S., Matthiessen, P., Neall, P., Sumpter, J. P., Tylor, T. and Zaman, N. (1997). Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. *Environ. Toxicol. Chem.* **16**, 534-542.

Hart, M. M., Swackhammer, E. S. and Straw, J. A. (1971). Studies on the site of action of *o,p'*-DDD in the dog adrenal cortex. II. TPNH- and corticosteroid precursor-stimulation of *o,p'*-DDD inhibited steroidogenesis. *Steroids* **17**, 575-586.

Hart, M. H., Reagan, R. L. and Adamson, R. H. (1973). The effect of isomers of DDD on ACTH-induced steroid output, histology and ultrastructure of the dog adrenal cortex. *Toxicol. Appl. Pharmacol.* **24**, 101-113.

Hartmann, J. and Quoss, H. (1993). Fecundity of whitefish (*Coregonus lavaretus*) during the eu- and oligotrophication of Lake Constance. *J. Fish Biol.* **43**, 81-87.

Hassall, K. A. (1982). "The Chemistry of Pesticides". MacMillan, London

Hatakeyama, S. and Yasuno, M. (1987). Chronic effects of Cd on the reproduction of the guppy (*Poecilia reticulata*) through Cd-accumulated midge larvae (*Chironomus yoshimatsui*). *Ecotoxicol. Environ. Saf.* **14**, 191-207.

Hattula, M. L., Sarkka, J., Janatuinen, J., Paasiverta, J. and Ross, A. (1978). Total mercury and methyl mercury contents in fish from Lake Paijanne. *Environ. Poll.* **17**, 19-29.

Haux, C., Larsson, Å., Lidman, U., Förlin, L., Hansson, T., Johansson-Sjöbeck, M-L. (1982). Sublethal physiological effects of chlorinated paraffins on the flounder, *Platichthys flesus* L. *Ecotoxicol. Environ. Saf.*. **6**, 49-59.

Haux, C., Björnsson, B. Th., Förlin, L., Larsson, Å. and Deftos, L. J. (1988). Influence of cadmium exposure on plasma calcium, vitellogenin and calcitonin in vitellogenic rainbow trout. *Mar. Environ. Res.* **24**, 199-202.

Hayes, T. B., Wu, T. H. and Gill, T. N. (1997). DDT-like effects as a result of corticosterone treatment in an anuran amphibian:Is DDT a corticoid mimic or stressor?. *Environ. Toxicol. Chem.* **16**, 1948-1953.

Heath, A. G. (1987). Effects of waterborne copper or zinc on the osmoregulatory response of bluegill to a hypertonic sodium chloride challenge. *Comp. Biochem. Physiol.* **88C**, 307-312.

Heisinger, J. F. and Green, W. (1975). Mercuric chloride uptake by eggs of the ricefish and resulting teratogenic effects. *Bull. Environ. Contam. Toxicol.* **14**, 665-673.

Hektoen, H., Bernhoft, A., Ingebrigtsen, K., Skaare, J. U. and Goksøyr, A. (1994). Response of hepatic xenobiotic metabolizing enzymes in rainbow trout (*Oncorhynchus mykiss*) and cod (*Gadus morhua*) to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). *Aquat. Toxicol.* **28**, 97-106.

Hellou, J. and Payne, J. F. (1993). Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in cod (*Gadus morhua*) from the Northwest Atlantic. *Mar. Environ. Res.* **36**, 117-128.

Hellou, J., Warren, W.G. and Payne, J.F. (1993). Organochlorines including polychlorinated biphenyls in muscle, liver, and ovaries of cod *Gadus morhua. Arch. Environ. Contam. Toxicol.* **25**, 497-505.

Hellou, J., Payne, J. F. and Hamilton, C. (1994). Polycyclic aromatic compounds in Northwest Atlantic cod (*Gadus morhua*). *Environ. Poll.* **84**, 197-202.

Hellou, J., Warren, W.G. and Mercer, G. (1995). Organochlorines in Pleuronectidae: Comparison between three tissues of three species inhabiting the northwest Atlantic. *Arch. Environ. Contam. Toxicol.* **29**, 302-308.

Henderson, I. W., and Kime, D. E. (1987). The adrenal cortical steroids. *In* "Vertebrate Endocrinology: Fundamentals and Biomedical Implications. Volume 2. Regulation of Water and Electrolytes", (P. K. T. Pang and M. P. Schriebman, Eds.), pp. 121-142. Academic Press, San Diego and London.

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

Hewitt, L. M., Munkittrick, K. R., Scott, I. M., Carey, J. H., Solomon, K. R. and Servos, M. R. (1996). Use of MFO-directed toxicity identification evaluation to isolate and characterize bioactive impurities from a lampricide formulation. *Environ. Toxicol. Chem.* **15**, 894-905.

Higgs, D. A., Fagerlund, U. H. M., Eales, J. G. and McBride, J. R. (1982). Application of thyroid and steroid hormones as anabolic agents in fish culture. *Comp. Biochem. Physiol.* **73B**, 143-176.

Hill, D. W., Hejtmancik, E. and Camp, B. J. (1976). Induction of hepatic microsomal enzymes by Arochlor© 1254 in *Ictalurus punctatus* (channel catfish). *Bull Environ. Contam. Toxicol.* **16**, 495-502.

Hilmy, A. M., Badawi, H. K. and Shabana, M. B. (1983a). Organochlorine pesticide residues in 12 freshwater Egyptian fish species with special emphasis on *Anguilla vulgaris* and *Mugil cephalus. Comp. Biochem. Physiol.* **76C**, 163-171.

Hilmy, A. M., Badawi, H. K. and Shabana, M. B. (1983b). Physiological mechanism of toxic action of DDT and endrin in two euryhaline freshwater fishes, *Anguilla vulgaris* and *Mugil cephalus. Comp. Biochem. Physiol.* **76C**, 173-179.

Hilton, J. W., Plisetskaya, E. M. and Leatherland, J. F. (1987). Does oral 3,5,3'-triiodo-L-thyronine affect dietary glucose utilization and plasma insulin levels in rainbow trout (*Salmo gairdneri*). *Fish Physiol. Biochem.* **4**, 113-120.

Hinton, D. E. and Laurén, D. J. (1990). Liver structural alterations accompanying chronic toxicity in fishes: Potential biomarkers of exposure. *In* " Biomarkers of Environmental Contamination", (J. F. McCarthy and L. R. Shugart, Eds.), pp. 17-57. Lewis Publishers, Boca Raton FL.

Hirose, K. (1975). Reproduction in medaka, *Oryzias latipes*, exposed to sublethal concentrations of γ-benzenehexachloride (BHC). *Bull. Tokai. Reg. Fish. Res. Lab.* **81**, 139-149.

Hodson, P. V., Maj, M. K., Efler, S., Burnison, B. K., van Heiningen, A. R. P., Girard, R. and Carey, J. H. (1997). MFO induction in fish by spent cooking liquors from kraft pulp mills. *Environ. Toxicol. Chem.* **16**, 908-916.

Hoffman, E. J., Mills, G. L., Latimer, J. S. and Quinn, J. G. (1984). Urban runoff as a source of polycyclic aromatic hydrocarbons to coastal waters. *Environ. Sci. Technol.* **18**, 580-587.

Holdway, D. A. and Dixon, D. G. (1986a). Impact of pulse exposure to methoxychlor on flagfish (*Jordanella floridae*) over one reproductive cycle. *Can. J. Fish. Aquat. Sci.* **43**, 1410-1415.

Holdway, D. A. and Dixon, D. G. (1986b). Effects of methoxychlor exposure of flagfish eggs (*Jordanella floridae*) on hatchability, juvenile methoxychlor tolerance and whole-body levels of tryptophan, serotonin and 5-hydroxyindole acetic acid. *Water Res.* **20**, 893-897.

Holm, G., Norrgren, L. and Linden, O. (1991). Reproductive and histopathological effects of long-term experimental exposure to bis(tributyltin)oxide (TBTO) on the three-spined stickleback, *Gasterosteus aculeatus* Linnaeus. *J. Fish. Biol.* **38**, 373-386.

Holm, G., Norrgren, L., Andersson, T. and Thurén, A. (1993). Effects of exposure to food contaminated with PBDE, PCN or PCB on reproduction, liver morphology and cytochrome P450 activity in the three-spined stickleback, *Gasterosteus aculeatus. Aquat. Toxicol.* **27**, 33-50.

Holm, G., Lundström, J., Andersson, T. and Norrgren, L. (1994). Influences of halogenated organic substances on ovarian development and hepatic EROD activity in the three-spined stickleback, *Gasterosteus aculeatus*, and the rainbow trout, *Oncorhynchus mykiss. Aquat. Toxicol.* **29**, 241-256.

Hontela, A. (1997). Endocrine and physiological responses of fish to xenobiotics: Role of glucocorticosteroid hormones. *Rev. Toxicol.* **1**, 159-206.

Hontela, A. (1998). Interrenal dysfunction in fish from contaminated sites:*in vivo* and *in vitro* assessment. *Environ. Toxicol. Chem.* **17**, 44-48.

Hontela, A., Roy, Y., van Coillie, R., Lederis, K. and Chevalier, G. (1989). Differential effects of low pH and aluminium on the caudal neurosecretory system of the brook trout, *Salvelinus fontinalis. J. Fish Biol.* **35**, 265-273.

Hontela, A., Rasmussen, J. B., Ko, D., Lederis, K. and Chevalier, G. (1991). Arginine vasotocin, an osmoregulatory hormone, as a potential indicator of acid stress in fish. *Can. J. Fish. Aquat. Sci.* **48**, 238-242.

Hontela, A., Rasmussen, J. B., Audet, C. and Chevalier, G. (1992). Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. *Arch. Environ. Contam. Toxicol.* **22**, 278-283.

Hontela, A., Rasmussen, J. B. and Chevalier, G. (1993a). Endocrine responses as indicators of sublethal toxic stress in fish from polluted environments. *Water Poll. Res. J. Can.* **28**, 767-780.

Hontela, A., Rasmussen, J. B., Lederis, K., Tra, H. V. and Chevalier, G. (1993b). Elevated levels of arginine vasotocin in the brain of brook trout (*Salvelinus fontinalis*) from acid lakes: a field test of a potential biomarker for acid stress. *Can. J. Fish. Aquat. Sci.* **50**, 1717-1727.

Hontela, A., Dumont, P., Duclos, D. and Fortin, R. (1995). Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence river. *Environ. Toxicol. Chem.* **14**, 725-731.

Hontela, A., Daniel, C. and Ricard, A.C. (1996). Effects of acute and subacute exposures to cadmium on the interrenal and thyroid function in rainbow trout, *Oncorhynchus mykiss*. *Aquat. Toxicol.* **35**, 171-182.

Hontela, A., Daniel, C. and Rasmussen, J. B. (1997). Structural and functional impairment of the hypothalamo-pituitary-interrenal axis in fish exposed to bleached kraft mill effluent in the St. Maurice River, Quebec. *Ecotoxicol.* **6**, 1-12.

Hopkins, C. L., Solly, S. R. B. and Ritchie, A. R. (1969). DDT in trout and its possible effect on reproductive potential. *N. Z. J. Mar. Freshw. Res.* **3**, 220-229.

Hose, J. E., Hannah, J. B., Landolt, M. L., Miller, B. S., Felton, S. P. and Iwaoka, W. T. (1981). Uptake of benzo[*a*]pyrene by gonadal tissue of flatfish (family Pleuronectidae) and its effects on subsequent egg development. *J. Toxicol. Environ. Health,* **7**, 991-1000.

Hose, J. E., Cross, J. N., Smith, S. G. and Diehl, D. (1989). Reproductive impairment in a fish inhabiting a contaminated coastal environment off southern California. *Environ. Poll.* **57**, 139-148.

Howard, P. H. (1991). "Handbook of Environmental Fate and Exposure Data for Organic Chemicals", Vol. III, pp. 309-318. Lewis, Michigan.

Howell, W. M. and Denton, T. E. (1989). Gonopodial morphogenesis in female mosquitofish, *Gambusia affinis affinis* masculinized by exposure to degradation products from plant sterols. *Environ. Biol. Fish.* **24**, 43-51.

Howell, W. M., Black, D. A. and Bortone, S. A. (1980). Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia affinis holbrooki*: Evidence for environmentally-induced masculinization. *Copeia* **4**, 676-681.

Huffman, N. N., Jones, R. W. and Katzberg, A. A. (1957). Antimitotic steroidal estrogens. *Cancer*, **10**, 707-710.

Humphrey, H. E. B. (1988). Chemical contaminants in the Great Lakes: The human health aspect. *In* "Toxic Contaminants and Ecosystem Health: A Great Lakes Focus", (M. S. Evans, Ed.), pp. 153-165. Wiley, New York.

Hunter, G. A. and Donaldson, E. M. (1983). Hormonal sex control and its application to fish culture. *In* "Fish Physiology, Volume IXB, Behaviour and Fertility Control", (W. S. Hoar, D. J. Randall and E. M. Donaldson, Eds.), pp. 223-303. Academic Press, New York.

Hunter, J. R. and Macewicz, B. J. (1985). Measurement of spawning frequency in multiple spawning fish. *In* "An Egg Production Method for Estimating Spawning Biomass of Pelagic fish: Application to the Northern Anchovy, *Engraulis mordax*" (R. Lasker, Ed.), pp. 79-94. NOAA Tech. Rep. NMFS **36**.

Huschenbeth, E. (1986). Zur kontamination von fischen der Nord- und Ostee sowie der Unterelbe mit organochlorpestiziden und polychlorierten Biphenylen. *Arch. Fisch. Wiss.* **36**, 269-286.

Hutson, D. H. and Roberts, T. R. (Eds.) (1985). "Insecticides". Progress in Pesticide Biochemistry and Toxicology Vol. 5., Wiley, Chichester.

Hwang, P. P and Tsai, Y. N. (1993). Effects of arsenic on osmoregulation in the tilapia *Oreochromis mossambicus* reared in seawater. *Mar. Biol.* **117**, 551-558.

Hylland, K., Haux, C., Hogstrand, C., Sletten, K. and Andersen, R. A. (1994). Properties of cod metallothionein, its presence in different tissues and effects of cadmium and zinc treatment. *Fish Physiol. Biochem.* **13**, 81-91.

Hyllner, S. J., Andersson, T., Haux, C. and Olsson, P. E. (1989). Cortisol induction of metallothionein in primary culture of rainbow trout hepatocytes. *J. Cell. Physiol.* **139**, 24-28.

Idler, D. R. and Truscott, B. (1963). *In vivo* metabolism of steroid hormones by sockeye salmon. (A) Impaired hormone clearance in mature and spawned Pacific salmon (*O. nerka*), (B) Precursors of 11-ketotestosterone. *Can. J. Biochem. Physiol.* **41**, 875-887.

Idler, D. R., Crim, L. W. and Walsh, J. M. (Eds.) (1987). "Proceedings of the Third International Symposium on Reproductive Physiology of Fish, St. John's, Newfoundland, Canada, August 1987". Marine Sciences Research Lab., St. Johns.

Iger, Y. (1992). Adaptive reactions in the skin of the common carp (*Cyprinus carpio*) under the impact of ecological factors and wounding. PhD Thesis, The Hebrew University of Jerusalem, Jerusalem, Israel.

Iger, Y., Balm, P. H. M. and Wendelaar Bonga, S. E. (1994). Cellular responses of the skin and changes in plasma cortisol levels of trout (*Oncorhynchus mykiss*) exposed to acidified water. *Cell. Tiss. Res.* **278**, 535-542.

Ilan, Z. and Yaron, Z. (1980). Suppression by organochlorines of the response to adrenocorticotrophin of the interrenal tissue in *Sarotherodon aureaus* (Teleostei). *J. Endocrinol.* **87**, 185-193.

Ilan, Z. and Yaron, Z. (1983). Interference of *o,p'*-DDD with interrenal function and cortisol metabolism in *Sarotherodon aureus* (Steindachner). *J. Fish Biol.* **22**, 657-669.

Ito, Y. and Murata, T. (1980). Studies on the influence of polychlorinated biphenyls (PCB) on aquatic organisms. III. Influence of PCB on drug-metabolising enzyme systems in carp. *Eisei Kagaku* **26**, 21-27.

Jacobson, J. L. and Jacobson, S. W. (1988). New methodologies for assessing the effects of prenatal toxic exposure on cognitive functioning in humans. *In* "Toxic Contaminants and Ecosystem Health: A Great Lakes Focus" (M. S. Evans, Ed.), pp. 373-509, Wiley, New York.

Jacobson, J. L. and Jacobson, S. W. (1993). A 4-year follow-up study of children born to consumers of Lake Michigan fish. *J. Great Lakes Res.* **19**, 776-783.

Jacobson, J. L. and Jacobson, S. W. (1996). Intellectual impairment in children expose to polychlorinated biphenyls *in utero*. *New England J. Med.* **335**, 783-789.

Jacobson, J. L., Jacobson, S. W. and Humphry, H. E. B. (1990). Effects of *in utero* exposure to polychlorinated biphenyls and other contaminants on cognitive functioning in young children. *J. Pediatr.* **116**, 38-45.

Jakoby, W. B. and Habig, W. H. (1980). Glutathione transferases. *In* "Enzymatic Basis of Detoxification", (W. B. Jakoby, Ed.), Vol. II, pp. 63-94. Academic Press, New York.

James, M. O. (1989). Biotransformation and disposition of PAH in aquatic invertebrates. *In* " Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment", (U. Varanasi, Ed.), pp. 69-91. CRC Press, Boca Raton.

James, M. O. and Bend, J. R. (1980). Polycyclic aromatic hydrocarbon induction of cytochrome P-450-dependent mixed-function oxidases in marine fish. *Toxicol. Appl. Pharmacol.* **54**, 117-133.

James, V. A. and Wigham, T. (1986). The effects of cadmium on prolactin cell activity and plasma cortisol levels in the rainbow trout (*Salmo gairdneri*). *Aquat. Toxicol.* **8**, 273-280.

James, M. O., Heard, C. S., and Hawkins, W. E. (1988). Effect of 3-methylcholanthrene on monooxygenase epoxide hydroxylase and glutathione S-transferase activities in small estuarine and freshwater fish. *Aquat. Toxicol.* **12**, 1-15.

Janssen, P. A. H. (1996). Reproduction of the flounder, *Platichthys flesus* (L.), in relation to environmental pollution: Steroids and vitellogenesis. *Ph. D. Thesis, University of Utrecht, The Netherlands.*

Janssen, P. A. H., Lambert, J. G. D. and Goos, H. J. Th. (1995). The annual ovarian cycle and the influence of pollution on vitellogenesis in the flounder, *Pleuronectes flesus. J. Fish Biol.* **47**, 509-523.

Janssen, P. A. H., Lambert, J. G. D., Vethaak, A. D. and Goos, H. J. Th. (1997). Environmental pollution caused elevated concentrations of oestradiol and vitellogenin in the female flounder, *Platichthys flesus* (L.). *Aquat. Toxicol.* **39**, 195-214

Janz, D. M. and Bellward, G. D. (1997). Effects of acute 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure on plasma thyroid and sex steroid hormone concentrations and estrogen receptor levels in adult great blue herons. *Environ. Toxicol. Chem.* **16**, 985-989.

Jardine, J. J., van der Kraak, G. J. and Munkittrick, K. R. (1996). Capture and confinement stress in white sucker exposed to bleached kraft pulp mill effluent. *Ecotoxicol. Environ. Saf.* **33**, 287-298.

Jimenez, B. D., Oikari, A., Adams, S. M., Hinton, D. E. and McCarthy, J. F. (1990). Hepatic enzymes as biomarkers: Interpreting the effects of environmental, physiological and toxicological variables. *In* "Biomarkers of Environmental Contamination", (J. F. McCarthy and L.R. Shugart, Eds.), pp. 123-142. Lewis Publishers, Boca Raton FL.

Jobling, S. and Sumpter, J. P. (1994). Detergent components in sewage effluent are weakly oestrogenic to fish: An *in vitro* study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquat. Toxicol.* **27**, 361-372.

Jobling, S., Reynolds, T., White, R., Parker, M. G. and Sumpter, J. P. (1995). A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. *Environ. Health Persp.* **103**, 582-587.

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

Jobling, S., Nolan, M., Tyler, C. R., Brighty, G. and Sumpter, J. P. (1998). Widespread sexual disruption in wild fish. Environ. Sci. Technol. (In Press).

Johnson, L. L., Casillas, E., Collier, T. K., McCain, B. B. and Varanasi, U. (1988). Contaminant effects on ovarian development in English sole (*Parophrys vetulus*) from Puget Sound, Washington. *Can. J. Fish. Aquat. Sci.* **45**, 2133-2146.

Johnson, L., Casillas, E., Sol, S., Collier, T., Stein, J. and Varanasi, U. (1993). Contaminant effects on reproductive success in selected benthic fish. *Mar. Environ. Res.* **35**, 165-170.

Johnson, L. L., Sol, S. Y., Lomax, D. P., Nelson, G. M., Sloan, C. A. and Casillas, E. (1996). Fecundity and egg weight in English sole *Pleuronectes vetulus* from Puget Sound, Washington. Influence of nutritional status and chemical contaminants. *Fish. Bull.* **95**, 231-249.

Jones, J. C. and Reynolds, J. D. (1997). Effects of pollution on reproductive behaviour of fishes. *Rev. Fish. Biol. Fisheries* **7**, 463-491.

Jones, R. W., Rhone, J. R. and Huffman, M. N. (1960). Cytostatic activities of steroidal estrogens against zebrafish embryos. *Proc. Soc. exp. Biol. Med.* **104**, 190-191.

Jones, K. A., Brown, S. B. and Hara, T. J. (1987). Behavioural and biochemical studies of onset and recovery from acid stress in arctic char (*Salvelinus alpinus*). *Can. J. Fish. Aquat. Sci.* **44**, 373-381.

Joshi, H. S. and Raeside, J. I. (1973). Synergistic effects of testosterone and oestrogens on accessory sex glands and sexual behaviour of the boar. *J. Reprod. Fertil.* **33**, 411-423.

Joy, K. P. and Kirubagaran, R. (1989). An immunocytochemical study on the pituitary gonadotropic and thyrotropic cells in the catfish, *Clarias batrachus* after mercury treatment. *Biol. Struct. Morphol.* **2**, 67-70.

Kammann, U., Friedrich, M. and Steinhart, H. (1996). Isolation of a metal-binding protein from ovaries of dab (*Limanda limanda* L.) distinct from metallothionein: Effect of cadmium exposure. *Ecotox. Environ. Saf.* **33**, 281-288.

Kanazawa, J. (1978). Bioconcentration ratio of diazinon by freshwater fish and snail. *Bull. Environ. Contam. Toxicol.* **20**, 613-617.

Kapur, K., Kamaldeep, K. and Toor, H. S. (1978). The effect of fenitrothion on reproduction of a teleost fish, *Cyprinus carpio* communis Linn.: A biochemical study. *Bull. Environ. Contam. Toxicol.* **20**, 438-442.

Karels, A. E., Soimasuo, R., Lappivaara, J., Leppannen, H., Santti, R. and Oikari, A. (1997). Effects of ECF bleached kraft mill effluent on reproductive steroids and liver MFO activity in perch and roach. *SETAC-Europe 7th Annual Meeting, Amsterdam, April 6-10, 1997.* Abstract P12.10, p. 257.

Katti, S. R. and Sathyanesan, A. G. (1986). Changes in the hypothalamo-neurohypophysial complex of lead treated teleostean fish *Clarias batrachus* (L.). *Z. mikrosk. anat. Forsch., Leipzig*, **100**, 347-352.

Katti, S. R. and Sathyanesan, A. G. (1987a). Lead nitrate-induced nuclear inclusions in the oocytes of the catfish *Clarias batrachus* (L). *Environ. Res.* **44**, 238-240.

Katti, S. R. and Sathyanesan, A. G. (1987b). Lead nitrate induced changes in the thyroid physiology of the catfish, *Clarias batrachus* (L.). *Ecotoxicol. Environ. Saf.* **13**, 1-6.

Kaur, K. and Toor, H. S. (1977). Toxicity of pesticides to embryonic stages of *Cyprinus carpio communis* Linn. *Ind. J. Exp. Biol.* **15**, 193-196.

Kavlock, R. J., Daston, G. P., DeRosa, C., Fenner-Crisp, P., Gray, L. E., Kaatari, S., Lucier, G., Luster, L., Mac, M. J., Macza, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheehan, D. M., Sinks, T. and 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. *Environ. Health. Persp.* **104, Suppl. 4**. 715-740.

Keenleyside, M. H. A. (1979). "Diversity and Adaptation in Fish Behaviour." Springer Verlag, Berlin.

Keizer, J., D'Agostino, G. and Vittozzi, L. (1991). The importance of biotransformation in the toxicity of xenobiotics to fish. 1. Toxicity and bioaccumulation of diazinon in guppy (*Poecilia reticulata*) and zebra fish (*Brachydanio rerio*). *Aquat. Toxicol.* **21**, 239-254.

Kelce, W. R., Lambright, C. R., Gray, L. E. and Roberts, K. P. (1994). Vinclozodin and *p,p'*-DDE alter androgenic gene expression: *In vivo* confirmation of an androgen receptor mediated mechanism. *Toxicol. Appl. Pharmacol.* **142**, 192-200.

Kelce, W. R., Stone, C. R., Laws, S. C., Gray, L. E., Kemppainen, J. A , and Wilson, E. M. (1995). Persistent DDT metabolite *p,p'*-DDE is a potent androgen receptor antagonist. *Nature* **375**, 581-585.

Kelly, A. G., and Campbell, L. A. (1994). Organochlorine contaminants in liver of cod (*Gadus morhua*) and muscle of herring (*Clupea harengus*) from Scottish waters. *Mar. Poll. Bull.* **28**, 103-108.

Kelso, J. R. M. and Frank, R. (1974). Organochlorine residues, mercury, copper and cadmium in yellow perch, white bass and smallmouth bass, Long Point Bay, Lake Erie. *Trans. Am. Fish. Soc.* **103**, 577-581.

Kenzic, N., Britvic, S., Protic, M., Simmons, J. E., Rijavec, M., Zahn, R. K. and Kurelec, B. (1983). Activity of benzo(*a*)pyrene monooxygenase in fish from the Sava River, Yugoslavia; correlation with pollution. *Sci. Total Environ.* **27**, 59-69.

Kersten, M., Dicke, M., Kriews, M., Naumann, K., Schmidt, D., Schulz, M., Schwikowski, M. and Steiger, M. (1988). Distribution and fate of heavy metals in the North Sea. *In* "Pollution of the North Sea, an Assessment", (W. Salomons, B. L. Bayne, E. K. Duursma and U. Forstner, Eds.), pp. 300-347. Springer Verlag, Berlin.

Khan, A. T. and Weis, J. S. (1987a). Toxic effects of mercuric chloride on sperm and egg viability of two populations of mummichog, *Fundulus heteroclitus. Environ. Poll.* **48**, 263-273.

Khan, A. T. and Weis, J. S. (1987b). Effects of methylmercury on sperm and egg viability of two populations of killifish (*Fundulus heteroclitus*). *Arch. Environ. Contam. Toxicol.* **16**, 499-505.

Khan, A. T. and Weis, J. S. (1987c). Effect of methyl mercury on egg and juvenile viability in two populations of killifish *Fundulus heteroclitus. Environ. Res.* **44**, 272-278.

Khan, A. T. and Weis, J. S. (1993). Differential effects of organic and inorganic mercury on the micropyle of the eggs of *Fundulus heteroclitus. Environ. Biol. Fish.* **37**, 323-327.

Khan, M. A. Q, Al-Ghais, S. M. and Al-Marri, S (1995). Petroleum-hydrocarbons in fish from the Arabian Gulf. *Arch. Environ. Contam. Toxicol.* **29**, 517-522.

Khosa, D. and Chandrasekhar, K. (1972). Effect of copper acetate and asphalt on gonadal activities and the correlated changes in the pre-optic nucleus of two genera of teleostean fishes. *Proc. Ind. Acad. Sci.* **76B**, 1225.

Kihlström, J. E. and Berglund, E. (1978). An estimation of the amounts of polychlorinated biphenyls in the biomass of the Baltic. *Ambio* 7, 175-178.

Kihlström, J. E., Lundberg, C. and Hulth, L. (1971). Number of eggs and young produced by zebrafishes (*Brachydanio rerio*, Ham. - Buch.) spawning in water containing small amounts of phenylmercuric acetate. *Environ. Res.* **4**, 355-359.

Kille, P., Kay, J., Leaver, M. and George, S. (1992). Induction of piscine metallothionein as a primary response to heavy-metal pollutants - applicability of new sensitive molecular probes. *Aquat. Toxicol.* **22**, 279-286.

Kime, D. E. (1984). The effect of cadmium on steroidogenesis by testes of the rainbow trout, *Salmo gairdneri. Toxicol. Lett.* **22**, 83-88.

Kime, D. E. (1993). "Classical" and "non-classical" steroids in teleost fish. *Rev. Fish. Biol. Fisheries.* **3**, 160-180.

Kime, D. E. (1995). The effects of pollution on reproduction in fish. *Rev. Fish Biol. Fisheries* **5**, 52-95.

Kime, D. E. and Nash, J. P. (1998). Gamete viability as an indicator of reproductive endocrine disruption in fish. *Sci. Total. Environ.* (In Press)

Kime, D. E., and Singh, P. B. (1996). *In vitro* effects of γ-hexachlorocyclohexane on *in vitro* biosynthesis and metabolism of steroids in goldfish, *Carassius auratus. Ecotox. Environ. Saf.* **34**, 165-173.

Kime, D. E., Vinson, G. P., Major, P. W., and Kilpatrick, R. (1980). Adrenal-gonad relationships. *In* "General, Comparative and Clinical Endocrinology of the Adrenal Cortex", (I. Chester Jones and I. W. Henderson, Eds.), Vol. 3, pp. 183-264. Academic Press, New York and London.

Kime, D. E., Lone, K., and Al-Marzouk, A. (1991). Seasonal changes in serum steroid hormones in a protandrous teleost, the sobaity (*Sparidentex hasta* Valenciennes). *J. Fish Biol.* **39**, 745-753.

Kime, D. E., Ebrahimi, M., Nysten, K., Roelants, I., Rurangwa, E., Moore, H. D. M., and Ollevier, F. (1996). Use of computer assisted sperm analysis (CASA) for monitoring the effects of pollution on sperm quality of fish; application to effects of heavy metals. *Aquat. Toxicol.* **36**, 223-237.

Kime, D. E., Nash, J. P. and Scott, A. P. (1998). Vitellogenesis as a biomarker of reproductive disruption by xenobiotics. *Aquaculture*, (In Press).

Kirubagaran, R. and Joy, K. P. (1988a). Inhibition of testicular 3β-hydroxy-Δ^5-steroid dehydrogenase (3β-HSD) activity in catfish *Clarias batrachus* (L.) by mercurials. *Ind. J. Exp. Biol.* **26**, 907-908.

Kirubagaran, R. and Joy, K. P. (1988b). Toxic effects of mercuric chloride, methylmercuric chloride, and emisan 6 (an organic mercurial fungicide) on ovarian recrudescence in the catfish *Clarias batrachus* (L.). *Bull. Environ. Contam. Toxicol.* **41**, 902-909.

Kirubagaran, R. and Joy, K. P. (1988c). Toxic effects of three mercurial compounds on survival, and histology of the kidney of the catfish *Clarias batrachus* (L.). *Ecotoxicol. Environ. Saf.* **15**, 171-179.

Kirubagaran, R. and Joy, K. P. (1989). Toxic effects of mercurials on thyroid function of the catfish, *Clarias batrachus* (L.). *Ecotox. Environ. Saf.* **17**, 265-271.

Kirubagaran, R. and Joy, K. P. (1991). Changes in adrenocortical-pituitary activity in the catfish, *Clarias batrachus* (L.), after mercury treatment. *Ecotoxicol. Environ. Saf.* **22**, 36-44.

Kirubagaran, R. and Joy, K. P. (1992). Toxic effects of mercury on testicular activity in the freshwater teleost, *Clarias batrachus* (L.). *J. Fish. Biol.* **41**, 305-315.

Kirubagaran, R. and Joy, K. P. (1994). Effects of short-term exposure to methylmercury chloride and its withdrawal on serum levels of thyroid hormones in the catfish *Clarias batrachus. Bull. Environ. Contam. Toxicol.* **53**, 166-170.

Kito, H., Ose, Y., Mizuhira, Z., Sato, T., Ishikawa, T. and Tazawa, T. (1982). Separation and purification of (Cd, Cu, Zn)-metallothionein in carp hepatopancreas. *Comp. Biochem. Physiol.* **73C**, 121-127.

Klaverkamp, J. F., Macdonald, W. A., Duncan, D. A. and Wageman, R. (1984). Metallothionein and acclimation to heavy metals in fish: A review. *In* "Contaminant Effects on Fisheries", (V. W. Cairns, P. V. Hodson and J. O. Nriagu, Eds.), pp. 99-113. Wiley, New York.

Kleinow, K. H., Haasch, M. L. and Lech, J. J. (1986). The effect of tricaine anesthesia upon induction of select P-450 dependent monooxygenase activities in rainbow trout (*Salmo gairdneri*). *Aquat. Toxicol.* **8**, 231-242.

Kline, E. R., Figuera, R. A., Rogers, J. H. Jr. and Dorn, P. B. (1996). Effects of nonionic surfactant (C14-15 AE7) on fish survival, growth and reproduction in the laboratory and in outdoor stream mesocosms. *Environ. Toxicol. Chem.* **15**, 997-1002.

Kling, D. (1981). Total atresia of the ovaries of *Tilapia leucosticta* (Cichlidae) after intoxication with the insecticide Lebaycid. *Experientia* **37**, 73-74.

Klontz, G. W. (1984). Epidemiology of diseases in wild fish populations. *In* "Contaminant Effects on Fisheries", (V. W. Cairns, P. V. Hodson, and J. O. Nriagu, Eds.), pp. 9-18. Wiley, New York.

Klotz, A. V., Stegeman, J. J. and Walsh, C. (1983). An aryl hydrocarbon hydroxylating hepatic cytochrome P-450 from the marine fish *Stenotomus chrysops. Arch. Biochem. Biophys.* **226**, 578-592.

Klotz, A. V., Stegeman, J. J., Woodin, B. R., Snowberger, E. A., Thomas, P. E. and Walsh, C. (1986). Cytochrome P-450 isozymes from the marine teleost *Stenotomus chrysops*: Their roles in steroid hydroxylation and the influence of cytochrome b_5. *Arch. Biochem. Biophys.* **249**, 326-338.

Knickmeyer, R. and Steinhart, H. (1990). Patterns of cyclic organochlorine contamination in livers of male Pleuronectiformes from the North Sea, Winter 1987. *Mar. Poll. Bull.* **21**, 187-189.

Koistinen, J., Paasivirta, J. and Vuorinen, P. J. (1989). Dioxins and other planar polychloroaromatic compounds in Baltic, Finnish and Arctic fish samples. *Chemosphere*, **19**, 527-530.

Koivusaari, U., Harri, M. and Hanninen, O. (1981). Seasonal variation of hepatic biotransformation in female and male rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **70C**, 149-157.

Kondal, J. K., Saxena, P. K. and Soni, G. L. (1989). Effect of vegetable oil factory effluent on the gonads of a freshwater teleost, *Heteropneustes fossilis* (Bl.) - a biochemical study. *Environ. Poll.* **57**, 117-125.

Koster, M.D., Ryckman, D.P., Weseloh, D.V.C. and Struger, J. (1996). Mercury levels in Great Lakes herring gull (*Larus argentatus*) eggs, 1972-1992. *Environ. Poll.* **93**, 261-270.

Krahn, M. M., Rhodes, L. D., Myers, M. S., Moore, L. K., MacLeod Jr., D. and Malins, D. C. (1986). Associations between metabolites of aromatic compounds in bile and the occurrence of hepatic lesions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. *Arch. Environ. Contam. Toxicol.* **15**, 61-67.

Krahn, M. M., Burrows, D. G., Yitalo, G. M., Brown, D. W., Wigren, C. A., Collier, T. K., Chan, S.-L. and Varanasi, U. (1992). Mass spectrometric analysis for aromatic compounds in bile of fish sampled after the Exxon Valdez oil spill. *Environ. Sci. Technol.* **26**, 116-126.

Krishnan, A. V., Stathis, P., Permuth, S. F., and Tokes, L. (1993). Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinol.* **132**, 2279-2286.

Kristensen, P. (1994). Sensitivity of embryos and larvae in relation to other stages in the life cycle of fish: a literature review. *In* "Sublethal and Chronic Effects of Pollutants on Freshwater Fish", (R. Muller and R. Lloyd, Eds.), pp. 155-166. Blackwell, Oxford.

Kruuk, H. and Conroy, J.W.H. (1996). Concentrations of some organochlorines in otters *(Lutra lutra* L.) in Scotland: implications for populations. *Environ. Poll.* 92, 165-171.

Kuehl, D. W., Butterworth, B. C., DeVita, W. M. and Sauer, C. P. (1987). Environmental contamination by polychlorinated dibenzo-*p*-dioxins and dibenzofurans associated with pulp and paper mill discharge. *Biomed. Environ. Mass. Spectrom.* **14**, 443-447.

Kulshrestha, S. K. and Arora, N. (1984). Impairments induced by sublethal doses of two pesticides in the ovaries of a freshwater teleost *Channa striatus* Bloch. *Toxicol. Lett.* **20**, 93-98.

Kumar, V. and Mukherjee, D. (1988). Phenol and sulfide induced changes in the ovary and liver of sexually maturing common carp, *Cyprinus carpio. Aquat. Toxicol.* **13**, 53-60.

Kumar, S. and Pant, S. C. (1984). Comparative effects of the sublethal poisoning of zinc, copper and lead on the gonads of the teleost *Puntius conchonius* Ham. *Toxicol. Lett.* **23**, 189-194.

Kumar, S. and Pant, S. C. (1988). Comparative sublethal ovarian pathology of some pesticides in the teleost, *Puntius conchonius* Hamilton. *Bull. Environ. Contam. Toxicol.* **41**, 227-232.

Kumari, M. and Dutt, N. H. G. (1991). Cadmium induced histomorphological changes in the testis and pituitary gonadotrophic hormone secreting cells of the cyprinid *Puntius sarana. Boll. Zool.* **58**, 71-76.

Kurelec, B., Protic, M., Britvic, S., Kenic, N., Rijavec, M. and Zahn, R. K. (1981). Toxic effects in fish and the mutagenic capacity of water from the Sava river in Yugoslavia. *Bull. Environ. Contam. Toxicol.* **26**, 179-187.

Laignelet, L., Narbonne, J.-F., Lhuguenot, J. C. and Riviére, J.-L. (1989). Induction and inhibition of rat liver cytochrome(s) P-450 by an imidazole fungicide (prochloraz). *Toxicol.* **59**, 271-284.

Lal, B. and Singh, T. P. (1987). The effect of malathion and γ-BHC on the lipid metabolism in relation to reproduction in the tropical teleost, *Clarias batrachus. Environ. Poll.* **48**, 37-47.

Lam, T. J. (1983). Environmental influences on gonadal activity in fish. *In* "Fish Reproduction Vol. IXB. Reproduction: Behaviour and Fertility Control", (W. S. Hoar, D. J. Randall and E. M. Donaldson, Eds.), pp. 65-116. Academic Press, New York.

Landner, L., Neilsson, A. H., Sörensen, L., Tärnholm, A. and Viktor, T. (1985). Short-term test for predicting the potential of xenobiotics to impair reproductive success in fish. *Ecotoxicol. Environ. Saf.* **9**, 282-293.

Larson, B. A. and Bern, H. A. (1987). The urophysis and osmoregulation. In "Vertebrate Endocrinology: Fundamentals and Biomedical Implications. Volume 2. Regulation of Water and Electrolytes" (P. K. T. Pang and M. P. Schreibman, Eds.), pp. 143-156, Academic Press, San Diego.

Larsson, P. (1984). Uptake of sediment released PCBs by the eel, *Anguilla anguilla*, in static model systems. *Ecol. Bull.* **36**, 62-67.

Law, F. C. P. and Addison, R. F. (1981). Response of trout hepatic mixed function oxidases to experimental feeding of ten known or possible chlorinated environmental contaminants. *Bull. Environ. Contam. Toxicol.* **27**, 605-609.

Leah, R. T., Evans, S. J. and Johnson, M. S. (1992). Mercury in flounder (*Platichthys flesus* L.) from estuaries and coastal waters of the northeast Irish Sea. *Environ. Poll.* **75**, 317-322.

Leatherland, J. F. (1992). Endocrine and reproductive function in Great Lakes salmon. *In* "Chemically-induced Alterations in Sexual and Functional Development: The Wildlife-Human Connection", (T. Colborn and C. Clement, Eds.), pp. 129-145. Princeton Scientific Publishing Co., Princeton

Leatherland J. F. (1993). Field observations on reproductive and developmental dysfunction in introduced and native salmonids from the Great Lakes. *J. Great Lakes Res.* **19**, 737-751.

Leatherland, J. F. and Sonstegard, R. A. (1979). Effects of dietary Mirex and PCB (Arochlor 1254) on thyroid activity and lipid reserves in rainbow trout, *Salmo gairdneri*. *J. Fish Dis.* **2**, 43-48.

Leatherland, J. F. and Sonstegard, R. A. (1980a). Seasonal changes in thyroid hyperplasia, serum thyroid hormone and lipid concentrations, and pituitary gland structure in Lake Ontario coho salmon (*Oncorhynchus kisutch*) and a comparison with coho salmon from Lakes Michigan and Erie. *J. Fish Biol.* **16**, 539-562.

Leatherland, J. F. and Sonstegard, R. A. (1980b). Effect of dietary polychlorinated biphenyls (PCB's) or Mirex in combination with food deprivation and testosterone administration on serum thyroid hormone concentration and bioaccumulation of organochlorines in rainbow trout (*Salmo gairdneri*). *J. Fish. Dis.* **3**, 115-124.

Leatherland, J. F. and Sonstegard, R. A. (1980c). Structure of thyroid and adrenal glands in rats fed diets of Great Lakes coho salmon. *Environ. Res.* **23**, 77-86.

Leatherland, J. F. and Sonstegard, R. A. (1981). Thyroid function, pituitary structure and serum lipids in Great Lakes coho salmon, *Oncorhynchus kisutch* Walbaum, 'jacks' compared with sexually immature spring salmon. *J. Fish Biol.* **18**, 643-654.

Leatherland, J. F. and Sonstegard, R. A. (1982). Bioaccumulation of organochlorines by yearling coho salmon (*Oncorhynchus kisutch* Walbaum) fed diets containing Great Lakes coho salmon, and the pathophysiological responses of the recipients. *Comp. Biochem. Physiol.* **72C**, 91-99.

Leatherland, J. F. and Sonstegard, R. A. (1987). Comparative fecundity and egg survival in two stocks of goitred coho salmon (*Oncorhynchus kisutch* Walbaum) from Lake Erie. *Can. J. Zool.* **65**, 2780-2785.

Leatherland, J. F. , Sonstegard, R. A. and Holdrinet, M. V. (1979). Effect of dietary Mirex and PCB's on hepatosomatic index, liver lipid, and carcass lipid and PCB and Mirex

bioaccumulation in yearling coho salmon, *Oncorhynchus kisutch. Comp. Biochem. Physiol.* **63C**, 243-246.

Leatherland, J. F., Copeland, P., Sumpter, J. P. and Sonstegard, R. A. (1982). Hormonal control of gonad maturation and development of secondary sexual characteristics in coho salmon, *Oncorhynchus kisutch*, from Lakes Ontario, Erie and Michigan. *Gen. Comp. Endocrinol.* **48**, 196-204.

Lech, J. J., Lewis, S. K. and Ren, L. (1996). *In vivo* estrogenic activity of nonylphenol in rainbow trout. *Fund. Appl. Toxicol.* **30**, 292-232.

Lee, R. M. and Gerking, S. D. (1980). Survival and reproductive performance of the desert pupfish, *Cyprinodon n. nevadensis* (Eigenmann and Eigenmann) in acid waters. *J. Fish Biol.* **17**, 507-515.

Leloup-Hatey, J. and Hardy, A. (1985). Effect of hydrocarbon pollution on interrenal function in eel (*Anguilla anguilla* L.). *Ichtyophysiol. Acta* **9**, 39-50.

LeMaire-Gony, S. and LeMaire, P. (1992). Interactive effects of cadmium and benzo[*a*]pyrene on cellular structure and biotransformation enzymes of the liver of the European eel *Anguilla anguilla. Aquat. Toxicol.* **22**, 145-160.

Lesniak, J. A. and Ruby, S. M. (1982). Histological and quantitative effects of sublethal cyanide exposure on oocyte development in the rainbow trout. *Arch. Environ. Contam. Toxicol.* **11**, 343-352.

Levi, P. E., Hollingworth, R. M. and Hodgson, E. (1988). Differences in oxidative dearylation and desulfuration of fenitrothion by cytochrome P-450 isoenzymes and in the inhibition of monooxygenase activity. *Pestic. Biochem. Physiol.* **32**, 224-231.

Lewis, D. F., Rodrigues, A. D., Ionnides, C., and Parke, D. V. (1989). Adverse reactions of imidazole antifungal agents: Computer graphic studies of cytochrome P-450 interactions. *J. Biochem. Toxicol.* **4**, 231-234.

Lindström-Seppä, P. (1985). Seasonal variation of the xenobiotic metabolising enzyme activities in the liver of male and female vendace (*Coregonus albula* L.). *Aquat. Toxicol.* **6**, 323-331.

Lindström-Seppä, P. (1988). Biomonitoring of oil spill in a boreal archipelago by xenobiotic biotransformation in perch (*Perca fluviatilis*). *Ecotox. Environ. Saf.* **15**, 162-170.

Lindström-Seppä, P. and Oikari, A. (1989a). Bleached kraft pulpmill effluents and TCDD: Effects on biotransformation and testosterone status of mature male rainbow trout (*Salmo gairdneri*). *Mar. Environ. Res.* **28**, 99-103.

Lindström-Seppä, P. and Oikari, A. (1989b). Biotransformation and other physiological responses in white-fish caged in a lake receiving pulp and paper mill effluents. *Ecotoxicol. Environ. Saf.* **18**, 191-203.

Lindström-Seppä, P. and Oikari, A. (1990a). Biotransformation, other toxicological and physiological responses in rainbow trout (*Salmo gairdneri* Richardson) caged in a lake receiving effluents of pulp and paper industry. *Aquat. Toxicol.* **16**, 187-204.

Lindström-Seppä, P. and Oikari, A. (1990b). Biotransformation activities of feral fish in waters receiving bleached pulp mill effluents. *Environ. Toxicol. Chem.* **9**, 1415-1424.

Lindström-Seppä, P., Korytko, P.J., Hahn, M. E., Stegeman, J. J. (1994). Uptake of waterborne 3,3',4,4'-tetrachlorobiphenyl and organ and cell-specific induction of cytochrome P4501A in adult and larval fathead minnow *Pimephales promelas. Aquat. Toxicol.* **28**, 147-167.

Lockhart, W. L., Uthe, J. F., Kenny, A. R. and Mehrle. P. M. (1972). Methyl mercury in northern pike (*Esox lucius*): Distribution, elimination, and some biochemical characteristics of contaminated fish. *J. Fish. Res. Bd. Can.* **29**, 1519-1523.

Lonky, E., Reihman, J., Darvill, T., Mather, J. Sr. and Daly, H. (1996). Neonatal behavioral assessment scale performance in humans influenced by maternal consumption of environmentally contaminated Lake Ontario fish. *J. Great Lakes Res.* **22**, 198-212.

Lorz, H. W. and McPherson, B. P. (1976). Effects of copper or zinc in freshwater on the adaptation to seawater and ATPase activity, and the effects of copper on migratory disposition of coho salmon (*Oncorhynchus kisutch*). *J. Fish. Res. Bd. Can.* **33**, 2023-2030.

Luckas, B. and Harms, U. (1987). Characteristic levels of chlorinated hydrocarbons and trace metals in fish from coastal waters of North and Baltic Sea. *Int. J. Environ. Anal. Chem.* **29**, 215-225.

Luksienė, D. (1978). Effect of thermic regimen of the basin-condenser of the Lithuanian State Regional Electric Power Plant on fish reproduction: 1. Characteristics of the sexual cycle of the bream. *Lithuanian SSR Acad. Sci. Ser. C.*. **4(84)**, 81-91 (In Russian). (*Biol. Abstr.* **68**, 59654 (1979)).

Luksienė, D. (1981). Effect of thermic regimen of the basin-condenser of the Lithuanian State Regional Electric Power Plant on fish reproduction: 2. Features of oogenesis in the roach *Rutilus rutilus. Lithuanian SSR Acad. Sci. Ser. B.*, **1(73)**, 61-73 (In Russian). (*Biol. Abstr.* **74**, 42665 (1982)).

Luksienė, D. (1982). Effect of thermic regimen of the basin-condenser of the Lithuanian State Regional Electric Power Plant on fish reproduction: 3. Characteristics of oogenesis of the perch *Perca fluviatilis* and the pike *Esox lucius Lithuanian SSR Acad. Sci. Ser. B.*, **2(78)**, 32-46, (In Russian). (*Biol. Abstr.* **76**, 40368 (1983)).

Luksiene, D. and Kontautas, A. (1988). Morphophysiological and biochemical characteristics of mature female bream in the Lithuanian central steam power station cooling water reservoir. *Acta Hydrobiol. Lituanica*, **8**, 131-138.

Luksiene, D. and Sandström, O. (1994). Reproductive disturbance in a roach (*Rutilus rutilus*) population affected by cooling water discharge. *J. Fish Biol.* **45**, 613-625.

Luxon, L., Hodson, P. V. and Borgmann, U. (1987). Hepatic aryl hydrocarbon hydroxylase activity of lake trout (*Salvelinus namaycush*) as an indicator of organic pollution. *Environ. Toxicol. Chem.* **6**, 649-657.

Lye, C. M., Frid, C. L. J., Gill, M. E. and McCormick, D. (1997). Abnormalities in the reproductive health of flounder *Platichthys flesus* exposed to effluent from a sewage treatment works. *Mar. Poll. Bull.* **34**, 34-41.

Mac, M. J. and Schwartz, T. R. (1992). Investigations into the effects of PCB congeners on reproduction in lake trout from the Great Lakes. *Chemosphere* **25**, 189-192.

Macek, K. J. (1968). Reproduction in brook trout (*Salvelinus fontinalis*) fed sublethal concentrations of DDT. *J. Fish. Res. Bd. Can.* **25**, 1787-1796.

MacFarlane, R. B. and Benville, P. E. Jr. (1986). Primary and secondary stress responses of striped bass (*Morone saxatilis*) exposed to benzene. *Mar. Biol.* **92**, 245-254.

MacLatchy, D. L. and Van der Kraak, G. (1995). The phytoestrogen β-sitosterol alters the reproductive endocrine status of goldfish. *Toxicol. Appl. Pharmacol.* **134**, 305-312.

MacLatchy, D., Peters, L., Nickle, J. and Van der Kraak, G. (1997). Exposure to β-sitosterol alters the endocrine status of goldfish differently than 17β-estradiol. *Environ. Toxicol. Chem.* **16**, 1895-1904.

MAFF (1991). Monitoring and survellance of non-radioactive contaminants in the aquatic environment. *Aquatic Environment Monitoring Report* **24**, MAFF, Lowestoft.

Malins, D. C., McCain, B. B., Brown, D. W., Chan, S.-L., Meyers, M. S., Landahl, J. T., Prohaska, P. G., Friedman, A. J., Rhodes, L. D., Burrows, D. G., Gronlund, W. D. and Hodgins, H. O. (1984). Chemical pollutants in sediments and diseases of bottom-dwelling fish in Puget Sound, Washington. *Environ. Sci. Technol.* **18**, 705-713.

Malins, D. C., Krahn, M. M., Myers, M. S., Rhodes, L. D., Brown, D. W., Krone, C. A., McCain, C. C. and Chan S.-L. (1985). Toxic chemicals in sediments and biota from a creosote-polluted harbour: Relationships with hepatic neoplasms and other hepatic lesions in English sole (*Parophrys vetulus*). *Carcinogenesis* **6**, 1463-1469.

Mani, K. and Saxena, P. K. (1985). Effect of safe concentrations of some pesticides on ovarian recrudescence in the freshwater murrel, *Channa punctatus* (Bl.): A quantitative study. *Ecotoxicol. Environ. Saf.* **9**, 241-249.

Mann, H. and Schmid, O. J. (1961). Der Einfluss von Detergentien auf Sperm, Befruchtung und Entwicklung bei der Forelle. *Int. Revue. ges. Hydrobiol.* **46**, 419-426.

Mannervik, B. (1985). The isozymes of glutathione transferase. *In* "Advances in Enzymology and Related Areas of Molecular Biology", (A. Meister, Ed.), pp. 357-417., Wiley, New York.

Manning , M. J. and Nakanishi, T. (1996). The specific immune system: cellular defenses. *In* "The Fish Immune System. Organism, Pathogen and Environment", (G. Iwama and T. Nakanishi, Eds.), pp. 159-205. Academic Press, San Diego.

Martel, P. H., Kovacs, T. G., O'Connor, B. I. and Voss, R. H. (1997). Source and identity of compounds in a thermomechanical pulp mill effluent inducing hepatic mixed-function oxygenase activity in fish. *Environ. Toxicol. Chem.* **16**, 2375-2383.

Martin, R. C., Dixon, D. G., Maguire, R. J., Hodson, P. V. and Tkacz, R. J. (1989). Acute toxicity, uptake, depuration and tissue distribution of tri-*n*-butyltin in rainbow trout, *Salmo gairdneri*. *Aquat. Toxicol.* **15**, 37-52.

Matsunaga, K. (1975). Concentration of mercury by three species of fish from Japanese rivers. *Nature* **257**, 49-50.

Matta, M. B., Cairncross, C. and Kocan, R. M. (1998). Possible effects of polychlorinated biphenyls on sex determination in rainbow trout. *Environ. Toxicol. Chem.* **17**, 26-29.

Matthiessen, P. and Brafield, A. E. (1977). Uptake and loss of dissolved zinc by the stickleback *Gasterosteus aculeatus* L. *J. Fish Biol.* **10**, 399-410.

Matthiessen, P. and Logan, J. W. M. (1984). Low concentration effects of endosulfan insecticide on reproductive behaviour in the tropical cichlid fish *Sarotherodon mossambicus*. *Bull. Environ. Contam. Toxicol.* **33**, 575-583.

Matty, A. J. (1985). "Fish Endocrinology". Croom Helm, London.

Mayer-Gostan, N., Wendelaar Bonga, S. E. and Balm, P. H. M. (1987). Mechanisms of hormone actions on gill transport. *In* "Vertebrate Endocrinology: Fundamentals and Biomedical Implications. Volume 2. Regulation of Water and Electrolytes", (P. K. T. Pang and M. P. Schreibman, Eds.), pp. 211-238. Academic Press, San Diego.

McCarter, J. A., Matheson, A. T., Roch, M., Olafson, R. W. and Buckley, J. T. (1982). Chronic exposure of coho salmon to sublethal concentrations of copper. II. Distribution of copper between high- and low-molecular-weight proteins in liver cytosol and the possible role of metallothionein in detoxification. *Comp. Biochem. Physiol.* 72C, 21-26.

McCormick, J. H., Stokes, G. N. and Hermanutz, R. O. (1989). Oocyte atresia and reproductive success in fathead minnows (*Pimephales promelas*) exposed to acidified hardwater environments. *Arch. Environ. Contam. Toxicol.* **18**, 207-214.

McDonald, T. O. and Fingerman, S. W. (1979). Effects of DDT and parathion on goldfish retina/pigment/epithelium/choroid concentrations of serotonin, dopamine and norepinephrine. *Toxicol. Appl. Pharmcol.* **48**, A199.

McElroy, A. E., Farrington, J. W., and Teal, J. M. (1989). Bioavailability of PAH in the aquatic environment. *In* " Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment", (U. Varanasi, Ed.), pp. 1-40, CRC Press, Boca Raton.

McEwan, F. L. and Stephenson, G. R. (1979). "The Use and Significance of Pesticides in the Environment". Wiley, New York.

McIntyre, J. D. (1973). Toxicity of methyl mercury for steelhead trout sperm. *Bull. Environ. Contam. Toxicol.* **9**, 98-99.

McKim, J. M. and Benoit, D. A. (1971). Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Bd. Can.* **28**, 655-662.

McKinney, J. D., Chae, K., Oatley, S. J. and Blake, C. C. F. (1985a). Molecular interactions of toxic chlorinated dibenzo-p-dioxins and dibenzofurans with thyroxine binding prealbumin. *J. Med. Chem.* **28**, 375-381.

McKinney, J. D., Fawkes, J., Jordan, S., Chae, K., Oatley, S., Coleman, R. E. and Briner, W. (1985b). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) as a potent and persistent thyroxine agonist: A mechanistic model for toxicity based on molecular reactivity. *Environ. Health Perspect.* **61**, 41-53.

McKinney, J. D., Chae, K., McConnell, E. E. and Birnbaum, L. S. (1985c). Structure-induction versus structure-toxicity relationships for polychlorinated biphenyls and related aromatic hydrocarbons. *Environ. Health Perspect.* **60**, 41-53.

McKinney, J. D., Fannin, R., Jordan, S., Chae, K., Rickenbacher, U. and Pedersen, L. (1987). Polychlorinated biphenyls and related compound interactions with specific binding sites for thyroxine in rat liver nuclear extracts. *J. Med. Chem.* **30**, 79-86.

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

McMaster, M. E., Van der Kraak, G. J., Portt, C. B., Munkittrick, K. R., Sibley, P. K., Smith, I. R. and Dixon, D. G. (1991). Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid levels and age at maturity of a white sucker (*Catostomus commersoni*) population exposed to bleached kraft pulp mill effluent. *Aquat. Toxicol.* **21**, 199-218.

McMaster, M. E., Portt, C. B., Munkittrick, K. R. and Dixon, D. G. (1992). Milt characteristics, reproductive performance, and larval survival and development of white sucker exposed to bleached kraft mill effluent. *Ecotoxicol. Environ. Saf.* **23**, 103-117.

McMaster, M. E., Munkittrick, K. R., Luxon, P. L. and van der Kraak, G. J. (1994). Impact of low-level sampling stress on interpretation of physiological responses of white sucker exposed to effluent from a bleached kraft pulp mill. *Ecotox. Environ. Saf.* **27**, 251-264.

Meinelt, T. and Staaks, G. (1994). The embryo-larval test with zebrafish (*Brachydanio rerio*): validity, limits and perspectives. *In* "Sublethal and Chronic Effects of Pollutants on Freshwater Fish", (R. Muller and R. Lloyd, Eds.), pp. 167-174. Blackwell, Oxford.

Melancon, M. J. and Lech, J. J. (1983). Dose-effect relationship for induction of hepatic monooxygenase activity in rainbow trout and carp by Arochlor 1254. *Aquat. Toxicol.* **4**, 51-61.

Melancon, M. J., Elcombe, C. R., Vodicnik, M. J. and Lech, J. J. (1981). Induction of cytochromes P-450 and mixed function oxidase activity by polychlorinated biphenyls and β-naphthoflavone in carp (*Cyprinus carpio*). *Comp. Biochem. Physiol.* **69C**, 219-226.

Melancon, M. J., Binder, R. L. and Lech, J. J. (1988). Environmental induction of monooxygenase activity in fish. *In* "Toxic Contaminants amd Ecosystem Health: A Great Lakes Focus", (M. S. Evans, Ed.), pp. 215-255. Wiley, New York.

Mellanen, P., Petänen, T., Lehtimäki, J., Mäkelä, S., Bylund, G., Holmbom, B., Mannila, E., Oikari, A. and Santti, R. (1996). Wood-derived estrogens: Studies *in vitro* with breast cancer cell lines and *in vivo* in trout. *Toxicol. Appl. Pharmacol.* **136**, 381-388.

Merlini, M. and Pozzi, G. (1977). Lead and freshwater fishes: Part 1, Lead accumulation and water pH. *Environ. Poll.* **12**, 167-172.

Metayer, C., Amiard, J. C. and Amiard-Triquet, C. (1982). Accumulation de quelques elements traces metalliques (Cd, Pd, Cu et Zn) chez la sole (*Solea solea*) et le flet

(*Platichthys flesus*): Variations en fonction de l'age et organotropisme. *Rev. Internationale d'Oceanographie Medicale* **66-67**, 33-45.

Meteyer, M. J., Wright, D. -A. and Martin F. D. (1988). Effect of cadmium on early developmental stages of the sheepshead minnow (*Cyprinodon variegatus*). *Environ. Toxicol. Chem.* **7**, 321-328.

Miettinen, V., Verta, M., Erkomaa, K. and Jarvinen, O. (1985). Chlorinated hydrocarbons and heavy metals in fish in the Finnish coastal areas of the Gulf of Finland. *Finn. Fish. Res.* **6**, 77-80.

Miller, M. A. (1993). Maternal transfer of organochlorine compounds in salmonines to their eggs. *Can. J. Fish. Aquat. Sci.* **50**, 1405-1413.

Miller, M. A., Kassulke, N. M. and Walkowski, M. D. (1993). Organochlorine concentrations in Laurentian Great Lakes salmonines: Implications for fisheries management. *Arch. Environ. Contam. Toxicol.* **25**, 212-219.

Mills, K. H. (1984). Fish population responses to experimental acidification of a small Ontario lake. *In* "Early Biotic Responses to Advancing Lake Acidification", (G. Hendry, Ed.), pp. 117-132 . Butterworth, Stoneham MA

Minganti, V., Fiorentino, F., Depellegrini, R., Capelli, R. (1994.). Bioaccumulation of mercury in the Antarctic bony fish *Pagothenia bernacchii. Int. J. Environ. Anal. Chem.* **55**, 197-202.

Misra, M. and Pandey, K. (1985). Effect of thiourea on the testicular steroidogenesis and secondary sexual characters of a tropical freshwater fish, *Mystus (M) vittatus* (Bloch). *Ann. d'Endocrinol. (Paris)* **46**, 421-425.

Mohr, L. C. and Chalanchuk, S. M. (1985). The effect of pH on sperm motility of white suckers, *Catostomus commersoni*, in the Experimental Lakes Area. *Environ. Biol. Fish.* **14**, 309-314.

Monod, G. (1985). Egg mortality of Lake Geneva Charr (*Salvelinus alpinus* L.) contaminated by PCB and DDT derivatives. *Bull Environ. Contam. Toxicol.* **35**, 531-536.

Monod, G., de Mones, A., and Fostier, A. (1993). Inhibition of ovarian microsomal aromatase and follicular oestradiol secretion by imidazole fungicides in rainbow trout. *Mar. Environ. Res.* **35**, 147-152.

Monosson, E., Fleming, W. J. and Sullivan, C. V. (1994). Effects of planar PCB 3,3',4,4'-tetrachlorobiphenyl (TCB) on ovarian development, plasma levels of sex steroid hormones and vitellogenin, and progeny survival in the white perch (*Morone americana*). *Aquat. Toxicol.* **29**, 1-19.

Moore, A. (1994). An electrophysiological study on the effects of pH on olfaction in mature male Atlantic salmon (*Salmo salar*) parr. *J. Fish Biol.* **45**, 493-502.

Moore, A. and Waring, C. P. (1996a). Sub-lethal effects of the pesticide diazinon on olfactory function in mature male Atlantic salmon (*Salmo salar* L.) parr. *J. Fish. Biol.* **48**, 758-775.

Moore, A. and Waring, C. P. (1996b). The effects of water quality on olfaction in the Atlantic salmon (*Salmo salar* L.). *In* "Fish Pheromones; Origins and Modes of Action", (A. V. M Canario and D. M. Power, Eds.), pp. 24-32 University of the Algarve, Faro, Portugal.

Morcillo, Y. and Porte, C. (1997). Interaction of tributyl- and triphenyltin with the microsomal monooxygenase system of molluscs and fish from the Western Mediterranean. *Aquat. Toxicol.* **38**, 35-46.

Morganstern, R., Lundquist, G., Andersson, G., Balk, L. and DePierre, J. W. (1984). The distribution of microsomal glutathione transferase among different organelles, different organs, and different organisms. *Biochem. Pharmacol.* **33**, 3609-3614.

Morrison, P. F., Leatherland, J. F. and Sonstegard, R. A. (1985a). Proximate composition and organochlorine and heavy metal contamination of eggs from Lake Ontario, Lake Erie and Lake Michigan coho salmon (*Oncorhynchus kisutch* Walbaum) in relation to egg survival. *Aquat. Toxicol.* **6**, 73-86.

Morrison, P. F., Leatherland, J. F. and Sonstegard, R. A. (1985b). Plasma cortisol and sex steroid levels in Great Lakes coho salmon (*Oncorhynchus kisutch* Walbaum) in relation to fecundity and egg survival. *Comp. Biochem. Physiol.* **80A**, 61-68.

Mount, D. R., Hocket, J. R. and Gern, W. A. (1988a). Effect of long-term exposure to acid, aluminium, and low calcium on adult brook trout (*Salvelinus fontinalis*). 2. Vitellogenesis and osmoregulation. *Can. J. Fish Aquat. Sci.* **45**, 1633-1642.

Mount, D. R., Ingersoll, C. G., Gulley, D. D., Fernandez, J. D., LaPoint, T. W. and Bergman, H. L. (1988b). Effect of long-term exposure to acid, aluminium, and low calcium on adult brook trout (*Salvelinus fontinalis*). 1. Survival, growth, fecundity, and progeny survival. *Can. J. Fish Aquat. Sci.* **45**, 1623-1632.

Muir, D.C.G., Ford, C.A., Rosenberg, B., Norstrom, R.J., Simon, M. and Béland, P. (1996a). Persistent organochlorines in beluga whales (*Delphinapterus leucas*) from the St Lawrence River estuary - I. concentrations and patterns of specific PCBs, chlorinated pesticides and polychlorinated dibenzo-p-dioxins and dibenzofurans. *Environ. Poll.* **93**, 219-234.

Muir, D.C.G., Koczanski, K., Rosenberg, B. and Béland, P. (1996b). Persistent organochlorines in beluga whales (*Delphinapterus leucas*) from the St Lawrence River estuary - II. Temporal trends, 1982-1994. *Environ. Poll.* **93**, 235-245.

Mukherjee, D., Guha, D., Kumar, V. and Chakrabarty, S. (1991). Impairment of steroidogenesis and reproduction in sexually mature *Cyprinus carpio* by phenol and sulfide under laboratory conditions. *Aquat. Toxicol.* **21**, 29-40.

Munkittrick, K. R. and Dixon, D. G. (1988a). Evidence for a maternal yolk factor associated with increased resistance and tolerance of white sucker (*Catastomus commersoni*) to waterborne copper. *Ecotox. Environ. Saf.* **15**, 7-20.

Munkittrick, K. R. and Dixon, D. G. (1988b). Growth, fecundity, and energy stores of white sucker (*Catastomus commersoni*) from lakes containing elevated levels of copper and zinc. *Can. J. Fish. Aquat. Sci.* **45**, 1355-1365.

Munkittrick, K. R. and Dixon, D. G. (1989). Effects of natural exposure to copper and zinc on egg size and larval copper tolerance in white sucker (*Catastomus commersoni*). *Ecotox. Environ. Saf.* **18**, 15-26.

Munkittrick, K. R. and Leatherland, J. F. (1984). Abnormal pituitary-gonad function in two feral populations of goldfish (*Carassius auratus* L.) suffering epizootics of an ulcerative disease. *J. Fish Dis.* 7, 433-447

Munkittrick, K. R., Portt, C. B., van der Kraak, G. J., Smith, I. R. and Rokosh, D. A. (1991). Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steroid levels of a Lake Superior white sucker (*Catastomus commersoni*) population. *Can. J. Fish. Aquat. Sci.* **48**, 1371-1380.

Munkittrick, K. R., McMaster, M. E., Portt, C. B., Smith, I. R. and Dixon, D. G. (1992). Changes in maturity, plasma sex steroid levels, hepatic mixed-function oxygenase activity, and the presence of external lesions in Lake Whitefish (*Coregonus clupeaformis*) exposed to bleached kraft mill effluent. *Can. J. Fish. Aquat. Sci.* **49**, 1560-1569.

Munkittrick, K. R., van der Kraak, G. J., McMaster, M. E., Portt, C. B., van den Heuvel, M. R. and Servos, M. R. (1994). Survey of receiving-water environmental impacts associated with discharges from pulp mills. 2. Gonad size, liver size, hepatic EROD activity and plasma sex steroid levels in white sucker. *Environ. Toxicol. Chem.* **13**, 1089-1101.

Murchelano, R. A. and Woelke, R. E. (1985). Epizootic carcinoma in the winter flounder *Pseudopleuronectes americanus. Science* **228**, 587-589.

Murugesan, A. G. and Haniffa, M. A. (1992). Histopathological and histochemical changes in the oocytes of the air-breathing fish *Heteropneustes fossilis* (Bloch) exposed to textile-mill effluent. *Bull. Environ. Contam. Toxicol.* **48**, 929-936.

Myers, M. S.,Landahl, J. T., Krahn, M. M. and McCain, B. B. (1991). Relationship between hepatic neoplasms and related lesions and exposure to toxic chemicals in marine fish from U. S. West Coast. *Environ. Health. Persp.* **90**, 7-15.

Nagahama, Y. (1983). The functional morphology of teleost gonads. *In* "Fish Reproduction, Vol. IXA Reproduction: Endocrine tissues and hormones", (W. S. Hoar, D. J. Randall and E. M. Donaldson, Eds.), pp. 223-276. Academic Press, New York.

Nagler, J. J., Aysola, P. and Ruby, S. M. (1986). Effect of sublethal pentachlorophenol on early oogenesis in maturing female rainbow trout (*Salmo gairdneri*). *Arch. Environ. Contam. Toxicol.* **15**, 549-555.

Nash, J. P., Winn-Brown, P. and Kime, D. E. (1997). Embryonic mortality caused by parental exposure to low levels of ethynylestradiol in the zebra danio (*Danio rerio*). *Abstr. SETAC-Europe 7th Annual Meeting, Amsterdam, 6-10 April 1997,* p. 256.

Neff, J. M. (1979). "Polycyclic aromatic hydrocarbons in the aquatic environment." Applied Science Publishers Ltd., London.

Nelson, J. A. (1974). Effects of dichlorodiphenyltrichloroethane (DDT) analogs and polychlorinated biphenyl (OCB) mixtures on 17β[^{3}H]estradiol binding to rat uterine receptor. *Biochem. Pharmacol.* **23**, 447-451.

Nelson, J. A., Struck, R. F. and James, R. (1978). Estrogenic activities of chlorinated hydrocarbons. *J. Toxicol. Environ. Health* **4**, 325-339.

Nichols, J. W., Wedemeyer, G. A., Mayer, F. L., Dickhoff, W. W., Gregory, S. V., Yasutake, W. T. and Smith, S. D. (1984). Effects of freshwater exposure to arsenic trioxide on the parr-smolt transformation of coho salmon (*Oncorhynchus kisutch*). *Environ. Toxicol. Chem.* **3**, 143-149.

Nimrod, A. C. and Benson,, W. H. (1996). Estrogenic responses to xenobiotics in channel catfish (*Ictalurus punctatus*). *Mar. Environ. Res.* **42**, 155-160.

Norrgren, L., Andersson, T., Bergqvist, P. -A. and Bjorklund, I. (1993). Chemical, physiological and morphological studies of feral Baltic salmon (*Salmo salar*) suffering from abnormal fry mortality. *Environ. Toxicol. Chem.* **12**, 2065-2075

Norris, D. O. (1969). Depression of growth following radiothyroidectomy of larval chinook salmon and steelhead trout. *Trans. Am. Fish. Soc.* **98**, 104-106.

Norstrom, R. J., Muir, D. C. G., Ford, C. A., Simon, M., MacDonald, C. R. and Béland, P. (1992). Indications of P450 monooxygenase activities in beluga (*Delphinapterus leucas*) and narwhal (*Monodon monocerus*). *Mar. Environ. Res.* **34**, 267-272.

Notter, M. F. D., Mudge, J. E., Neff, W. H. and Anthony, A. (1976). Cytophotometric analysis of RNA changes in prolactin and Stannius corpuscles cells of acid-stressed brook trout. *Gen. Comp. Endocrinol.* **30**, 273-284.

Oesch, F. and Golan, M. (1980). Specificity of mouse liver cytosolic epoxide hydrolase for K-region epoxides derived from polycyclic aromatic hydrocarbons. *Cancer Lett.* **9**, 169-173.

Oikari, A. and Jiminez, B. (1992). Effects of hepatotoxicants on the induction of microsomal monooxygenase activity in sunfish liver by β-naphthoflavone and benzo[*a*]pyrene. *Ecotox. Environ. Saf.* **23**, 89-102.

Olivereau, M. and Olivereau, J. (1979). Estradiol positive feedback on gonadotropic (GTH) cells in freshwater male silver eels. *Gen. Comp. Endocrinol.* **39**, 247-261.

Olsson, M. and Reutergardh, L. (1986). DDT and PCB pollution trends in the Swedish aquatic environment. *Ambio* **15**, 103-109.

Olsson, P. E., Haux, C. and Förlin, L. (1987). Variations in hepatic metallothionein, zinc and copper levels during an annual reproductive cycle in rainbow trout, *Salmo gairdneri*. *Fish Physiol. Biochem.* **3**, 39-47.

Olsson, P. E., Zafarullah, M. and Gedamu, L. (1989a). A role of metallothionein in zinc regulation after estradiol induction of vitellogenin synthesis in rainbow trout, *Salmo gairdneri*. *Biochem. J.* **257**, 555-559.

Olsson, P., Larsson, A., Maage, A., Haux, C., Bonham, K., Zafarullah, M. and Gedamu, L. (1989b). Induction of metallothionein synthesis in rainbow trout, *Salmo gairdneri*, during long term exposure to waterborne cadmium. *Fish. Physiol. Biochem.* **6**, 221-229.

Olsson, P-E., Kling, P., Petterson, C. and Silversand, C. (1995). Interaction of cadmium and ostradiol-17β on metallothionein and vitellogenin synthesis in rainbow trout (*Oncorhynchus mykiss*). *Biochem. J.* **397**, 197-203.

Oulmi, Y., Negele, R. D. and Braunbeck, T. (1995a). Cytopathology of liver and kidney in rainbow trout *Oncorhynchus mykiss* after long-term exposure to sublethal concentrations of linuron. *Dis. Aquat. Org.* **21**, 35-52.

Oulmi, Y., Negele, R-D. and Braunbeck, T. (1995b). Segment-specificity of the cytological response in rainbow trout (*Oncorhynchus mykiss*) renal tubules following prolonged exposure to sublethal concentrations of atrazine. *Ecotoxicol. Environ. Saf.* **32**, 39-50.

Overnell, J., McIntosh, R. and Fletcher, T. C. (1987a). The levels of liver metallothionein and zinc in plaice, *Pleuronectes platessa* L., during the breeding season, and the effect of estradiol injection. *J. Fish Biol.* **30**, 539-546.

Overnell, J., McIntosh, R. and Fletcher, T. C. (1987b). The enhanced induction of metallothionein by zinc, its half-life in the marine fish *Pleuronectes platessa*, and the influence of stress factors on metallothionein levels. *Experientia* **43**, 178-181.

Owens, J. W. (1991). The hazard assessment of pulp and paper effluents in the aquatic environment: A review. *Environ. Toxicol. Chem.* **10**, 1511-1540.

Paasivarta, J. and Rantio, T. (1991). Chloroterpenes and other organochlorines in Baltic finfish and Arctic wildlife. *Chemosphere* **22**, 47-55.

Pajor, A. M., Stegeman, J. J., Thomas, P. and Woodin, B. (1990). Feminization of the hepatic microsomal cytochrome P-450 system in brook trout by estradiol, testosterone, and pituitary factors. *J. Exp. Zool.* **253**, 51-60.

Palace, V. P., Klaverkamp, J. F., Lockhart, W. L., Metner, D. A., Muir, D. C. G. and Brown, S. B. (1996). Mixed-function oxidase enzyme activity and oxidative stress in lake trout (*Salvelinus namaycush*) exposed to 3,3',4,4',5-pentachlorobiphenyl (PCB126). *Environ. Toxicol. Chem.* **6**, 955-960.

Pandey, A. K. and Shukla, L. (1980). Effect of benzene hexachloride - an insecticide on the testicular histology in *Tilapia mossambica*. *Geobios* **7**, 251-253.

Pandey, A. K. and Shukla, L. (1982). Effect of an organophosphorus insecticide malathion on the testicular histophysiology in *Sarotherodon mossambicus*. *Nat. Acad. Sci. Letters* **5**, 141-143.

Pandey, A. K, George, K. C. and Mohamed, M. P. (1993). Effect of mercuric chloride on thyroid gland of *Liza parsia* (Hamilton-Buchanan). *J. Adv. Zool.* **14**, 15-19.

Pawar, K. R. and Katdare, M. (1983). Effect of sumithion on the ovaries of freshwater fish *Garra mullya* (Sykes). *Curr. Sci.* **16**, 784-785.

Payne, J. F. and Penrose, W. R. (1975). Induction of aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase in fish by petroleum. *Bull. Environ. Contam. Toxicol.* **14**, 112-116.

Payne, J. F., Fancy, L. L., Rahimtula, A. D. and Porter, E. L. (1987). Review and perspective on the use of mixed function oxygenase enzymes in biological monitoring. *Comp. Biochem. Physiol.* **86C**, 233-245.

Peakall, D. B., Fox, G. A., Gilman, A. P., Hallett, D. J. and Norstrom, R. J. (1980). Reproductive success of herring gulls as an indicator of Great Lakes Water Quality. *In* "Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment", (B. K. Afghan and D. Mackay, Eds.), pp. 337-344. Plenum, New York.

Pearce, F. (1997). Northern exposure. *New Scientist,* **31 May**, 24-27.

Pelgrom, S. M. G. J. (1995). Interactions between copper and cadmium in fish - metal accumulation, physiology and endocrine regulation. Ph.D. Thesis, Catholic University of Nijmegen, The Netherlands, Chapter 6.

Pelgrom, S. M. G. J., Lamers, L. P. M., Haaijman, A., Balm, P. H. M., Lock, R. A. C. and Wendelaar Bonga, S. E. (1994). Interactions between copper and cadmium during single or combined metal exposures in the teleost fish *Oreochromis mossambicus*: heavy metal accumulation and endocrine events. *In* "Sublethal and Chronic Effects of Pollutants on Freshwater Fish", (R. Muller and R. Lloyd, Eds.), pp. 62-74. Blackwell, Oxford.

Pelgrom, S. M. G. J., Lock, R. A. C., Balm, P. H. M. and Wendelaar Bonga, S. E. (1995a). Effects of combined waterborne Cd and Cu exposures on ionic composition and plasma cortisol in tilapia, *Oreochromis mossambicus. Comp. Biochem. Physiol.* **111C**, 227-235.

Pelgrom, S. M. G. J., Lock, R. A. C., Balm, P. H. M and Wendelaar Bonga, S. E. (1995b). Integrated physiological responses of tilapia, *Oreochromis mossambicus*, to sublethal copper exposure. *Aquat. Toxicol.* **32**, 303-320.

Pelgrom, S. M. G. J., Lock, R. A. C., Balm, P. H. M. and Wendelaar Bonga, S. E. (1997). Calcium fluxes in juvenle tilapia, *Oreochromis mossambicus*, exposed to sublethal waterborne Cd, Cu or mixtures of these metals. *Environ. Toxicol. Chem.* **16**, 770-774.

Pelissero, C. and Sumpter, J. P. (1992). Steroids and "steroid-like" substances in fish diets. *Aquaculture* **107**, 283-301.

Pelissero, C., Cuisset, B. and Le Menn, F. (1989). The influence of sex steroid in commercial fish meals and fish diets on plasma concentration of estrogens and vitellogenin in cultured Siberian sturgeon, *Acipenser baeri. Aquat. Living Resour.* **2**, 161-168.

Pelissero, C., Le Menn, F. and Kaushik, S. (1991a). Estrogenic activity of soy-bean meal in the Siberian sturgeon (*Acipenser baeri*). *Gen. Comp. Endocrinol.* **83**, 447-457.

Pelissero, C., Bennetau, B., Babin, P., Le Menn, F. and Dunogués, J. (1991b). Estrogenic activity of certain phytoestrogens in the Siberian sturgeon *Acipenser baeri*). *J. Steroid Biochem.* **38**, 293-299.

Pelissero, C., Flouriot, G., Foucher, J. L., Bennetau, B., Dunogues, J., Le Gac, F. and Sumpter, J. P. (1993). Vitellogenin synthesis in cultured hepatocytes; an *in vitro* test for the estrogenic potency of chemicals. *J. Steroid Biochem. Mol. Biol.* **44**, 263-272.

Pereira, J. J., Ziskowski, J., Mercaldo-Allen, R., Luedke, D. and Gould, E. (1992). Vitellogenin studies in winter flounder (*Pleuronectes americanus*) from Long Island Sound and Boston Harbor. *Estuaries* **15**, 289-297.

Pereira, J. J., Mercaldo-Allen, R., Kuropat, C., Luedke, D. and Sennefelder, G. (1993). Effect of cadmium accumulation on serum vitellogenin levels and hepatosomatic and gonadosomatic indices of winter flounder (*Pleuronectes americanus*). *Arch. Environ. Contam. Toxicol.* **24**, 427-431.

Peterson, R. H. and Martin-Robichaud, D. J. (1982). Water uptake by Atlantic salmon ova as affected by low pH. *Trans. Am. Fish. Soc.* **111**, 772-774.

Peterson, R. H., Daye, P. G. and Metcalfe, J. L. (1980). Inhibition of Atlantic salmon hatching at low pH. *Can. J. Fish. Aquat. Sci.* **37**, 770-774.

Peterson, R. E., Theobald, H. M. and Kimmel, G. L. (1993). Developmental and reproductive toxicity of dioxins and related compounds: Cross-species comparisons. *Critical Rev. Toxicol.* **23,** 283-335.

Petit, F., Le Goff, P., Cravedi, J.-P, Valotaire, Y. and Pakdel, F. (1996). Recombinant yeast for estrogen receptor: A bioassay for screening the estrogenic potentiality of xenobiotics. *Ann. d'endocrinol.* **57 Suppl.** 59.

Phillips, G. R. and Russo, R. C. (1978). "Metal Bioaccumulation in fishes and aquatic invertebrates: A literature review." 116 pp. Environmental Research Laboratory, Duluth, MN.

Pickering, A. D. (Ed.) (1981). "Stress and Fish". Academic Press, New York.

Pickering, A. D.and Duston, J. (1983). Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *J. Fish Biol.* **23,** 163-175.

Pickering, A. D. and Pottinger, T. G. (1989). Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. *Fish Physiol. Biochem.* **7,** 253-258.

Pickering, Q., Brungs, W. and Gast, M. (1977). Effect of exposure time and copper concentration on reproduction of the fathead minnow (*Pimephales promelas*). *Water Res.* **11,** 1079-1083.

Pierson, K. B. (1981). Effects of chronic zinc exposure on the growth, sexual maturity, reproduction, and bioaccumulation of the guppy, *Poecilia reticulata. Can. J. Fish. Aquat. Sci.* **38,** 23-31.

Piferrer, F. and Donaldson, E. M. (1992). The comparative effectiveness of the natural and a synthetic estrogen for the direct feminization of chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture*, **106,** 183-193.

Piferrer, F., Zanuy, S., Carrillo, M., Solar, I. I., Devlin, R. H., and Donaldson, E. M. (1994). Brief treatment with an aromatase inhibitor during sex differentiation causes chromosomally female salmon to develop as normal, functional males. *J. Exp. Zool.* **270,** 255-262.

Povlsen, A. F., Korsgaard, B. and Bjerregaard, P. (1990). The effect of cadmium on vitellogenin metabolism in estradiol-induced flounder (*Platichthys flesus* (L.)) males and females. *Aquat. Toxicol.* **17,** 253-262.

Pratap, H. B. and Wendelaar Bonga, S. E. (1990). Effects of water-borne cadmium on plasma cortisol and glucose in the cichlid fish *Oreochromis mossambicus. Comp. Biochem. Physiol.* **95C,** 313-318.

Pruell, R. J. and Englehardt, F. R. (1980). Liver cadmium uptake, catalase inhibition and cadmium thionein production in the killifish (*Fundulus heteroclitus*) induced by cadmium exposure. *Mar. Environ. Res.* **3,** 101-111.

Pulliainen, E., Korhonen, K., Kankaaranta, L. and Mäki, K. (1992). Non-spawning burbot on the northern coast of the Bothnian Bay. *Ambio* **21,** 170-175.

Pundir, R. and Saxena, A. B. (1990). Seasonal changes in the testes of fish *Puntius ticto*, and their relation to heavy metal toxicity. *Bull. Environ. Contam. Toxicol.* **45,** 288-293.

Pundir, R. and Saxena, A. B. (1992). Chronic toxic exposure of cadmium on the pituitary gland of fish *Puntius ticto* and pattern of recoupment. *J. Environ. Biol.* **13,** 69-74.

Purdom, C. E., Hardiman, P. A., Bye, V. J., Eno, N. C., Tyler, C. R. and Sumpter, J. P. (1994). Estrogenic effects of effluents from sewage treatment works. *Chem. Ecol.* **8,** 275-285.

Ram, R. N. and Joy, K. P. (1988). Mercurial induced changes in the hypothalamo-neurohypophysial complex in relation to reproduction in the teleostean fish, *Channa punctatus* (Bloch). *Bull. Environ. Contam. Toxicol.* **41,** 329-336.

Ram, R. J. and Sathyanesan, A. G. (1983). Effect of mercuric chloride on the reproductive cycle of the teleostean fish *Channa punctatus*. *Bull. Environ. Contam. Toxicol.* **30**, 24-27.

Ram, R. J. and Sathyanesan, A. G. (1984). Mercuric chloride induced changes in the protein, lipid and cholesterol levels of the liver and ovary of the fish *Channa punctatus*. *Environ. Ecol.* **2**, 113-117.

Ram, R. J. and Sathyanesan, A. G. (1987a). Effect of a mercurial fungicide on the gonadal development of the teleostean fish *Channa punctatus* (Bloch). *Ecotoxicol. Environ. Saf.* **11**, 352-360.

Ram, R. J. and Sathyanesan, A. G. (1987b). Effects of long-term exposure to Cythion on the reproduction of the teleost fish, *Channa punctatus* (Bloch). *Environ. Poll.* **44**, 49-60.

Ram, R. N. and Sathyanesan, A. G. (1987c). Histopathological changes in liver and thyroid of the teleostean fish, *Channa punctatus* (Bloch), in response to ammonium sulphate fertilizer treatment. *Ecotoxicol. Environ. Saf.* **13**, 185-190.

Rask, M. (1992). Changes in the density, population structure, growth and reproduction of perch, *Perca fluviatilis* L. in two acidic forest lakes in southern Finland. *Environ. Poll.* **78**, 121-125.

Rask, M. (1983). The effect of low pH on perch, *Perca fluviatilis* L. I. effects of low pH on the development of eggs of perch. *Ann. Zool. Fennici* **20**, 73-76.

Rask, M., Vuorinen, P. J. and Vuorinen, M. (1990). Delayed spawning of perch, *Perca fluviatilis* L., in acidified lakes. *J. Fish Biol.* **36**, 317-325.

Rastogi, A. and Kulshrestha, S. K. (1990). Effect of sublethal doses of three pesticides on the ovary of a carp minnow *Rasbora daniconius*. *Bull. Environ. Contam. Toxicol.* **45**, 742-747.

Razani, H. and Hanyu, I. (1986). Effects of continued short photoperiod at warm temperature and following changes of regimes on gonadal maturation of goldfish. *Bull. Jap. Soc. Sci. Fish.* **52**, 2069-2078.

Reader, J. P., Dalzeil, T. R. K. and Morris, R. (1988). Growth, mineral uptake and skeletal calcium deposition in brown trout, *Salmo trutta* L. yolk-sac fry exposed to aluminium and manganese in soft acid water. *J. Fish Biol.* **32**, 607-624.

Reader, S., Saint Louis, R., Pelletier, E. and Denizeau, F. (1996). Accumulation and biotransformation of tri-*n*-butyltin by isolated rainbow trout hepatocytes. *Environ. Toxicol. Chem.* **15**, 2049-2052.

Reijnders, P. J. H. (1986). Reproductive failure in common seals feeding on fish from polluted coastal waters. *Nature* **324**, 456-457.

Reimold, R. J. and Shealy Jr., M. H. (1976). Chlorinated hydrocarbon pesticides and mercury in coastal young-of-the year finfish, South Carolina and Georgia 1972-74. *Pestic. Monit. J.* **9**, 170-175.

Reinert, R. E. (1979). Pesticide concentrations in Great Lakes fish. *Pestic. Monit. J.* **3**, 233-240.

Reis-Henriques, M. A., Cruz, M. M. and Pereira, J. O. (1997). The modulating effect of vitellogenin on the synthesis of 17β-estradiol by rainbow trout ovary. *Fish Physiol. Biochem.* **16**, 181-186.

Ren, L., Lattier, J. and Lech, J. J. (1996). Estrogenic activity in rainbow trout determined with a new c-DNA probe for vitellogenin psg5Vg. *Bull. Environ. Contam. Toxicol.* **56**, 287-294.

Ribeyre, F. and Boudou, A. (1984). The bioaccumulation and distribution of mercury (mercuric chloride and methylmercurous chloride) in the tissues of *Salmo gairdneri* after direct contamination. *Water Air Soil Poll.* **23**, 169-186.

Richter, C. J. J. and Goos, H. J. Th. (compilers) (1982). "Proceedings of the International Symposium on Reproductive Physiology of Fish, Wageningen, The Netherlands, 2-6 August 1982". Pudoc, Wageningen.

Roberts, K. S., Cryer, A., Kay, J., Solbe, J. F. De L., Wharfe, J. R. and Simpson, W. R. (1979). The effects of exposure to sub-lethal concentrations of cadmium on enzyme activities and accumulation of the metal in tissues and organs of rainbow and brown trout (*Salmo gairdneri*, Richardson and *Salmo trutta fario* L.). *Comp. Biochem. Physiol.* **62**, 135-140.

Robertson, O. H., Hane, S., Wexler, B. C. and Rinfret, A. P. (1963). The effect of hydrocortisone on immature rainbow trout (*Salmo gairdnerii*). *Gen. Comp. Endocrinol.* **3**, 422-436.

Roch, M. and McCarter, J. A. (1984a). Hepatic metallothionein production and resistance to heavy metals by rainbow trout (*Salmo gairdneri*) - I. Exposed to an artificial mixture of zinc, copper and cadmium. *Comp. Biochem. Physiol.* **77C** 71-75.

Roch, M. and McCarter, J. M. (1984b). Hepatic metallothionein production and resistance to heavy metals by rainbow trout (*Salmo gairdneri*) - II. Held in a series of contaminated lakes. *Comp. Biochem. Physiol.* **77C**, 77-82.

Roch, M., McCarter, J. M., Matheson, A. T., Clark, M. J. R. and Olafson, R. W. (1982). Hepatic metallothionein in rainbow trout (*Salmo gairdneri*) as an indicator of metal pollution in the Campbell River system. *Can. J. Fish. Aquat. Sci.* **39**, 1596-1601.

Rombough, P. J. and Garside, E.T. (1982). Cadmium toxicity and accumulation in eggs and alevins of Atlantic salmon (*Salmo salar*). *Can. J. Zool.* **60**, 2006-2014

Routledge, E. J. and Sumpter, J. P. (1996). Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environ. Toxicol. Chem.* **15**, 241-248.

Routledge, E. J., Sheahan, D., Desbrow, C., Brighty, G., Waldock, M. and Sumpter, J. P. (1998). Identification of estrogenic chemicals in STW effluent. 2. *In vivo* responses in trout and roach. *Environ. Sci. Technol.* 32, 1559-1565.

Roy, R. L., Ruby, S. M., Idler, D. R. and So, Y. (1990). Plasma vitellogenin levels in pre-spawning rainbow trout, *Oncorhynchus mykiss,* during acid exposure. *Arch. Environ. Contam. Toxicol.* **19**, 803-806.

Rubinstein, N. I., Gilliam, W. T. and Gregory, N. R. (1984). Dietary accumulation of PCBs from a contaminated sediment source by a demersal fish (*Leiostomus xanthurus*) *Aquat. Toxicol.* **5**, 331-342.

Ruby, S. M., Aczel, J. and Craig, G. R. (1977). The effects of depressed pH on oogenesis in flagfish *Jordanella floridae*. *Water Res.* **11**, 757-762.

Ruby, S. M., Aczel, J. and Craig, G. R. (1978). The effects of depressed pH on spermatogenesis in flagfish *Jordanella floridae*. *Water Res.* **12**, 621-626.

Ruby, S. M., Idler, D. R. and So, Y. P. (1986). The effect of sublethal cyanide exposure on plasma vitellogenin levels in rainbow trout (*Salmo gairdneri*) during early vitellogenesis. *Arch. Environ. Contam. Toxicol.* **15**, 603-607.

Ruby, S. M., Idler, D. R. and So, Y. P. (1987). Changes in plasma, liver, and ovary vitellogenin in landlocked Atlantic salmon following exposure to sublethal cyanide. *Arch. Environ. Contam. Toxicol.* **16**, 507-510.

Ruby, S. M., Jaroslawski, P. and Hull, R. (1993a). Lead and cyanide toxicity in sexually maturing rainbow trout, *Oncorhynchus mykiss* during spermatogenesis. *Aquat. Toxicol.* **26**, 225-238.

Ruby, S. M., Idler, D. R. and So, Y. P. (1993b). Plasma vitellogenin, 17β-estradiol, T_3 and T_4 levels in sexually maturing rainbow trout *Oncorhynchus mykiss* following sublethal HCN exposure. *Aquat.Toxicol.* **26**, 91-102.

Runn, P. N., Johansson, N. and Milbrink, G. (1977). Some effects of low pH on the hatchability of eggs of perch, *Perca fluviatilis* L. *Zoon* **5**, 115-125.

Rurangwa, E., Roelants, I., Huyskens, G., Ebrahimi, M., Kime, D. E. and Ollevier, F. (1998). The minimum effective spermatozoa to egg ratio for artificial insemination and the effects of mercury on sperm motility and fertilization ability in the African catfish (*Clarias gariepinus*, Burchell 1822). *J. Fish Biol.* **53**, 402-413.

Sadhu, A. R. and Mukhopadhyay, P. K. (1985). Comparative effect of two pesticides malathion and carbofuran on testes of *Clarias batrachus* (Linn.). *J. Environ. Biol.* **6**, 217-222.

Sadler, K. and Lynam, S. (1987). Some effects on the growth of brown trout from the exposure to aluminium at different pH levels. *J. Fish Biol.* **31**, 209-219.

Safe, S., Safe, L. and Mullin, M. (1985). Congener-specific analysis of a commercial mixture and a human milk extract. *J. Agric. Food. Chem.* **33**, 24-29.

Sage, M. and Bromage, N. R. (1970). The activity of the pituitary cells of the teleost Poecilia during the gestation cycle and the control of the gonadotropic cells. *Gen. Comp. Endocrinol.* **14**, 127-136.

Salomons, W., Bayne, B. L., Duursma, E. K. and Forstner, U. (Eds.) (1988). "Pollution of the North Sea, an Assessment". Springer Verlag, Berlin.

Samis, A.J.W., Colgan, P. W. and Johansen, P. H. (1991). A comparison of the effects of subchronic and acute spill-mimicking pentachlorophenol exposures on growth of bluegill sunfish (*Lepomis macrochirus*). *Aquat. Toxicol.* **19**, 231-240.

Sancho, E., Ferrando, M. D., Andreu, E. and Gamon, M. (1993). Bioconcentration and excretion of diazinon by eel. *Bull. Environ. Contam. Toxicol.* **50**, 578-585.

Sandström, O., Neuman, E. and Karås, P. (1988). Effects of a bleached pulp mill effluent on growth and gonad function in Baltic coastal fish. *Water. Sci. Tech.* **20**, 107-118.

Sangalang, G. B. and Freeman, H. C. (1974). Effects of sublethal cadmium on maturation and testosterone and 11-ketotestosterone production *in vivo* in brook trout. *Biol. Reprod.* **11**, 429-435.

Sangalang, G. B. and Freeman, H. C. (1979). Tissue uptake of cadmium in brook trout during chronic sublethal exposure. *Arch. Environ. Contam. Toxicol.* **8**, 77-84.

Sangalang, G. B. and O'Halloran, M. J. (1972). Cadmium-induced testicular injury and alterations of androgen synthesis in brook trout. *Nature* **240**, 470-471.

Sangalang, G. B. and O'Halloran, M. J. (1973). Adverse effects of cadmium on brook trout testis and on *in vitro* testicular androgen synthesis. *Biol. Reprod.* **9**, 394-403.

Sangalang, G. B., Freeman, H. C. and Crowell, R. (1981). Testicular abnormalities in cod (*Gadus morhua*) fed Arochlor 1254. *Arch. Environ. Contam. Toxicol.* **10**, 617-626.

Sastry, K. V. and Rao, D. R. (1981). Enzymological and biochemical changes produced by mercuric chloride in a teleost fish, *Channa punctatus*. *Toxicol. Lett.* **9**, 321-326.

Sauer, G. R. and Watabe, N. (1988). The effects of heavy metals and metabolic inhibitors on calcium uptake by gills and scales of *Fundulus heteroclitus in vitro*. *Comp. Biochem. Physiol.* **91C**, 473-478.

Saxena, P. K. and Bhatia, R. (1983). Effect of vegetable oil factory effluent on ovarian recrudescence in the freshwater teleost, *Channa punctatus* (Bl.). *Water Air Soil Poll.* **20**, 55-61.

Saxena, P. K. and Garg, M. (1978). Effect of insecticidal pollution on ovarian recrudescence in the freshwater teleost, *Channa punctatus* Bl. *Ind. J. Exp. Biol.* **16**, 690-691.

Saxena, P. K. and Mani, K. (1985). Quantitative study of testicular recrudescence in the fresh water teleost, *Channa punctatus* (Bl.) exposed to pesticides. *Bull. Environ. Contam. Toxicol.* **34**, 597-607.

Saxena, P. K. and Mani, K. (1987). Effect of safe concentrations of some pesticides on testicular recrudescence in the freshwater murrel, *Channa punctatus* (Bl.): a morphological study. *Ecotoxicol. Environ. Saf.* **14**, 56-63.

Saxena, P. K. and Mani, K. (1988). Effect of safe concentrations of some pesticides on thyroid in the freshwater murrel, *Channa punctatus*: A histopathological study. *Environ. Poll.* **55**, 97-106.

Saxena, P. K., Mani, K. and Kondal, J. K. (1986). Effect of safe application rate (SAR) concentrations of some biocides on the gonads of the fresh water murrel, *Channa punctatus* (Bl.) - a biochemical study. *Ecotoxicol. Environ. Saf.* **12**, 1-14.

Schell, J. D. Jr., Cooper, K. O. and Cooper, K. R. (1987). Hepatic microsomal mixed-function oxidase activity in the Japanese medaka (*Oryzias latipes*). *Environ. Toxicol. Chem.* **6**, 717-722.

Schlebusch, H., Wagner, U., van der Ven, H., Al-Hasani, S., Diedrich, K. and Krebs, D. (1989). Polychlorinated biphenyls: the occurrence of the main congeners in follicular and sperm fluids. *J. Clin. Chem. Biochem.* **27**, 663-667.

Schneider, R. (1982). Polychlorinated biphenyls (PCBs) in cod tissues from Western Baltic: Significance of equilibrium partitioning and lipid composition in the bioaccumulation of lipophilic pollutants in gill-breathing animals. *Meeresforsch.* **29**, 69-79.

Schreck, C. B. (1996). Immunomodulation: Endogenous factors. *In* "The fish immune system. Organism, pathogen and environment", (G. Iwama and T. Nakanishi, Eds.), pp. 311-337. Academic Press, San Diego.

Schreck, C. B. and Lorz, H. W. (1978). Stress response of coho salmon (*Oncorhynchus kisutch*) elicited by cadmium andcopper and the potential use of cortisol as an indicator of stress. *J. Fish. Res. Bd. Can.* **35**, 1124-1129.

Schröder, J. H. and Peters, K. (1988). Differential courtship activity and alterations of reproductive success of competing guppy males (*Poecilia reticulata* Peters; Pisces: Poecilidae) as an indicator for low concentrations of aquatic pollutants. *Bull. Environ. Contam. Toxicol.* **41**, 385-390.

Schwaiger, J., Worle, B., Kalbfus, W. and Negele, R. D. (1997). Effects of nonylphenol on fish: Test design and preliminary results. *Workshop: Effects of Endocrine Disrupters in the Environment on Neuronal Development and Behaviour, Berlin, Feb 17-18, 1997.* p. 14.

Schwarzenberger, F., Toole, G. S., Christie, H. L. and Raeside, J. I. (1993). Plasma levels of several androgens and estrogens from birth to puberty in male domestic pigs. *Acta Endocrinol.* **128**, 173-177.

Scott, A. P., Sumpter, J. P., Kime, D. E. and Rolfe, M. S. (Eds.) (1991). "Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, University of East Anglia, Norwich, U. K., 7-12 July 1991". FishSymp91, Sheffield.

Sehgal, R. and Pandey, A. K. (1984). Effect of cadmium chloride on testicular activities in guppy *Lebistes reticulatus*. *Comp. Physiol. Ecol.* **9**, 225-230.

Sehgal, R., Tomar, V. and Pandey, A. K. (1984). Comparative effects of two heavy metallic salts on the testis of viviparous teleost, *Lebistes reticularis* (Peters). *J. Environ. Biol.* **5**, 185-192.

Selye, H. (1950). Stress and the general adaptation syndrome. *Brit. Med. J.* **1950**, 1383-1392.

Servizi, J. A., Gordon, R. W., Martens, D. W., Lockhart, W. L., Metner, D. A., Rogers, I. H., McBride, J. R. and Norstrom, R. J. (1993). Effects of biotreated bleached kraft mill effluent on fingerling chinook salmon (*Oncorhynchus tschawytscha*). *Can. J. Fish. Aquat. Sci.* **50**, 846-857.

Sharpe, R. M. and Skakkebaek, N. E. (1993). Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* **B41**, 1392-1395.

Sherwood, N. M., Kyle, A. L., Kreiberg, H., Warby, C. M., Magnus, T. H., Carolsfeld, J. and Price, W. S. (1991). Partial characterisation of a spawning pheromone in the herring *Clupea harengus pallasi. Can. J. Zool.* **69**, 104-110.

Short, R. V. (1982). Sex determination and differentiation. *In* "Reproduction in Mammals. Book 2: Embryonic and Fetal Development", 2nd Edition, (C. R. Austin and R. V. Short, Eds.), pp. 70-113. Cambridge University Press, Cambridge.

Shukla, J. P. and Pandey, K. (1984a). Effects of three insecticides on the histoarchitechture of pituitary gland in *Sarotherodon mossambicus. Uttar Pradesh J. Zool.* **4**, 201-204.

Shukla, J. P. and Pandey, K. (1984b). Impaired spermatogenesis in arsenic treated freshwater fish, *Colisa fasciatus* (Bl. and Sch.). *Toxicol. Lett.* **21**, 191-195.

Shukla, J. P. and Pandey, K. (1984c). Arsenic induced cellular and biochemical changes during the testicular cycle of a freshwater perch, *Colisa fasciatus* (Bl. & Sch.). *Cell. Mol. Biol.* **30**, 227-231.

Shukla, J. P. and Pandey, K. (1984d). Carbamide induced histological alterations during different phases of the testicular cycle of a freshwater perch, *Colisa fasciatus* (Bl. & Sch.). *Bull. Inst. Zool. Acad. Sinica* **23**, 181-186.

Shukla, J. P. and Pandey, K. (1984e). Arsenic induced structural changes during the ovarian cycle of a freshwater perch, *Colisa fasciatus* (Bloch and Schneider). *Bull. Inst. Zool., Acad. Sinica* **23**, 69-74.

Shukla, J. P. and Pandey, K. (1985). Ovarian recrudescence in a freshwater teleost, *Sarotherodon mossambicus* in DDT treatment and pattern of recoupment. *J. Environ. Biol.* **6**, 195-204.

Shukla, J. P. and Pandey, K. (1986a) Effects of endosulphan on the hypothalamo-hypophysial complex and fish reproductive physiology. *Bull. Environ. Contam. Toxicol.* **36**, 122-131.

Shukla, L. and Pandey, A. K. (1986b). Restitution of thyroid activity in the DDT exposed *Sarotherodon mossambicus*: A histological and histochemical profile. *Water Air Soil Poll.* **27**, 225-236.

Shukla, L., Shrivastava, A., Merwani, D. and Pandey, A. K. (1984). Effect of sublethal malathion on ovarian histophysiology in *Sarotherodon mossambicus. Comp. Physiol. Ecol.* **9**, 13-17.

Sijm, D. T. H. M. and Opperhuizen, A. (1989). Biotransformation of organic chemicals by fish: enzyme activities and reactions. *In* "Handbook of Environmental Chemistry", (O. Hutzinger, Ed.), pp. 163-235. Springer, Berlin

Singh, H. (1989). Interaction of xenobiotics with reproductive endocrine functions in a protogynous teleost, *Monopterus albus. Mar. Environ. Res.* **28**, 285-289.

Singh, P. B. (1992). Impact of malathion and γ-BHC on lipid metabolism in the freshwater female catfish, *Heteropneustes fossilis. Ecotoxicol. Environ. Saf.*. **23**, 22-32.

Singh, H. (1993). Effects of malathion on steroidogenesis and sex reversal in *Monopterus albus. Mar. Environ. Res.* **35**, 159-164.

Singh, P. B. and Kime, D. E. (1994). *In vivo* incorporation of [1-^{14}C]acetic acid into liver lipids of goldfish, *Carassius auratus*, during γ-hexachlorocyclohexane exposure. *Aquat. Toxicol.* **30**, 237-248.

Singh, P. B. and Kime, D. E (1995). Impact of γ-hexachlorocyclohexane on the *in vitro* production of steroids from endogenous and exogenous precursors in the spermiating roach, *Rutilus rutilus. Aquat. Toxicol.* **31**, 231-240.

Singh, H. and Singh, T. P. (1980a). Effects of two pesticides on testicular ^{32}P uptake, gonadotrophic potency, lipid and cholesterol content of testis, liver and blood serum during spawning phase in *Heteropneustes fossilis* (Bloch). *Endokrinologie* **76**, 288-296.

Singh, H. and Singh, T. P. (1980b). Short-term effect of two pesticides on the survival, ovarian ^{32}P uptake and gonadotrophic potency in a freshwater catfish, *Heteropneustes fossilis* (Bloch). *J. Endocrinol.* **85**, 193-199.

Singh, H. and Singh, T. P. (1980c). Short-term effect of two pesticides on lipid and cholesterol content of liver, ovary and blood serum during the pre-spawning phase in the freshwater teleost, *Heteropneustes fossilis* (Bloch). *Environ. Poll.* **22**, 85-90.

Singh, H. and Singh, T. P. (1980d). Effect of two pesticides on total lipid and cholesterol contents of ovary, liver and blood serum during different phases of the annual reproductive cycle in the freshwater teleost *Heteropneustes fossilis* (Bloch). *Environ. Poll.* **23**, 9-17.

Singh, H. and Singh, T. P. (1980e). Effect of two pesticides on ovarian ^{32}P uptake and gonadotrophin concentration during different phases of annual reproductive cycle in the freshwater catfish, *Heteropneustes fossilis* (Bloch). *Environ. Res.* **22**, 190-200.

Singh, H. and Singh, T. P. (1981). Effect of parathion and aldrin on survival, ovarian ^{32}P-uptake and gonadotrophic potency in a freshwater catfish, *Heteropneustes fossilis* (Bloch). *Endokrinologie* 77, 173-178.

Singh, H. and Singh, T. P. (1982a). Effect of some pesticides on hypothalamo-hypophyseal-ovarian axis in the freshwater catfish *Heteropneustes fossilis* (Bloch). *Environ. Poll.* **27A**, 283-288.

Singh, H. and Singh, T. P. (1982b). Effects of pesticides on fish reproduction. *Ichthyologia* **15**, 71-81.

Singh, S. and Singh, T. P. (1987a). Evaluation of toxicity limit and sex hormone production in response to Cythion and BHC in the vitellogenic catfish *Clarias batrachus*. *Environ. Res.* **42**, 482-488.

Singh, S. and Singh, T. P. (1987b). Impact of malathion and hexachlorocyclohexane on plasma profiles of three sex hormones during different phases of the reproductive cycle in *Clarias batrachus*. *Pesticide Biochem. Physiol.* **27**, 301-308.

Singh, P. B. and Singh, T. P. (1991). Impact of γ-BHC on sex steroid levels and their modulation by ovine luteinizing hormone-releasing hormone and *Mystus* gonadotropin in the freshwater catfish, *Heteropneustes fossilis*. *Aquat. Toxicol.* **21**, 93-102.

Singh, P. B. and Singh, T. P. (1992a). Impact of γ-BHC on lipid class levels and their modulation by reproductive hormones in the freshwater catfish, *Heteropneustes fossilis*. *Bull. Environ. Contam. Toxicol.* **48**, 23-30.

Singh, P. B. and Singh, T. P. (1992b). Impact of malathion and γ-BHC on steroidogenesis in the freshwater catfish, *Heteropneustes fossilis*. *Aquat. Toxicol.* **22**, 69-80.

Singh, P. B. and Singh, T. P. (1992c). Modulatory actions of ovine luteinizing hormone-releasing hormone and *Mystus* gonadotropin on γ-BHC-induced changes in lipid levels in the freshwater catfish, *Heteropneustes fossilis*. *Ecotoxicol. Environ. Saf.* **24**, 192-202.

Singh, T. P., Yadav, A. K., Lal, B. and Singh, S. (1989). Impact of pesticide on fish reproduction: a review. *Sci. Res. J.* **1**, 9-20.

Singh, P. B., Kime, D. E. and Singh, T. P. (1993). Modulatory actions of *Mystus* gonadotropin on γ-BHC-induced histological changes, cholesterol, and sex steroid levels in *Heteropneustes fossilis*. *Ecotoxicol. Environ. Safety* **25**, 141-153.

Singh, P. B., Kime, D. E., Epler, P. and Chyb, J. (1994). Impact of γ-hexachlorocyclohexane exposure on plasma gonadotropin levels and *in vitro* stimulation of gonadal steroid production by carp hypophyseal homogenate in *Carassius auratus*. *J. Fish Biol.* **44**, 195-204.

Sinha, N., Lal, B. and Singh, T. P. (1991a). Pesticides induced changes in circulating thyroid hormones in the freshwater catfish *Clarias batrachus. Comp. Biochem. Physiol.* **100C**, 107-110.

Sinha, N., Lal, B., and Singh, T. P. (1991b). Effect of endosulfan on thyroid physiology in the freshwater catfish, *Clarias batrachus. Toxicology* **67**, 187-197.

Sinha, N., Lal, B and Singh, T. P. (1992a). Thyroid physiology impairment by malathion in the freshwater catfish *Clarias batrachus. Ecotox. Environ. Saf.* **24**, 17-25.

Sinha, N., Lal, B. and Singh, T. P. (1992b). Carbaryl-induced thyroid dysfunction in the freshwater catfish, *Clarias batrachus. Ecotoxicol. Environ. Saf.* **21**, 240-247.

Sivarajah, K., Franklin, C. S. and Williams, W. P. (1978a). Some histopathological effects of Arochlor 1254 on the liver and gonads of rainbow trout, *Salmo gairdneri* and carp, *Cyprinus carpio. J. Fish Biol.* **13**, 411-414.

Sivarajah, K., Franklin, C. S. and Williams, W. P. (1978b). The effects of polychlorinated biphenyls on plasma steroid levels and hepatic microsomal enzymes in fish. *J. Fish Biol.* **13**, 401-409.

Sivarajah, K., Franklin, C. S. and Williams, W. P. (1979). Some studies on the hepatic microsomal enzyme activities and steroid hormone levels in carp, *Cyprinus carpio* exposed for six months. *J. Fish Biol.* **15**, 249-253.

Skare, J. U., Stenersen, J., Kveseth, N. and Polder, A. (1985). Time trends of organochlorine chemical residues in seven sedentary marine fish species from a Norwegian Fjord during the period 1972-1982. *Arch. Environ. Contam. Toxicol.* **14**, 33-41.

Slemr, F. and Langer, E. (1992). Increase in global atmospheric concentrations of mercury inferred from measurements over the Atlantic Ocean. *Nature*, **355**, 434-437.

Smedstad, O. M. (1994). Torsk, hysing og hvitting i Nordsjøen. In"Annual Report on Fishery Resources in Norwegian Waters" Fisken og Havet 1, 63-65 (In Norwegian).

Smith, R. M. and Cole, C. F. (1973). Effects of egg concentrations of DDT and dieldrin on development in winter flounder (*Pseudopleuronectes americanus*). *J. Fish. Res. Bd. Can.* **30**, 1894-1898.

Soimasuo, R., Jokinen, I., Kukkonen, J., Petänen, T., Ristola, T. and Oikari, A. (1995). Biomarker responses along a pollution gradient - effects of pulp and paper-mill effluents on caged whitefish. *Aquat. Toxicol.* **31**, 329-345.

Solbakken, J. E., Tilseth, S., and Palmork, K. H. (1984). Uptake and elimination of aromatic hydrocarbons and chlorinated biphenyl in eggs and larvae of cod *Gadus morhua. Mar. Ecol.* **16**, 297-301.

Somero, G. N., Chow, T. J., Yancey, P. H. and Snyder, C. B. (1977). Lead accumulation rates in tissues of the estuarine teleost fish, *Gillichthys mirabilis*: salinity and temperature effects. *Arch. Environ. Contam. Toxicol.* **6**, 337-348.

Sonstegard, R. A. and Leatherland, J. F. (1979). Aquatic organism pathobiology as a sentinel system to monitor environmental mutagens. *In* "Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment", (B. K. Afghan and D. Mackay, Eds.), pp. 513-520. Plenum Press, New York.

Speranza. A. W., Seeley, R. J., Seeley, V. A. and Perlmutter, A. (1977). The effect of sublethal concentrations of zinc on reproduction in the zebrafish, *Brachydanio rerio* Hamilton-Buchanan. *Environ. Poll.* **12**, 217-222.

Spies, R. B. and Rice, D. W. Jr. (1988). Effects of organic contaminants on reproduction of the starry flounder *Platichthys stellatus* in San Francisco Bay. II. Reproductive success of fish captured in San Francisco Bay and spawned in the laboratory. *Mar. Biol.* **98**, 191-200.

Spies, R. B., Rice, D. W. and Ireland, R. R. (1984). Preliminary studies of growth, reproduction and activity of hepatic mixed-function oxidase in *Platichthys stellatus*. *Mar. Environ. Res.* **14**, 426-428.

Spies, R. B., Rice, D. W., Montagna, P. A. and Ireland, R. R. (1985). Reproductive success, xenobiotic contaminants and hepatic mixed function oxidase (MFO) activity in *Platichthys stellatus* populations from San Francisco Bay. *Mar. Environ. Res.* **17**, 117-121.

Spies, R. B., Rice, D. W. Jr. and Felton, J. (1988). Effects of organic contaminants on reproduction of the starry flounder *Platichthys stellatus* in San Francisco Bay. I. Hepatic contamination and mixed function oxidase (MFO) activity during the reproductive season. *Mar. Biol.* **98**, 181-190.

Spies, R. B., Stegeman, J. J., Rice, D. W. Jr., Woodin, B., Thomas, P., Hose, J. E., Cross, J. N. and Prieto, M. (1990). Sublethal responses of *Platichthys stellatus* to organic contamination in San Francisco Bay with emphasis on reproduction. *In* " Biomarkers of Environmental Contamination", (J. F. McCarthy and L. R. Shugart, Eds.), pp. 87-121. Lewis Publishers, Boca Raton FL.

Spry, D. J. and Wood, C. M. (1989). The influence of dietary and waterbourne zinc on heat-stable metal ligands in rainbow trout, *Salmo gairdneri* Richardson: Quantification by ^{109}Cd radioassay and evaluation of the assay. *J. Fish Biol.* **35**, 557-576.

Srivastava, A. K. (1987). Changes induced by lead in fish testis. *J. Environ. Biol.* **8**, 329-332.

Srivastava, A. K. and Srivastava, A. K. (1994). Effects of chlordecone on the gonads of freshwater catfish, *Heteropneustes fossilis*. *Bull. Environ. Contam. Toxicol.* **53**, 186-191.

Stacey, N. E. (1984). Control of the timing of ovulation by exogenous and endogenous factors. *In* "Fish Reproduction: Strategies and Tactics", (G. W. Potts and R. J. Wootton, Eds.), pp. 207-222. Academic Press, London.

Stacey, N. E. (1991). Hormonal pheromones in fish: Status and prospects. *In* "Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, University of East Anglia, Norwich, U. K., 7-12 July 1991", (A. P. Scott, J. P. Sumpter, D. E. Kime, and M. S. Rolfe, Eds.), pp 177-181. FishSymp91, Sheffield.

Stacey, N. E. and Cardwell, J. R. (1995). Hormones as sex pheromones in fish: Widespread distribution among freshwater species. *In* "Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, University of Texas at Austin, Austin, Texas, 2-8 July, 1995", (F. W. Goetz and P. Thomas), pp. 244-248. Fish Symposium 95, Austin.

Stacey, N. E., Kyle, A. L. and Liley, N. R. (1986). Fish reproductive pheromones. *In* "Chemical Signals in Vertebrates, 4. Ecology, Evolution and Comparative Biology", (D. Duvall, D. Muller-Schwarze and R. M. Silverstein, Eds.), pp.117-133. Plenum Press, New York and London.

Stagg, R. M., Rusin, J. and Brown, F. (1992). Sodium, potassium ATPase activity in the gills of the flounder (*Platichthys flesus*) in relation to mercury contamination in the Firth of Forth. *Mar. Environ. Res.* **33**, 255-266.

Stave, J. W. and Roberson, B. S. (1985). Hydrocortisone suppresses the chemiluminescent response of striped bass phagocytes. *Dev. Comp. Immun.* **9**, 77-84.

Staveland, G. and Marthinsen, I. (1989). Growth, condition and PCB content of cod (*Gadus morhua*) and flounder (*Platichthys flesus*) in the Hvaler area, Southern Norway. *ICES Marine Environmental Quality Committee, E:3, 14 pp.*

Stegeman, J. J. and Kloepper-Sams, P. J. (1987). Cytochrome P-450 monooxygenase activity in aquatic animals. *Environ. Health Perspect.* **71**, 87-95.

Stein, J. E., Hom, T. Sanborn, H. R. and Varanasi, U. (1991). Effects of exposure to a contaminated-sediment extract on the metabolism and disposition of 17β-estradiol in English sole (*Parophrys vetulus*). *Comp. Biochem. Physiol.* **99C**, 231-240.

Stephanou, E. and Giger, W. (1982). Persistent organic chemicals in sewage effluents. 2. Quantitative determinations of nonylphenols and nonylphenol ethoxylates by glass capillary gas chromatography. *Environ. Sci. Technol.* **16**, 800-805.

Stephens, S. M., Alkindi, A. Y. A., Waring, C. P. and Brown, J. A. (1997). Corticosteroid and thyroid responses of larval and juvenile turbot exposed to the water soluble fraction of crude oil. *J. Fish Biol.* **50**, 953-964.

Sterligova, O. P., Pavlovskij, S. A. and Komulainen, S. F. (1988). Reproduction of coregonids in the eutrophied Lake Sjamozero, Karelian ASSR. *Finnish Fish. Res.* **9**, 485-488.

Stock, J. N. and Cope, O. B. (1969). Some effects of TEPA, an insect chemosterilant, on the guppy, *Poecilia reticulata. Trans. Am. Fish. Soc.* **2**, 280-287.

Stone, H. and Overnell, J. (1985). Non-metallothionein cadmium binding proteins. *Comp. Biochem. Physiol.* **80C**, 9-14.

Strüssmann, C. A. and Patiño, R. (1995). Temperature manipulation of sex differentiation in fish. *In* "Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, Austin, Texas, 2-8 July, 1995", (F. W. Goetz and P. Thomas, Eds.), pp. 153-157. Fish Symposium 95, Austin.

Sukumar, A. and Karpagaganapathy, P. R. (1992). Pesticide-induced atresia in ovary of a freshwater fish, *Colisa ialia* (Hamilton-Buchanan). *Bull. Environ. Contam. Toxicol.* **48**, 457-462.

Sumpter, J. P. (1990). General concepts of seasonal reproduction. *In* "Reproductive Seasonality in Teleosts", (A. D. Munro, A. P. Scott and T. J. Lam, Eds.), pp. 14-31. CRC Press, Boca Raton.

Sumpter, J. P. and Jobling, S. (1993). Male sexual development in a 'sea of oestrogen'. *The Lancet* **342**, 124-125.

Sumpter, J. P. and Jobling, S. (1996). Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environ. Health Perspect.* **103 Suppl. 7**, 173-178.

Sumpter, J. P., Jobling, S. and Tyler, C. R. (1996). Oestrogenic substances in the aquatic environment and their potential impact on animals, particularly fish. *In* " Toxicology of Aquatic Pollution. Physiological, Molecular and Cellular Approaches", (E. W. Taylor, Ed.), pp. 206-224. Soc. Exp. Biol. Seminar Series **57**, Cambridge University Press, Cambridge

Sundararaj, B. I. and Goswami, S. V. (1966). Effects of mammalian hypophysial hormones, placental gonadotrophins, gonadal hormones, and adrenal corticosteroids on ovulation and spawning in hypophysectomized catfish, *Heteropneustes fossilis* (Bloch). *J. Exp. Zool.* **161**, 287-296.

Swanson, P. (1991). Salmon gonadotropins: Reconciling old and new ideas. *In* "Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, University of East Anglia, Norwich, U. K., 7-12 July 1991", (A. P. Scott, J. P. Sumpter, D. E. Kime and M. S. Rolfe, Eds.), pp. 2-7., FishSymp91, Sheffield, U. K.

Swift, D. J. (1981). Changes in selected blood component concentrations of rainbow trout, *Salmo gairdneri* Richardson, exposed to hypoxia or sublethal concentrations of phenol or ammonia. *J. Fish Biol.* **19**, 45-61.

Szabo, A., Ruby, S. M., Rogan, F. and Amit, Z. (1991). Changes in brain dopamine levels, oocyte growth and spermatogenesis in rainbow trout, *Oncorhynchus mykiss*, following sublethal cyanide exposure. *Arch. Environ Contam. Toxicol.* **21**, 152-157.

Tachikawa, M., Sawamura, R., Takeda, A., Okada, S., and Nara, M. (1987). Differences between fresh- and sea-water fishes in the accumulation and effect of environmental chemical pollutants. II. Intakes of chlordane and pentachlorophenol by freshwater acclimated mullet (*Mugil cephalus*). *Eisei Kagaku* **3**, 405-408.

Takeda, H. and Shimizu, C. (1982). Purification of metallothionein from the liver of skipjack and its properties. *Bull. Jap. Soc. Sci. Fish.* **48**, 717-723.

Tam, W. H. and Payson, P. D. (1986). Effects of chronic exposure to sublethal pH on growth, egg production, and ovulation in brook trout, *Salvelinus fontinalis*. *Can. J. Fish. Aquat. Sci.* **43**, 275-280.

Tam, W. H., Birkett, L., Makaran, R., Payson, P. D., Whitney, D. K. and Yu, C. K.-C. (1987). Modification of carbohydrate metabolism and liver vitellogenic function in brook trout (*Salvelinus fontinalis*) by exposure to low pH. *Can. J. Fish Aquat. Sci.* **44**, 630-635.

Tam, W. H., Fryer, J. N., Valentine, B. and Roy, R. J. J. (1990). Reduction in oocyte production and gonadotrope activity, and plasma levels of estrogens and vitellogenin, in brook trout exposed to low environmental pH. *Can. J. Zool.* **68**, 2468-2476.

Tanasichuk, R. W. and Ware, D. M. (1987). Influence of interannual variations in water temperature on fecundity and egg size in Pacific herring (*Clupea harengus pallasi*). *Can. J. Aquat. Sci.* **44**, 1485-1495.

Tanner, D. K. and Knuth, M. L. (1996). Effects of esfenvalerate on the reproductive success of the bluegill sunfish, Lepomis macrochirus in littoral enclosure. *Arch. Environ. Contam. Toxicol.* **31**, 244-251.

Tate, L. G. (1988). Characterization of phase-I and phase-II drug metabolism and the effect of β-naphthoflavone in the liver and posterior kidney of the channel catfish, *Ictalurus punctatus*. *Arch. Environ. Contam. Toxicol.* **17**, 325-354.

Tattersfield, L., Matthiessen, P., Campbell, P., Grandy, N. and Lange, R. (Eds.). (1997). SETAC-Europe/OECD/EC Expert Workshop on Endocrine Modulators and Wildlife: Assessment and Testing (EMWAT), Veldhoven, The Netherlands, 10-13 April 1997. SETAC-Europe, Amsterdam.

Tesch F. W. (1977). "The eel: biology and management of anguilloid eels." (English Edition, P. H. Greenwood, Ed.), Chapman and Hall, London.

Thomas, P. (1988). Reproductive endocrine function in female Atlantic croaker exposed to pollutants. *Mar. Environ. Res.* **24**, 179-183.

Thomas, P. (1989). Effects of Arochlor 1254 and cadmium on reproductive endocrine function and ovarian growth in Atlantic croaker. *Mar. Environ. Res.* **28**, 499-503.

Thomas, P. (1990a). Teleost model for studying the effects of chemicals on female reproductive endocrine function. *J. Exp. Zool.* **Suppl. 4**, 126-128.

Thomas, P. (1990b). Molecular and biochemical responses of fish to stressors and their potential use in environmental monitoring. *Am. Fish Soc. Symp.* **8**, 9-28.

Thomas, P. (1993). Effects of cadmium on gonadotropin secretion from Atlantic croaker pituitaries incubated *in vitro*. *Mar. Environ. Res.* **35**, 141-145.

Thomas, P. and Smith, J. (1993). Binding of xenobiotics to the estrogen receptor of spotted seatrout: a screening assay for potential estrogenic effects. *Mar. Environ. Res.* **35**, 147-151.

Thomas, P. and Wofford, H. W. (1993). Effects of cadmium and Arochlor 1254 on lipid peroxidation, glutathione peroxidase activity, and selected antioxidants in Atlantic croaker tissue. *Aquat. Toxicol.* **27**, 159-178.

Thomas, P., Woodin, B. R. and Neff, J. M. (1980). Biochemical responses of the striped mullett *Mugil cephalus* to oil exposure I. Acute responses - interrenal activations and secondary stress responses. *Mar. Biol.* **59**, 141-149.

Thomas, P., Wofford, H. W. and Neff, J. M. (1981). Biochemical stress response of striped mullet to fluorene analogs. *Aquat. Toxicol.* **1**, 329-342.

Thomas, D. G., Solbe, J. F. de L. G., Kay, J. and Cryer, A. (1983). Environmental cadmium is not sequestered by metallothionein in rainbow trout. *Biochem. Biophys. Res. Comm.* **110**, 584-592.

Timmers, R. J. M. and Lambert, J. G. D (1987). Measurement of aromatase activity in the brain of the African catfish, *Clarias gariepinus* - a comparison of two assay methods. *Comp. Biochem. Physiol.* **88B**, 453-456.

Toledo, M. C. F. and Jonsson, C. M. (1992). Bioaccumulation and elimination of endosulfan in zebra fish (*Brachydanio rerio*). *Pest. Sci.* **36**, 207-211.

Tomasso, J. R., Davis, K. B. and Simco, B. A. (1981). Plasma corticosteroid dynamics in channel catfish (*Ictalurus punctatus*) exposed to ammonia and nitrate. *Can. J. Fish. Aquat. Sci.* **38**, 1106-1112.

Tremblay, L., Yao, Z. and Van der Kraak, G. (1995). Interactions of the environmental estrogens nonylphenol and β-sitosterol with liver estrogen receptors in fish. *In* "Proc. 5th Int. Symp. Reprod. Physiol. Fish, Austin Texas July 2-8, 1995" (F. W. Goetz and P. Thomas, Eds.), p. 202, Fish Symposium 95, Austin.

Tripp, R. A., Maule, A. G., Schreck, C. B. and Kaattari, S. L. (1987). Cortisol-mediated suppression of salmonid lymphocyte responses *in vitro*. *Dev. Comp. Immunol.* **11**, 565-576.

Truscott, B., Walsh, J. M., Burton, M. P., Payne, J. F. and Idler, D. R. (1983). Effect of acute exposure to crude petroleum on some reproductive hormones in salmon and flounder. *Comp. Biochem. Physiol.* **75C**, 121-130.

Truscott, B., Idler, D. R. and Fletcher, G. L. (1992). Alteration of reproductive steroids of male winter flounder (*Pleuronectes americanus*) chronically exposed to low levels of crude oil in sediments. *Can. J. Fish. Aquat. Sci.* **49**, 2190-2195.

Tsuda, T., Aoki, S., Kojima, M. and Harada, H. (1989). Bioconcentration and excretion of diazinon, IBP, malathion and fenitrothion by willow shiner. *Toxicol. Environ. Chem.* **24**, 185-190.

Tsuda, T., Aoki, S., Kojima, M. and Harada, H. (1990). Bioconcentration and excretion of diazinon, IBP, malathion and fenitrothion by carp. *Comp. Biochem. Physiol.* **96C**, 23-26.

Tsuda, T., Aoki, S., Inoue, T. and Kojima, M. (1994). Accumulation and excretion of pesticides used as insecticides or fungicides in agricultural products by the willow shiner *Gnathopogon caerulescens*. *Comp. Biochem. Physiol.* **107C**, 469-473,

Tulasi, S. J., Reddy, P. U. M. and Rao, J. V. R. (1989). Effects of lead on the spawning potential of the fresh water fish, *Anabas testudineus*. *Bull. Environ. Contam. Toxicol.* **43**, 858-863.

Tyler, C. R. and Sumpter, J. P. (1996). Oocyte growth and development in teleosts. *Rev. Fish Biol. Fisheries.* **6**, 287-318.

Tyler, C. R., Sumpter, J. P., Kawauchi, H. and Swanson, P. (1991). Involvement of gonadotropin in the uptake of vitellogenin into vitellogenic oocytes. *Gen. Comp. Endocrinol.* **84**, 291-299.

Uthe, J. F., Chou, C. L. and Musial, C. J. (1984). Research in fisheries contaminants and toxicology conducted by the contaminants research unit, Fisheries and Environmental Sciences Division, Halifax Component in 1983. *Can. Man. Rep. Fish Aquat. Sci. No. 1757,* 12 pp.

Van den Berg, M., Blank, F., Heeremans, C.,, Wagenaar, H. and Olie, K. (1987). Presence of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in fih-eating birds and fish from the Netherlands. *Arch. Environ. Contam. Toxicol.* **16**, 149-158.

Van den Heever, D. J. and Frey, B. J. (1994). Human health aspects of the metal zinc and copper in tissue of the African sharptooth catfish, *Clarias gariepinus*, kept in treated sewage effluent and in the Krugersdrift Dam. *Water S. A.* **20**, 205-212.

Van den Hurk, R. and Lambert, J. G. D. (1983). Ovarian steroid glucuronides function as sex pheromones for male zebrafish, *Brachydanio rerio*. *Can. J. Zool.* **61**, 2381-2387.

Van den Hurk, R., Schoonen, W. G. E. J., van Zoelen, G. A. and Lambert, J. G. D. (1987). The biosynthesis of steroid glucuronides in the testis of the zebrafish, *Brachydanio rerio*, and their pheromonal function as ovulation inducers. *Gen. Comp. Endocrinol.* **68**, 179-188.

Van der Kraak, G. J., Munkittrick, K. R., McMaster, M. E., Portt, C. B. and Chang, J. P. (1992). Exposure to bleached kraft pulp mill effluent disrupts the pituitary-gonadal axis of white sucker at multiple sites. *Toxicol. Appl. Pharmacol.* **115**, 224-233.

Van der Weiden, M. E. J., Celander, M., Seinen, W., van den Berg, M., Goksoyr, A. and Forlin, L. (1993). Induction of cytochrome P450 1A in fish treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or chemically contaminated sediment. *Environ. Toxicol. Chem.* **12**, 989-999.

Van der Weiden, M. E. J., de Vries, L. P., Fase, L. P., Celander, M., Seinen, W. and Van den Berg, M. (1994). Relative potencies of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) for cytochrome P450 1A induction in the mirror carp (*Cyprinus carpio*). *Aquat. Toxicol.* **29**, 163-182.

Varanasi, U., Stein, J. E. and Nishimoto, M. (1989). Biotransformation and disposition of PAH in fish. *In* "Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment", (U. Varanasi, Ed.), pp. 93-150. CRC Press, Boca Raton.

Verbost, P. M., Flik, G., Lock, R. A. C. and Wendelaar Bonga, S. E. (1987). Cadmium inhibition of Ca^{2+} uptake in rainbow trout gills. *Am. J. Physiol.* **253**, R216-221.

Verma, S. R. and Tonk, I. P. (1983). Effect of sublethal concentrations of mercury on the composition of liver, muscles and ovary of *Notopterus notopterus*. *Water Air Soil Poll.* **20**, 287-292.

Viarengo, A., Bettella, E., Fabbri, R., Burlando, B. and Lafaurie, M. (1997). Heavy metal inhibition of EROD activity in liver microsomes from the bass *Dicentrarchus labrax* exposed to organic xenobiotics: Role of GSH in the reduction of heavy metal effects. *Mar. Environ. Res.* **44**, 1-11.

Victor, B., Mahalingam, S. and Sarojini, R. (1986). Toxicity of mercury and cadmium on oocyte differentiation and vitellogenesis of the teleost, *Lepidocephalichthys thermalis* (Bleeker). *J. Environ. Biol.* **7**, 209-214.

Vignier, V., Berthon, F., Dreano, Y. and Folch, H. H. (1985). Dibenzothiophen sulphoxidation: a new and fast high-performance liquid chromatographic assay of mixed-function oxidation. *Xenobiotica* **15**, 991-999.

Vignier, V., Vandermeulen, J. H. and Fraser, A. J. (1992). Growth and food conversion by Atlantic salmon parr during 40 days' exposure to crude oil. *Trans. Am. Fish. Soc.* **121**, 322-332.

Vijayan, M. M., Flett, P. A. and Leatherland, J. F. (1988). Effects of cortisol on the *in vitro* hepatic conversion of thyroxine to tri-iodothyronine in brook charr (*Salvelinus fontinalis* Mitchill). *Gen. Comp. Endocrinol.* **70**, 312-318.

Vijayan, M. M., Feist, G., Otto, D. M. E., Schreck, C. B. and Moon, T. W. (1997). 3,3',4,4'-tetrachlorobiphenyl affects cortisol dynamics and hepatic function in rainbow trout. *Aquat. Toxicol.* **37**, 87-98.

Villeneuve, D. L., Crunkilton, R. L. and DeVita, W. M. (1997). Aryl hydrocarbon receptor-mediated toxic potency of dissolved lipophilic organic contaminants collected from

Lincoln Creek, milwaukee, Wisconsin, USA, to PLHC-1 (*Poeciliopsis lucida*) fish hepatoma cells. *Environ. Toxicol. Chem.* **16**, 977-984.

Vindimian, E., Namour, P., Migeon, B. and Garric, J. (1991). *In situ* pollution induced cytochrome P450 activity of freshwater fish - barbel (*Barbus barbus*), chub (*Leuciscus cephalus*) and nase (*Chondrostoma nasus*). *Aquat. Toxicol.* **21**, 255-266.

Vodicnik, M. J., Elcombe, C. R. and Lech, J. J. (1981). The effect of various types of inducing agents on hepatic microsomal monooxygenase activity in rainbow trout. *Toxicol. Appl. Pharmacol.* **59**, 553-558.

Vom Saal, F. S., Montano, M. M. and Wang, M. H. (1992). Sexual differentiation in mammals. *In* "Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection", (T. Colborn and C. Clement, Eds.), pp.17-82. Princeton Scientific Publishing, Princeton.

Von der Decken, A., Olin, T., and Bergqvist, P.-A. (1992). Physiological properties of liver, gonads and muscle during maturation of female Atlantic salmon, *Salmo salar*. Comparison between a control and xenobiotics containing fish feed. *Comp. Biochem. Physiol.* **101C**, 525-529.

Von Westernhagen, H. D., Rosenthal, H., Dethlefsen, V., Ernst, W., Harms, U. and Hansen, P. D. (1981). Bioaccumulating substances and reproductive success in Baltic flounder, *Platichthys flesus*. *Aquat. Toxicol.* **1**, 85-99.

Von Westernhagen, H., Dethlefsen, V., Cameron, P. and Janssen, D. (1987). Chlorinated hydrocarbon residues in gonads of marine fish and effects on reproduction. *Sarsia* **72**, 419-422.

Von Westernhagen, H., Cameron, P., Dethlefsen, V. and Janssen, D. (1989). Chlorinated hydrocarbons in North Sea whiting (*Merlangus merlangus* L.), and effects on reproduction: I. Tissue burden and hatching success. *Helgolaender Meeresuntersuchungen* **43**, 45-60.

Vuorinen, P. J. and Vuorinen, M. (1985). Effects of bleached kraft mill effluent on reproduction of brown trout (*Salmo trutta* L.) on a restricted diet. *Finnish Fish. Res.* **6**, 92-105.

Vuorinen, P. J., Vuorinen, M. and Peuranen, S. (1990). Long-term exposure of adult whitefish (*Coregonus watmanni*) to low pH/aluminium: effects on reproduction, growth, blood composition and gills. *In* " Acidification in Finland", (P. Kauppi, P. Anttila and K. Kenttämies, Eds.), pp. 941-961. Springer Verlag, London.

Vuorinen, P. J., Vuorinen, M., Peuranen, S., Rask, M., Lappalainen, A. and Raitaniemi, J. (1992). Reproductive status, blood chemistry, gill histology and growth of perch (*Perca fluviatilis*) in three acidic lakes. *Environ. Poll.* **78**, 19-27.

Walczak, B. Z., Hammer, U. T. and Huang, P. M. (1986). Ecophysiology and mercury accumulation of rainbow trout (*Salmo gairdneri*) when exposed to mercury in various concentrations of chloride. *Can. J. Fish. Aquat. Sci.* **43**, 710-714

Walker, M. K., Spitsbergen, J. M., Olsen, J. R. and Petersen, R. E. (1991). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity during early life stage development of lake trout (*Salvelinus namaycush*). *Can. J. Fish. Aquat. Sci.* **48**, 875-883.

Walton, D. G., Fancy, L. L., Greens, J. M., Kiceniuk, J. W. and Penrose, W. R. (1983). Seasonal changes in aryl hydrocarbon hydroxylase activity of a marine fish *Tautogolabrus adspersus* with and without petroleum exposure. *Comp. Biochem. Physiol.* **76C**, 246-253.

Wang, X., Porter, W., Krishnan, V., Narasimhan, T. R. and Safe, S. (1993). Mechanism of 2,3,7,8-tetrachlorodibenzodioxin (TCDD)-mediated decrease of the nuclear estrogen receptor in MCF-7 human breast cancer cells. *Mol. Cell. Endocrinol.* **96**, 159-166.

Wannemacher, R., Rebstock, A., Kulzer, E., Schrenk, D. and Bock, K. W. (1992). Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on reproduction and oogenesis in zebrafish (*Brachydanio rerio*). *Chemosphere* **24**, 1361-1368.

Waring, C. P. and Moore, A. (1997). Sublethal effects of a carbamate pesticide on pheromonal mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. *Fish Physiol. Biochem.* **17**, 203-211

Waring, C. P., Brown, J. A., Collins, J. E. and Prunet, P. (1996a). Plasma prolactin, cortisol, and thyroid responses of the brown trout (*Salmo trutta*) exposed to lethal and sublethal aluminium in acidic soft waters. *Gen. Comp. Endocrinol.* **102**, 377-385.

Waring, C. P., Stagg, R. M., Fretwell, K., McLay, H. A. and Costello, M. J. (1996b). The impact of sewage sludge exposure on the reproduction of the sand goby, *Pomatoschistus minutus*. *Environ. Poll.* **93**, 17-25.

Warner, R. R. (1997). Sperm allocation in coral reef fishes: Strategies for coping with demands on sperm production. *Bioscience* **47**, 561-564

Warner, R. R., Sharpio, D. Y., Marcanato, A. and Petersen, C. W. (1995). Sexual conflict: Males with highest mating success convey the lowest fertilization benefits to females. *Proc. Roy. Soc. London Ser. B* **262**, 135-139

Watanabe, S., Shimada, T., Nakamura, S., Nishiyama, N., Yamashita, N., Tanabe, S. and Tatsukawa, R. (1989). Specific profile of liver microsomal cytochrome P450 in dolphin and whales. *Mar. Environ. Res.* **27**, 51-65.

Weber, D. N., Eisch, S., Spieler, R. E. and Petering, D. H. (1992). Metal redistribution in largemouth bass (*Micropterus salmoides*) in response to restrainment stress and dietary cadmium: Role of metallothionein and other metal-binding proteins. *Comp. Biochem. Physiol.* **101C**, 255-262.

Wedemeyer, G. (1968). Uptake and distribution of Zn^{65} in the coho salmon egg (*Oncorhynchus kisutch*). *Comp. Biochem. Physiol.* **26**, 271-279.

Weiner, G. S., Schreck, C. B. and Li, H. W. (1986). Effects of low pH on reproduction of rainbow trout. *Trans. Am. Fish. Soc.* **115**, 75-82.

Weis, P. (1974). Ultrastructural changes induced by low concentrations of DDT in the livers of the zebrafish and the guppy. *Chem. Biol. Interact.* **8**, 25-30.

Weis, J. S. and Khan, A. A. (1990). Effects of mercury on the feeding behavior of the mummichog, *Fundulus heteroclitus* from a polluted habitat. *Mar. Environ. Res.* **30**, 243-250.

Weisbart, M., Chakraborti, P. K., Gottschall and Freeman, H. C. (1989). Effect of acid environments on cortisol and cortisol receptor activity in Atlantic salmon, *Salmo salar*. *Fish Physiol. Biochem.* **7**, 267-272.

Wester, P. W. (1991). Histopathological effects of environmental pollutants β-HCH and methyl mercury on reproductive organs in freshwater fish. *Comp. Biochem. Physiol.* **100C**, 237-239.

Wester, P. W. and Canton, J. H. (1986). Histopathological study of *Oryzias latipes* (medaka) after long-term β-hexachlorocyclohexane exposure. *Aquat. Toxiocol.* **9**, 21-45.

Wester, P. W. and Canton, J. H. (1987). Histopathological study of *Poecilia reticulata* (guppy) after long-term exposure to bis(tri-n-butyltin)oxide (TBTO) and di-n-butyltindichloride (DBTC). *Aquat. Toxicol.* **10**, 143-165.

Wester, P. W. and Canton, J. H. (1992). Histopathological effects in *Poecilia reticulata* (guppy) exposed to methyl mercury chloride. *Toxicol. Pathol.* **20**, 81-92.

Wester, P. W. and Roghair, C. J. (1994). Monitoring of sublethal chronic effects in fish: the pathomorphological approach. *In* "Sublethal and Chronic Effects of Pollution on Freshwater Fish", (R. Müller and R. Lloyd, Eds.), pp. 7-14. Fishing News Books, Oxford.

Wester, P. W., Canton, J. H. and Bisschop, A. (1985). Histopathological study of *Poecilia reticulata* (guppy) after long-term β-hexachlorocyclohexane exposure. *Aquat. Toxicol.* **6**, 271-296.

White, R. D., Hahn, M. E., Lockhart, W. L. and Stegeman, J. J. (1994). Catalytic and immunochemcal characterization of hepatic microsomal cytochromes P450 in beluga whale (*Delphinapterus leucas*). *Toxicol. Appl. Pharmacol.* **126**, 45-57.

White-Stevens, R. (Ed.) (1971). "Pesticides in the Environment". Vol. 1, Part I. Marcel Dekker, New York

Whitehead, C. and Brown, J. A. (1989). Endocrine responses of brown trout, *Salmo trutta* L., to acid, aluminium and lime dosing in a Welsh hill stream. *J. Fish. Biol.* **35**, 59-71.

Whittier, J. M. and Crews, D. (1987). Seasonal reproduction: Patterns and control. *In* "Hormones and Reproduction in Fishes, Amphibians and Reptiles", (D. O. Norris and R. E. Jones, Eds.), pp. 385-409. Plenum Press, New York.

WHO (World Health Organization). (1986). Organophosphorus pesticides: A general introduction. *In* "Environmental Health Criteria", Vol. 63. WHO, Geneva.

Wicklund, A., Runn, P. and Norrgren, L. (1988). Cadmium and zinc interactions in fish: Effects of zinc on the uptake, organ distribution, and elimination of cadmium-109 in the zebrafish, *Brachydanio rerio. Arch. Environ. Contam. Toxicol.* **17**, 345-354.

Wicklund, A., Norrgren, L, and Runn, P. (1990). The influence of cadmium and zinc on cadmium turnover in the zebrafish, *Brachydanio rerio. Arch Environ. Contam. Toxicol.* **19**, 348-353

Wickstrom, K. and Pyysalo, H. (1981). Organochlorine compounds in liver of cod (*Gadus morhua*) in the Northern Baltic. *Chemosphere* **10**, 999-1004.

Williams, D. E. and Buhler, D. R. (1982). Purification of cytochromes P-448 from β-naphthoflavone-treated rainbow trout. *Biochim. Biophys. Acta.* **717**, 398-404.

Williams, D. E. and Buhler, D. R. (1984). Benzo[*a*]pyrene hydroxylase catalyzed by purified isozymes of cytochrome P-450 from β-naphthoflavone-fed rainbow trout. *Biochem. Pharmacol.* **33**, 3743-3753.

Wilson, C. E., Crim, L. W. and Morgan, M. J. (1995). The effects of stress on spawning performance and larval development in Atlantic cod, *Gadus morhua. In* "Proceedings of the 5th International Symposium on Reproductive Physiology of Fish, Austin 1995", (F. W. Goetz and P. Thomas, Eds.), p. 198. Fish Symposium 95, Austin.

Wofford, H. W. and Thomas, P. (1984). Interactions of cadmium with sulfhydryl-containing compounds in striped mullet (*Mugil cephalus* L.). *Mar. Environ. Res.* **14**, 119-137.

Wofford, H. W. and Thomas, P. (1988a). Effect of xenobiotics on peroxidation of hepatic microsomal lipids from striped mullet (*Mugil cephalus*) and Atlantic croaker (*Micropogonias undulatus*). *Mar. Environ. Res.* **24**, 285-289.

Wofford, H. W. and Thomas, P. (1988b). Peroxidation of mullet and rat liver lipids *in vitro*: effects of pyridine nucleotides, iron, incubation buffer, and xenobiotics. *Comp. Biochem. Physiol.* **89C**, 201-205.

Wren, C. D. and MacCrimmon, H. R. (1986). Comparative bioaccumulation of mercury in two adjacent freshwater ecosystems. *Water Res.* **20**, 763-770.

Yadav, A. K. and Singh, T. P. (1986). Effect of pesticide on circulating thyroid hormone levels in the freshwater catfish, *Heteropneustes fossilis* (Bloch). *Environ. Res.* **39**, 136-142.

Yadav, A. K. and Singh, T. P. (1987a). Pesticide induced changes in peripheral thyroid hormone levels during different reproductive phases in *Heteropneustes fossilis. Ecotox. Environ. Saf.* **13**, 97-103.

Yadav, A. K. and Singh, T. P. (1987b). Pesticide-induced impairment of thyroid physiology in the freshwater catfish, *Heteropneustes fossilis. Environ. Poll.* **43**, 29-38.

Yadav, A. K. and Singh, T. P. (1987c). Effect of pesticides on thyroid peroxidase activity in *Heteropneustes fossilis* (Teleostei, Clariidae): An *in vitro* study. *Zool. Jb. Physiol.* **91**, 333-336.

Yamamoto, T. (1969). Sex differentiation. *In* "Fish Physiology ,Vol. 3", (W. S. Hoar and D. J. Randall, Eds.), pp.117-175. Academic Press, New York and London.

Yano, T. and Matsuyama, H. (1986). Stimulatory effect of PCB on the metabolism of sex hormones in carp hepatopancreas. *Bull. Jap. Soc. Sci. Fish.* **52** 1847-1852.

Yao, Z., Van der Kraak, G. J. and Squires, E. J. (1996). Effect of heavy metals on *in vitro* vitellogenin production from cultured hepatocytes of goldfish. *Proc. 3rd Int. Symp. Fish Endocrinol., Hakodate, 27-31 May, 1996*, p. 172.

Yasuno, M., Hatakeyama, S. and Miyashita, M. (1980). Effects on reproduction in the guppy (*Poecilia reticulata*) under chronic exposure to temephos and fenitrothion. *Bull. Environ. Contam. Toxicol.* **25**, 29-33.

Yeoh, C-G., Schreck, C. B., Fitzpatrick, M. S. and Feist. G.W. (1996a). *In vivo* steroid metabolism in embryonic and newly hatched steelhead trout (*Oncorhynchus mykiss*). *Gen. Comp. Endocrinol.* **102**, 197-209.

Yeoh, C-G., Schreck, C. B., Feist. G.W. and Fitzpatrick, M. S. (1996b). Endogenous steroid metabolism is indicated by fluctuations of endogenous steroid and steroid glucuronide levels in early development of the steelhead trout *(Oncorhynchus mykiss). Gen. Comp. Endocrinol.* **103**, 107-114.

Young, G., Brown, C. L., Nishioka, R. S., Folmar, L. C., Andrews, M., Cashman, J. R. and Bern, H. A. (1994). Histopathology, blood chemistry, and physiological status of normal and moribund striped bass (*Morone saxatilis*) involved in summer mortality ('die-off') in the Sacramento-San Joaquin Delta of California. *J. Fish Biol.* **44**, 491-512.

Zabel, E. W. and Peterson, R. E. (1996). TCDD-like activity of 2,3,6,7-tetrachloroxanthene in rainbow trout early life stages and in a rainbow trout gonadal cell line (RTG-2). *Environ. Toxicol. Chem.* **15**, 2305-2309.

Zabel, E. W., Pollenz, R. and Peterson, R. E. (1996). Relative potencies of individual polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and biphenyl congeners and congener mixtures based on induction of cytochrome P4501A mRNA in a rainbow trout gonadal cell line. *Environ. Toxicol. Chem.* **15**, 2310-2348.

Zacharewski, T., Campbell, P., Routledge, E., Cooper, R., Kime , D., Wenzel, A. and Huet, M-C. (1997). Current approaches for the use of *in vitro* tests in identifying the hazard of endocrine modulating chemicals to wildlife., *In* "SETAC-EUROPE/OECD/EC Expert Workshop on Endocrine Modulators and Wildlife - Assessment and Testing (EMWAT), Veldhoven, The Netherlands 10-13 April 1997." (L. Tattersfield, P. Matthiessen, P. Campbell, N. Grandy and R. Lange,Eds.), Chapter 3, pp.41-58. SETAC-Europe, Amsterdam.

Zahn, T., Hauck, C. and Braunbeck, T. (1993). Cytological alterations in fish fibrocytic R1 cells as an alternative test system for the detection of sublethal effects of environmental pollutants: A case-study with 4-chloroaniline. *In* "Fish in Ecotoxicology and Ecophysiology", (T. Braunberger, W. Hanke and H. Segner, Eds.), pp. 103-126. VCH, Weinheim and New York.

Zahn, T. Hauck, C., Holzschuh and Braunbeck, T. (1995). Acute and sublethal toxicity of seepage water from garbage dumps to permanent cell lines and primary cultures of hepatocytes from rainbow trout (*Oncorhynchus mykiss*): A novel approach to environmental risk assessment for chemicals and chemical mixtures. *Zbl. Hyg.* **722**, 1-22

Zitko, V. and Saunders, R. L. (1979). Effect of PCBs and other organochlorine compounds on the hatchability of Atlantic salmon (*Salmo salar*) eggs. *Bull. Environ. Contam. Toxicol.* **21**, 125-130.

Species Key

Abramis brama = bream
Acipenser baeri = Siberian sturgeon
Ambloplites rupestri = rock bass
Anguilla anguilla = European eel

Bass,
- rock = *Ambloplites rupestris*
- sea = *Centropristis striata*
- smallmouth = *Micropterus dolomieui*
- white = *Morone chrysops*

Bluegill = see *Lepomis macrochirus*
Brachydanio rerio, see Danio rerio
Bream = *Abramis brama*
Bullhead = *Cottus gobio*
- brown = *Ictalurus nebulosus*

Burbot = *Lota lota*

Carassius auratus = goldfish
Carp, common = *Cyprinus carpio*
Catastomus commersoni = white sucker
Catfish
- African = *Clarias gariepinus*
- channel = *Ictalurus punctatus*
- Indian = *Heteropneuestes fossilis*
- walking = *Clarias batrachus*

Centropristis striatus = sea bass
Channa punctatus = murrel
Charr, Lake Geneva = *Salvelinus alpinus*
Chub, lake = *Couesius plumbeus*
Clarias batrachus = walking catfish
Clarias gariepinus = African catfish
Clupea harengus = herring
Cod = *Gadus morhua*
Colisa fasciatus = perch
Coregonus clupeaformis = lake whitefish
Coregonus lavaretus = whitefish
Coregonus watmanni = whitefish
Cottus gobio = bullhead
Couesius plumbeus = lake chub
Croaker,
- Atlantic= *Micropogonias undulatus*
- White = *Genyonemus lineatus*

Cynoscion nebulosus = spotted sea trout
Cyprinidon nevadensis = pupfish
Cyprinidon variegatus = sheepshead minnow

Cyprinus carpio = common carp

Dab = *Limanda limanda*
Danio rerio = zebrafish, zebra danio

Eel
  European = *Anguilla anguilla*
  ricefield = *Monopterus albus*
Esox lucius = northern pike

Flagfish, American = *Jordanella floridae*
Flounder,
  = *Platichthys flesus*
  = *Pleuronectes flesus*
  starry = *Platichthys stellatus*
  winter = *Pleuronectes americanus*
Fundulus heteroclitus = killifish
  = mummichog

Gadus morhua = cod
Gambusia affinis = mosquitofish
Gasterosteus aculeatus = stickleback
Genyonemus lineatus = white croaker
Goldfish = *Carassius auratus*
Guppy = *Poecilia reticulata*

Herring = *Clupea harengus*
  lake = *Coregonus artedii*
  Pacific = *Clupea harengus*
Heteropneuestes fossilis = Indian catfish
Hippoglossoides elassodon = flathead sole

Ictalurus nebulosus = brown bullhead
Ictalurus punctatus = channel catfish

Jordanella floridae = American flagfish

Killifish = *Fundulus heteroclitus*
  least = *Heterandia formosa*
Leiostomus xanthurus = spot
Lepidopsetta bilineata = rock sole
Lepomis auritus = redbreast sunfish
Lepomis gibbosus = pumpkinseed sunfish
Lepomis macrochirus = bluegill sunfish
Lota lota = bubot

Medaka = *Oryzias latipes*
Micropogonias undulatus = Atlantic croaker
Micropterus dolomieui = smallmouth bass
Minnow = *Phoxinus phoxinus*
  fathead = *Pimephales promelas*
  sheepshead = *Cyprinidon variegatus*
Merlangus merlangus = whiting
Monopterus albus = ricefield eel
Morone americana = white perch
Morone chrysops = white bass
Mosquitofish = *Gambusia affinis*
Mugil cephalus = striped mullet
Mullet, striped = *Mugil cephalus*
Mummichog = *Fundulus heteroclitus*
Murrel = *Channa punctatus*

Oncorhynchus kitsutch = coho salmon
Oncorhynchus mykiss= rainbow trout
Oncorhynchus tshawytscha = chinook salmon
Oreochromis aureaus = tilapia
Oreochromis mossambicus = tilapia
Oryzias latipes = medaka

Paddlefish = *Polyodon spathula*
Parophrys vetulus = English sole
Perca flavescens = *yellow perch*

Perca fluviatilis = perch
Perch
= *Perca fluviatilis*
= *Colisa fasciatus*
white = *Morone americanus*
yellow = *Perca flavescens*
Percopsis omiscomaycus = troutperch
Phoxinus phoxinus = minnow
Pike, northern = *Esox lucius*
Pimephales promelas = fathead minnow
Platichthys flesus = flounder
Platichthys stellatus = starry flounder
Pleuronectes americanus = winter flounder
Pleuronectes flesus = flounder
Poecilia reticulata = guppy
Polyodon spathula = paddlefish
Pseudopleuronectes americanus, see *Pleuronectes americanus*
Pupfish = *Cyprinidon nevadensis*

Roach = *Rutilus rutilus*
Rutilus rutilus = roach

Salmo gairdneri, see Oncorhynchus mykiss
Salmo salmo = Atlantic salmon
Salmo trutta = brown trout
Salmon
Atlantic = *Salmo salmo*
chinook = *Oncorhynchus tshawytscha*
coho = *Oncorhynchus kitsutch*
Salvelinus alpinus
Salvelinus fontinalis = brook trout
Salvelinus namaycush = lake trout
Sauger = *Stizostedion canadense*
Scup = *Stenotomus chrysops*
Sole = *Solea solea*
English = *Parophrys vetulus*
flathead = *Hippoglossoides elassodon*
rock = *Lepidopsetta bilineata*
Solea solea = sole
Spot = *Leiostomus xanthurus*
Stenotomus chrysops = scup
Stickleback = *Gasterosteus aculeatus*
Stizostedion canadense = sauger
Stizostedion vitreum = walleye
Sturgeon, Siberian = *Acipenser baeri*
Sucker, white = *Catastomus commersoni*
Sunfish,
bluegill = *Lepomis macrochirus*
pumpkinseed = *Lepomis gibbosus*
redbreast = *Lepomis auritus*

Tilapia,
= *Oreochromis aureaus*
= *Oreochromis mossambicus*
= *Tilapia nilotica*
= *Tilapia leucosticta*
Tilapia leucosticta = tilapia
Tilapia nilotica = tilapia
Trout,
brook = *Salvelinus fontinalis*
brown = *Salmo trutta*
lake = *Salvelinus namaycush*
rainbow = *Oncorhynchus mykiss*
steelhead = *Oncorhynchus mykiss*
spotted sea = *Cynoscion nebulosus*
Troutperch = *Percopsis omiscomaycus*

Walleye = *Stizostedion vitreum*
Whitefish,
= *Coregonus lavaretus*
= *Coregonus watmanni*
lake = *Coregonus clupeaformis*
Whiting = *Merlangus merlangus*

Zebrafish = D*anio rerio*

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