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Biomarkers for Environmental Monitoring

Suggestions for Norwegian monitoring programs

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Biomarkers for Environmental Monitoring - Suggestions for Norwegian monitoring programmes

Sammendrag - summary

In Norway, monitoring of chemical contamination is performed within four different environmental monitoring programs; monitoring of hazardous substances in Norwegian fjords and coastal waters (MILKYS), monitoring of pollutants in large lakes, monitoring of pollutants in urbanized fjords, and monitoring of pollutants in terrestrial and urbanized environments. Besides chemical concentrations, a number of biological markers (biomarkers) are included in MILKYS.

This report evaluates if the used biomarkers are useful in relation to the aim of the program and if biomarkers could be useful within the three other programs. A large number of biomarkers were assessed and ranked according to specificity for chemicals, ecological relevance, ability to provide early warning, ability to detect different types of chemicals and mixtures, current status in environmental monitoring and feasibility of the analysis. The following biomarkers are suggested to be used:

Fjords and coastal waters (MILKYS): EROD (or CYP1A), PAH-metabolites in bile, vitellogenin in fish, aromatase in fish and liver somatic index (LSI) in fish
Large lakes: EROD (or CYP1A), vitellogenin in fish, aromatase in fish, liver somatic index (LSI) in fish and macroscopic liver neoplasms in fish
Urbanized fjords: Micronuclei test in fish, skeletal deformities in fish, antioxidants in fish liver (Cat, GST, GR), macroscopic liver neoplasms in fish and total oxyradical scavenging capacity (TOSC) in blue mussel
Terrestrial and urbanized environment: Congenital malformations in birds, egg shell thickness for carnivorous birds, hatching success and brood size for birds, EROD in birds and gonadal aromatase activity in brown rat

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Preface

EnviroPlanning AB has been commissioned by the Norwegian Environment Agency (Miljødirektoratet) to perform a literature study of biomarkers in order to identify and suggest biomarkers for Norwegian environmental monitoring of pollutants. The aim of the study was to find biomarkers that:

- are relevant for biological effects relating to changes high in the biological hierarchy (individual, population, community or ecosystem)
- are relevant for the current Norwegian monitoring programs in fjords and coastal waters, large lakes, urbanized fjords and terrestrial and urbanized environments
- include long-term effects
- are relevant for mixture toxicity

The report has been written by Ph.D. Niklas Hanson, M.Sc. Maja Halling and M.Sc. Helena Norin. The report has been reviewed by Professor Åke Larsson and Ph.D. Tim Hipkiss.

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Göteborg, november 2013

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Sammendrag

Overvåking av miljøgifter fremskaffer informasjon om forekomst og tidstrend av kjemikalier i miljøet. Slik miljøovervåking blir i dag gjennomført gjennom fire statlige programmer som fokuserer på ulike naturmiljøer: "Miljøgifter langs kysten" (MILKYS), "Store innsjøer", "Miljøgifter i en urban fjord" og "Miljøgifter i terresterisk og bynært miljø". MILKYS er det mest omfattende av disse, og er en del av norske rapporteringsforpliktelser til Oslo Paris konvensjonen (OSPAR) for Nordøst-Atlanteren. I tillegg til kjemiske nivåer, rapporteres også en rekke biologiske effektmålinger (biomarkører). Formålet med denne rapporten har vært å evaluere om biomarkørene som har blitt benyttet har bidratt med nyttig informasjon i tråd med målsetningene i overvåkingsprogrammet, og om biomarkører kan være til nytte i miljøgiftovervåkingens øvrige tre måleprogrammer.

Miljøovervåking i andre land har med hell benyttet biomarkører for deteksjon av eksponeringsgrad og kartlegging av biologiske effekter. Utallige biomarkører har blitt benyttet, mange er under utvikling, og alle har de sine styrker og svakheter. I denne rapporten har en lang rekke biomarkører blitt gjennomgått for å avgjøre potensiell nytte i norsk statlig miljøovervåking. Biomarkørene er hentet fra ulike miljøovervåkingsprogrammer i utlandet, samt fra vitenskapelig litteratur. De ble videre poengsatt ut fra fem sentrale kriterier: spesifisitet for kjemikalier, økologisk relevanse, anvendelse som tidlig varslingssignal, anvendelse for blandingsgiftighet og analytisk gjennomførbarhet. Kriteriene ble videre benyttet til å rangere de ulike biomarkørenes nytteverdi innenfor miljøovervåking. Ettersom de ulike overvåkingsprogrammene kan ha ulike forurensningsproblemstillinger, ble det utarbeidet rangerte biomarkørlister med hovedvekt på tre ulike scenarioer: Høy økologisk relevans, anvendelse for blandingsgiftighet og anvendelse som tidlig varslingssignal. Foreslåtte biomarkører for de fire forskjellige overvåkingsprogrammene er oppsummert i tabellen under:

Foreslåtte biomarkører for norsk miljøovervåking

Miljøgifter langs kysten (MILKYS)	Miljøgifter i store innsjøer	Miljøgifter i en urban fjord	Miljøgifter I terrestrisk og bynært miljø
EROD (eller CYP1A)	EROD (eller CYP1A)	Micronucleus test i fisk	Misdannelser hos fugl
PAH-metabolitter i galle	Vitellogenin i fisk	Skjelettdeformasjon i fisk	Eggskalltykkelse hos rovfugl
Vitellogenin i fisk	Aromatase i fisk	Antioksidantenzymer i fiskelever (Cat, GST, GR)	Klekkesuksess og kullstørrelse hos fugl
Aromatase i fisk	Leversomatisk indeks (LSI) i fisk	Makroskopiske celleforandringer i fiskelever	EROD i fugl
Leversomatisk indeks (LSI) i fisk	Makroskopiske celleforandringer i fiskelever	Total oxyradical scavenging capacity (TOSC) i blåskjell	Aromatase i rotte

Summary

Environmental monitoring of chemicals is performed to provide information about the distribution of chemicals in the environment and their temporal trends. In Norway, monitoring of chemical contamination is performed within four different programs, which are focused on different environments. The programs are monitoring of hazardous substances in Norwegian fjords and coastal waters (MILKYS), monitoring of pollutants in large lakes, monitoring of pollutants in urbanized fjords, and monitoring of pollutants in terrestrial and urbanized environments. MILKYS is the largest of the programs, and the results are reported to OSPAR. Besides chemical concentrations, a number of biological markers (biomarkers) are included in MILKYS. In the present report, it is evaluated if the biomarkers that are used provide useful information in relation to the aim of the program. Furthermore, it is evaluated if biomarkers could be useful within the three other programs.

Biomarkers have successfully been used in environmental monitoring and assessment around the world to detect exposure to and effects of chemicals. Many different biomarkers have been used or are under development, and they all have different strengths and weaknesses. In the present report, a large number of biomarkers were assessed for their potential use in Norwegian monitoring programs. The biomarkers were collected from monitoring programs in various countries and from the scientific literature. The biomarkers were given scores for five properties that were considered important for environmental monitoring. Those were specificity for chemicals, ecological relevance, ability to provide early warning, ability to detect different types of chemicals and mixtures, current status in environmental monitoring, and feasibility of the analysis. Based on the properties, the biomarkers were then ranked for usefulness in environmental monitoring. As different properties are valued in different monitoring scenarios, three different rankings of biomarkers were created. In the different rankings, higher weight was put on ecological relevance, ability to detect mixture effects, or ability to act as an early warning signal. Based on the results, biomarkers were suggested for the four different monitoring programs according to the table below.

Suggested biomarkers for Norwegian environmental monitoring

Fjords and coastal waters (MILKYS)	Large lakes	Urbanized fjords	Terrestrial and urbanized environment
EROD (or CYP1A)	EROD (or CYP1A)	Micronuclei test in fish	Congenital malformations in birds
PAH-metabolites in bile	Vitellogenin in fish	Skeletal deformities in fish	Egg shell thickness for carnivorous birds
Vitellogenin in fish	Aromatase in fish	Antioxidants in fish liver (Cat, GST, GR)	Hatching success and brood size for birds
Aromatase in fish	Liver somatic index (LSI) in fish	Macroscopic liver neoplasms in fish	EROD in birds
Liver somatic index (LSI) in fish	Macroscopic liver neoplasms in fish	Total oxyradical scavenging capacity (TOSC) in blue mussel	Gonadal aromatase activity in brown rat

1. Introduction

1.1 Ecological risk of chemicals

Although most chemicals have contributed positively to human society, e.g., by providing food and welfare, there are also numerous examples of chemicals that have caused harm to human health as well as the ecosystem. For ecosystems around the world this has, for example, resulted in loss of biodiversity (Preston and Shackelford 2002). Ecological risk assessment (ERA) is performed, to reduce the risk for environmental harm (Suter 1993). ERA is most often based on simple toxicity tests, such as acute mortality for small crustaceans and fish, and growth inhibition for algae. From the tests, a presumed safe concentration is established. If the environmental concentration is not expected to exceed this concentration following normal use, it is assumed that the chemical can be used without harming the environment. However, there are substantial difficulties involved in extrapolating results from short experiments in the laboratory to long term effects in the environment (Cairns and Mount 1990). To deal with this, safety factors are used. It has been shown that although these safety factors are usually protective, they can also sometimes be underproductive (Ahlers et al 2006). It could be argued that if ERA does not fully protect the environment, higher safety factors should be used. However, this is complicated as it would also mean that more chemicals that do not pose an environmental risk are unnecessarily restricted or banned (Hanson and Stark 2012). As a result of current ERA practices, it cannot be expected that the environment is protected from all potential chemical threats. Furthermore, ERA is performed for one chemical at a time, while they occur in mixtures in the environment. The total toxicity of those mixtures is often unknown. There are also many chemicals for which ERA has never been performed. Environmental monitoring is a necessary tool to detect environmental threats that, for whatever reason, have not been detected by ERA.

1.2 Environmental monitoring

Environmental monitoring of chemicals is carried out to provide information about the distribution of chemicals in the environment and their temporal trends. Monitoring can be performed by measuring concentrations of the actual substances of interest (contamination) or by using biological endpoints to estimate pollution from chemicals. The different strategies are described below.

1.2.1 Monitoring of chemical concentrations

Monitoring of chemical concentrations is the most frequently used method for environmental monitoring of chemicals. However, there are limitations with this approach that reduces the usefulness for environmental management. The most important limitation is that chemical measurements only provide information about the chemicals that are included in the analysis (Wernersson 2012). Although it is possible to monitor a large number of substances, the cost soon becomes unmanageable. Furthermore, the substances that were emitted to the environment may have been transformed so that they are no longer covered by the analytical method, and for many substances no analytical method has been developed. This means that monitoring of chemicals in the environment cannot be expected to provide information about unexpected or unknown environmental threats. In simpler monitoring situations, such as a single industrial discharge, this may not be a problem as the most important pollutants are often known. However, for diffuse pollution or complex mixtures of chemicals, the mentioned limitations can be considerable.

One example that highlights the limitations with chemical monitoring is the recent interest in perfluorooctanesulfonic acid (PFOS), which was brought to attention in the early 2000s (Lehmler 2005). This attention resulted in that PFOS was included in environmental monitoring programs and that it was regulated to reduce risk. In 2010, a retrospective

analysis of PFOS in guillemot (*Uria aalge*) eggs from Stora Karlsö in the Baltic Proper showed a dramatic increase in PFOS from 1970 to 2010 (Figure 1). However, the increase occurred before PFOS was recognized as an environmental risk and included in monitoring programs. Environmental monitoring did, thus, not provide any valuable information about the increasing risk posed by PFOS. It is highly likely that other chemicals are increasing in the environment in a similar way today, but are missed by routine chemical monitoring as they have not yet been recognized as environmental threats.

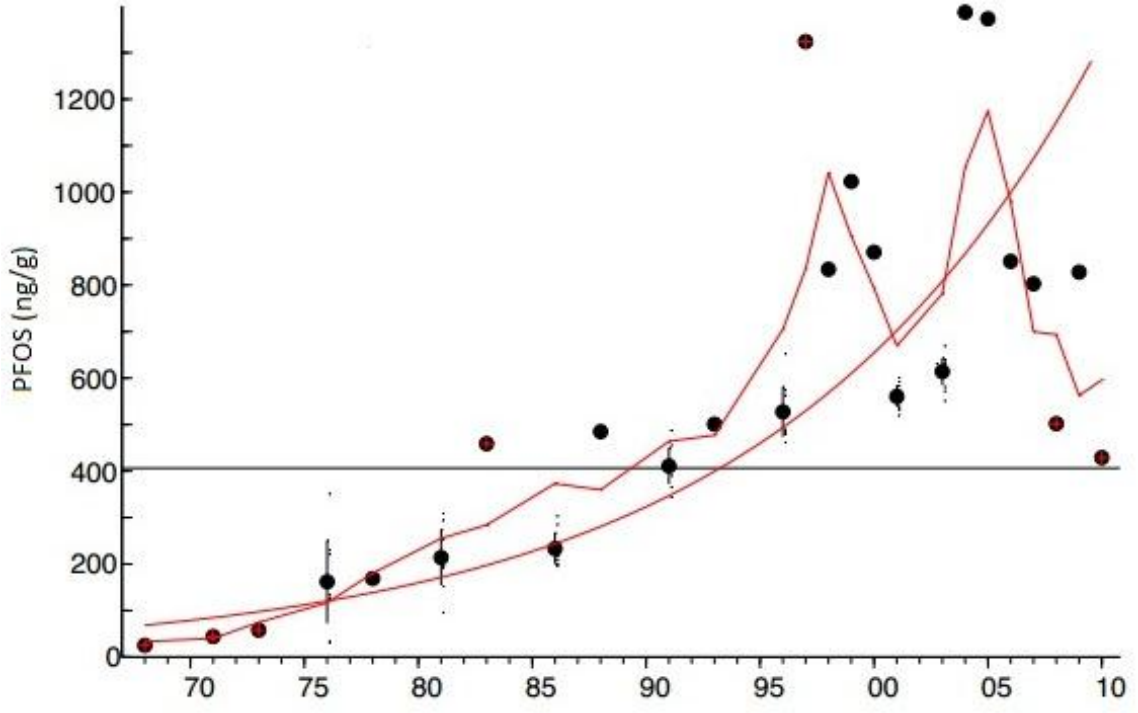


Figure 1. The change in concentration of perfluorooctanesulfonic acid (PFOS) over time. The measurements were performed retrospectively in guillemot eggs from Stora Karlsö in the Baltic Proper. The red dots in the figure are based on pooled samples or mean values of individual samples. The curves show logarithmic increase and the moving average. The figure is modified from Bignert et al (2012).

Another limitation with environmental monitoring of chemical concentrations is that the same concentration in two different areas may have very different effects on the ecosystem. This is because environmental and ecological factors may affect the uptake and toxicity of chemicals (Whitfield 2001). Those factors include temperature, salinity, age structure and diversity. Interactions with environmental factors, or with other chemicals in the environment, may be both synergistic and antagonistic (Cairns and Mount 1990).

In every monitoring program, it is essential to have pre-defined assessment criteria to which the results are compared (unless the aim is solely to monitor for trends). For concentrations of chemicals, it is relatively easy to set such criteria. For many chemicals, toxicity data are available that can be used to determine safe environmental concentrations. For other chemicals, where such data are not available, background levels from reference sites can be used as guidance. If the reference sites retain satisfactory ecological values, it is assumed that those concentrations are safe. For species that are used for human consumption, safe concentrations are often based on human exposure through food.

Table 1 lists the most important benefits and drawbacks with environmental monitoring based on chemical measurements.

Table 1
Benefits and drawbacks with environmental monitoring based on chemical measurements

Benefits	Drawbacks
Provides detailed information about the concentrations of specific chemicals	Provides no information about chemicals other than those included in the analysis, and often not even about degradation products of the monitored chemicals
Easily related to legal limit values / Easy to define limit values	Mixture effects and interactions between chemicals (synergistic as well as antagonistic) are not included
Easily standardized methods with high precision and repeatability	Interactions with the environment (temperature, pH, turbidity etc) are not included

1.2.2 Biological monitoring

As emphasis is increasingly shifting from known point sources to diffuse pollution (Crathorne et al 1996) and mixtures of known and unknown pollutants, chemical monitoring may not be satisfactory in all situations. An alternative to chemical monitoring is biological monitoring, or biomonitoring. Most commonly, the endpoint of biological monitoring is abundance of a species or a measure of biodiversity. For example, community structure for benthic macrofauna has been widely used to determine environmental stress in the aquatic environment (Ingole et al 2006) and biotic indices based on the presence or absence of certain indicator species are often used to simplify the interpretation (Bustos-Baez and Frid 2003, Roberts et al 1998).

The major benefit of biological monitoring is that the ecological relevance is high, which also means that relevance for environmental management is high. Other benefits of biological monitoring are that bioavailability always is included, and mixture effects of known and unknown chemicals often are included. Furthermore, interactions with the environment are automatically included in the biological response, which may be beneficial as it increases the ecological relevance.

There are also a number of drawbacks with biomonitoring, compared to chemical monitoring. In most cases, it is difficult to determine the cause of an observed alteration. Thereby, it may be difficult to determine the most cost effective action to reduce risk. Methods for biomonitoring are also often difficult to standardize, leaving more room for subjective interpretations of the results. The establishment of assessment criteria, or critical effect size (CES), is also more complicated due to the inherent variability in biological systems (Munkittrick et al 2009). This means that there is a substantial risk of reaching the wrong conclusion (Hanson 2010). A final drawback with biological monitoring is that effects are not seen until the populations or communities are significantly affected. It is desirable to have methods that provide early warning so that effects on higher levels can be avoided.

Table 2 lists the most important benefits and drawbacks with biomonitoring that is based on abundance and/or diversity.

Table 2
Benefits and drawbacks with biomonitoring that is based on abundance and/or diversity

Benefits	Drawbacks
High ecological relevance	Difficult to link effects to specific chemicals or sources
Includes mixture effects and interactions between chemicals (synergistic as well as antagonistic)	Confounding factors (temperature, pH, turbidity etc)
Includes bioavailability and interactions with the environment	Methods may be difficult to standardize (depending on ecosystem and species). Precision and repeatability are often low.
	Difficult to define critical effect size (CES).

1.2.3 Biomarkers

The concept of biological monitoring can be extended to include sub-organismal measurements. These are often referred to as biomarkers (or biological markers) and include biochemical responses as well as physiological and histopathological alterations caused by chemicals (Shugart 1996). The use of biomarkers has its origin in human toxicology where they have proved to be very useful as measures of exposure to chemicals as well as to provide early warning signals for specific diseases (Timbrell 1998). Biomarkers can be considered as a shortcut where the mode of action itself is monitored, rather than monitoring all the chemicals that have that particular mode of action. Thereby, the number of monitored parameters can be significantly reduced. For example, the biomarker EROD (ethoxyresorufin O-deethylase) provides information about the exposure to dioxins, planar PCBs, certain PAHs and other chemicals with similar structures. Thereby, one single measure can replace many chemical measurements.

As with other kinds of environmental monitoring, there are both benefits and drawbacks with the use of biomarkers. Because of the diversity among biomarkers, however, it is not possible to list benefits and drawbacks as for chemical monitoring based on concentrations (Table 1) and biomonitoring based on abundance (Table 2). As a general rule, it can be said that biomarkers that are measured at higher levels of biological organization have higher ecological relevance and lower specificity (Figure 2). However, there are exceptions to this. As chemicals have their primary effect on lower levels of organization, such as the protein that they interact with in the cell, measurements at lower levels of organization can be assumed to act as early warning signals for effects at higher levels (Figure 2).

It must be noted that not all biomarkers are good biomarkers and that it is necessary to be aware of potential confounding factors (Forbes et al 2006). The confounding factors can be both biological (e.g., species, age, sex, genetic population, feeding status, reproductive phase) and environmental (e.g., temperature, oxygen concentration, pH, salinity). Because of these factors, it is necessary to standardize monitoring and only use well studied species in well studied environments. For example, Traven et al (2013) did not see an induction in EROD-activity in adult sea bass (*Dicentrarchus labrax*) at a site that was highly contaminated with PAHs. Numerous other studies have shown that EROD is very sensitive to PAH contamination in a number of species (van der Oost et al 2003). It is likely that the different responses are caused by confounding biological and/or environmental factors. When such factors are not controlled for (e.g., by standardization), the study cannot be expected to provide useful information for management (McCarty and Munkittrick 1996).

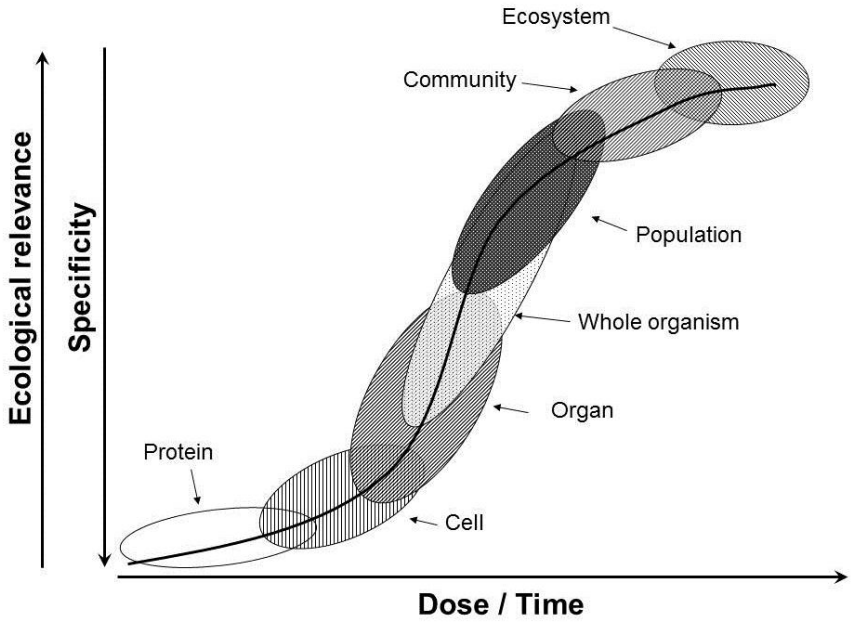


Figure 2. Biomarkers with high ecological relevance are often found at higher hierarchical levels. Biomarkers with high specificity are often found at lower levels of biological organization.

In the present report, biomarkers are defined as measurements in whole organisms or at lower levels of biological organization. Measurements that are based on counting or sex determination of broods are here considered as sub-organismal measurements that represent reproduction of individuals.

2. Environmental monitoring in Norway

Here a short description of relevant Norwegian monitoring programs is presented. In the monitoring program for hazardous substances in fjords and coastal waters (MILKYS), a few biomarkers are already in use (described below). How well these biomarkers work in relation to the goals of the program is evaluated in a separate section of this report.

2.1 Monitoring in fjords and coastal waters (MILKYS)

The monitoring program for hazardous substances in Norwegian fjords and coastal waters (MILKYS) is the Norwegian contribution to OSPAR's coordinated monitoring program (CEMP). The aim of CEMP is to provide data that can aid to assess anthropogenic impact on the marine environment in the North East Atlantic, and provide a basis for management. The Norwegian contribution to CEMP focuses on levels, trends and effects of hazardous substances. The program includes areas exposed to known point sources, areas exposed to diffuse pollution, and areas that can be expected to have background levels of contaminants. The chemicals that are monitored are mainly traditional pollutants such as metals, dioxins, PCB and brominated flame retardants. Of the monitored sites, 78.5% were classified as insignificantly polluted. Only 0.5% were classified as extremely polluted (the highest level of concern). From a total of 1035 time series, 52 positive trends (i.e. indicating increasing levels of contaminants) and 277 negative (decreasing) trends are observed (NIVA, 2012). If the

monitored substances are representative for the total environmental load of contaminants, this could be taken as evidence of decreasing contamination. As discussed above, there is an obvious bias in environmental monitoring towards chemicals that are confirmed environmental risks. This means that the monitored chemicals often are already regulated, or even banned. Therefore, decreasing trends are often expected and may not be representative of the total environmental exposure. However, they show if environmental management, such as restrictions of chemicals, provides the expected results.

The monitoring program for hazardous substances in fjords and coastal waters (MILKYS) also include four biomarkers in fish (Atlantic cod, *Gadus morhua*). These are OH-pyrene (PAH-metabolite), activity of the enzyme δ-aminolevulinic acid dehydratase (ALA-D) in blood, concentration of the protein CYP1A in liver, and EROD-activity in liver. These biomarkers were selected for specificity, robustness and because they are recommended by international organizations (OSPAR, ICES). All of these can be said to be very specific biomarkers of exposure. OH-pyrene is specific to PAHs, ALA-D is relatively specific to lead, and CYP1A and EROD are specific to chemicals that bind to the aryl hydrocarbon (Ah) receptor (planar organic chemicals such as dioxin, PCBs and PAHs). OH-pyrene, CYP1A and EROD all indicates higher exposure levels in the inner Oslo fjord compared to more pristine areas. OH-pyrene also indicates higher exposure levels in Sørfjord. ALA-D indicates higher exposure levels in both inner Oslo fjord and Sørfjord, compared to reference sites (NIVA 2012).

Imposex in dog whelk (*Nucella lapillus*) is another biomarker that is used in the monitoring program. This is a measure of the degree of masculinization of female dog whelk. Imposex is a highly specific biomarker for tributyltin (TBT). However, imposex has also very high ecological relevance and is, thus, an exception to the general rule presented in Figure 2. Imposex trends are generally negative, indicating that the ban on TBT is working.

2.2 Other Norwegian monitoring programs

Besides the larger program for monitoring of hazardous substances in Norwegian fjords and coastal waters (MILKYS), there are three more specific monitoring programs ("Monitoring of pollutants in large lakes", "Pollutants in urbanized fjords" and "Pollutants in terrestrial and urbanized environments") where biomarkers may add value. The three programs are described below. These programs are all newly started and there is, hence, no data available. None of these programs presently include biomarkers.

2.2.1 Monitoring of pollutants in large lakes

The program is intended to investigate the presence of mercury, PCB, perfluorinated compounds (PFC) and polybrominated diphenyl ethers (PBDE) in four larger lakes in Norway. Furthermore, siloxanes and chloroparaffins will be monitored in one lake each. In three of these lakes, at least two trophic levels will be examined to estimate biomagnification and other food web related effects. These levels include zooplankton, smelts, whitefish and trout. Stable isotopes will be used to determine trophic levels.

2.2.2 Pollutants in urbanized fjords

This monitoring program is intended to detect pollutants that are used in the human society and, by different processes, reach the environment. The program covers four sites in the Oslo fjord and will include zooplankton, shrimp, flatfish, blue mussels and polychaetes. As different trophic levels are monitored, it will be possible to investigate biomagnification and other food web related effects. The program will also include measurements in stormwater. The pollutants that will be monitored are metals, DDT, PCB, perfluorinated alkylated substances (PFAS), hexabromocyclododecane (HBCDD), PBDE, chloroparaffins, siloxanes, phosphorus flame retardants (PFR), bisphenol A, tetrabromobisphenol A, octyl-/nonylphenol and antifouling chemicals.

2.2.3 Pollutants in terrestrial and urbanized environments

This is the only program that does not focus on the aquatic environment. The aim of the program is to provide information regarding the risks for health and environment in terrestrial and urbanized areas. Several trophic levels will be used to provide information about biomagnification of pollutants in the food chain. The data from the program is also intended to provide information that can be used to estimate the total toxic load and mixture effects for terrestrial animals. The species to be monitored are earthworms, European pied flycatcher, red fox, brown rat, golden eagle and herring gull. The analyzes will include metals, PCB, PBDE, PFC, siloxanes and chloroparaffins. Stable isotopes will be analyzed to determine trophic levels of the different species.

3. Environmental monitoring with biomarkers in other countries

Besides Norway, biomarkers are used in national and regional monitoring programs in several countries around the world. Biomarkers have also been used in numerous research projects where the aim has been to investigate the presence of contaminants in the environment. Below are examples of environmental monitoring programs that included biomarkers.

3.1 Sweden

Biomarkers have been used in Sweden for monitoring of coastal fish since 1988. The program now includes four sites along the Swedish coast and the two species perch (*Perca fluviatilis*) and eelpout (*Zoarces viviparous*). All sites are chosen to represent background levels of pollution. This means that they are located far from known large point sources and large population centers. Approximately 25 biomarkers are analyzed annually, although there have been some changes throughout the years. Table 3 shows the biomarkers that are or have been used within the program, roughly divided into categories.

Table 3
Biomarkers used in Swedish monitoring of coastal fish

Repro-duction	De-toxification	Oxidative stress	Geno-toxicity	Metabolism / Energy partitioning	Immune defense	Oxygen transport (blood)	Ion balance
Gonad somatic index (GSI)	EROD-activity[1]	GR-activity[3]	DNA-adducts	Blood glucose	White blood cells	Hematocrit (HT)	Cl^-
Vitellogenin in males	MT[2]	GST activity[4]		Liver somatic index (LSI)	Macrophage centers	Hemoglobin (Hb)	Ca^{2+}
Vitellogenin in females		Catalase				Immature red blood cells (iRBC)	Na^+
Primary sex ratio							K^+

1 Ethoxyresorufin O-deethylase activity
2 Metallothionein
3 Glutathione reductase activity
4 Glutathione S-transferase activity

About ten of these biomarkers have shown significant temporal trends for at least one site for each species. One example of such a biomarker is EROD, which has been increasing significantly in four out of five time series, and the fifth is almost significant (Larsson et al 2012). This indicates an increasing exposure to contaminants that bind to the Ah-receptor. It is noticeable that this time trend is seen at geographically very distinct monitoring sites. Follow up studies have shown that EROD-activity correlated with run-off from land (Hanson et al 2009a) and to the occurrence of PAH-metabolites in bile (Hanson et al 2009b and unpublished data). However, more recent studies have also shown that recolonization of soft bottom fauna in the area correlates to the highest peaks in EROD-activity. A plausible explanation is that "old" contaminants are released due to bioturbation. Among the other biomarkers in fish that show significant time trends at several sites are GSI (reduced at two sites), GST (reduced at four sites) and the relative number of white blood cells (reduced at three sites). The trend in biomarkers cannot be explained by any of the traditional contaminants that are measured in the areas, for example metals, DDT, PCB and HCH (hexachlorocyclohexane). EROD and GSI have been considered as the most useful biomarkers in this program. This is because they, toghether, provide both high precision and high ecological relevance. DNA-adducts has been omitted from the program as it has not shown any effects and is relatively expensive. Also MT has been omitted from the program, but due to analytical difficulties.

Imposex in netted dog whelk (*Hinia nitida*) is used as a biomarker for exposure to and effects of TBT in Swedish coastal waters. This monitoring program is focused on areas where the levels of TBT can be expected to be high, such as marinas. As in Norwegian monitoring, trends in imposex are generally negative after the ban of TBT in antifouling paints (Magnusson et al 2012). Simultaneous measurements of TBT concentrations show the same time trends as imposex.

The small amphipods *Monoporeia affinis* and *Pontoporeia femorata* are used to monitor contamination of sediments in the Baltic Sea. The two species are important from an ecological perspective as they serve as food for many species and thereby can introduce chemicals into the food chain. Five sites are monitored in the Bothnian Bay and nine in the Baltic Proper. Besides population density, a number of biomarkers that are related to reproduction are used, including frequency of malformed embryos, egg production and frequency of dead broods/dead eggs. The monitoring program has shown differences between the Bothnian Bay and the Baltic Proper, indicating higher exposure levels in the Baltic Proper (Sundelin et al 2007). No chemical measurements are performed on the amphipods.

The white tailed sea eagle (*Haliaeetus albicilla*) is a suitable indicator of bioaccumulating chemicals as it is found high in the food chain. The brood size of the eagles is used as an indicator of reduced fertility. Data on brood size for sea eagles have been collected since the mid-1800s. Although the older data was not intended to detect pollution, it provides a valuable reference level. The brood size fell dramatically in the 1950s and 1960s as a result of organic pollution (mostly DDT) (Helander 2000). After the ban on DDT, brood sizes became larger and are now close to the reference level (pre 1950) at most sites. However, there are indications that the positive trend has been halted or even reversed during the last few years, with reduced brood sizes (Helander and Bignert 2012). There is presently no explanation available for this trend. However, there are a number of hypotheses that are being tested. One hypothesis is that bioturbation after recolonization of marine sediments has brought old contaminants back to the surface and into the food web. Another hypothesis is that the eagles have shifted their food preference to a higher trophic level, leading to higher contamination though biomagnification.

Seals that are shot or found dead in Sweden are analyzed for a number of health related parameters that can be used as biomarkers. Examples of parameters that are analyzed are blubber thickness and the frequency of colonic ulcers. Most of the seals are grey seals (*Halichoerus grypus*) from the Baltic Sea. The blubber thickness has shown a decreasing trend since 1996 (Härkönen et al 2012). The trend may be caused by toxicants, as increased energy

costs for detoxification means less energy can be stored in blubber. It is also possible that chemicals have direct effects on fat metabolism. However, other environmental factors may contribute, such as changes in food quality or quantity. Colonic ulcers are exclusively occurring in the Baltic Sea and may be related to the larger pollution load there compared to other areas. The frequency increased from the 1980s to about 2000, followed by a small reduction (Härkönen et al 2012). It is likely that the ulcers are caused by a parasite, and that the increase in frequency is due to a reduced ability to heal the wounds, i.e. impaired immune defense. This impairment may also be caused by the higher contamination load in the Baltic Sea.

3.2 United Kingdom

The UK Clean Seas Environment Monitoring Program (CSEMP) uses a network of sites around the UK coast where different marine agencies analyse a common set of biomarkers using agreed protocols and standards. Samples of fish and benthos are taken from a total of around 45 fixed stations in intermediate and open sea areas around England and Wales. The program continuously evolves to incorporate new legislative requirements and improve the ability to detect trends. As trends are established and risks are confirmed or disproved, effort is focused on the higher risk areas.

EROD, PAH metabolites in bile, and DNA-adducts are measured in fish to estimate exposure to and effects of PAHs and other contaminants (CSEMP 2012). These biomarkers are used in an integrated assessment with chemical concentrations in sediment and biota, and biological responses at higher levels of organization (e.g. abundance). The induction of EROD activity has been consistent with the levels of certain chemicals, such as PCBs (CEFAS 2012). Fish disease and liver pathology are used as indicators of effects caused by organic contaminants (CEFAS 2012, CSEMP 2012). Table 4 lists the biomarkers in fish that are listed in the CSEMP green book. However, the monitoring program is revised continually and both biomarkers and monitored species are added and excluded (CSEMP 2012).

Table 4
Biomarkers used in UK fish monitoring

Reproduction	Detoxification	Genotoxicity	Metabolism / Energy partitioning	Liver function	Growth	Neuro-toxicity
Gonad somatic index (GSI)	EROD-activity	DNA-adducts	Liver somatic index (LSI)	Liver Pathology	Length	Acetylcholinesterase (AchE)
Vitellogenin in males	PAH bile metabolites		Fish Disease	Liver lipid content	weight	

Imposex and intersex are used as biomarkers for exposure to and effects of TBT and other contaminants in UK coastal waters. Imposex are monitored in several species, including dog whelks. Intersex is monitored in the common periwinkle (*Littorina littorea)*. As in Norway and Sweden, the incidence and severity of imposex is declining following the ban on TBT.

Metallothionein (MT), the lysosomal neutral red retention (NRR) assay to determine lysosomal membrane stability, scope for growth, and acetylcholinesterase (AChE) are monitored in blue mussel (CSEMP 2012). Information concerning clear trends or significant differences between sites is sparse. However, the generally low contaminant concentrations present in the Celtic Sea and English Channel are reflected in low biological effects.

3.3 Canada

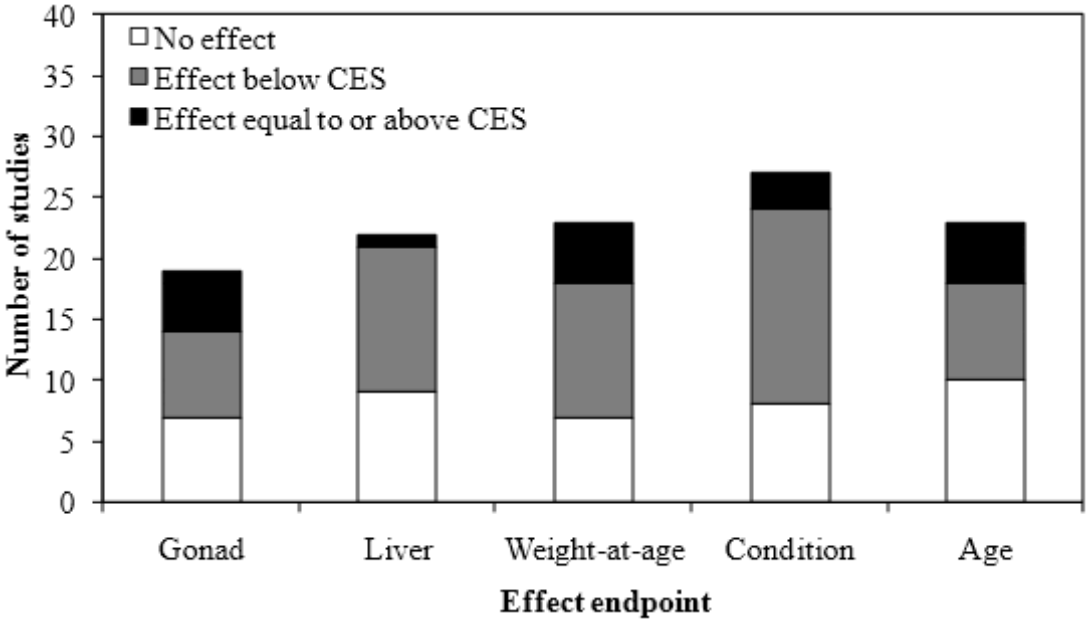
In Canada, pulp and paper and metal mining industries are required to provide data to assess their impact on the aquatic environment. Environment Canada has developed guidance documents on how to conduct the monitoring. The monitoring includes survival, growth, reproduction and condition of fish in the recipient (Table 5). The standard adult fish survey design recommends the collection of adult males and females of two sentinel species to determine if there are changes in the effect indicators between the exposure and reference areas, or along an effluent concentration gradient (Environment Canada 2010).

Table 5
Fish population survey – effect indicators and endpoints.

Effect Indicators	Effect Endpoints	Critical effect size[1]
Survival	Age	± 25%
Growth (energy use)	Size-at-age (body weight relative to age)	± 25%
Reproduction (energy use)	Relative gonad size (gonad weight to body weight)	± 25%
Condition (energy storage)	Condition (body weight to length)	± 10%
	Relative liver size (liver weight to body weight)	± 25%

[1] Percent (%) of reference mean.

Pulp and paper mills in Canada have completed five cycles of monitoring since the start in 1992. In the most recent cycle, 24 of the 32 pulp and paper mills that conducted biological monitoring studies reported an effect in at least one of the effect indicators and 12 reported at least one effect with a magnitude equal to or greater than the critical effect size (CES) (Figure 3). The number, magnitude and type of effects observed were similar to those observed in previous cycles and illustrated the same common patterns of effects, which were nutrient enrichment and metabolic disruption (gonad reduction) in fish (Environment Canada 2012a).



Figur 3. The number of fish studies in recipients to pulp and paper mill effluent that had effects above or below the critical effect size (CES). The figure is modified from Environment Canada (2012a).

Metal mining industries have conducted environmental effect monitoring since 2002. Fish collected in areas exposed to effluent showed significantly reduced condition, liver size, and

growth rate, as well as some reductions in gonad size. There were also significant effects on the population level (changed age structure). These response patterns may reflect direct effects of the effluent, or indirect effects such as habitat alteration or impact on food sources (Environment Canada 2012b).

4. "Omics" – biomarkers under development

Omics comprises a range of techniques that have in common that they study how an individual's entire genome, proteome or metabolome translates into biological functions. Omics have great potential for use as biomarkers in environmental monitoring and assessment. Omic techniques may also be important to elucidate mode of action (MOA) of the chemicals (or other stressor). The mode of action can be mechanism of toxicity as well as the biological mechanism of adaptation or response to the environmental changes. Omics will thereby be central in the validation and development of new biomarkers. However, at present the available techniques are not themselves suitable for monitoring purposes. For example, there is a need for standardization and inter-laboratory comparison (ECETOC 2010, Dowd 2012). Therefore the omics techniques are excluded from the evaluation of biomarkers for monitoring purposes. Instead, a short description of the different techniques is given below.

4.1 Transcriptomics

Transcriptomics is the study of the structure and function of the transcriptome, which is the set of all RNA molecules produced in one cell or a group of cells. By measuring the amount of RNA from a particular gene or group of genes, it is possible to estimate both exposure and effect of chemicals. The most common technologies used to investigate gene expression changes are DNA microarrays and quantitative real time polymerase chain reaction (Bourlat 2013). If the cells have been exposed to pollutants, the expression of a certain gene or genes may increase. As the sum of all RNA mirrors the genes that are actively expressed in a cell (or an organism) at a given time, they can provide very specific information about the responses to different stressors. The sum of all RNA changes can be used as a fingerprint that provides information about the specific stressor. However, it has not yet been possible to link this to health related effects (Schirmer et al 2010).

4.2 Proteomics

Environmental proteomics aims to analyse the entire set of proteins (the proteome) in organisms and to identify variations in the proteins that may be induced by chemicals. Proteomics has the potential to identify not only single proteins, but also to generate protein fingerprints that are specific for certain pollutants (Bourlat 2013). Proteomics has great potential as a technic for identifying modes of action and for developing new biomarkers. Proteins are predicted to be less transient than gene expression patterns and more stable as potential biomarkers (Denslow et al 2012). However, proteins are technically more difficult to identify than, for example, gene expression patterns.

4.3 Metabolomics

Metabolomics is the systematic study of metabolites. A fingerprint of metabolites can provide information regarding the specific cellular processes that have occurred. Exposure to

chemicals can change those processes, and thereby the fingerprint of metabolites. By using samples from urine, blood and saliva, metabolomics can be conducted without killing the organism. Metabolomics can consequently be evaluated over time in the same individual, and can be correlated to changes in the organism's physiology. With time-of-flight mass spectrometry (MS-TOF), it has become easier to identify the metabolites, and relate the results to different physiological processes. It is thereby possible to make functional interpretations of the metabolomics results. However, several challenges remain for metabolomics. For example, it is still difficult to extract meaningful data from background changes that are caused by normal metabolism (Denslow et al 2012). Despite the difficulties, Hines and co-workers (2010) managed to identify metabolic signatures that provided information about molecular MOA and predicted scope for growth (SFG) in blue mussels. This indicates that besides providing information about mechanisms, metabolomics can also provide information about health related effects.

5. Evaluation of biomarkers used in Norway

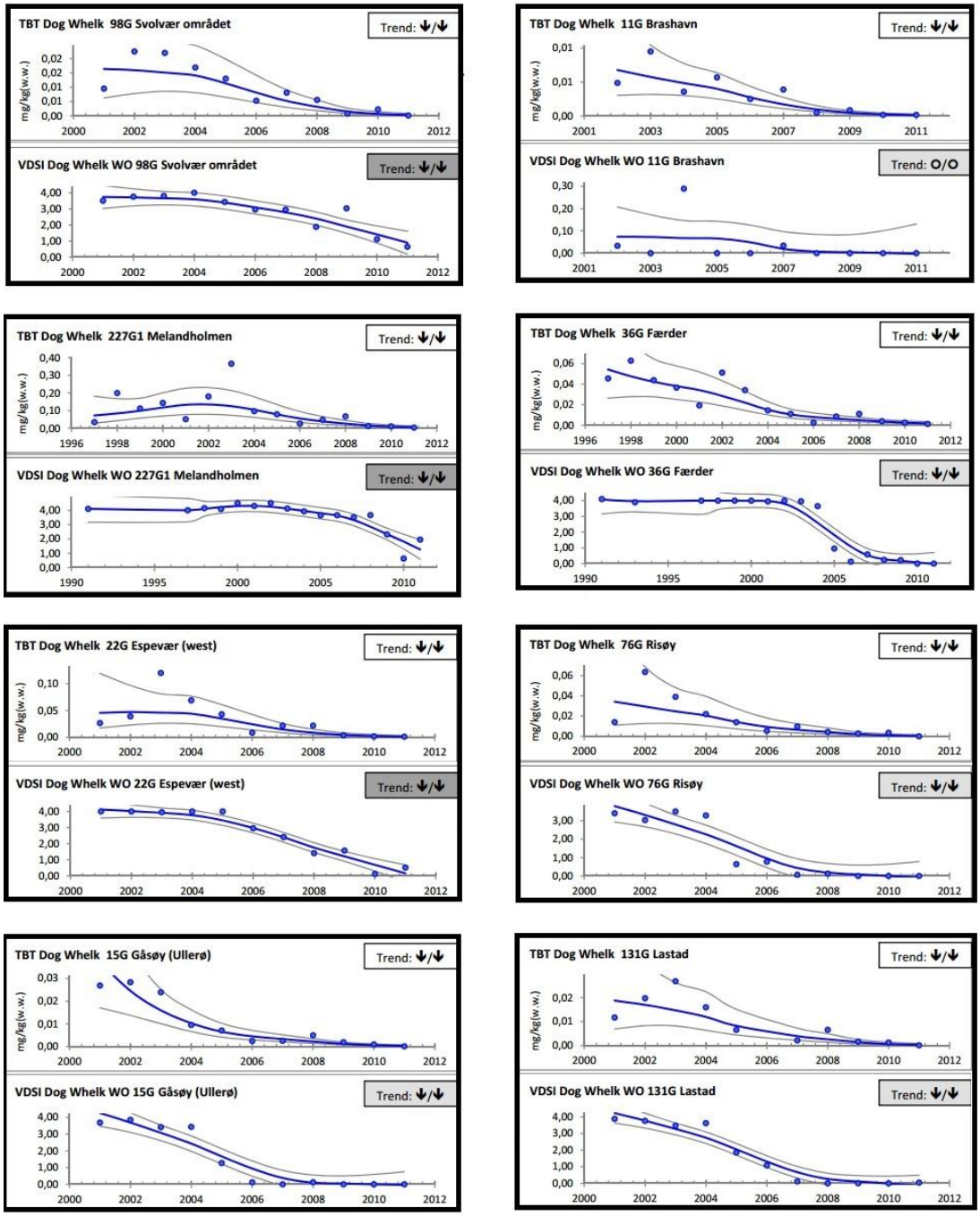
The program for environmental monitoring of hazardous substances in Norwegian fjords and coastal waters (MILKYS) includes five biological endpoints that all fall under the definition of biomarkers. Four of the biomarkers are measured in different tissues in Atlantic cod (*Gadhus morhua*). These are OH-pyrene (PAH-metabolite) in bile, activity of the enzyme δ-aminolevulinic acid dehydratase (ALA-D) in blood, concentration of the protein CYP1A in liver, and ethoxyresorufin O-deethylase (EROD) activity in liver. The fifth biomarker is imposex in dog whelk (*Nucella lapillus*), measured as the vas deferens sequence index (VDSI). The five biomarkers were selected for specificity, robustness and because they are recommended by international organizations (OSPAR, ICES). The biomarkers are included to evaluate whether organisms are exposed to contaminants in concentrations that trigger biological effects. Furthermore, the biomarkers may provide information about exposures to other chemicals than those that are monitored. The biomarkers are also expected to provide information about bioaccumulation (NIVA 2012). The five biomarkers, and their benefits and drawbacks, are discussed below.

5.1 Imposex in dog whelk

Imposex and TBT concentrations are monitored in dog whelk at eight stations. Significant negative trends in VDSI have been observed at seven stations, and negative trends in TBT concentrations have been observed at all stations (Figure 4).

The only station without a negative trend in VDSI was also the station with the lowest VDSI levels and the lowest TBT concentrations throughout the monitoring period. From Figure 4, it can be seen that after a period of about 3-4 years with TBT concentrations under 0,005 mg/kg, imposex disappears. The delayed effect on imposex can be explained by the fact that only mature dog whelks can be used to determine VDSI, while the effects may be initiated earlier during the life cycle. The delay is well in agreement with the 3-5 years generation time of dog whelk (Feare 1970). This shows that the tissue concentrations of TBT are more sensitive to changes in exposure than VDSI is. Based on this, it cannot be said that imposex is a very good early warning signal for population level effects of TBT exposure. Instead, the

main strength of imposex as a biomarker is that it provides evidence that links exposure to biological effects. This may be important in communication with stakeholders as well as the general public. Although it has been shown that other types of exposure may affect imposex (e.g., Santos et al 2008), it is clear that exposure to TBT is the main factor in environmentally realistic scenarios. Therefore, it can also not be expected that imposex is a good measure of mixture effects that include unknown chemicals. The biomarker can be said to have medium costs. Although no expensive apparatus is needed, highly skilled personnel is needed to determine the level of masculinization of the female dog whelk.



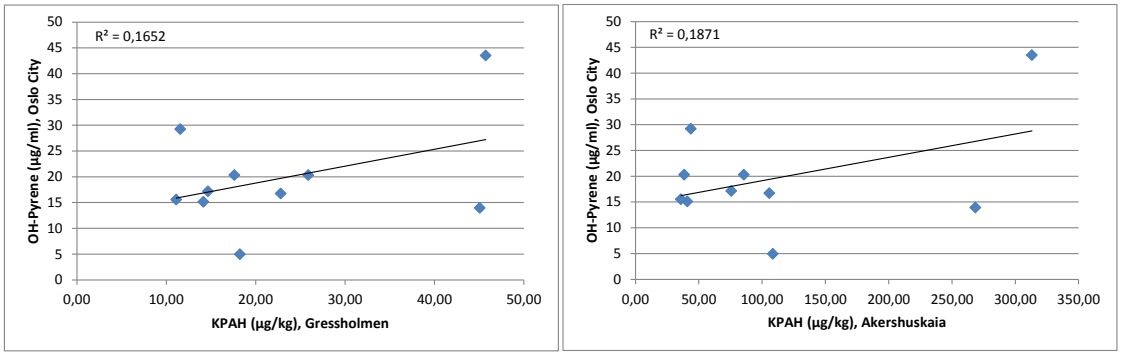
Figur 4. TBT concentrations and imposex in dog whelk. Following the ban of TBT in anti-fouling paints, both TBT concentrations and imposex has decreased dramatically. The figure is modified from NIVA (2012).

5.2 OH-pyrene in Atlantic cod bile

Measuring metabolites in bile provides information regarding chemicals that are readily metabolized and, therefore, do not bioaccumulate in tissue. PAHs are examples of such chemicals. Prescence of PAH-metabolites in bile is a commonly used biomarker for PAH-exposure. OH-pyrene is a very specific biomarker as it measures the presence of metabolized pyrene only. The main confounding factor that may affect the results is the feeding status of the fish. When the organism feeds, bile is excreted through the digestive tract. During periods between feeding, bile is accumulated in the bile bladder. When the bladder has reached its maximum volume, water is excreted from the bladder so that the volume is constant. However, endogenous or exogenous metabolites are still added to the bile, resulting in increased concentrations (Hanson and Larsson 2008). This may lead to both over- and underestimated concentrations at specific sampling occasions. However, due to the standardized method that is used within the monitoring program (Hylland et al 2009), it is unlikely that this will affect the results in terms of consistent differences between sites or temporal trends.

OH-pyrene in Atlantic cod bile is measured at four stations (Inner Oslo fjord, Inner Sørfjord, Karihavet and Ullerø). No significant temporal trends have been observed at any of the sites. The concentration of OH-pyrene has been consistently higher in the inner Oslo fjord than in the other areas where it is measured. Because of the quick metabolism of PAHs in fish, it is not possible to get reliable data on tissue concentrations of PAHs in Atlantic cod from this area. However, PAHs have been measured in blue mussel from the inner Oslo fjord during the same period.

Figure 5 shows correlations between concentrations of carcinogenic PAHs in blue mussel at two sites in the inner Oslo fjord and OH-pyrene in Atlantic cod bile. In both cases, there was a positive correlation that explained about 17-19% of the variation. However, the individual correlations were not significant (p=0.21-0.24). When all data were used in one correlation, and site was controlled for, the correlation became somewhat stronger but still not significant (p=0.15).



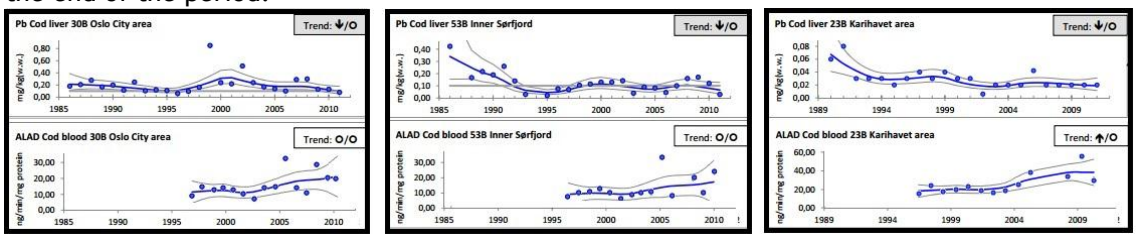
Figur 5. Correlations between carcinogenic PAHs (KPAH) in blue mussel and OH-pyrene in Atlantic cod bile. None of the correlations are significant.

The lack of significant correlations should not be taken as evidence that OH-pyrene in Atlantic cod bile does not reflect relevant PAH exposure. Atlantic cod and mussels live in different environments and are exposed through different pathways. The measurements also integrate over different time periods. Bile reflects exposure since the last feeding (days), while tissue concentrations in blue mussel integrate over a significantly longer time period. Furthermore, the PAH measurements include other PAHs. Although many PAHs have the same origin and similar environmental fate, there are also PAHs that behave differently. PAH measurements in mussels can therefore not be expected to be very good estimates of pyrene exposure for Atlantic cod, and OH-pyrene in bile cannot be expected to be a very good estimate of total PAH exposure.

OH-pyrene in bile is a very sensitive biomarker that has high specificity and can be used as an early warning signal. Furthermore, it is a readily available method with low costs that is frequently used in environmental monitoring. However, the ecological relevance is low and it cannot provide any information about mixtures or unknown chemicals.

5.3 ALA-D (δ-aminolevulinic acid dehydratase) in Atlantic cod blood

ALA-D is a biomarker that is very specific to lead, although other contaminants (e.g., zinc) have also been shown to affect it (Schmitt et al 2002, Finelli et al 1975). In the monitoring program, ALA-D activities have been shown to be lower in Atlantic cod from the inner Oslo fjord area and inner Sørfjord compared to Atlantic cod from the more pristine Karihavet area (Figure 6). Lower ALA-D activity is an indication of lead exposure. Throughout the monitoring period, lead concentrations have been higher in the Inner Oslo fjord area and inner Sørfjord compared to the Karihavet area (Figure 6). The ALA-D levels at the different sites are therefore in line with the lead exposure. Furthermore, there are tendencies toward increasing ALA-D activities over time at all sites, although these temporal trends are not significant (NIVA 2012). Also lead concentrations have been decreasing, although slower at the end of the period.



Figur 6. Lead concentrations in Atlantic cod liver and ALA-D activity in Atlantic cod blood at three monitoring stations. The figure is modified from NIVA (2012).

ALA-D is a sensitive biomarker with low cost that has high specificity and can be used as an early warning signal. However, ALA-D cannot be expected to provide valuable information regarding mixtures of chemicals or unexpected chemicals. Furthermore, the ecological relevance is intermediate, as impairment in hemoglobin synthesis may lead to health effects, but there is no obvious link between ALA-D and population level effects.

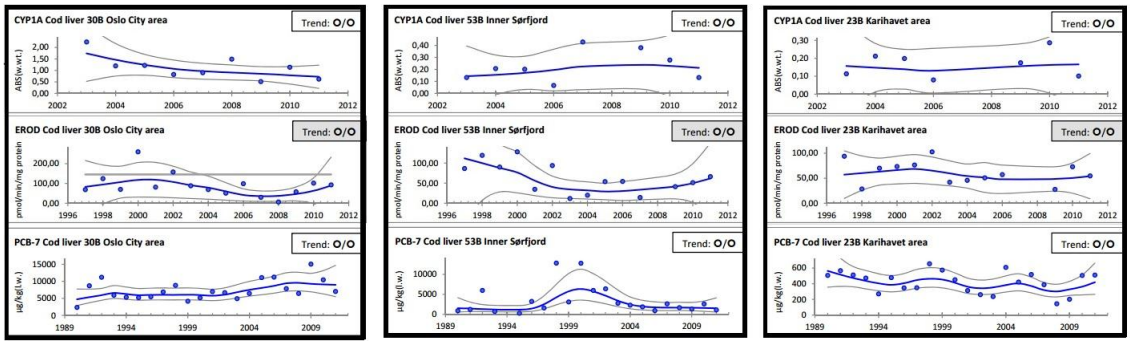
5.4 CYP1A and ethoxyresorufin O-deethylase (EROD) in Atlantic cod liver

CYP1A and EROD are both measurements of exposure to chemicals that bind to the aryl hydrocarbon (Ah) receptor. This includes chemicals such as dioxins, PAHs and planar PCBs. When a chemical binds to the Ah-receptor, a chain of events is initiated that results in the production of CYP1A enzyme. CYP1A then catalyzes metabolism of the exogenous substance, often making it less toxic and more hydrophilic (easier to excrete). Measuring CYP1A is thereby an indirect measurement of all chemicals that bind to the Ah-receptor. Besides metabolizing contaminants, CYP1A can transform 7-ethoxyresorufin to resorufin. As resorufin is fluorescent, the transformation of 7-ethoxyresorufin to resorufin can be used to estimate the presence of CYP1A enzyme. This is called the ethoxyresorufin O-deethylase activity, or EROD-activity. The extra step in EROD compared to CYP1A means that there are more potential confounding factors. At very high exposure levels, for example, most CYP1A enzyme may interact with contaminant molecules. In such cases, less CYP1A are available for transformation of 7-ethoxyresorufin to resorufin, and the EROD activity is lower. It has also

been shown that certain organic contaminants (Willett et al 2001) as well as metals (Martín-Díaz et al 2005) may inhibit EROD-activity.

Since the start of the monitoring period, the concentration of CYP1A has been higher in the inner Oslo fjord area compared to inner Sørfjord and the Karihavet area (Figure 7). There is an almost significant negative trend in CYP1A concentration in the Inner Oslo fjord area (p=0.058). In inner Sørfjord and the Karihavet areas, however, there are no indications of time trends in CYP1A concentration. In seven of the eight last years when all stations have been analyzed, EROD-activity in the inner Oslo fjord area was higher than at the other sites. In 2005, EROD was slightly higher in Inner Sørfjord. EROD activities, thus, provide the same general picture of differences in pollution between the three sites as CYP1A does.

Planar PCBs are known to bind to the Ah-receptor, and have been analyzed in Atlantic cod liver at the same sites (Figure 7). The PCB concentrations also indicate that the Inner Oslo fjord area is more polluted than the other two sites, although there was a peak in Inner Sørfjord area around year 2000 with similar concentrations as in the Inner Oslo fjord area. There is a tendency towards increasing PCB concentrations in the Inner Oslo fjord area, which contradicts the tendency towards reduced CYP1A concentration. This indicates that other contaminants than PCBs are important in the CYP1A induction.



Figur 7. CYP1A concentrations, EROD-activity and PCB concentrations in Atlantic cod liver. Note that the monitoring periods differ. The figure is modified from NIVA (2012).

Although both CYP1A and EROD essentially monitor the same type of contamination, the data presented in Figure 7 clearly shows that CYP1A is more sensitive. The differences between sites are clearer and an almost significant negative trend in CYP1A in the Inner Oslo fjord area can be seen. Both biomarkers can be considered as early warning signals for contaminants that bind to the Ah-receptor. As there is no clear link to growth, reproduction or survival, the ecological relevance must be considered as low. The costs for both methods are relatively low.

6. Ranking of biomarkers

Within the time frame of the present project, it was not possible to examine all biomarkers in detail. Instead, prioritizations were made to focus on those biomarkers that are most likely to add value to environmental management. To prioritize among biomarkers, they were ranked according to their different properties. The properties that were used are presented below.

6.1 Properties used for ranking of biomarkers

The rank order is based on specificity for chemicals, ecological relevance, ability to act as an early warning signal, ability to include mixture effects, current status in environmental assessment, and analysis (reliability, availability, cost). Each of these properties were given a score of 2, 1 or 0 to reflect the biomarkers usefulness in environmental monitoring (Table 6).

Table 6
Score system for ranking of biomarkers

Score	Specificity	Relevance	Early Warning	Mixture	Status	Analysis
2p	Specific for contaminants	Link to reproduction, survival or growth	Responds before effects on health are seen	Responds to at least two groups of contaminants (described below)	Recommended by ICES or OSPAR	Standardized method, high availability, low cost
1p	Other factors than chemicals are known to affect the biomarker (confounding)	Link to condition or metabolism	Minor health effects, no effects on population	Responds to several contaminants within one group	Used frequently in environmental monitoring or has shown promising results	Accepted analysis that does not fulfill the requirements for 2p
0p	Other factors are more important	No link to fitness or health	Fitness already affected	Responds to one or a few chemicals within one group	Not used for environmental assessment	Method development necessary

In different monitoring scenarios, different properties may be important. Furthermore, it may be desirable to use a battery of biomarkers with different properties (Sandström et al 2005). To obtain biomarkers with the properties that are most highly valued in a specific monitoring situation, the desired properties can be given more weight in the overall assessment. Here, three alternatives are presented where focus is put on ecological relevance, ability to respond to different chemicals and mixtures, and ability to work as an early warning signal (Table 7).

Table 7
Relative weight of the different properties in the ranking of biomarkers

Type of monitoring	Specificity	Relevance	Early Warning	Mixture	Status	Analysis
Ecological relevance	25%	35%	10%	10%	10%	10%
Mixture effects	20%	25%	10%	25%	10%	10%
Early warning signal	25%	20%	25%	10%	10%	10%

Below is a short description of how the different biomarker properties were used for the ranking.

6.1.1 Specificity for chemicals

One of the goals with environmental monitoring is to retrieve information that can be useful for environmental management. In the case of monitoring for environmental pollution, it is essential that the effects that are seen actually are caused by pollution. Some biomarkers are very specific to chemicals, or even certain groups of chemicals. These biomarkers are often, but not always, found at lower levels of biological organizations, such as the protein level. A response in such a biomarker can often be taken as a strong evidence for exposure to chemicals. Other biomarkers can be affected by confounding factors such as temperature, UV-radiance, salinity and food availability. These biomarkers are often found at higher levels of biological organization.

Because specificity is a desired property among biomarkers that are used in monitoring, the highest score (2p) was given to biomarkers that are highly specific for contaminants (Table 6). Biomarkers that are somewhat affected by confounding factors were given 1p. Endpoints that are mainly driven by non-chemical factors were given zero points.

6.1.2 Ecological relevance

Environmental monitoring is carried out with the aim of protecting populations of species, and thereby also biodiversity and ecological services. This means that it is important to use measures that provide relevant information about the risk for populations. Some biomarkers have a strong link to survival rates or reproductive output, and are thereby good indicators of ecological risk. Such biomarkers are often found at higher level of biological organization. An example of such a biomarker is imposex in gastropods. Imposex often results in sterility, with subsequent population crashes.

In the ranking of biomarkers, the highest score (2p) was given to biomarkers that reflect reproduction, survival and growth (i.e. fitness) (Table 6). A lower score (1p) was given to biomarkers that reflect general effects on health condition or metabolism. No points were given to biomarkers of exposure that cannot be linked to health effects.

6.1.3 Early warning signal

It is often argued that biomarkers respond before effects are seen at higher levels of biological organization. This means that biomarkers act as early warning signals for effects at the population level. Thereby, biomarker responses may help environmental managers to take action before populations are affected. However, this is not equally true for all biomarkers. Some biomarkers are, indeed, very sensitive and responses are seen at concentrations that do not contribute to any population level risk. Other biomarkers do not show any effects until reproduction, survival or growth already are affected.

Biomarkers that respond before any effects can be expected on the organisms health were given the highest score (2p) (Table 6). Measurements of physiological effects that are not directly linked to fitness were given a lower score (1p). Biomarkers that do not react until the health of the organism is already significantly impaired were given zero points.

6.1.4 Mixture effects

Mixture effects, also popularly referred to as cocktail effects, have gained increasing attention during the past 5-10 years. Mixture effects refer to the total toxicity of all contaminants in a recipient. Although each chemical may be present in safe concentrations, it is possible that the sum of all chemicals still cause an unacceptable risk. The toxicity of mixtures has proven very difficult to predict as chemicals have different modes of action and may interact both synergistically or antagonistically. Biomarkers may provide a shortcut by measuring the total effect of all pollutants in an area, without necessarily knowing exactly which chemicals are included in the mixture or how they interact. However, all biomarkers are not equally suited for this. Some biomarkers are specific to only one or a few chemicals. It can be argued that such specificity is an advantage as it is easy to identify the cause and take action to reduce the risk. However, when the causal link is already established, this information can also be retrieved from chemical measurements. In many monitoring

situations, it is more important to include a broad spectrum of chemicals, including unexpected or even unknown contaminants.

Here, the higher score (2p) was given to biomarkers that respond to at least two different groups of chemicals (metals, endocrine disruptors, dioxin-like compounds, pesticides, other organic contaminants) (Table 6). A lower score (1p) was given to biomarkers that are specific for a certain groups of chemicals, such as metals. No points were given to biomarkers that are mainly associated with only one or a few chemicals.

6.1.5 Current status

Some biomarkers have been used extensively in monitoring programs or for environmental assessment around the world. This means that the method is well understood and described in the literature, that potential confounding factors are likely to have been identified, and that some sort of consensus is likely to have developed regarding the interpretation of results. For newer biomarkers, or biomarkers that for some reason have not been adopted in monitoring programs, new knowledge and experience is generated more slowly, and the risk for unpleasant surprises is higher.

To take this into account, the biomarkers have been given a score to reflect their current status in environmental monitoring and assessment. The highest score (2p) was given to biomarkers that are recommended by ICES or OSPAR (Table 6). Other biomarkers that are used frequently for environmental monitoring or have shown to be useful in single scientific studies were given a lower score (1p). No points were given to biomarkers that have not been used for environmental assessments.

6.1.6 Analysis

For biomarkers to be truly useful in environmental monitoring, it is necessary that there is an agreed method for the analysis so that results are comparable (over time and between labs). Furthermore, it is beneficial if the method is readily available and that the cost per analysis is low. For many biomarkers that have been used in environmental monitoring for several decades, there are international standard protocols to follow (ISO, OECD). Other biomarkers may only have been used once by a single research group.

The highest score (2p) is given to biomarkers for which international standards exists, the analysis is easy to perform or possible to buy, and the costs are relatively low. Methods that are agreed upon within ecotoxicological research, but do not fulfill the requirements for the highest score are given the intermediate score (1p). This could, for example, be biomarkers that have been used frequently in ecotoxicological research but no intercalibration has been performed and the method has not been adopted for environmental management. No points are given to biomarkers for which methods are not agreed upon, the analysis is difficult to perform and not available to buy, and the method requires expensive equipment or highly skilled personnel.

6.2 Ranking of selected biomarkers

Biomarkers that were included in the ranking were collected from national monitoring programs as well as from the scientific literature. The monitoring programs that were included were those discussed in chapters 5 and 6 (Norway, UK, Sweden, Canada). The scientific literature was scanned for biomarkers by searching the keywords “biomarker” and “environmental monitoring” or “biomonitoring” in the scientific data base Web of Science. This rendered a list of about 100 biomarkers, which are presented in Appendix A, along with references where the biomarkers are described in more detail. For some biomarkers, however, it was not possible to find information about the properties that were used to rank the biomarkers. Those biomarkers were then omitted from the ranking, leaving approximately 80 biomarkers that could be ranked. The biomarkers that were ranked are presented in Appendix B, together with their scores for the different properties.

Below is a more detailed description of the ten highest ranked biomarkers within each category.

6.2.1 Biomarkers with high ecological relevance

The biomarkers that ranked highest for ecological relevance are presented in Table 8, including the score for each property. This list is dominated by biomarkers that are linked to reproduction and respond to endocrine disruptors. This is because relatively high weight was placed on specificity and ecological relevance. The endocrine system is often quite robust to other stressors than chemicals that mimic endogenous hormones, and the ecological relevance of impaired reproduction is obviously high. Biomarkers that affect other traits with high ecological relevance, such as growth and immune defence, often scored low on specificity.

For biomarkers with high ecological relevance, imposex in gastropods was the only biomarker among the ten highest ranked that is presently used in Norwegian environmental monitoring.

Table 8
Highest ranked biomarkers with high ecological relevance

Rank	Biomarker	Specificity	Relevance	Early Warning	Mixture	Status	Analysis
1	Vitellogenin (VTG) in fish	2	2	2	1	2	2
2	Spiggin in three spined stickleback	2	2	1	1	1	1
2	Intersex in fish and gastropods	2	2	0	1	2	1
2	Egg shell thickness	2	2	0	0	1	2
5	Zona Radiata protein	2	2	1	1	0	1
5	Imposex in gastropods (e.g., VDSI)	2	2	0	0	2	1
7	Aromatase	1	2	1	2	1	1
7	Congenital malformations in birds	1	2	1	2	1	1
7	Macroscopic liver neoplasms (tumours)	1	2	1	1	2	1
7	Hatching success and brood size in birds	1	2	0	2	1	2
7	Skeletal deformities	1	2	1	2	1	1

Vitellogenin (VTG) in fish

Vitellogenin (VTG) in fish had the highest ranking among biomarkers for ecological relevance. VTG is an egg yolk precursor protein expressed in the females of nearly all oviparous species. In environmental monitoring, VTG in fish has been used extensively to monitor for estrogenic chemicals. The production of VTG is normally initiated by natural estrogen hormones. Females therefore have different levels of the protein depending on hormonal cycles, while males and juveniles have very low levels throughout the year. When males and juveniles are exposed to xenoestrogens, however, they start to produce VTG just like females. This biomarker has mainly proved useful to detect environmental effects of synthetically produced hormones (e.g., Larsson et al 1999). High points are given to VTG for most properties. VTG induction in males and juveniles is very specific to xenoestrogens. VTG inhibition in females

can be used to show anti-estrogenic effects. However, this is more complicated as there is a natural production of estrogens in females. Therefore, an observed effect could be a secondary response to other types of stress, including non-chemical stressors. The ecological relevance is high as VTG is linked to reproduction. In a study where a lake was contaminated by a synthetic estrogen, VTG was measured along with intersex, altered gonadal development, impact on kidneys, and population decline in four species. Induction of VTG was seen in all species before effects were seen on the other endpoints (Palace et al 2009). Therefore, it can be said that VTG is also an early warning signal. VTG reacts to all estrogenic chemicals, known or unknown, and is frequently used for environmental monitoring. The analysis is relatively cheap and there are commercial ELISA-kits (Enzyme-Linked Immunosorbent Assay) available for several fish species.

Spiggin in three spined stickleback

Next in the rank order was the production of the glue protein spiggin in three-spined stickleback. The protein is normally produced in male sticklebacks and used for building a nest. The production is normally initiated by natural androgens in male stickleback. When females are exposed to xenoandrogens, they also start to produce spiggin (Katsiadaki et al 2002). Furthermore, reduced spiggin in male sticklebacks can be used as an indicator for exposure to anti-androgens (Hogan et al 2012). The specificity to endocrine disruptors (androgens and anti-androgens) is high, as is the ecological relevance. It is likely that effects on spiggin occur before the fitness of the individual is affected. Spiggin is not used in monitoring programs as frequently as VTG, but has been used quite often in research projects. One reason for this may be that exposure to synthetic estrogens has been considered a larger environmental risk than androgens. Exposure to androgens has mainly been restricted to effluents from pulp and paper industry (Wartman et al 2009). There is a commercial ELISA-kit available, which keeps the costs to a relatively low level. The analysis is, however, limited in that it can only be used in ecosystems that contain the three-spined stickleback.

Intersex in fish and gastropods

Intersex had the same total score as spiggin. Intersex describes a condition where both male and female tissue occurs in the reproductive organs of one individual of fish or gastropod. As a biomarker, it has been used to detect estrogenic effects in male fish (Jobling et al 1998) and androgenic effects in female gastropods (Barroso et al 2000). The specificity of intersex is high as the effects are very specific to endocrine disruptors and intersex does not occur within what can be considered as natural variability. Ecological relevance is also high as intersex is a measure of actual alterations of reproductive organs. However, as effects already have occurred, it is not a very good early warning signal. For example, when lake trout were exposed to a synthetic estrogen in an experimental lake, no effects were seen on intersex until the population started to decline (Palace et al 2009). Intersex in male fish can be caused by a number of estrogenic chemicals, including synthetic estrogens that reach the environment through sewage treatment plants. Intersex in gastropods is mainly caused by TBT, through the same mechanism that causes imposex. Intersex in both fish and gastropods is used frequently in monitoring programs and is recommended by ICES. The analysis requires skilled personnel, but no advanced technical equipment.

Egg shell thickness

Egg shell thickness has mainly been associated with DDT exposure. Dramatic decreases in egg shell thickness were observed in the 1950s and 1960s, followed by an increase after the bans on DDT in the 1970s (Bignert et al 1994). Egg shell thickness scores are high as it is a biomarker with high specificity and ecological relevance, which has been used in environmental monitoring and assessment and has low cost. It may act as an early warning signal if the reduction in egg shell thickness is detected before the thickness is below the critical level. However, egg shell thickness cannot be expected to provide information about mixtures of chemicals.

Zona radiata protein

Zona radiata protein (ZRP) is another biomarker that is sensitive to estrogenic chemicals. ZRP is an eggshell protein that, just like VTG, is induced by endogenous estrogens (Arukwe 1997). Dose-response studies have shown that ZRP and VTG are approximately equally sensitive to estrogenic exposure (Arukwe et al 2000). As ZRP can be linked to reproduction, there are also reasons to believe that the level of ecological relevance is high. However, ZRP has not been used frequently in environmental monitoring or environmental assessments. The analysis is relatively simple, but is not as commercially available as VTG.

Imposex in gastropods

Imposex in the gastropod dog whelk is already in use for environmental monitoring of hazardous substances in Norwegian fjords and coastal waters. Imposex is a measure of the development of male genetalia in females. Imposex affect reproduction and has been linked to population decline in gastropods (Oehlmann et al 1996). It is very specific for exposure to TBT, but not a useful early warning signals as the organisms are already seriously affected (often to the degree of sterility). Furthermore, it cannot be expected to react to other chemicals than TBT. Imposex is recommended by ICES and the analysis requires skilled personnel.

Aromatase

Aromatase is an enzyme that is involved in the aromatization of androgens into estrogens. Aromatase is, thus, important for maintaining the hormonal balance. Tissues with high concentrations of aromatase include brain and gonads. Several types of contaminants have been linked to aromatase inhibition (Noaksson et al 2002, Lavado et al 2004). However, due to the complexity of the hormonal system, including different feedback mechanisms, interpretation of the results is complicated and other factors cannot be excluded. Aromatase may act as an early warning signal in some exposure situations, but it cannot always be expected. Aromatase in fish is used in environmental monitoring and assessments (e.g., in the UK) but is not recommended by ICES or OSPAR.

Congenital malformations in birds

Congenital malformations (birth defects, for example crossed bills) in fish-eating birds and other birds of prey can be used to detect biologically significant exposure to developmental toxins in the food chain (Fox et al. 1991). Congenital malformations have, for example, been used for identifying the presence of embryotoxic contaminants in agricultural drainwater (Ohlendorf et al 1986) and in the Great Lakes ecosystems (Kubiak et at 1989). The ecological relevance is obviously very high as such impairment may significantly reduce fitness. As the phenomenon is rare in pristine areas, it can also be concluded that it is relatively specific to chemical stress (although other factors cannot be excluded). Congenital malformations have mostly been associated with severely polluted areas. Therefore, it is likely that other effects have already been observed, and that it is not a very good early warning signal. The effects may be caused by a large number of chemicals. Congenital malformations have been used in different research projects and environmental assessments, but are not frequently used for environmental monitoring. The analysis is relatively straight forward, but it may be time consuming to collect the necessary material.

Macroscopic liver neoplasms

Macroscopic liver neoplasms (tumours) can be used to investigate exposure to and effects of chemicals (Koehler 2004). The effects can be caused directly by carcinogenic chemicals, but can also be an indirect effect of chronic stress caused by chemicals. The ecological relevance is high, as it can be expected that the fitness of the individual is significantly impaired. However, it cannot be excluded that other factors contribute to the effect. The method may provide some value as an early warning signal and the cost is moderate. The method is recommended by OSPAR.

Hatching success and brood size in birds

Another reproduction related biomarker is hatching success in birds. Early studies in the great lakes showed that chemicals could reduce hatching success via intrinsic (embryotoxic) and extrinsic (adult behavioral) mechanisms (Keith 1966, Gilbertson and Hale 1974). Hatching success, and subsequent brood size, is used for monitoring as well as research purposes (Helander and Bignert 2012). The ecological relevance is very high, while the specificity for chemicals is somewhat lower. The analysis is simple with a low cost.

Skeletal deformities

Skeletal deformities have been observed in several fish species that have been exposed to complex mixtures of chemicals (Lindesjöö 1992, Bengtsson et al 1985, Hanson and Larsson 2011). Such deformities are likely to affect fitness, and thereby have high ecological relevance. The specificity for chemicals is intermediate as other environmental or biological factors cannot be excluded. Skeletal deformities are likely to respond to a large variety of chemicals. The analysis is simple, but has not been used frequently for environmental monitoring.

6.2.2 Biomarkers for mixture effects

The biomarkers for mixture effects that ranked highest are presented in Table 9, including the score for each property. Among the ten biomarkers that ranked highest, seven were also ranked high for ecological relevance. These were VTG in fish, aromatase, congenital malformations in birds, skeletal deformities, hatching success and brood size for birds, spiggin in three-spined stickleback, and intersex in fish and gastropods. The large overlap can be explained by the fact that the properties ecological relevance and ability to respond to different types of chemicals are both associated with endpoints at higher levels of biological organization. The biomarkers that are presented in the previous section (and Table 8) are not presented again in this section. Below is a presentation of the remaining three biomarkers.

No biomarkers on this list are presently used in Norwegian environmental monitoring.

Table 9
Highest ranked biomarkers for detecting mixture effects

Rank	Biomarker	Specificity	Relevance	Early Warning	Mixture	Status	Analysis
1	Vitellogenin (VTG) in fish	2	2	2	1	2	2
2	Aromatase	1	2	1	2	1	1
2	Congenital malformations in birds	1	2	1	2	1	1
2	Skeletal deformities	1	2	1	2	1	1
2	Hatching success and brood size for birds	1	2	0	2	1	2
6	Spiggin in three spined stickleback	2	2	1	1	1	1
6	Micronuclei test	1	2	1	1	2	1
6	Intersex in fish and gastropods	2	2	0	1	2	1
9	Liver somatic index (LSI)	1	1	1	2	2	2
10	Blubber thickness in seals	0	2	1	2	1	2

Micronuclei test

The micronuclei test provides information about the accumulated genetic damage during the lifespan of the cells. For monitoring purposes, the micronuclei test has mostly been used on bivalves and fish, although other groups of organisms can also be used. The test has shown to respond dose dependently to many chemicals, although there are also other biotic and environmental factors involved (Bolognesi and Hayashi 2011). The ecological relevance must be considered as high as genetic damage may affect several generations. The micronucleus test responds before damage to the populations, but effects on the individual's health cannot be excluded. Therefore, it gets the intermediate score for early warning. It is recommended by ICES to use the micronuclei test for environmental monitoring and assessment. The procedure is technically easier and more rapid than other tests for chromosomal damage, but still complex compared to many other biomarkers.

Liver somatic index

The liver somatic index (LSI) is the weight of the liver in relation to the somatic weight. The biomarker is mostly used in fish, although other organisms could also be used (e.g., birds). LSI has been linked to contaminants from a number of sources, for example pulp and paper mill effluents (Hodson et al 1992, Förlin et al 1995), landfills (Noaksson et al 2001) and wastewater treatment plants (Kosmala et al 1998). However, there are also confounding factors that may influence liver size. These include seasonal variations (Förlin and Haux 1990) as well as feeding status, age, and temperature (George et al 1990). A change in liver size may reflect somewhat impaired function. The ecological relevance was therefore set to the intermediate level. Effects on LSI can be expected to be seen before the health is significantly affected. However, it is not likely to respond as quickly as the protein based biomarkers. Therefore, also early warning was set to the intermediate score. A biomarker at the organ level, such as LSI, can be expected to respond to many different chemicals. The highest score was therefore given for the ability to detect mixture effects. LSI in fish is used in many monitoring programs (e.g., Sweden, UK and Canada) and is recommended as a supplemental biomarker (ICES 2012). The analysis is extremely simple.

Blubber thickness in seals

A reduced layer of blubber in seals can be an indication of exposure to chemicals. The reduction may be caused by increased energy costs for detoxification, which means less energy to be stored in blubber. It is also possible that chemicals can have a direct effect on the fat metabolism. Taken together, a large number of chemicals may affect blubber thickness. However, other environmental factors are likely to be even more important for blubber thickness, e.g., food quality and quantity. The ecological relevance is likely to be high as the blubber serves as both energy storage and insulation. Small reductions in blubber thickness are likely to occur before the health of the seal is significantly impaired. Therefore, it can serve as something of an early warning signal. Blubber thickness is used for environmental monitoring in the Baltic Sea, where a significant decreasing trend since 1996 has been observed (Härkönen et al 2012). The analysis is simple, but requires that dead seals are collected.

6.2.3 Biomarkers that are good early warning signals

All biomarkers that ranked among the ten best for early warning are presented in Table 10, including the score for each property. Six of the biomarkers that ranked among the ten best biomarkers for early warning were also ranked high for ecological relevance or mixture effects. Those were VTG in fish, spiggin in three spined stickleback, zona radiata protein, macroscopic liver neoplasms (tumours), intersex in fish and gastropods, and the micronuclei test. Intersex in fish was omitted from the list as it scored zero in early warning. The reason that it still made the top-10 was the relatively high scores for all other properties. A more detailed description of the six biomarkers is given in the previous two sections about biomarkers with high ecological relevance and biomarkers to detect mixture effects.

Among the ten highest ranked biomarkers for early warning were three biomarkers that are presently used in Norwegian environmental monitoring. Those were the Ah-receptor mediated responses (CYP1A and EROD) and PAH-metabolites in bile (including OH-pyren).

Table 10
Highest ranked biomarkers that are good early warning signals

Rank	Biomarker	Specificity	Relevance	Early Warning	Mixture	Status	Analysis
1	Vitellogenin (VTG) in fish	2	2	2	1	2	2
2	Increase in CYP1A. Ethoxyresorufin-O-deethylase (EROD) activity	2	0	2	1	2	2
2	PAH-metabolites in bile	2	0	2	1	2	2
4	Spiggin in three-spined stickleback	2	2	1	1	1	1
4	Egg shell thickness	2	2	0	0	1	2
6	Micronuclei test	1	2	1	1	2	1
7	Total oxyradical scavenging capacity (TOSC)	1	1	2	2	1	1
7	Zona Radiata protein (protein and mRNA)	2	2	1	1	0	1
7	Macroscopic liver neoplasms (tumours)	2	1	1	1	2	1
7	Antioxidant enzymes	1	0	2	2	2	2

CYP1A and EROD

CYP1A and EROD have similar properties and are therefore analyzed as one biomarker. The specificity for chemicals is high as the response is induced by binding to the Ah-receptor (e.g., dioxins, PAHs, PCBs). Although high EROD-levels have been linked to health effects, statistically detectable CYP1A or EROD-induction is often well within the span that the organism can cope with, thus not leading to health effects. Therefore, ecological relevance was considered low. CYP1A and EROD are very good early warning signals for chemicals that bind to the Ah-receptor. EROD is frequently used for environmental monitoring and is recommended by both ICES and OSPAR. The analysis has a low cost and is widely available.

PAH-metabolites in bile

Equally high scores were given to PAH-metabolites in bile. OH-pyrene, which is included in the program for monitoring of hazardous substances in Norwegian fjords and coastal waters, is an example of a PAH-metabolite. There are different methods to measure PAH-metabolites in bile. A cheap semi-quantitative method is to use fixed wavelength fluorescence. By using different wavelengths, the PAHs can be roughly divided into 2-, 4-, and 5-ringed (Hanson and Larsson 2008). The ratio between 2- and 4-ringed PAHs can be used to roughly determine the dominating source of the PAHs (pyrogenic or petrogenic) (Hanson et al 2009b). However, specific PAHs cannot be determined. A method with higher precision is to use HPLC. This is the method that is currently used in the program for monitoring of hazardous substances in Norwegian fjords and coastal waters. The cost of the higher precision is that other PAHs are not included in the analysis. Regardless of method used, PAH-metabolites in bile has very high specificity for chemicals and low ecological relevance.

Total oxiradical scavenging capacity (TOSC)

Total oxiradical scavenging capacity (TOSC) assay describes the biological resistance to various kinds of oxyradicals. When the assay is used for monitoring, the capability to neutralize cellular oxidants is measured on field collected organisms, e.g., mussels (Regoli 2000). Thereby, the assay shows whether the organisms have been exposed to chemicals that impair the resistance to oxyradicals. At high pollution levels, many species are subjected to increased intracellular flux of oxyradicals produced by the Fenton reaction in the presence of transition metals or by the redox cycle of several organic compounds (Winston, 1991). The specificity of the assay is intermediate as both chemicals and other factors may affect the results (Regoli 2000). The assay is a direct measure of an organisms ability to resist oxidative stess. However, there is no obvious link to fitness or population level effects. The assay has shown to be sensitive and is therefore a good early warning signal. The analysis has been used for different research projects and environmental assessments, but is not recommended by ICES or OSPAR. The analysis is relatively straight forward, but not widely available.

Antioxidant enzymes

A number of antioxidant enzymes have been used for environmental monitoring and assessment. Examples of such biomarkers are catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), and glutathione peroxidase (GPOX). The measurements have mainly been used in fish (e.g., Monteiro et al 2006), but other organisms can also be used. Although there is clear evidence showing that antioxidant biomarkers respond to chemicals, there are also other factors that affect the results, e.g., solar radiance (Hanson et al 2006). Therefore, the results may sometimes be difficult to interpret. The ecological relevance is low as there is no link to health or fitness. Antioxidant biomarkers are very good early warning signals as measurable effects occur before health is impaired. They can respond to a variety of chemicals with antioxidant properties and are therefore suitable for detecting effects of mixtures that include unknown or unexpected chemicals. CAT and GR are recommended by ICES and the analyses are relatively cheap.

7. Recommendations for Norwegian monitoring

Based on the findings in this literature study, specific recommendations for the different Norwegian monitoring programs are presented below. For each program, five biomarkers are suggested. However, it must be noted that these are just examples of biomarkers that are likely to add value to the monitoring programs. In Appendix A and B, there are many other biomarkers that may be just as good, or better, depending on the specific goals, cost related issues and available species. The reasoning behind the suggestions is given in the following sections.

7.1 Monitoring in fjords and coastal waters (MILKYS)

The biomarkers presently used for monitoring in fjords and coastal waters (MILKYS) are all very or relatively specific to contaminants, or groups of contaminants, that are already quite well monitored. It is very likely that it would be possible to gain more information about the exposure to and effect of chemicals by using a broader spectrum of biomarkers.

Imposex has very high ecological relevance and was ranked high among biomarkers for ecological relevance. However, it is very specific for TBT, and it can be questioned if it provides valuable information for environmental management that is not also retrieved from chemical measurements of TBT. However, it is possible that other contaminants with similar properties will appear. Monitoring imposex may therefore be motivated for other reasons than just following the decrease in TBT.

ALA-D is another biomarker with high specificity (lead). Again, it can be questioned whether the biomarker provides information for environmental management that is not already retrieved from chemical measurements. Unlike imposex, ALA-D is not a biomarker with high ecological relevance. It can therefore be questioned whether ALA-D provides any valuable information in continuous monitoring. Instead, ALA-D may be most useful in retrospective risk assessments where lead is suspected of having contributed to a biological effect (e.g. fish death). This is because the effect on ALA-D remains for a relatively long period, so that it can be shown that the organisms have been exposed to lead even after the lead has left the ecosystem.

CYP1A and EROD rank high among biomarkers for exposure and they provide information that can be considered as very useful for environmental monitoring. However, they both respond to chemicals that bind to the Ah-receptor. It could, therefore, be argued that it would be sufficient to only use one of them.

OH-pyrene in bile measures exposure to another chemical that binds to the Ah-recpetor, and thus induce CYP1A and EROD (Zapata-Pérez et al 2002). For general monitoring of diffuse pollution or biological effects, it may be satisfactory to monitor CYP1A or EROD. The drawback is that it is not known if a response is caused by PAHs or other contaminants. If there is a specific interest in pyrene or other PAHs, it may be motivated to monitor PAH-metabolites in bile even if CYP1A or EROD are also monitored. PAHs in blue mussel are already monitored at several sites. However, it cannot be expected that mussels provide a good estimate of exposure to pelagic fish (see correlations in Figure 5). It is therefore motivated to measure in both blue mussel and Atlantic cod bile. One way of making the different measurements in mussel and fish complement each other would be to use the semi-quantitative method based on fluorescence for bile. This would mean that both specific and broad PAH-analyses are included.

In Table 11, the biomarkers that are used within the program for monitoring of hazardous substances in fjords and coastal waters are shown with their scores for the six properties. The sum of the scores for all biomarkers is shown in the last row of Table 11. As can be seen, it is ecological relevance and the ability to detect mixture effects that have the lowest scores. The only score for ecological relevance is for imposex, and that is mainly for one singe pollutant.

Table 11
Biomarkers used for monitoring in fjords and coastal waters (MILKYS)

Biomarker	Specificity	Relevance	Early Warning	Mixture	Status	Analysis
Imposex in gastropods (e.g., VDSI)	2	2	0	0	2	1
δ-aminolevulinic acid dehydratase inhibition (ALA-D)	1	0	2	0	1	2
CYP1A levels	2	0	2	1	2	2
EROD	2	0	2	1	2	2
PAH-metabolites in bile	2	0	2	1	2	2
Total:	9	2	8	3	9	9

There are several biomarkers in Tables 8 and 9 that could be added to increase the score for ecological relevance and ability to detect mixtures. As VTG is ranked highest in both ecological relevance and to detect mixtures, it is a logical choice. Aromatase had maximum scores in both ecological relevance and ability to detect mixture effects, and would add a nice complement to the other biomarkers. As the liver of the fish is already dissected, LSI can be added at a negligible cost. LSI has the highest score in ability to detect mixture effects, and because of the relatively high scores in other properties, it was also ranked among the ten best for mixtures.

In Table 12, the scores for the different properties are presented for the suggested biomarkers, including the total score for each property. By comparing Tables 11 and 12, it can be seen that ecological relevance and ability to detect mixture effects is higher, while the scores for the other properties is relatively unchanged.

Table 12
Suggested biomarkers for monitoring in fjords and coastal waters (MILKYS)

Biomarker	Specificity	Relevance	Early Warning	Mixture	Status	Analysis
EROD (or CYP1A)	2	0	2	1	2	2
PAH-metabolites in bile	2	0	2	1	2	2
Vitellogenin in fish	2	2	2	1	2	2
Aromatase in fish	1	2	1	2	1	1
Liver somatic index (LSI) in fish	1	1	1	2	2	2
Total:	8	5	8	7	9	9

7.2 Monitoring of pollutants in large lakes

The monitoring program for pollutants in large lakes includes chemical measurements in fish from different trophic levels. The purpose of this approach is to assess biomagnification through the food web. By adding biomarkers in at least two of the fish species that are included (smelts, whitefish and trout), it can be seen if food web effects in concentrations are also followed by biological effects. This is interesting not only from a management perspective, but also from a scientific perspective. Considering the aim and the approach of the program, it is advisable to add biomarkers that rank high for ecological relevance and for detecting mixture effects. This means that the biomarkers that were suggested in the previous paragraph (for MILKYS) are likely to also add value for monitoring of pollutants in large lakes. However, in this program it is suggested that macroscopic liver neoplasms is added and PAH-metabolites is removed. This change is because it is likely that PAHs are already covered to a large extent by EROD, and it is unlikely that they will accumulate through the food chain. Liver neoplasms, on the other hand, are more likely to be caused by chemicals with bioaccumulating properties. The scores on the different properties for the suggested biomarkers are presented in Table 13, including the total score for each property. Again, the scores of the different properties are relatively balanced.

Table 13
Suggested biomarkers for monitoring of pollutants in large lakes

Biomarker	Specificity	Relevance	Early Warning	Mixture	Status	Analysis
EROD (or CYP1A)	2	0	2	1	2	2
Vitellogenin in fish	2	2	2	1	2	2
Aromatase in fish	1	2	1	2	1	1
Liver somatic index (LSI) in fish	1	1	1	2	2	2
Macroscopic liver neoplasms in fish	2	1	1	1	2	1
Total:	8	6	7	7	9	8

7.3 Monitoring of pollutants in urbanized fjords

Also this monitoring program is focused on the aquatic environment and includes measurements in several groups of species at different trophic levels. However, in this case there is only one fish species. The other groups of species include zooplankton, shrimp, blue mussel and polycheates. To keep the program similar to the other aquatic programs, it is advisable to use biomarkers in fish in this program as well. However, because of the fact that the program focuses on the urbanized environment, it may be valuable to focus a bit more on providing early warning for contaminants that are likely to origin from human activities. This can be new chemicals with unexpected ecological impact, or well-known chemicals that are released through new behaviors. Therefore, focus was set on biomarkers with an ability to act as an early warning and to provide information about a large number of chemicals (mixtures). Furthermore, the biomarkers were chosen to complement those that are used in MILKYS, and thereby already analyzed in the Oslo fjord.

In Table 14, the suggested biomarkers are presented, along with the scores on the different properties.

Table 14
Suggested biomarkers for monitoring of pollutants in urbanized fjords

Biomarker	Specificity	Relevance	Early Warning	Mixture	Status	Analysis
Micronuclei test in fish	1	2	1	1	2	1
Skeletal deformities in fish	1	2	1	2	1	1
Antioxidants in fish liver (Cat, GST, GR)	1	0	2	2	2	2
Macroscopic liver neoplasms in fish	2	1	1	1	2	1
Total oxyradical scavenging capacity (TOSC) in blue mussel	1	1	2	2	1	1
Total:	6	6	7	8	8	6

7.4 Monitoring of pollutants in terrestrial and urbanized environments

The organisms that are included in this program are earthworms, European pied flycatcher, red fox, brown rat, golden eagle and herring gull. Although it is mainly the aquatic environment that has been monitored with biomarkers, there are a number of biomarkers that may add valuable information for this monitoring program. However, because of the limited use of biomarkers in the terrestrial environment, it was difficult to obtain enough information so that those biomarkers that have been used could be properly ranked. Instead, a condensed discussion about the present status of biomarkers for environmental monitoring in terrestrial organisms is given. Table 15 lists a number of biomarkers for the terrestrial environment (no ranking) of which several may provide value for the program.

In the environmental monitoring programs that were reviewed, few biomarkers were used for terrestrial species other than birds. There may be many reasons for this, such as ethical considerations related to mammals and that it is the aquatic environment that is usually most affected by pollution. In Tables 8-10, three biomarkers based on bird reproduction were the only ones that were not mainly aquatic. However, several of the highly ranked biomarkers may be possible to use in the terrestrial environment, although it has not been done to any considerable extent (e.g., EROD and aromatase).

Table 15
Biomarkers suitable for terrestrial species

Organism class	Biomarker
Earthworm (*Lumbricus terrestris*)	Granulocyte morphometric alterations Blood haemoglobin Eleocyte riboflavin concentration Lysosomal membrane stability
Birds of prey, fish-eating birds	Brood size Number of eggs per female Embryo death Hatching success Congenital malformations (birth defects) Lipid weight Shell thickness
Birds	EROD Porphyrin
Mammals	Ovary and follicle size Epithelial height of uterine cells Ovulation frequency Number and morphology of Sertoli cells Sperm number and mobility Ability of the sperm to penetrate oocytes Gonadal aromatase activity Ano-genital distance

For birds, biomarkers have been used for a long time. For peregrine falcons (*Falco peregrinus*), eggshell thickness has been negatively correlated to the concentration of DDT as far back as 1947. For osprey (*Pandion haliaetus*), egg shell thickness was affected by DDT and mercury. Other birds of prey show similar patterns. Reduced eggshell thickness led to increased risk of the eggs breaking, resulting in reduced brood size (Naturvårdsverket, 2008).

Eggshell thickness of the Baltic guillemot is included in the Swedish environmental monitoring program. For pied flycatcher (*Ficedula hypoleuca*) biomarkers such as EROD, tarsus length and breeding success were studied in the contamination gradient from a copper smelter and showed dose-dependent results (Eeva et al. 2000). Other studies have also shown promising results with EROD in birds (Barton et al 2013) Porphyrin levels have been suggested as a biomarker for polyhalogenated hydrocarbons and heavy metals in birds by Casini (2003). Celis et al (2012) showed that lead and mercury also affected the porphyrin levels in penguin excrements. Martinez-Haro et al (2013) investigated biomarkers for lead exposure in excrements from greylag geese (*Anser anser*) and purple gallinule (*Porphyrio porphyria*) ten years after a mine spill. Concentrations of faecal porphyrins and biliverdin were determined as non-invasive biomarkers for studying lead exposure.

Different biomarkers in feathers have been examined. Le Tortorec et al (2012) examined the influence of habitat fragmentation on feather growth bar length in the free-living Eurasian treecreeper (*Certhia familiaris*) but found no correlation. Fairhurst et al (2013) showed that corticosterone in feathers from tree swallows (*Tachycineta bicolor*) can reflect plasma corticosterone, but correlations may not always be expected. Koren et al (2012) measured testosterone, corticosterone and cortisol in the feathers of house sparrows (*Passer domesticus*). They found that concentrations of testosterone, corticosterone and cortisol in feathers correlated to winter mortality. Feather steroids can hence be possible biomarkers to predict the future survival of individuals in the wild. However, these studies have not examined any correlation to pollutants. Many other studies examined the chemical content of feathers, such as mercury and PBDEs. Bourgeon et al (2012) examined biomarkers in Great skua (*Stercorarius skua*). No consistent within-colony relationships between feather corticosterone, plasma immunoglobulin Y levels and oxidative stress and POPs such as organochlorines and PBDEs could be shown. The authors suggested that other ecological factors such as food availability could constrain physiological indicators more than anthropogenic contaminants.

In mammals, the effects of environmental contaminants have largely focused on disruptions of the gonad. In females, some of the most common indices of xenoestrogen exposure include reductions in ovary and follicle size, epithelial height of uterine cells, and ovulation frequency. Testicular endpoints are most common in males, including number and morphology of Sertoli cells, sperm number and mobility, and the ability of the sperm to penetrate oocytes. Gonadal aromatase activity can be used as a marker for both males and females. Aromatase is an enzyme that converts testosterone to estradiol. This biomarker has been shown to correlate to exposure for chemicals such as methyl tertiary-butyl ether (MTBE) (de Peyster et al 2003), bisphenol-A (Akingbemi 2004), and triazine herbicides (Sanderson et al 2001). The ano-genital distance have also been used to assess exposure to endocrine disrupting chemicals (Hamlin and Guillette Jr 2010), e.g. phtalates (e.g., Swan 2008).

Impaired tooth development of bank vole (*Clethrionomys glareolus*) proved to be a sensitive endpoint of dioxin exposure (Kattainen et al. 2001). Size of molar teeth has been suggested as a sensitive and robust biomarker for PCDD/Fs exposure (Miettinen 2006).

Fecal porphyrin levels from rabbit have been shown to be a good biomarker for exposure to the insecticide diazinon (Hernández-Moreno 2012). One advantage of this biomarker is that it does not require that the animal is killed.

Earthworms are important for soil formation and organic matter breakdown in most terrestrial environments. Earthworms are significantly affected by pollution from intensive use of biocides in agriculture, industrial activities, and atmospheric deposition. Hence, earthworms are valuable bioindicators of soil pollution (Lionetto 2012). Different pollutants, such as metals and pesticides, have been shown to influence biomarkers in earthworms (e.g. Lukkari et al. 2004, Laszczyca et al. 2004).

It is advisable to include biomarkers in birds of prey or fish eating birds as they are found high in the food web and are, therefore, extra sensitive due to biomagnification of contaminants. Congenital malformations, egg shell thickness and hatching success/brood size were all ranked high, and can therefore be recommended. All three have very high ecological relevance, and congenital malformations and hatching success/brood size can be expected to respond to mixtures of different types of chemicals. Although effects on eggshell thickness have been reduced following the ban on DDT, it is possible that new chemicals will have the same effect (although the MOA may be different). Furthermore, old releases of DDT may reach the food chain again due to environmental or biological processes (Helander and Bignert 2012). EROD has been used to a limited extent in birds, and is ranked high among biomarkers that are good early warning signals. However, it may be difficult to include EROD in birds in a monitoring program as it requires that the organism is killed. Already dead animals cannot be used as the enzymatic activity ceases when oxygen is no longer transported to the tissue. Aromatase activity in gonads can be used to detect endocrine disruption in mammals. For aromatase analyses, brown rat is suggested. However, as for birds it may be difficult to collect the animals (which needs to be alive at sampling).

In Table 16, the suggested biomarkers in terrestrial and urbanized environments are presented. However, only the first three have been properly assessed in this report. EROD in birds and aromatase in brown rat are assumed to provide the desired information based on the experience with these enzyme systems in fish, and the limited use that is reported in birds and mammals, respectively.

Table 16
Suggested biomarkers for monitoring of pollutants in terrestrial and urbanized environments

Biomarker	Specificity	Relevance	Early Warning	Mixture	Status	Analysis
Congenital malformations in birds	1	2	1	2	1	1
Egg shell thickness for carnivorous birds	2	2	1	0	1	2
Hatching success and brood size for birds	1	2	0	2	1	2
EROD in birds	2	0	2	1	2	2
Gonadal aromatase activity in brown rat	1	2	1	2	1	1
Total:	7	8	4	8	8	6

8. References

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Appendix A – List of biomarkers

Appendix A List of biomarkers			
Biomarker	**Biomarker**	**Organism class**	**References**
Ache Inhibition of cholinesterase activity nmol.min-1 mg prot-1	freshwater, marine, terrestrial	vertebrates (Flounder, Dab, Red mullet, Eelpout) & invertebrates (Mytilus edulis, Mytilus galloprovincialis, crustaceans)	ICES, 1999; Kirby et al., 2000; BEEP, 2001; Trudeau & Sans Cartier, 2001; Spurgeon et al., 2002
Alkylphenol bile metabolites	freshwater, marine, terrestrial	Vertebrates (fish), invertebrates	Beyer, 2013
ALT, alanine transaminase alanine aminotransferase (serum)	freshwater, marine	bivalve, fish, verterbrate	van der Oost, 2003; Cajaraville, 2000
Antioxidant enzymes e.g. Superoxide dismutase (SOD), catalase CAT, Glutathione peroxidase (GPOX), Glutathione reductase (GRED)	freshwater, marine, terrestrial	vertebrates (fish), invertebrates & plants	Siesko et al., 1997; ICES, 1999; BEEP, 2001; Hartley-Whitaker & Meharg, 2001; Lindstrom-Seppa et al., 2001; MacFarlane, 2002; Spurgeon et al., 2002
Aromatase	freshwater, marine	fish	Lavado, 2004
AST, Aspartate aminotransferase (serum);	freshwater, marine, terrestrial	vertebrate	Pérez 2010; van der Oost, 2003
Blood glucose	freshwater, marine	fish	Wernersson AS, 2012
Blood hemoglobin concentration	terrester	earthworm Lumbricus terrestris	Calisi, 2013
Blubber thickness	marine	Baltic seals	Wernersson AS, 2012
Brood size	freshwater, marine, terrestrial	raptors, viviparous fish	Wernersson AS, 2012

Appendix A
List of biomarkers

Biomarker	Biomarker	Organism class	References
CEA, Cellular energy allocation	freshwater, marine, terrestrial	invertebrates and small fish	Beyer, 2013
CF, condition factor (wholebody weight to length)	freshwater, marine	fish	Environment Canada, 2010
Changes in vitamin levels e.g. Vitamin A (retinoids), Vitamin C (ascorbic acid)	freshwater, marine, terrestrial	vertebrates, invertebrates & plants	Peakall, 1992; Fox, 1993; Lytle & Lytle, 1997; Brown, 1999; Ryckman et al., 2001
Colonic ulcers	marine	Baltic seals	Wernersson AS, 2012
Congenital malformations (birth defects) in birds	terrester	bird	Fox, 1993
Delayed reproduction/gonadal maturation in fish	freshwater, marine	fish	CSEMP, 2012
δ-aminolevulinic acid dehydratase inhibition (ALAD)	freshwater, marine, terrestrial	haemoglobin-carrying organisms, fish	Peakall, 1992; Gompertz et al., 1996; ICES, 1999; Spurgeon et al., 2002
Developmental disorders of teeth, third lower molar	terrester	wild vole species bank vole (Clethrionomys glareolus)	Murtomaa, 2007
DNA adduct **Increase in DNA Adduct formation**	freshwater, marine, terrestrial	vertebrates, invertebrates & plants fish: Dab, Flounder, Long Rough Dab, Halibut, Herring and sprat, Cod, Haddock	Peakall, 1992; Gompertz et al., 1996; ICES, 1999; Spurgeon et al., 2002; Hamers, 2002
DNA repair capacity	freshwater, marine, terrestrial	fish	Kienzler 2013
DNA Strand Breakage (e.g. COMET assay) and other chromosomal aberrations	freshwater, marine, terrestrial	vertebrates (fish), invertebrates (mussels) & plants	Peakall, 1992; ICES, 1999; Mitchelmore & Chipman, 1998; Spurgeon et al., 2002
Egg shell thickness	terrester	guillemot eggs, white tailed eagle	Bignert, 1995
Egg size	freshwater, marine	fish	Environment Canada, 2010,

Appendix A
List of biomarkers

Biomarker	Biomarker	Organism class	References
Elevation of Serum Enzyme activity (e.g. Sorbitol dehydrogenase, glutamate dehydrogenase, glutamate pyruvate transaminase, lactate dehydrogenase)	freshwater, marine, terrestrial	vertebrates & invertebrates	Peakall, 1992, Peakall & McBee, 2001
Embryo aberrations in field collected amphipod crustaceans	freshwater, marine	amphipods crustaceans	HaV, Wernersson, 2012
Hatching success and brood size for birds	terrester	bird	Fox, 1993
Embryonal development in Monoporeia affinis and Pontoporeia femorata **Frequency of malformed embryos, egg production and frequency of dead broods/dead eggs.**	marine	benthic invertebrate	Wernersson AS, 2012
External damage/visible diseases - Fish disease Index **Ac, Acanthochondria cornuta** **Ep, Epidermal hyperplasia/ papilloma** **Fi, Acute/healing fin rot/erosion; Hp, Hyperpigmentation;** **Le, Lepeophtheirus sp.;** **Ly, Lymphocystis;** **St, Stephanostomum baccatum;** **Ul, Acute/healing skin ulcerations;** **Xc, X-cell gill disease**	freshwater, marine	dab	ICES, 2011
fin erosion	freshwater, marine	fish	ICES, 2011

Appendix A
List of biomarkers

Biomarker	Biomarker	Organism class	References
Fluctuating asymmetry (fa)	freshwater, marine, terrestrial	invertebrates (fish) and plants	Spurgeon et al., 2002
Glycogen (liver&muscle)	freshwater, marine, terrestrial	fish, rat, lizard	Roche, 2002
Granulocyte morphometric alterations	freshwater, marine, terrestrial	earthworm Lumbricus terrestris Mussel	Calisi, 2013
Growth Weight at age	freshwater, marine	fish	Environment Canada, 2010
GSI, gonad somatic index	freshwater, marine	fish	CSEMP, 2012
Haptoglobin and ferritin	terrester	common guillemot	Troisi, 2007
Heat Shock (or Stress) Protein expression, e.g. HSP60, HSP70 & HSP 90	freshwater, marine, terrestrial	vertebrates, invertebrates & plants	Ernst & Peterson, 1994; Siesko et al., 1997; Spurgeon et al., 2002; Gupta, 2010
Hematocrite	freshwater, marine	fish	Wernersson AS, 2012
Histopathological changes contaminanat specific and nonspecific eg liver nodules or tumour and lesion formation	freshwater, marine, terrestrial	vertebrates (fish: bad, flounder), invertebrates (Mytilus) & plants	Berg 2013; Gompertz et al., 1996; Marine Pollution Monitoring Management Group, 1998; Hahn, 1999; ICES, 1999; Schmitt et al., 1999; Schmitt & Dethloff, 2000; Spurgeon et al., 2002
HSI, LSI, hepatic somatic index	freshwater, marine	fish	Wernersson AS, 2012; CSEMP, 2012
Immature red blood cells (irbc)	freshwater, marine	fish	Wernersson AS, 2012
Immune system activity e.g. Phagocytosis, lymphocyte viability white blood cell count, macrophage activity	freshwater, marine, terrestrial	vertebrates (fish) & invertebrates	ICES, 1999; Schmitt et al., 1999; Schmitt & Dethloff, 2000; Peakall & McBee, 2001; Spurgeon et al., 2002
Impairment of normal neuroendocrine stress response (e.g. Cortisol, adrenocorticotrophic	freshwater, marine	vertebrates	Hontela, 1998; Hontela et al., 1999; Pottinger et al., 2002

Appendix A
List of biomarkers

Biomarker	Biomarker	Organism class	References
hormone (ACTH)			
Impairment of Reproductive steroids (e.g. Luteinizing hormone (LH), follicle stimulating hormone (FSH), 11-ketotestosterone and progesterone)	freshwater, marine, terrestrial	vertebrates	Peakall, 1992; Schmitt et al., 1999; Schmitt & Dethloff, 2000
Imposex development in gastropods Indices: **VDSI (Vas Deferens Sequence Index)** **Changes in gonad** **RPLI (based on penis lengths of females and males respectively)**	freshwater, marine	invertebrates netted dogwhelk (Hinia nitida) (Nucella lapillus), Nassarius reticulata, Buccinum undatum and Neptunea antiqua	Marine Pollution Monitoring Management Group, 1998; ICES, 1999; Osborn et al., 2000; Strand & Jacobsen, 2002
Increase in CYP1A. Ethoxyresorufin-O-deethylase (EROD) activity	freshwater, marine, terrestrial	vertebrates & invertebrates Dab, Flounder, Plaice, Cod, Plaice, Four spotted megrim, Dragonet, Red mullet, Eelpout, Clethrionomys rufocanus	Fossi et al., 1992; Gompertz et al., 1996; Marine Pollution Monitoring Management Group, 1998; Marsili et al., 1998; Elliott et al., 1999; Hahn, 1999; ICES, 1999; Jeffery et al., 1999; BEEP, 2001; Rattner & Melancon, 2001; Ryckman et al., 2001; Schmitt et al., 1999; Schmitt & Dethloff, 2000; Spurgeon et al., 2002; Fossi et al., 2003
Increase in Glutathione S-transferase (GST) activity	freshwater, marine, terrestrial	vertebrates (fish), invertebrates & plants	ICES, 1999; Lindstrom-Seppa et al., 2001; Spurgeon et al., 2002
Increase in Kidney Epithelial Cell Height (KEH)	freshwater, marine	stickleback (Gasterosteus aculeatus L.)	Allen et al., 2002
Increase in Porphyrins	freshwater, marine, terrestrial	vertebrates, invertebrates & plants	Peakall, 1992, Fox, 1993 & Peakall & McBee, 2001
Induction/inhibition of	freshwater, marine	Mytilys edulis, fish, molluscs	Brooks, 2009

Appendix A List of biomarkers			
Biomarker	**Biomarker**	**Organism class**	**References**
multidrug/multixenobiotic resistance (MDR/MXR)			
Inhibition of Calcium atpase activity	freshwater, marine, terrestrial	vertebrates & invertebrates	Gastaldi et al., 2002; Viarengo et al., 2002 a&b
Intersex development in fish % prevalence	freshwater, marine	vertebrates Dab, Flounder, Cod, Red mullet, Eelpout	Marine Pollution Monitoring Management Group, 1998; ICES, 1999; Schmitt et al., 1999; Osborn et al., 2000; Strand & Jacobsen, 2002
Intersex development in gastropod	marine	Littorina littorea	Bryan, 1986; ICES Advice 2011
Ions (Na, K and Ca) in blood plasma	freshwater, marine	fish	Wernersson AS, 2012
Kidney alterations (glomeruli changes, tubular cell proliferations)	marine	Seal	Wernersson AS, 2012
Lactate in blood plasma	freshwater, marine, terrestrial	fish	Wernersson AS, 2012
Lipid weight of soft tissues muscle lipid content storage disorders (lipid accumulation, NL)	marine	Blue mussels	Marigomez, 2013
Liver lipid content	freshwater, marine	fish	Berg 2013
LMS Lysosomal membrane stability minutes Cytochemical; liver all species Neutral Red Retention: all species	freshwater, marine, terrestrial	vertebrates(fish), invertebrates (Mytilus, oyster) & plants	ICES, 1999; BEEP, 2001; Capri et al., 2002; Spurgeon et al., 2002; Viarengo et al., 2002a
LPF Accumulation of lipofuscins	marine	mussels	Marigomez, 2013
Macroscopic liver neoplasms	freshwater, marine	fish: dab,flounder	Koehler 2007
Metabolic profiling using high resolution nuclear magnetic	freshwater, marine, terrestrial	vertebrates & invertebrates	Griffin et al., 2000a, b & c; Griffin et al., 2001 a & b; Spurgeon et al., 2002

Appendix A List of biomarkers			
Biomarker	**Biomarker**	**Organism class**	**References**
resonance spectroscopy (NMR)			
Metallothionein induction Hepatic metallothionein ìg/g (w.w.)	freshwater, marine, terrestrial	vertebrates (Fish), invertebrates (Mytilus) & plants	Elliott et al., 1999; ICES, 1999; BEEP, 2001; Spurgeon et al., 2002; Viarengo et al., 2002a
Micronucleated and polynucleated alveolar macrophages and micronucleated polychromatic erythrocytes in bone marrow	freshwater, marine, terrestrial	invertebrate and vertebrate	Izzotti 2001
Micronuclei test	freshwater, marine, terrestrial	European pond turtles	Bolognesi and Hayashi, 2011
MMC Melano Macrophage centers	freshwater, marine	invertebrate (mussel) and vertebrate (fish)	Marigomez, 2013; Agius, 2003
Morphologically intermediate papilla syndrome (mips)	freshwater, marine	Fish - specifically used in the Sand Goby	Allen et al., 2002; Kirby, 2003
Morphometrical alterations of lysosomes volume density (vvlys) and numerical density (nvlys) of lysosomes & Lysosomal enlargement; µm3/µm3 (quantitative)	freshwater, marine, terrestrial	mollusc, fish	Cajaraville et al., 2000;
Necrosis/degenerated liver cells	freshwater, marine	fish: dab,flounder	Wernersson AS, 2012
NKA, Na+,K+-atpase activity	freshwater, marine	fish	Stagg et al 1992
PAH-metabolites 1-hydroxypyrene (1-HP) OH-pyren	freshwater, marine, terrestrial	vertebrates fish	Kamman, 2013
PAH-metabolites in bile	freshwater, marine	fish:	Elliott et al., 1999; ICES, 1999; Defra, 2000b;

Appendix A List of biomarkers			
Biomarker	**Biomarker**	**Organism class**	**References**
		Dab, Cod, Flounder, Haddock, Eelpout , Herring	BEEP, 2001
Peroxisomal acyl-coa oxidase (AOX) activity and peroxisomal volume	freshwater, marine, terrestrial	fish, invertebrates	Cajaraville, 2000; Marigomez 2013
Phytochelatin concentration, changes	freshwater, marine, terrestrial	plants	Ernst & Peterson, 1994; Hartley-Whitaker & Meharg, 2001
Pre/neoplastic lesions by NADPH producing enzymes	freshwater, marine	fish	Fricke et al.;2012
Proteomics **Changes in protein expression**	freshwater, marine, terrestrial	vertebrates, invertebrates & plants	Spurgeon et al., 2002
Reproductive success in Eelpout, **Zoarces viviparous** **Malformed fry** **Late dead fry** **Early dead fry** **Total abnormal fry**	freshwater, marine	Eelpout	Wernersson AS, 2012
Sex ratio	freshwater, marine	gastropod, viviparous fish	Wernersson AS, 2012; CSEMP, 2012
SFG Scope for Growth **Joules/hr/g dry wt.**	freshwater, marine	invertebrates bivalve mollusc	ICES, 1999; Defra, 2000a
Shell height	freshwater, marine	gastropod	CSEMP, 2012
Skeletal deformities	freshwater, marine	fish	Sassi, 2010
Spiggin in three spined stickleback	freshwater, marine	female three-spined stickleback (Gasterosteus aculeatus L.)	Allen et al., 2002; Katsiadaki et al., 2002; Thomas et al., 2002 ; Hogan , 2012
Structural changes of the digestive tubule epithelium MLR/MET:ratio of	freshwater, marine	mussels, molluscs	Marigomez, 2013

Appendix A
List of biomarkers

Biomarker	Biomarker	Organism class	References
mean lumen radius to mean epithelial thickness Digestive tubule epithelial atrophy and thinning; µm/µm (quantitative) MLR Mean luminal radius ratio of mean epithelial thickness to mean diverticula radius (MET/MDR)			
Total antioxidant scavenging capacity (TOSC)	freshwater, marine, terrestrial	invertebrate and vertebrate	Regoli et al, 2002; Regoli, 2000
Total number of eggs per female	terrester, aquatic	raptors, fish	Environment Canada, 2010,
Transcriptomics - oligonucleotide and cdna microarrays	freshwater, marine, terrestrial	invertebrate and vertebrate	ICES, 2002; Spurgeon et al., 2002; Viarengo et al., 2002b
White blood cell ratios (lymphocytes, granulocytes, trombocytes)	freshwater, marine	fish	Wernersson AS, 2012
Vitellogenin (VTG) protein induction in fish	freshwater, marine	fish and crustaceans (assays are being developed for birds)	Gompertz et al., 1996; ICES, 1999; Schmitt et al., 1999; Schmitt & Dethloff, 2000; Defra, 2000b; Pottinger et al., 2002; Palace 2009
VTG mrna expression in male fish	freshwater, marine	fish - including sand goby, viviparous blenny, sheepshead minnow & rainbow trout (assays are being developed for birds)	Allen et al., 2002
Vvbas: Volume density of basophilic cells- increase due to exposure Cell type composition of digestive gland epithelium; µm3/µm3	freshwater, marine	mussel	Cajaraville, 1990

Appendix A List of biomarkers			
Biomarker	**Biomarker**	**Organism class**	**References**
(quantitative)			
Zona Radiata protein (protein and mrna)	freshwater, marine	fish	Arukwe, 1997; Allen et al., 2002

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Appendix B – Scores biomarkers

Appendix B
Scores - biomarkers

Biomarker	Specificity	Relevance	Early warning	Mixture	Status	Analysis
1-hydroxypyrene (1-HP) OH-pyren	2	0	2	0	1	2
Ache Inhibition of cholinesterase activity nmol.min-1 mg prot-1	1	0	2	1	2	2
Alkylphenol bile metabolites	2	1	2	0	0	2
ALT, alanine transaminase alanine aminotransferase (serum)	0	1	1	2	0	2
Antioxidant enzymes e.g. Superoxide dismutase (SOD), catalase CAT, Glutathione peroxidase (GPOX), Glutathione reductase (GRED)	1	0	2	2	2	2
Aromatase	1	2	1	2	1	1
AST, Aspartate aminotransferase (serum);	0	1	1	2	0	2
Blood glucose	0	1	1	1	1	2
Blood hemoglobin concentration	1	1	1	1	1	2
Blubber thickness	0	2	1	2	1	2

Appendix B
Scores - biomarkers

Biomarker	Specificity	Relevance	Early warning	Mixture	Status	Analysis
Brood size viviparous fish	0	2	0	2	1	2
CEA, Cellular energy allocation	1	1	1	2	0	1
CF, condition factor (whole body weight to length)	0	2	0	2	1	2
Changes in Immune system activity e.g. Phagocytosis, lymphocyte viability white blood cell count, macrophage activity	0	1	1	2	1	2
Changes in Phytochelatin concentration	1	0	1	1	0	2
Changes in vitamin levels e.g. Vitamin A (retinoids), Vitamin C (ascorbic acid)	0	2	1	2	1	1
Colonic ulcers	1	1	1	1	1	1
Congenital malformations (birth defects) in birds	1	2	1	2	1	1
Delayed reproduction/gonadal maturation in fish	0	2	0	2	1	2
δ-aminolevulinic acid dehydratase inhibition (ALAD)	1	0	2	0	1	2
DNA repair capacity	1	1	1	2	0	1
Egg shell thickness	2	2	1	0	1	2
Egg size	0	2	1	2	1	1

Appendix B
Scores - biomarkers

Biomarker	Specificity	Relevance	Early warning	Mixture	Status	Analysis
Elevation of Serum Enzyme activity (e.g. Sorbitol dehydrogenase, glutamate dehydrogenase, glutamate pyruvate transaminase, lactate dehydrogenase)	0	0	1	2	1	2
Embryo aberrations in field collected amphipod crustaceans	0	2	1	2	0	2
Embryonal development in Monoporeia affinis and Pontoporeia femorata Frequency of malformed embryos, egg production and frequency of dead broods/dead eggs.	0	2	0	2	1	1
External damage/visible diseases - Fish disease Index Ac, Acanthochondria cornuta Ep, Epidermal hyperplasia/ papilloma Fi, Acute/healing fin rot/erosion; Hp, Hyperpigmentation; Le, Lepeophtheirus sp.; Ly, Lymphocystis; St, Stephanostomum baccatum; Ul, Acute/healing skin ulcerations; Xc, X-cell gill disease	0	1	1	2	2	1

Appendix B
Scores - biomarkers

Biomarker	Specificity	Relevance	Early warning	Mixture	Status	Analysis
fin erosion	1	1	1	1	2	2
Fluctuating asymmetry (fa)	0	1	1	2	0	0
Glycogen (liver&muscle)	1	1	1	2	1	1
Granulocyte morphometric alterations	1	1	1	2	0	1
Growth Weight at age	0	2	0	2	1	2
GSI, gonad somatic index	0	2	0	2	1	2
Hatching success and brood size for birds	1	2	0	2	1	2
Heat Shock (or Stress) Protein expression, e.g. HSP60, HSP70 & HSP 90	0	0	2	2	0	2
Hematocrite	1	1	1	1	1	2
Histopathological changes contaminant specific and nonspecific eg liver nodules or tumour and lesion formation	1	1	1	2	2	1
Immature red blood cells (irbc)	0	1	1	1	1	1
Impairment of normal neuroendocrine stress response (e.g. Cortisol, adrenocorticotrophic hormone (ACTH)	0	1	1	2	1	1

Appendix B
Scores - biomarkers

Biomarker	Specificity	Relevance	Early warning	Mixture	Status	Analysis
Impairment of Reproductive steroids (e.g. Luteinizing hormone (LH), follicle stimulating hormone (FSH), 11-ketotestosterone and progesterone)	0	2	1	2	1	1
Imposex development in gastropods Indices: VDSI (Vas Deferens Sequence Index) Changes in gonad RPLI (based on penis lengths of females and males respectively)	2	2	0	0	2	1
Increase in CYP1A. Ethoxyresorufin-O-deethylase (EROD) activity	2	0	2	1	2	2
Increase in DNA Adduct formation nm adducts mol DNA	1	1	1	1	2	1
Increase in DNA Strand Breakage (e.g. COMET assay) and other chromosomal aberrations	0	1	1	1	1	2
Increase in Glutathione S-transferase (GST) activity	1	0	1	1	1	2
Increase in Kidney Epithelial Cell Height (KEH)	0	1	1	1	0	1

Appendix B
Scores - biomarkers

Biomarker	Specificity	Relevance	Early warning	Mixture	Status	Analysis
Increase in Porphyrins	1	1	1	2	1	1
Induction/inhibition of multidrug/multixenobiotic resistance (MDR/MXR) in Mytilys edulis	1	0	1	2	2	1
Inhibition of Calcium atpase activity	0	0	1	2	1	1
Intersex in fish and gastropods	2	2	0	1	2	1
Ions (Na, K and Ca) in blood plasma	0	1	1	1	1	2
Kidney alterations (glomeruli changes, tubular cell proliferations)	1	1	1	1	1	1
Lactate in blood plasma	0	1	1	1	1	2
Lipid weight of soft tissues muscle lipid content storage disorders (lipid accumulation, NL	1	1	1	1	0	1
Liver lipid content	1	1	1	1	1	1
LMS Lysosomal membrane stability minutes Cytochemical; liver all species Neutral Red Retention: all species	1	1	1	2	2	1
LPF Accumulation of lipofuscins	1	1	1	2	1	1

Appendix B
Scores - biomarkers

Biomarker	Specificity	Relevance	Early warning	Mixture	Status	Analysis
LSI, liver somatic index HSI, hepatic somatic index	1	1	1	2	2	2
Macroscopic liver neoplasms (tumours)	1	2	1	1	2	1
Metallothionein induction Hepatic metallothionein ìg/g (w.w.) Fish	1	0	1	1	1	2
Micronuclei test	1	2	1	1	2	1
MMC Melano Macrophage centers	1	1	1	1	1	1
Morphologically intermediate papilla syndrome (mips)	1	2	1	1	0	1
Morphometrical alterations of lysosomes volume density (vvlys) and numerical density (nvlys) of lysosomes & Lysosomal enlargement; µm3/µm3 (quantitative)	1	1	1	2	2	1
Necrosis/degenerated liver cells	1	1	1	1	1	1
NKA, Na+,K+-atpase activity	0	1	1	1	0	1
PAH-metabolites in bile	2	0	2	1	2	2
Peroxisomal acyl-coa oxidase (AOX) activity and peroxisomal volume peroxisomal	1	1	1	2	0	1

Appendix B
Scores - biomarkers

Biomarker	Specificity	Relevance	Early warning	Mixture	Status	Analysis
proliferation						
Pre/neoplastic lesions by NADPH producing enzymes (fish)	1	1	1	1	2	2
Reproductive success in Eelpout, Zoarces viviparous Malformed fry Late dead fry Early dead fry Total abnormal fry	0	2	0	2	2	1
Sex ratio	1	2	0	0	1	1
Shell height	0	2	0	1	1	2
Skeletal deformities	1	2	1	2	1	1
Spiggin in three-spined stickleback	2	2	1	1	1	1
Structural changes of the digestive tubule epithelium MLR/MET:ratio of mean lumen radius to mean epithelial thickness Digestive tubule epithelial atrophy and thinning; µm/µm (quantitative) MLR Mean luminal radius ratio of mean epithelial thickness to mean diverticula radius (MET/MDR)	1	1	1	1	2	1

Appendix B
Scores - biomarkers

Biomarker	Specificity	Relevance	Early warning	Mixture	Status	Analysis
Total antioxidant scavenging capacity (TOSC)	1	1	2	2	1	1
Total number of eggs per female	0	2	0	2	1	2
White blood cell ratios (lymphocytes, granulocytes, trombocytes)	1	1	1	1	1	1
Vitellogenin (VTG) in fish	2	2	2	1	2	2
Vvbas: Volume density of basophilic cells- increase due to exposure Cell type composition of digestive gland epithelium; µm3/µm3 (quantitative)	1	1	1	2	2	1
Zona Radiata protein (protein and mrna)	2	2	1	1	0	1

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Miljødirektoratet ble opprettet 1. juli 2013 og er en sammenslåing av Direktoratet for naturforvaltning og Klima- og forurensningsdirektoratet (Klif).

Vi er et direktorat under Miljøverndepartementet med 700 ansatte i Trondheim og Oslo. Statens naturoppsyn er en del av direktoratet med over 60 lokalkontor.

Miljødirektoratet har sentrale oppgaver og ansvar i arbeidet med å redusere klimagassutslipp, forvalte norsk natur og hindre forurensning.

Våre viktigste funksjoner er å overvåke miljøtilstanden og formidle informasjon, være myndighetsutøver, styre og veilede regionalt og kommunalt nivå, samarbeide med berørte sektormyndigheter, være faglig rådgiver og bidra i internasjonalt miljøarbeid.