

LÄNSSTYRELSEN Västra götalands län





Swedish monitoring of hazardous substances in the aquatic environment - current vs required monitoring and potential developments





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Utökad sammanfattning och övergripande slutsatser

Syfte och uppbyggnad

Följande rapport baseras på en forskningsutredning som pågått under 2010-2011, med syfte att identifiera eventuella luckor i dagens övervakningssystem av miljöfarliga ämnen i den svenska akvatiska miljön, gentemot de krav som ställs eller åtaganden som gjorts främst internationellt, men även för att tillhandahålla det prioriteringsunderlag som behövs för åtgärdsarbete.

Rapporten består huvudsakligen av två delar där den första redogör för de övervaknings- och rapporteringskrav som ställs på Sverige, och hur övervakningen går till i dagsläget på nationell och regional nivå. Förslag ges också på hur man skulle kunna hantera några av de brister som identifierats. Den andra delen lägger särskild tonvikt på val av provtagningsmatris (vatten, sediment, biota) i samband med statusklassning inom vattenförvaltningen, för att förutsäga risk för negativa effekter i eller via vattenmiljön, samt användbarheten hos effektbaserad övervakningsmetodik för att bedöma effekter, eller risk för sådana, av miljöfarliga ämnen i den akvatiska miljön.

Även om flera resurs- och utvecklingsbehov har kunnat identifieras och kort beskrivs nedan, görs ändå bedömningen att Sverige har ett gott utgångsläge för att i framtiden tillgodose de behov som finns.

<u> Miljömålen – avsaknad av indikatorer</u>

Underlag i form av miljöövervakningsdata för miljöfarliga ämnen och deras effekter i den akvatiska miljön behövs i samband med utvärderingar av främst tre av de svenska miljömålen (Giftfri miljö, Hav i balans, Levande sjöar och vattendrag). I dagsläget finns dock bara en utvecklad indikator relaterad till halter av miljöfarliga ämnen i den akvatiska miljön. Ytterligare indikatorer skulle därför behöva utvecklas, för att i högre grad än tidigare även basera utvärderingarna på övervakningsdata på ett transparent och enkelt sätt. Pågående eller nyligen avslutade projekt på regional nivå för att utveckla sådana indikatorer är t ex relaterade till förekomst av tennorganiska föreningar i sediment i småbåtshamnar, och missbildningar hos kiselalger. Det är också rimligt att utgå ifrån att man framöver skulle kunna använda sig av den statusklassning som sker inom vattenförvaltningsarbetet. Eftersom det pågår en revision av den förteckning över s k prioriterade ämnen men även de bedömningsgrunder som ligger till grund för denna statusklassning, bör man dock avvakta med att använda sig av dessa klassningar som miljömålsindikatorer, till dess att de har hunnit revideras.

<u>Utökade och förändrade övervakningskrav genom implementeringen av nya</u> <u>direktiv</u>

Sverige har krav på sig att bedriva övervakning av miljöfarliga ämnen och effekter av dessa i den akvatiska miljön, genom både internationella konventioner och EU direktiv, såsom OSPAR och HELCOM samt ramdirektivet för vatten (2000/60/EG) och marina strategidirektivet (2008/56/EG). Genom implementeringen av de två sistnämnda i svensk lagstiftning (vattenförvaltningsförordningen resp havsmiljöförordningen) ökar de formella kraven på övervakning av akvatiska miljöer markant, och fokuserar i allt högre grad på att inte enbart kunna bedöma trender utan även möjliggöra en bedömning av huruvida uppmätta halter kan antas ge upphov till effekter i eller via den akvatiska miljön. Dessa bedömningar ska tillsammans med trendanalysen ligga till grund för prioriteringar inom åtgärdsarbetet. Övervakningen inom vattenförvaltningen ska ske i form av tre olika typer av program (kontrollerande, operativa resp undersökande program), vilket tydligare än tidigare ställer krav även på regional övervakning och att källor och orsaker till påverkan spåras.

Nationellt övervakningssystem är idag fokuserat på att uppskatta trender

Den övervakning av miljöfarliga ämnen i den akvatiska miljön som sker nationellt i Sverige idag har ett tydligt fokus på att studera trender och till viss del för att skönja geografiska skillnader mellan olika delar av Sverige. Trendövervakning behövs för att t ex avgöra om de åtgärder som har satts in har lett till någon tydlig återhämtning och om ytterligare insatser krävs för att nå målsättningarna i tid. I synnerhet uppåtgående trender påvisar risk för effekter i framtiden och därmed ett akut behov av åtgärder, eftersom återhämtningen för många miljöfarliga ämnen kan ta väldigt lång tid i anspråk ochge upphov till stora kostnader. Geografiska skillnader mellan större regioner kan påvisa skillnader i belastning, både till följd av t ex långväga atmosfärstransporter och regionala skillnader i användning men även olika känslighet mellan regionerna.

Övervakningen av miljöfarliga ämnen i biota är relativt väl utbyggd i både limnisk och marin miljö, i synnerhet för fisk. Det är främst klassiska föroreningar som analyseras (såsom metaller, PCB och dioxiner). Även några relativt nyligen uppmärksammade substanser såsom perfluorerade ämnen och polybromerade difenyletrar ingår dock i dagens nationella program. Sedimentövervakning bedrivs på utsjösediment, i Vänern och Vättern samt i några jordbruksområden. Nationell övervakning av miljöfarliga ämnen i vatten sker både i vattendrag och sjöar men inte alls i marin miljö, och det är uteslutande metaller och bekämpningsmedel som analyseras på vattenfasen. Några marina program har också med effektrelaterade parametrar, både för att mäta effekter av specifika substanser (tennorganiska föreningar) och för att undersöka mer generell påverkan (på fisk, evertebrater och säl).

<u>Behov av kompletterande variabler och program för att även uppfylla de nya</u> <u>krav som ställs och för att underlätta åtgärdsarbetet</u>

Enbart mindre luckor kunde identifieras vid jämförelse med de övervakningskrav eller åtaganden som gjorts enligt de regionala marina konventionerna (OSPAR och HELCOM), och den befintliga nationella övervakningen kan i hög grad även utnyttjas för att tillgodose de krav på kontrollerande övervakning som ställs inom ramen för vattenförvaltningen. Sverige har i synnerhet fokuserat övervakning av ackumulerande substanser på ackumulerande matriser (sediment och biota) vilket inte bara sparar resurser genom att övervakningen baseras på tidsintegrerade prover utan även är mer vetenskapligt motiverat. Genomgången av lämpligaste matris att undersöka visar på att det ofta är möjligt att göra trend och statusbedömningar baserade på data som genereras inom ett och samma program. På detta sätt kan stora ekonomiska vinster göras. Vissa tillägg skulle dock behöva göras.

<u>Substanser att lägga till</u>

Det saknas nationell övervakning för flera av de kandidater som föreslås inför revideringen av dotterdirektivet om prioriterade ämnen (t ex läkemedel). Idag övervakas dessutom ett flertal angelägna prioriterade substanser (alkylfenoler, ftalater) enbart i utsjösediment (dvs utanför det område som regleras av vattenförvaltningsförordningen).

<u>Tillägg av vävnad eller stödjande parametrar och framtagande av</u> omräkningsfaktorer

Dessutom analyseras i vissa fall inte den vävnad som man behöver kunna utvärdera med avseende på risk för effekter (status), vilket krävs enligt både vattenförvaltningsförordningen och havsmiljöförordningen. För några substanser kan denna bedömning troligen ändå göras genom att utveckla omräkningsfaktorer och lägga till ytterligare stödjande parametrar, men för vissa behövs eventuellt en utökning av parallella program för trend och statusövervakning baserade på olika vävnader eller matriser. PFOS analyseras idag t ex på lever medan risk för human hälsa behöver kunna utvärderas och då bör även analys av muskel ingå tills vidare.

Behov av limnisk övervakning av sediment och lägre trofinivåer

Prioriterade substanser som ackumuleras, men främst i sediment och organismer på lägre trofinivåer, är idag särskilt problematiska att bedöma i limnisk miljö eftersom limnisk nationell övervakning av andra arter än fisk saknas och limnisk regelbunden sedimentövervakning är begränsad till Vänern och Vättern. Ett tillägg av sådana program för den här typen av substanser är därför mycket angelägna. Detta skulle även underlätta jämförelser med data som tas fram på lokal och regional nivå t ex för att kunna avgöra behov av lokala åtgärdsinsatser.

Behov av att kunna bedöma status i och belastning från vattendrag

Avsaknad av nationella övervakningsprogram i vattendrag för andra miljöfarliga substanser än metaller och bekämpningsmedel innebär att underlaget för statusklassning av vattendrag är bristfälligt inom vattenförvaltningen. Det innebär också att det är problematiskt att uppskatta belastningen (samt trend) av ackumulerande substanser på havet via tillförsel från inlandsvatten. Sådan övervakning är angelägen i synnerhet för sådana substanser som uppvisar uppåtgående trender, men även sådana som inte minskar i tillräckligt hög grad för att uppsatta mål ska kunna nås i tid, för att identifiera de mest betydelsefulla källorna. Att övervaka hydrofoba ämnen i vatten är dock problematiskt. En strategi för hur både status och belastning från vattendrag ska kunna bedömas för ackumulerande ämnen behöver därför utarbetas och praktiska lärdomar kan troligen dras från andra medlemsländers övervakningssytem, där man hittills har lagt större vikt vid att analysera även ackumulerande substanser i vattenfas.

Regional övervakning mycket bristfällig

Regional regelbunden övervakning av miljöfarliga ämnen i den akvatiska miljön är mycket bristfällig, både med avseende på antalet platser och vilka variabler som övervakas. Substanser utöver de som normalt övervakas inom de nationella programmen ingår sällan trots att det i anslutning till lokala källor kan antas vara angeläget att övervaka sådana lokalt betydelsefulla substanser, snarare än att på detta sätt "förtäta" den nationella övervakningen av kända problemsubstanser om det inte kan antas finnas också lokala källor för dessa. De nuvarande övervakningsprogrammen uppfyller således inte de krav som rimligen kan ställas på operativ övervakning enligt vattenförvaltningsförordningen. Endast en mindre andel av de vattenförekomster som pekas ut i den påverkansanalys som gjordes under föregående förvaltningscykel övervakas överhuvudtaget med avseende på prioriterade substanser i någon matris. Det kan finnas flera orsakter till detta, varav resursbrist troligen är en viktig bidragande faktor, men även avsaknad av tydliga juridiska styrmedel och vägledning för hur sådana program ska läggas upp. Behoven behöver också ses över för att särskilt prioritera undersökningar på sådana områden där det finns en överhängande risk för effekter och lokalt/regionalt åtgärdsarbete är särskilt angeläget.

Behov av ekonomiska resurser och juridiska styrmedel

De regionala medlen för operativ övervakning av miljöfarliga ämnen är mycket begränsade i dagsläget. Man har i hög grad troligen förlitat sig till att detta ska skötas inom ramen för de s k samordnade recipientkontrollprogrammen (SRK). Det saknas dock styrmedel för att påverka dessa i tillräckligt hög grad om inte egna medel också kan skjutas till. Bara enskilda verksamhetsutövare kan föreläggas att bedriva recipientkontroll och de allmänna råd som finns i dagsläget innebär relativt begränsade skyldigheter. Det är särskilt otydligt om en verksamhetsutövare kan förväntas göra undersökningar av hälsorelaterade effekter via den akvatiska miljön men även sådana aspekter ska kunna bedömas i ett operativt övervakningsprogram. Påverkan från miljöfarliga ämnen kommer också många gånger från källor av mer diffus eller historisk karaktär varför det är komplicerat att identifiera en enskild ansvarig som rimligen kan förväntas stå för kostnader i samband med dessa utredningar.

<u>Prioritering av övervakningsinsatser – påverkansanalysverktyg och effektbaserade</u> <u>metoder kan underlätta</u>

Den påverkansanalys som gjordes under föregående förvaltningscykel var inte harmoniserad mellan de olika länen eller distrikten. Ett nationellt verktyg håller därför på att tas fram av vattenmyndigheterna, vilket är angeläget för att man på ett säkrare sätt ska kunna identifiera de övervakningsbehov som finns. För de vattenförekomster för vilka det identifierats en risk för att god status inte uppnås ska operativa övervakningsprogram etableras. Det är dock också tänkbart att ytterligare prioriteringsunderlag för att identifiera behov av löpande operativa övervakningsprogram behövs, i form av t ex kampanjvisa undersökningar. I detta sammanhang kan effektbaserad metodik vara särskilt användbar eftersom lokalt inriktat åtgärdsarbete rimligen bör prioritera områden där man redan nu kan observera effekter. Metoder som även beaktar den samlade påverkan från ett stort antal ämnen finns tillgängliga men används mycket sparsamt på regional nivå.

Behov av vägledning och expertstöd

Att lägga upp miljöövervakningsprogram för miljöfarliga ämnen på regional nivå kräver lokal och regional kännedom för att kunna avgöra var övervakning är särskilt angelägen och vilken typ av föroreningar som kan tänkas förekomma på den aktuella platsen. Denna bedömning görs därför bäst på lokal och regional nivå. Stöd behövs dock för att bedöma hur särskilt "olistade" substanser bör undersökas (provtagningsmetodik, frekvens, matris att övervaka) och för att även kunna tillämpa effektbaserade metoder i högre utsträckning än tidigare.

En tydlig och praktisk vägledning för hur operativ övervakning bör genomföras behöver tas fram, liksom vägledning i samband med utvärderingen av data. Statusklassningarna var i förra förvaltningscykeln inte heller harmoniserade till stor del beroende på brist på bedömningsgrunder för den matris för vilken det fanns data. I dessa fall har expertbedömningar använts men vägledning behövs även för hur man arbetar med dessa.

Ett projekt med syfte att ta fram ett vägledningsdokument för hur man lägger upp ett operativt program pågår med medverkande framför allt från sydlänen. Detta dokument kan ge viktigt stöd framöver, men vägledningen kommer att behöva revideras och kan inte ge svar på alla frågor som kan tänkas dyka upp. Inrättandet av en nationell referensgrupp på området, med sakkunniga inom de discipliner som behövs, skulle därför också kunna underlätta. Bedömningsgrunder och vägledning för expertbedömningar behöver också tas fram för att i högre grad harmonisera statusklassningarna framöver.

Miljöfarliga ämnen beaktas inte i samband med ekologisk statusklassning

<u>Behov av biologiska verktyg och bedömningsgrunder för att avgöra effekter på</u> <u>populations- och samhällsnivå</u>

I samband med ekologisk statusklassning inom vattenförvaltningsarbetet ska även miljöfarliga ämnen beaktas. De biologiska verktygen idag svarar dock i väldigt liten grad på effekter från miljöfarliga ämnen och har istället främst utvecklats för att kunna bedöma eutrofiering och försurning. Här behövs en utveckling och validering av nya metoder samt bedömningsgrunder för att även kunna direkt mäta effekter av miljöfarliga ämnen på ekologisk nivå (samhällen och populationer). Några lovande sådana metoder (såsom SPEAR, PICT och metagenomics) har identifierats för främst lägre trofinivåer, men de behöver antingen utvecklas vidare eller valideras för svenska förhållanden. Dessutom skulle några av de indikatorer som tas fram inom ramen för havsmiljöförordningen även kunna tillämpas i detta sammanhang, då de kan relateras till effekter av miljöfarliga ämnen, och i vissa fall är de tillämpbara för både marin och limnisk miljö. Vägledning och bedömningsgrunder behöver dock i så fall tas fram för att underlätta och harmonisera tillämpningen av dessa metoder vid expertbedömningar i samband med ekologisk statusklassning.

Angreppssätt för att beakta Särskilt förorenande ämnen

Dessutom ska s k särskilt förorenande ämnen (SFÄ), dvs andra substanser än de prioriterade men sådana som inte är "listade", beaktas i samband med den ekologiska statusklassningen. Till skillnad från de flesta andra medlemsländer har Sverige dock ännu inte tydligt gjort detta, trots att ett flertal substanser (främst metaller) redan under föregående förvaltningscykel har bedömts förekomma i sådana halter att de borde föranleda en sänkning av ekologisk status på ett relativt stort antal platser (>200 vattenförekomster). Genom att lyfta fram de substanser som på vattenförekomstnivå förekommer i halter som misstänks ge effekt erhålls viktigt stöd för prioriteringar av lokalt och regionalt åtgärdsarbete. Det är därför angeläget att implementeringen i lagstiftningen och dess tillämpning ses över. Det behövs även en revidering av befintliga förslag på bedömningsgrunder för några sådana substanser, liksom en strategi för hur man kan identifiera SFÄ i de fall då det är okänt vilka substanser som kan tänkas förekomma. Eftersom SFÄ skulle kunna omfatta ett obegränsat antal substanser, behövs kompletterande angreppssätt än enbart riktade kemiska analyser. I rapporten ges förslag på hur effektbaserad metodik skulle kunna användas i detta sammanhang (se även nedan).

Inrapportering och kvalitetssäkring av data behöver ses över

Miljöövervakningsdata för miljöfarliga ämnen är mycket kostsamma att ta fram, varför det är anmärkningsvärt att det påvisats brister i inrapporteringen. Detta har observerats särskilt på regional nivå men även nationell och internationell. En bristande inrapportering av data försvårar revideringen av befintliga övervakningsprogram, statusklassningar och åtgärdsprogram som behöver göras inom vatten- och havsmiljöförvaltning. För de nationella programmen finns en tydlig instruktion för vart data ska levereras och hur, men det är mer oklart för regionala data som kanske är av en annan karaktär (andra matriser och substanser undersöks eller är i form av kampanjer) och här förekommer inte heller samma krav på leverans ens för sådana data som finansieras av statliga medel. Datavärdskapen behöver därför ses över, liksom instruktioner och ansvarsfördelning på regional och nationell nivå för både inrapportering och kvalitetssäkring.

Behov av bedömningsgrunder och vägledning för att bedöma om ett utsläpp riskerar att leda till sänkt status

Det finns ett stort behov av bedömningsgrunder för miljöfarliga ämnen i många olika sammanhang, såsom statusklassning, efterbehandling, muddring. De bedömningsgrunder som ska användas för statusklassning inom både havsmiljöoch vattenförvaltning ska baseras på risk för negativa effekter. Det är dock olämpligt att strikt fastställa en koncentrationsnivå som inte får överskridas i miljön

eftersom samma halt på en plats kan vara problematisk medan den på en annan plats inte alls behöver ge upphov till oro. Det finns för många substanser stora osäkerheter vid beräkningen av de s k EQS väden som tagits fram, i synnerhet för marin miljö. De statusklasser som anges för prioriterade ämnen är dock bara två; "god status" respektive "uppnår ej god status", och det finns således bara ett EQS värde. Man får dock beakta t ex biotillgänglighet och för metaller även bakgrundshalt. För att ge stöd vid bedömningar men ändå förtydliga dessa osäkerheter skulle man därför kunna överväga att ta fram två nivåer för att bedöma risk för negativa effekter. Ett övre värde som om det överskrids anger "uppnår ej god status" och ett lägre värde, som om det underskrids anger "uppnår god status". Värden som hamnar i intervallet däremellan behöver däremot åtföljas av uppföljande studer innan man bör dra slutsatsen att ett åtgärdsbehov föreligger. Sådana intervall är särskilt angelägna att ta fram för sediment eftersom biotillgängligheten hos föroreningar i sediment ofta är svår att bedöma, och tillämpas redan i t ex Kanada. Även den reviderade europeiska vägledningen för framtagande av EQS föreslår en stegvis bedömning för sediment i de fall det förekommer osäkerheter.

De EQS värden som anges är avsedda att uppfyllas i recipienten men det behövs även stöd för att bedöma om ett utsläpp riskerar att leda till sänkt status. Sådana bedömningar är nödvändiga i samband med t ex fastställande av utsläppsvillkor men även i samband med prioriteringar av områden som bör övervakas på lokal nivå. De riktlinjer för hur man beräknar utsläppsvillkor utifrån en fastställd acceptabel blandningszon, och som har tagits fram på europeisk nivå, är inte tillräckliga för att ge detta stöd förutom för substanser som är vattenlösliga och nedbrytbara, dvs sådana substanser som inte ackumuleras i miljön.

Substanser och matriser att prioritera respektive stryka

Med tanke på att resurserna är knappa kan det vara angeläget att se över val av substanser och matris (se kap 7) vid etablering och revidering av övervakningsprogram. En preliminär bedömning av vilka befintliga prioriterade substanser som troligen inte behöver ingå i operativa program (och kanske även övervakas sparsamt på nationell nivå) för att bedöma status gjordes utifrån de data som fanns tillgängliga via datavärdar (se kap 6), liksom bakgrundsdokument och "fate" modellering. Följande substanser utgör t ex troligen inte något problem i vare sig vatten eller sediment, på regional eller nationell nivå, om det inte finns anledning att misstänka lokala källor: atrazin, diklormetan, simazin, triklorbensen, PCP och C10-C13 kloralkaner. Biota var mer problematiskt att bedöma men skulle i så fall troligen främst vara relevant att övervaka för de två sistnämnda (bedömt utifrån deras inneboende egenskaper).

Följande prioriterade substanser förekommer omvänt troligen i så pass höga halter att det är motiverat att sänka status på ett stort antal platser, i vissa fall på nationell nivå (och för vissa främst i marin miljö¹): TBT, PAH, antracen, fluoranten, oktylfenol, kvicksilver, kadmium, bly, PBDE. Denna bedömning baseras främst på deras förekomst i sediment och/eller biota (fisk/musslor). Det bör påpekas att

¹ Till viss del kan detta bero på att EQS för marina vatten ofta är lägre än för limnisk miljö, till följd av att det är bristande tillgång till data för marina organismer och man därför lägger till en högre säkerhetsmariginal.

slutsatserna är preliminära eftersom några bedömningsgrunder för dessa matriser ännu inte fastställts annat än för kvicksilver. Substanser som ger upphov till sänkt status i stort sett överallt är främst motiverat att övervaka inom nationella program, om det inte finns anledning att misstänka att betydelsefulla lokala källor förekommer. Detta kan vara svårt att avgöra på förhand och en undersökande övervakningsinsats kan därför vara motiverad.

Ovanstående har inte tagit hänsyn till trender. Det bör betonas att det även är särskilt viktigt att fortsätta undersöka trender av t ex HBCD, PFOS, dioxiner, Cd, Hg, Ni, Pb, Zn, Cu, då dessa inte tenderar att minska och vissa t o m uppvisar uppåtgående trender (främst observerats i marin biota). De tre förstnämnda är för närvarande kandidater till att bli prioriterade substanser.

Effektbaserad metodik kan vara särskilt användbar för att undersöka komplexa källor och påverkade miljöer

Det är i dagsläget mycket problematiskt att förutsäga risk för effekter av miljöfarliga substanser enbart med utgångspunkt från uppmätta halter, i synnerhet då flera substanser i hög grad förekommer samtidigt. I sådana komplext förorenade prover kan det dessutom vara problematiskt att veta vilka substanser man bör mäta.

Screening

Utöver den regelbundna nationella övervakningen finns ett program för screening, främst med fokus på att analysera enskilda substanser eller grupper av ämnen, för att undersöka deras förekomst och spridning i miljön. Det är också vanligt att Länsstyrelserna deltar i dessa screeningprogram genom att förtäta provtagningen på regional nivå. Screeningen är ett viktigt redskap för att generera underlag inför revidering av regelbundna program men kan även ge viktig information om betydelsefulla källor. Screeningaktiviteter på en sådan nationell nivå kan t ex användas för att identifiera storskaliga problemsubstanser eller påvisa mer klassiska ämnens förekomst i dittills dåligt undersökta matriser.

Vissa typer av källor eller substanser med viss användning har också undersökts, ofta i form av bredare analyser än riktad analys av ett fåtal ämnen, och sådana undersökningar kan ge stöd även åt regionala myndigheter att identifiera vissa typer av verksamheter som särskilt belastande. Ett problem med den här typen av screeningundersökningar är dock att man sällan har kunskap om de koncentrationer som påträffas borde ge anledning till oro samt att det är omöjligt att kemiskt analysera alla substanser som kan tänkas förekomma. Effektbaserade screeningmetoder (s k in vitrometodik) har än så länge förekommit mycket sparsamt men skulle kunna tänkas bidra med viktig information särskilt i samband med dessa bredare screeningundersökningar för att avgöra relativa betydelsen hos källor med komplexa utsläpp.

Avloppsvattenkaraktärisering

Sådana in vitro undersökningar är även användbara i samband med de karaktäriseringar av avloppsvatten som sker, främst inom ramen för villkorsprövningar. Amerikanska studier har visat på att t ex relativt harmlösa orsaker (salthalt och pH) ofta ligger bakom den toxicitet som kan påvisas vid användning av mer traditionella korttidstester på avloppsvatten, samtidigt som kroniska tester är kostsamma att utföra och därför bör övervägas främst då det är särskilt angeläget. Flera in vitro metoder existerar idag som täcker in ett stort antal verkningsmekanismer som är angelägna att undersöka och som är lovande för detta screeningändamål. I de fall negativa effekter kan påvisas är det också rimligt att orsaken identifieras och etablerad amerikansk vägledning för att ta reda på orsaker finns tillgänglig sedan länge, men angreppssättet tillämpas sällan/aldrig i Sverige. In vitro analyser omnämns inte heller i det BREF dokument som tagits fram för sammanhanget.

Recipientundersökningar och operativ övervakning

Även i den mottagande recipienten kan vissa typer av effektbaserade metoder vara användbara för att bedöma lokal påverkan till följd av en viss typ av källa med komplexa och till stor del okända utsläpp (såsom avloppsreningsverk men även spridning från historiska verksamheter och äldre deponier). Metoder och angreppssätt beskrivs övergripande i denna rapport och en europeisk vägledning för effektbaserade metoder i samband med vattendirektivsarbetet håller på att tas fram.

<u>Effektbaserad övervakning inom vattenförvaltningsarbetet ger även stöd för havsmiljöarbetet, och tvärtom</u>

Kraven på s k effektbaserad övervakning är främst tydliga i de regionala konventionerna och det marina strategidirektivet. Den effektbaserade nationella övervakningen av miljöfarliga ämnen bedrivs också uteslutande i marin miljö. Indikatorer baserade på effektbaserade metoder ska tas fram inom havsmiljöarbetet medan miljöfarliga ämnen i dagsläget inte alls beaktas i samband med ekologisk statusklassning (se nedan). Detta medför en risk för att den statusklassning som görs inom ramen för det marina direktivet kommer att skilja sig från den som görs för kusten inom vattenförvaltningsförordningen trots att områdena överlappar varandra, men även att bedömningen av påverkan på marin från limnisk miljö försvåras (möjligheter att identifiera påverkanskällor).

Integrerade angreppssätt ger bästa underlaget

Effektbaserade metoder av olika slag bör dock alltid kompletteras med kemiska analyser, för fortsatta prioriteringar av åtgärdsarbetet. För att underlätta utvärderingen bör övervakningen ske på ett integrerat sätt genom att samma individer eller åtminstone populationer undersöks med avseende på både effekter och halter. Precis som för kemiska analyser är det idag svårt att mäta alla tänkbara typer av effekter, och för att kunna dra slutsatser om orsaker, liksom för att bedöma risk för framtida påverkan på högre trofinivåer av substanser som biomagnifieras, behöver även kemiska analyser ingå. Effektstyrda kemiska analyser kan också vara särskilt användbara i vissa sammanhang för att identifiera vilka substanser som kan antas ge upphov till en observerad effekt. Hur sådana går till beskrivs också övergripande i rapporten.

English summary and overall conclusions

The major conclusions of each chapter can be found at the end of each chapter. The following English summary is therefore focused on overall findings and somewhat less extensive than the Swedish summary.

This report is based on a research investigation that took place in 2010-2011 with the aim to identify gaps in the current monitoring system of hazardous substances in the Swedish environment. This was assessed in relation to international requirements but also need for decision support for the work on control measures. The report is divided into two major parts. The first part is a description of requirements and how monitoring is performed at the moment on national and regional level. Some suggestions are also made on how to handle some of the identified gaps. The second part of the report is primarily focused on aspects to consider when choosing monitoring compartment in the context of status classifications related to the Water Framework Directive, to predict effects from hazardous substances in or via the aquatic environment. Also effect based monitoring tools and their usefulness in predicting/observing effects is described.

An overall conclusion is that in spite of several gaps and needs for resources and development have been identified and described below, Sweden has a good potential to adapt the monitoring system to fill the needs.

Environmental Quality Objectives – lack of indicators.

Monitoring data are needed in the evaluation of primarily three of the Swedish national environmental quality objectives, but so far there is only one indicator that is actually related to the concentrations of hazardous substances in the aquatic environment. Additional indicators would therefore need to be developed. Ongoing work on regional level is described, including tools related to the concentrations of organic tin compounds as well as diatom malformations. It can also be concluded that indicators based on the status classifications within the WFD context would preferably not be made until the revision of status has been performed.

Extended and altered monitoring requirements through the implementation of new EU directives

Sweden is required to monitor hazardous substances and the effects of these in the aquatic environment through international conventions and EU directives, including OSPAR, HELCOM, Water Framework Directive and the Marine Strategy Framework Directive. By the implementation of the last two, monitoring requirements have increased substantially and focus is to a large extent changing towards also generating data that can be used in absolute terms to assess effects in or via the aquatic environments and not only trends. The assessment of status and trends should together provide the basis for management. Within the WFD, monitoring should be performed in three different programs (surveillance, operational and investigative). Regional programs are therefore required, in order to identify sources.

National monitoring system currently primarily focused on trend analyses

The Swedish national monitoring programs of hazardous substances in the aquatic environment are largely focused on trend analyses and to a certain extent identify geographical differences between areas. The biota program is rather extensive and covers both the marine and limnic environment, at least for fish. Primarily well known substances are included but also some emerging substances are being monitored. Sediment is monitored off shore, and in Vänern and Vättern as well as in a few agricultural areas. Water is monitored in rivers and lakes but not in the marine environment and entirely focused om metals and pesticides. Some marine programs also include effect based monitoring, related to specific substances (imposex) and more general effects (in fish, invertebrates and seal).

Additional variables and programs needed to fulfill requirements and to facilitate the work on measures

Only minor gaps could be identified by comparison with requirements related to the regional conventions (OSPAR and HELCOM). Current national monitoring programs also to a high degree generate data that can be used to fulfill the requirements of surveillance monitoring within WFD. Current monitoring of accumulating substances is largely performed in accumulating compartments and in most cases data provided can be used for both evaluating trends and in the absolute assessments required for status classifications. However, some substances and supportive parameters would need to be added, and recalculation instructions be developed to assess risks to several objectives of protection. In some cases it is necessary to monitor trend and status in two parallel programs (including e.g. several tissues). A need to also monitor limnic sediment and limnic biota at lower trophic levels on national scale was identified. Such data would provide valuable support to evaluate need for local and regional management. To assess the status of and load from accumulating substances in rivers was also identified to be particularly complicated and a clear strategy would need to be developed to fulfill the needs related to both WFD and MSFD.

Regional monitoring of hazardous substances very limited

Large gaps were found between operational monitoring requirements and current regular regional monitoring programs registered. Gaps were identified related to both substances (number and choice of substances) and areas covered (in relation to water bodies being identified to be at risk of non compliance). There was also a lack of a harmonized approach for the assessment of risks (identifying water bodies that are considered at risk of non compliance) and in status classifications perfomed. A number of reasons can probably be found, including lack of resources, assessment criteria and guidance but also regulatory aspects. A national tool to assess risks is under development as well as a guidance document on operational monitoring. The latter would however need to be revised in the future and assessment criteria and guidance related to status classifications (when expert judgement is needed) is necessary.

Hazardous substances are not considered within ecological status classifications

Hazardous substances should be considered in the context of WFD ecological status classifications. Current biological assessment criteria do not cover such aspects and were primarily developed for the assessment of eutrophication and acidification. New tools and assessment criteria are therefore needed and some promising methods were identified in this investigation, and there is also probably potential to harmonise the approach with ongoing work within the MSFD, where such indicators are to be included (at least for such tools that could be applied in both marine and limnic environments). However, guidance on how to utilize such data would then be needed.

Within ecological status classification, river basin specific pollutants (RBSPs) should also be considered. However, in spite of the fact that several such substances were identified on county administrative board level and for about 200 water bodies, and suggested assessment criteria have been provided on national level, such criteria were not formally "developed" on river basin district levels. To highlight local management needs the issue of RBSPs is important and it is recommended to reconsider the legislative implementation as well as to develop clear procedures and a strategy for the future handlig of hazardous substances in this context. Because RBSPs could comprise a more or less unlimited number of compounds, alternatives to targeted chemical analyses are needed and the usefulness of effect based tools in this context are described.

Reporting and data quality control

Lack in the reporting of monitoring data to the national data hosts was identified on both national and – in particular – regional level. Lack in available data has implications not only on status classifications but also in the revision of current programs and management plans.

Assessment criteria and guidance related to emissions

There is an extensive need to develop assessment criteria in many contexts, such as status classifications, remediation, dredging, emission limit values, and link concentrations and amounts in emissions to risk of exceeding environmental standards. Because of the large uncertainties normally involved in environmental standards, it is recommended to consider establishing two levels (a span). Measured concentrations exceeding the higher value should be considered to indicate non compliance and values below compliance, whereas values inbetween should rather trigger additional studies.

Substances and compartments to select vs deselect

A preliminary assessment of the most prioritized compartment to monitor for the current priority substances was made, based on available monitoring data, background information and fate modelling. In addition, substances that probably are not necessary to include in any monitoring program were identified as well as potential "ubiquitous" substances that probably primarily should be monitored within surveillance monitoring unless significant local sources can be identified.

Effect based tools can be of particular value to investigate complex sources and exposed environments

It can be concluded that effect based tools are especially valuable for screening purposes and in whole effluent assessments as well as to assess effects and identify the needs for local management in complex exposure situations. Different types of effect based monitoring tools are described in this report and can be used for different purposes. Effect based indicators and monitoring are required in the MSFD and within the context of regional conventions (HELCOM, OSPAR). To also consider such tools in the limnic environment and within the WFD context could further harmonize the assessments.

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1 General introduction

1.1 Background and objective of the investigation

In order to assess progress towards reaching the goal of a non toxic aquatic environment, knowledge about how hazardous substances actually influence aquatic ecosystems is required. However, the monitoring of hazardous substances in the aquatic environment is usually very costly and the evaluation is complex, not the least because of the vast amont of potential substances to monitor and complexity of effects that can occur. There is thus a need for a coordinated monitoring system that is able to assess the extent of the problem on several scales, such as geographic (local-regional-national-international) and in time (also work proactively to prevent future problems), but still in a cost effective manner. The monitoring system should also be able to identify and evaluate the most effective /preventive/ measures.

With the implementation of the Water Framework Directive (WFD) and in particular its daughter directive 2008/105/EC related to priority substances as well as the Marine Strategy Framework Directive (MSFD), there was a need to review the current Swedish monitoring systems of hazardous substances for regulatory purposes. Therefore a research investigation on this topic was granted by the Swedish Environmental Protection Agency in 2010. The objective was to identify gaps and propose potential developments.

1.2 Approach and outline of the report

This report is divided into two main parts. Part I describes the requirements and current monitoring programs related to hazardous substances in the aqatic environment. Gaps are identified and some ways to fill these gaps are suggested.

Part II describes different approaches and tools to predict the effects from hazardous substances in the aquatic environment in absolute terms, focusing on chemical assessment of mixture effects, selection of compartment for WFD compliance checking and a review on available effect based monitoring tools. This part also includes identification of some research needs identified to improve the toolbox within the near future.

The contents of this report is primarily based on searching web pages of relevant authorities and organisations, and personal communication with national expertise in different areas including contacts at the Swedish Environmental Protection Agency, County Administrative Boards and River Basin District representatives in Sweden. Some literature searches in international scientific journals were also performed. Data was compiled from the database VISS and extracts from the national monitoring data hosts were retrieved. Such data was used to aid in the description of the current monitoring programs and status classifications, make conclusions about the most prioritized compartments and substances to monitor etc. Part II is also based on a questionnaire and discussions at a workshop organized on effect based tools, guidance documents developed within OSPAR and HELCOM, and personal communication with national and international experts in this area.

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2 List of abbreviations

AA Annual Average AChE Acetyl Cholinesterase AhR Arylhydrocarbon Receptor ALA-D Aminolaevulinic acid dehydratase AMAP Arctic Monitoring and Assessment Programme AOX Adsorbable Organic Halides AR Androgen Receptor AVS Acid Volatile Sulphides **BAC Background Assessment Concentration** BAT Best Available Technique **BCF Bioconcentration Factor** BDE Brominated diphenyl ether **BLM Biotic Ligand Model** BOD Biological Oxygen Demand BPD Biocidal Products Directive (98/8/EC) **BQI Benthic Quality Index BSAP Baltic Sea Action Plan** CA Concentration Addition CALUX Chemically Activated Luciferase Expression CAMP Comprehensive Atmospheric Monitoring Programme CEMP Co-ordinated Environmental Monitoring Programme cfu colony forming unit CIRCA Communication and Information Resource Administrator **CIS Common Implementation Strategy** CMEP Chemical Monitoring and Emerging Pollutants COD Chemical Oxygen Demand COHIBA Control of hazardous substances in the Baltic Sea region COM European Commission COMBINE Cooperative Monitoring in the Baltic Marine Environment **CR** Contamination Ratio CSIHS Core Set of Indicators for Hazardous Substances CYP Cytochrome P450 (CYP 1A: family 1 subfamily A) DBT dibutyltin DDT dichlorodiphenyltrichloroethane DEHP bis (2-ethylhexyl)phthalate **DR Dioxin Receptor** DYNAMEC Dynamic Selection and Prioritisation Mechanism for Hazardous Substance EAC Environmental Assessment Criteria EC Effect Concentration ECHA European Chemicals Agency EcoO Ecological Objective EcoQO Ecological Quality Objective **EDA Effects Directed Analysis** EDTA ethylenediaminetetraacetic acid EE-2 17-alpha ethinyl oestradiol EEA European Environment Agency ELISA enzyme-linked immunosorbent assay EMMA European Marine Monitoring and Assessment **EN European Norm** EqP Equilibrium Partitioning EQS Environmental Quality Standard ER Estrogen Receptor EROD Ethoxyresorufin-O-deethylase FDI Fish Disease Index FORMAS Forskningsrådet för miljö, areella näringar och samhällsbyggande (The Swedish Research Council FORMAS) GC Gas chromatography **GES Good Environmental Status**

GIS Geographic Information System GMP Global Monitoring Plan **GMP Good Manufacturing Practice** GR Glucocorticoid Receptor **GSI** Gonadosomatic Index GU Göteborg University HaV Havs och Vattenmyndigheten (Swedish Agency for Marine and Water Management) HBCD, hexabromocyclododecane HCB hexachlorobenzene HCBD hexachlorobutadiene HCH Hexachlorocyclohexane HELCOM Helsinki Commission HI Hazard Index HPLC High Pressure Liquid Chromatography HSC Hazardous Substances Committee IA Independent Action ICES International Council for the Exploration of the Sea ICP Inductively Coupled Plasma IKEU Integrerad Kalkeffektuppföljning (Integrated Studies of the Effects of Liming Acidified Waters) IMO International Maritime Organisation ISO International Standardisation Organisation ITM Institutet för Tillämpad Miljöforskning (Department of Applied Environmental Science at the Stockholm University) IVL Institutet för Vatten och Luftvårdsforskning (Swedish Environmental Research Institute) JAMP Joint Assessment and Monitoring Programme JRC Joint Research Centre of the European Commission KÖ Kommunal Övervakning Municipality Monitoring Koc Organic Carbon Coefficient Kow Octanol Water Coefficient LOD Level of Detection LOQ Level of Quantification LSI Liver Somatic Index MAC Maximum Allowed Concentration MBT monobutyltin MDR/MXR Multidrug/multixenobiotic Resistance MODELKEY Models for Assessing and Forecasting the Impact of Environmental Key Pollutants on Marine and Freshwater Ecosystems and Biodiversity MONAS Monitoring and Assessment MPC Maximum Permissible Concentration MS Mass Spectromotry **MS Member State** MSFD Marine Strategy Framework Directive (2008/56/EC) MT Metallothionein **NIP National Implementation Plan** NMÖ Nationell Miljöövervaknikng National Environmtenal Monitoring NOEC No Observed Effect Concentration NPE Nonylphenol Ethoxilates NRM Naturhistoriska Riksmuséet (The Swedish Museum of Natural History) OECD Organisation for Economic Co-operation and Development **OSPAR Oslo Paris Convention** PAH Polycyclic Aromatic Hydrocarbons PBDE Polybrominated Diphenyl ethers (/penta/) PCB Polychlorinated biphenyl PCDD/F Polychlorinated dibenzodioxins/furans PCP Pentachlorophenol PEC Predicted Environmental Concentration PFBA perfluorobutanoic acid PFBS perfluorobutane sulfonate PFCs perfluorinated compounds PFOA perfluorooctanoic acid

PFOS Perfluorooctanesulfonic acid PHS Priority Hazardous Substances PICT Pollution Induced Community Tolerance **PNEC Predicted No Effect Concentration** PODI Point of Departure POP Persistent Organic Pollutants PPAR Peroxisome Proliferator Activated Receptors PR Progesterone Receptor **PS Priority Substances** PTI Pesticide Toxicity Index QA Quality Assessment QA/QC directive" (2009/90/EC), **QS** Quality Standard QSR Quality Status Report **RBSP River Basin Specific Pollutants** REACH Registration Evaluation Authorisation and Restriction of Chemicals (EC/1907/2006) RID Comprehensive Study on Riverine Inputs and Direct Discharges **RMBP** River Basin Management Plan RMÖ Regional Miljöövervakning (Regional Environmental Monitoring) **RPF Relative Potency Factor RPSI** Relative penis size index RYA Recombinant Yeast Assay SCHER Scientific Committee on Health and Environmental Risks ScorePP Source Control Options for reducing emissions of priority pollutants SDS Substance Data Sheets SEM Simultaneously Extracted Metals SGU Sveriges Geologiska Undersökning (Geological Survey of Sweden) SLU Sveriges Lantbruksuniversitet (Swedish University of Agricultural Sciences) SOCOPSE Source Control of Priority Substances in Europe SoE State of the Environment SPE Solid Phase Extraction SPEAR Species At Risk SPIN Substances in Products in Nordic Countries SRK Samordnad Recipient kontroll (Coordinated recipient monitoring) STP Sewage Treatment Plant **TBT** tributyltin **TEF Toxicity Equivalent Factor TEQ Toxic Equivalent TIE Toxicity Identification Evaluation TOC Total Organic Carbon TR Thyroid Receptor TU Toxic Unit UN United Nations** VDSI (Vas Deference Sequence Index) VISS (VattenInformationsSystem Sverige) VTG Vitellogenin WATERS Waterbody Assessment Tools for Ecological Reference conditions and status in Sweden WEA Whole Effluent Assessment WFD Water Framework Directive (2000/60/EC) WGBEC Working Group on Biological Effects of Contaminants WGE Working Group E (Environment) WHO World Health Organisation WISE Water Information System for Europe WQG Water Quality Guidelines YAS Yeast Androgen Screen YES Yeast Estrogen Screen

PART I. REGULATORY FRAMEWORK AND REPORTING REQUIREMENTS vs CURRENT MONITORING

Environmental monitoring of hazardous substances fulfils many purposes. Long term regular monitoring programs frequently generate data that could be used to estimate trends of single substances in a particular compartment or effects in a particular organism. Monitoring data can be used to assess progress towards national environmental objectives on actions decided. Monitoring programs can act as early warning signals, provide knowledge that can be used to estimate risk to human health and ecosystems and as a ground for specific measures such as use restrictions. Monitoring data from reference areas can also be used to establish baseline levels that can be used when evaluating results from studies closer to local sources. The results also form the basis for several types of international reporting and official statistics about the state of the environment.

The objectives of part I of this report are to evaluate current regular monitoring programs related to hazardous substances *vs* the expected monitoring and reporting (voluntary and mandatory) related to national and international legislation and agreements. Gaps in the current monitoring system and potential developments are identified.

3 Monitoring requirements of hazardous substances in the aquatic environment – voluntary and mandatory

3.1 Sixteen national environmental quality objectives

Several current monitoring programs related to hazardous substances can be used to assess progress towards Swedish environmental objectives (box 3.1.). The national environmental objectives were established in 1999, and progress was reviewed annually since 2002. The current system is being revised at the moment and a report on the evaluation of the progress towards reaching the goals according the current system was therefore presented in May 2011 (Naturvårdsverket 2011). The "interim targets" will be dropped and are therefore not described in the current report.

Box 3.1. The sixteen Swedish environmental objectives	
1. Reduced Climate Impact	
2. Clean Air	
3. Natural Acidification Only	
4. A Non-Toxic Environment	
5. A Protective Ozone Layer	
6. A Safe Radiation Environment	
7. Zero Eutrophication	
8. Flourishing Lakes and Streams	
9. Good-Quality Groundwater	
10. A Balanced Marine Environment, Flourishing Coastal Areas and Archipelagos	
11. Thriving Wetlands	
12. Sustainable Forests	
13. A Varied Agricultural Landscape	
14. A Magnificent Mountain Landscape	
15. A Good Built Environment	
16. A Rich Diversity of Plant and Animal Life	

The environmental quality objective that is obviously related to hazardous substances in the aquatic environment is the fourth objective, "A non-toxic environment". Concentrations of naturally occurring substances in the environment should be close to background levels whereas concentrations of non-naturally occurring substances in the environment close to zero and their impacts on ecosystems negligible (Box 3.2.).

Box 3.2. The outcome of the objective "a non toxic environment" should include the following achievements:

All fish in Swedish seas, lakes and watercourses will be fit for human consumption with respect to their content of non-naturally occurring substances.

Overall exposure to substances of very high concern in the work environment, the external environment and the indoor environment will be close to zero, while exposure to other chemical substances will not be harmful to human health.

Contaminated sites will have been investigated and, where necessary, remediated.

However, also other water related objectives are of relevance to hazardous substances in the aquatic environment, especially objectives 8-10 ("Flourishing Lakes and Streams", "Good-Quality Groundwater", and "A Balanced Marine Environment, Flourishing Coastal Areas and Archipelagos") (Box 3.3.).

Box 3.3.

The outcome of the objective "Flourishing lakes and streams" should include the following achievements:

Nutrient and pollutant loadings will not adversely affect the basic conditions for biodiversity.

Fish and other species that live in or are directly dependent on lakes and watercourses will be able to survive in viable populations.

The habitats of threatened, rare or care-demanding species and naturally occurring habitats with features of conservation value will be maintained at a favourable conservation status.

Threatened species will be able to spread to new sites within their natural ranges, ensuring longterm viable populations.

Lakes and watercourses will achieve good surface water status with respect to species composition and chemical and physical conditions, as defined in the EU Water Framework Directive (2000/60/EC).

Biodiversity will be restored and conserved in lakes and watercourses.

The outcome of the objective "A Balanced Marine Environment, Flourishing Coastal Areas and Archipelagos" should include the following achievements:

Threatened species and stocks will be able to spread to new sites within their natural ranges, ensuring long-term viable populations.

The habitats of threatened, rare and care-demanding species and naturally occurring habitats of conservation value will be maintained at a favourable conservation status.

Fishing, shipping and other uses of seas and other areas of water as well as construction and other development in coastal and archipelago areas, will be undertaken with due consideration for the productive capacity, biodiversity, natural and cultural heritage assets and assets for outdoor recreation of the areas of water concerned.

All coastal waters will achieve good surface water status with respect to species composition and chemical and physical conditions, as defined in the EU Water Framework Directive (2000/60/EC).

3.1.1 Indicators related to monitoring

To facilitate the assessment of progress towards reaching the environmental objectives, several indicators have so far been established. The indicators specified in Box 3.4. are used to assess the progress towards achieving the environmental quality objective "A non-toxic environment".

Box 3.4. Current indicators formally related to the environmental quality objective "A non-toxic environment"

Allergenic chemical products CMR substances in products Organic livestock production Organic milk Land under organic cultivation Contaminated sites Chemical products hazardous to health Chemical products available to consumers Pollutants in breast milk Environmental management systems National emissions of CFC Nickel allergy Plant protection products Plant protection products In addition, there are several indicators linked to "Fluorishing Lakes and Streams" as well as "A Balanced Marine Environment, Flourishing Coastal Areas and Archipelagos" that could indirectly be assumed to be related to reaching the objective "A non-toxic environment". Examples are "Limited nutrient leaching – catch crops" and "Limited nutrient leaching – protection zones" as well as "Oil discharges in marine areas". The reduction in nutrient leaching probably would imply also a reduction in plant protection products leaching into surface water. A reduction in oil discharges means also less toxic substances, such as PAHs, in the aquatic environment.

However, it is clear that only two of the current indicators for "A non-toxic Environment" actually are based on *monitoring data* of hazardous substances in the environment: "Pollutants in breast milk" and "Plant protection products in surface water". The latter, described below (Box 3.5) and in more detail in chapter 8, is directly related to the aquatic environment but restricted to active substances in plant protection products. The former can be suspected to monitor concentrations related to many exposure paths, including also indoor exposure, other food items and personal use of products in addition to an exposure through the aquatic environment.

Box 3.5. Pesticide Toxicity Index

The indicator "Plant protection products in surface water" refers to the so called PTI (Pesticide Toxicity Index), developed to estimate the trends in total pesticide exposure. It is based on evaluated data from four sites within the national pesticide monitoring programme. The conclusions that can be made from the exposure situation and trends vary between sites monitored in Sweden, but in general one of the type areas has a lower PTI (County of Västra Götaland) than the other four (Graaf et al 2011). See also chapter 8.

3.1.1.1 Need to develop indicators to assess progress in the aquatic environment

Monitoring data have been used to assess the progress of achieving a non toxic environment. The purpose of several regular monitoring programs, both nationally and regionally, includes the generation of data to evaluate the progress towards reaching the environmental quality objectives. Easy accessible statistics on the trends observed for several hazardous substances in marine and limnic environments are available². However, if not having clearly defined indicators, the assessment will be less transparent. There is therefore a need to develop more formal indicators that focus on monitoring data of the aquatic environment and that cover other substances than active substances in plant protection products. The Swedish Chemicals Agency also suggests that concentrations of hazardous substances in e.g. fish and sewage sludge could be used as indicators for the evaluation of progress (Kemikalieinspektionen 2011). In selecting indicators it is

² <u>http://www.naturvardsverket.se/sv/Start/Statistik/Officiell-statistik/Statistik-efter-amne/Miljotillstandet-i-kust-och-hav/ and http://www.naturvardsverket.se/sv/Start/Statistik/Officiell-statistik/Statistik-efter-amne/Miljotillstandet-i-sotvatten/</u>

important that these focus on such substances or effects that are considered major threats but also that are able to measure progress in reducing these threats.

3.1.1.1.1 Should indicators focus on toxicants or toxicity (effects)?

The overall assessment made by the Environmental Objectives Council in May 2011 is that it will be very difficult, by 2020, to create conditions for achieving, in the longer term, the state of the environment which the objective "A non toxic environment" expresses.

The focus during the assessment is on the *presence* of hazardous substances and less on observed effects. In this context, it is important to point out that the Swedish wording of this quality objective is "Giftfri miljö" and should actually be translated into "Environment free of toxicants". The less awkward translation that is officially used, "A non-toxic environment", has a somewhat different meaning.

The new suggested wording to describe this objective also includes the following statements "....presence of substances *should not threaten human health or biodiversity*", "...the *impact* on human health and ecosystems is negligible". Thus, the goal to prevent effects may suggest an increased need to also develop indicators based on monitored effects.

3.1.1.1.2 Indicators currently being developed on regional level

In a recent report by the County Administrative Board in Västerbotten, the potential to develop indicators within the regional monitoring programmes was presented (Backlund 2011). In this report, the regional monitoring programme "Miljögifter i kustfisk" (Hazardous substances in coastal fish) is suggested to generate data that can be used also as an indicator for the environmental objective "a non toxic environment".

The County Administrative Board of Blekinge recently received grants to participate in a current validation study of diatom malformations in rivers in order to also assess the effects from hazardous substances within this context (see also chapter 9).

The County Administrative Board of Västra Götaland also received grants to develop an indicator based on TBT (tributyltin) concentrations in sediment from pleasure craft harbours, (Box 3.6.). In addition, several tools that are described in this report could probably be suitable to be used as indicators, also including effect based tools. However, in order to use effect based tools as indicators to estimate progress made, the ability to establish a cause and effect relationship is an important aspect to consider in order to facilitate working with the right measures.

Box 3.6. TBT in sediment from pleasure craft harbours as an indicator

A recent project aiming at developing a new indicator to measure progress towards a non-toxic aquatic environment is related to the presence of organotin compounds in pleasure craft harbour

sediments (Länsstyrelsen Västra Götaland 2011). Organotin compounds are still considered a major threat to aquatic ecosystems, in spite of bans implemented several decades ago.

In 1989, TBT was banned as an antifouling agent on pleasure crafts (<25 meters) and since 2003 there was a complete ban also for larger ships. Since 2008 anti fouling paints containing TBT need to be covered to reduce further release of the substance (782/2003/EC)³. However, Eklund et al (2008) recently reported that the concentrations of TBT in sediment from pleasure craft harbours from the East coast of Sweden were ten times higher in samples taken from the surface than one decimeter deeper. The sum of its degradation products monobutyltin and dibutyltin (MBT+DBT) was also lower than the actual TBT concentrations, suggesting that TBT is still being released to the harbour surface sediments at a higher rate than what is degraded. This is probably caused during boat uptake for storage and treatment, since the concentrations were higher at the spot for boat uptake (up to 2 mg/kg found) and extremely high (>60 mg/kg) in the sediment of boat wash equipment.

Although it is often assumed that the main uptake route for many gastropods is through water, Strand et al (2003) found a strong correlation between TBT concentrations in sediment and in Nuculana pernula, suggesting that TBT concentrations in sediment could provide a good estimate of current TBT exposure of gastropods. Analysing surface water for TBT is connected with large analytical problems because the Level of Quantification (LOQ) levels are higher than the current Environmental Quality Standard (EQS) value in 2008/105/EC. Within the regular national monitoring programme, imposex and tissue concentrations of organotin compounds are analysed. However, in several harbours, the usually monitored species (Nassarius nitidus on the West Coast and Hydrobia ulvae on the East Coast) might be absent for different reasons, including the possibility that they may have become locally extinct due to contamination.

Before dredging can take place in pleasure craft harbours, sampling and analysis of sediment is necessary according to current legislation. Although not restricted to the analysis of surface sediments, this fact increases the available data set of TBT concentrations in sediment for near shore areas. In order to investigate the usefulness of TBT concentrations in harbour sediment as an indicator, sediment from 40 pleasure craft harbours of different character along the west coast were recently sampled and with the same strategy that was used by Eklund et al (2008), i.e. sampling TBT and its degradation products both in the surface and in the 10cm layer. Several of the investigated sites were also selected because of upcoming installations of boat wash installations. Baseline data were therefore created to also enable future studies to check the effectiveness of these measures.

At 14 locations, TBT concentrations were higher than 100 ug/kg in the upper surface, and if also including DBT and MBT, more than half of the locations exceed 100 ug/kg. The ratio between TBT and its degradation products exceed 1 in about 1/3 of the surface samples and the TBT concentrations are higher in the surface than in historic layers also at about 1/3 of the locations.

This suggests that TBT is still released in significant amounts and that local maintenance activities such as boat wash installations and controlled disposal of effluents, sludge and dust from boat sanding are necessary to limit ongoing addition of pollutants to the marine environment. An indicator related to the exposure to organotin compounds aiming at the assessment of environmental quality objectives and need for supplementary measures should be based on the combined assessment of upper and lower sediment concentrations as well as the ratio between TBT and its degradation products.

³ Europaparlamentets och rådets förordning (EG) nr 782/2003 av den 14 april 2003 om förbud mot tennorganiska föreningar på fartyg. Artikel 5.

3.1.1.1.3 Indicators related to status classifications within WFD?

The objectives "Flourishing lakes and streams", and "A Balanced Marine Environment, Flourishing Coastal Areas and Archipelagos" include the goal to achieve "good surface water status with respect to species composition and chemical and physical conditions, as defined in the EU Water Framework Directive (2000/60/EC)."

The number of water bodies that fail to achieve good chemical and ecological status will be reported to the European Commission by the River Basin Districts every 6th year. It is easy to obtain statistics on different geographical scales, such as national, county or district by using the VISS (VattenInformationsSystem Sverige⁴) database. Using these values as indicators would therefore be relatively straightforward.

The chemical status is based on the concentrations of priority substances (further described later in this chapter). However, the status classification will be revised by 2014 at the latest, and then probably include other substances (due to a revision of the list of priority substances in Annex X of WFD) and new assessment criteria (the EQS values for some of the current priority substances are also being revised and assessment criteria for other matrices than water will become available). In addition, many counties and districts may so far have used different assessment criteria for substances that are normally not monitored in water but rather sediment and/or biota, due to the absence of national guidelines. It is therefore strongly recommended to start using chemical status as an indicator only after this revision and not based on current classification.

Ecological status classifications were so far not based on effects from hazardous substances. Current assessment criteria for the biological quality elements are also not good indicators of stress from hazardous substances. Ecological status based on current classifications are therefore not recommended to be used to assess progress towards reaching the objective "A non toxic environment". Some potenital developments in this area are described further in this report, in particular chapter 9.

3.2 International monitoring requirements

The previous chapter focused on the national environmental quality objectives and need for indicators that are based on monitoring data from the aquatic environment. There are also international reporting monitoring requirements related to hazardous substances in the aquatic environment. The following sections describe international monitoring and reporting obligations (both mandatory and voluntary) imposed on Sweden. Other related reporting may also be required, such as emissions of hazardous substances into the environment, and a list of legislations and conventions requiring such reporting is included in the appendix (chapter 10).

⁴ VISS homepage: <u>http://www.viss.lst.se/</u>

3.2.1 Reporting to the European Commission (DG Environment)

With the implementation of several EU directives, there are also requirements to report certain type of data to the European Commission (DG Environment). In order to identify gaps in current monitoring systems *vs* the required monitoring, international obligations are described. With the implementation of the WFD, the European monitoring obligations related to hazardous substances have increased substantially.

3.2.1.1 Bathing water directive

The bathing water directive (76/160/EEC) does not regulate any hazardous substances but rather microbiological parameters. The following parameters need to be measured: concentration of Intestinal *Entercocci* and *Escherichia coli* (in "colony forming unit" per 100 ml; cfu/100ml). Any short term⁵ pollution events that occur during the bathing season also need to be reported and the next report is due December 2011.

3.2.1.2 Shellfish directive

The shellfish directive (79/923/EEC) applies to coastal and brackish waters that have been designated by the Member States as "needing protection or improvement in order to support shellfish (bivalve and gasteropod molluscs) life and growth and thus to contribute to the high quality of shellfish products directly edible by man". The shellfish and fish directives are regulated in the Swedish legislation by "Förordningen (2001:554) om miljökvalitetsnormer för fisk- och musselvatten".

The directive includes some parameters that are related to hazardous substances: petroleum hydrocarbons, metals and organohalogenated substances. However, the mandatory quality criteria for the hydrocarbons are vague and the method of analysis is by visual inspection:

"Hydrocarbons must not be present in the shellfish water in such quantities as to:

- produce a visible film on the surface of the water and/or a deposit on the shellfish,
- have harmful effects on the shellfish"

Although the methods of analysis for organohalogenated substances and metals are by GC (Gas chromatography) and Atomic absorption spectrometry respectively, the mandatory quality criteria are again vague:

- For organohalogenated substances: "The concentration of each substance in the shellfish water or in shellfish flesh must not reach or exceed a level which has harmful effects on the shellfish and larvae"
- For metals: "The concentration of each substance in the shellfish water or in the shellfish flesh must not exceed a level which gives rise to harmful effects

⁵ Defined as "microbiological contamination that has clearly identifiable causes, is not normally expected to affect bathing water quality for more than approximately 72 hours after the bathing water quality is first affected and for which the competent authority has established procedures to predict and deal with"

on the shellfish and their larvae. The synergic effects of these metals must be taken into consideration"

According to article 7 of the directive, monitoring is not necessary if it can be assumed that these criteria are not exceeded. In Sweden, there are 33 designated shellfish areas, all in the Skagerrak and Kattegat River Basin District and the County of Västra Götaland⁶. None of these sites are monitored regularly because the locations are considered "safe". Next report is due September 2011.

3.2.1.3 Fish directive

The Fish Directive (2006/44/EC) concerns the "quality of fresh waters and shall apply to those waters designated by the Member States as needing protection or improvement in order to support fish life". It lists physical and chemical parameters in its Annex I. The directive includes some parameters that can be considered hazardous substances: copper and zink⁷.

In addition, phenolic compounds "must not be present in such concentrations that they adversely affect fish flavour". Petroleum products "must not be present in water in such quantities that they:

- form a visible film on the surface of the water or form coatings on the beds of water-courses and lakes,
- impart a detectable 'hydrocarbon' taste to fish,
- produce harmful effects in fish"

These are analysed by visual examination and examination by taste.

In Sweden, there are 480 designated "fish waters" (according to information registered in VISS). The shellfish and fish directives are regulated in the Swedish legislation by "Förordningen (2001:554) om miljökvalitetsnormer för fisk- och musselvatten". The fish directive will be repealed by the Water Framework Directive 2000/60/EEC 13 years after its entry into force.

3.2.1.4 Drinking Water Directive

The Drinking water directive (98/83/EC), implemented by SLVFS 2001:30⁸, concerns the quality of water intended for human consumption and several hazardous substances are included in the parameters that should be monitored. The frequency of monitoring depends on the purpose. "Check monitoring" needs to be performed more frequently but only for a small number of parameters, whereas

⁶ Regulated by "Länsstyrelsens i Västra Götalands län föreskrift avseende förteckning över musselvatten som skall skyddas enligt förordningen (SFS 2001:554) om miljökvalitetsnormer för fisk- och musselvatten; beslutade den 5 juli 2007." <u>http://www5.o.lst.se/VISInformWebsite/asp/fs/14FS_2007_554.pdf</u>

⁷ The Fish Directive (2006/44/EC) contains guidance values for dissolved copper concentrations that shoud not be exceeded. For water hardness 10, 15, 100 and 300 mg/l CaCO3 respectively: 0,005; 0,022; 0,04 and 0,112 mg/l cannot be exceeded respectively. There are also mandatory values for total zink concentrations that cannot be exceeded and these are, for the corresponding water hardness levels: 0,03; 0,2; 0,3 and 0,5 mg/l for salmonid waters and 0,3; 0,7; 1,0 and 2,0 mg/l for cyprinid waters.

⁸ SLVFS 2001:30. Livsmedelsverkets föreskrifter om dricksvatten.

"audit monitoring" is performed less frequently but on all parameters. Criteria should be fulfilled at the tap. In Sweden there are more than 200 surface water bodies (162 lakes and 48 rivers) that are designated for drinking water purposes, in addition to the 857 groundwaters for the same purpose (according to information registered in VISS). Data related to water quality from these areas is generally available to the county administrative boards. Exact locations of monitoring stations are confidential, although it is possible to find out whether the sample taken is from the raw water or at the tap.

Next reporting to the DG Environment is due December 2011.

3.2.1.5 Water Framework Directive

With the implementation of the water framework directive (WFD, 2000/60/EC), monitoring of the aquatic environment is required, in order to assess the status of European waters and to provide basis for the implementation of measures. The overall goal is that all waters should fulfil the specified requirements defining good status by 2015, although exceptions to the deadline can be made under certain circumstances. Every 6th year a "River Basin Management Plan" (RMBP) needs to be established, stating what measures should be implemented to fulfil quality goals of the respective water bodies. The first RBMP was published in December 2009 and by mid 2012 the current monitoring programs need to be revised to fulfil the needs of the WFD. Although the main focus is on ecological status, being based on so called biological quality elements, also chemicophysical quality elements need to be taken into account to assess ecological status. An indicative list of "main pollutants" to include in this assessment can be found in annex VIII of the WFD and the first nine groups are related to hazardous substances (box 3.9). Also chemical status needs to be assessed, based on the presence of so called priority substances identified in the daughter directive 2008/105/EC (also listed in Annex X of the WFD). Hazardous substances that should be monitored according to the WFD are therefore defined in both annex VIII and annex X of the WFD.

Five of the County Administrative Boards are appointed "river basin district authorities", responsible for the river basin management plans of their districts. However, according to annex II of Naturvårdsverket 2009, it is recommended that the Swedish Environmental Protection Agency should be the main responsible for the surveillance monitoring programs of chemical status (largely based on national monitoring programs, traditionally financed by the Swedish EPA). The River Basin Districts should be responsible for the Operational monitoring programs. Nevertheless, the river basin districts make formal decisions on all WFD monitoring programs to be reported to the European Commission, but lack their own budget to support regular monitoring programs. In addition, also reference stations within regional monitoring programs (County Administrative Board level) could be utilized for the purposes of surveillance monitoring. Since July 2011, a new national agency, "Havs och vattenmyndigheten (Swedish Agency for Marine and Water Management)⁹, has taken over most of the responsibilities related to the WFD and

⁹ <u>http://www.havochvatten.se/</u>

MSFD from the Swedish Environmental Protection Agency. However, the national monitoring of hazardous substances will remain a responsibility of the Swedish Environmental Protection Agency and further divisions between the two agencies are still unclear at the time of writing this report.

3.2.1.5.1 Guidance documents

There are several European guidance and policy documents¹⁰ related to the implementation of the WFD, developed within a Common Implementation Strategy, CIS. Of particular importance to the monitoring and reporting of hazardous substances are the following European guidance documents:

No 3. Analysis of pressures and impacts

- No 7. Monitoring
- No 13. Classification of ecological status and ecological potential
- No 19. Surface water chemical monitoring
- No 21. Reporting
- No 25. Chemical monitoring of sediment and biota
- No 27. Deriving of Environmental Quality Standards

There are also separate "Technical guidelines for the identification of mixing zones pursuant to art. 4(4) of the directive 2008/105/EC".

Two Swedish handbooks (Naturvårdsverket 2008a and Naturvårdsverket 2007) also include chapters related to hazardous substances.

3.2.1.5.2 Three different types of monitoring programs

The WFD makes a distinction between three different types of monitoring: surveillance, operational and investigative. Based on the pressures and impact assessment (regulated by Article 5 and Annex II of WFD; box 3.7.), surveillance and operational monitoring programmes need to be established for each management cycle.

Box 3.7. Analysis of pressures and impacts of hazardous substances

The analysis of pressures and assessment of impacts is an important prerequisite in determing the need for monitoring. For water bodies at risk of failing specified objectives, an implementation of additional monitoring and programme of measures need to be considered. In CIS 3, a pressure and impact assessment is defined as a four step process. In the first step the driving forces are described. Especially land use patterns (industrial, urban, agricultural, forestry etc) indicate areas in which specific pressures are located. In the second step, pressures (point and diffuse souces of hazardous substances) are identified that could have a possible impact on water bodies. This can be done by considering the magnitude of the pressure and the susceptibility of the water body. The impacts resulting from the pressure is assessed as a third step. This can be based on other information such as the results of monitoring data. Finally the likelihood of failing to meet the

¹⁰ All the CIS guidance documents can be downloaded from

http://circa.europa.eu/Public/irc/env/wfd/library?l=/framework_directive/guidance_documents&vm=detailed &sb=Title

objectives are evaluated in the fourth step. The significant pressures and water bodies at risk of failing the objectives need to be identified and reported.

In Sweden, the County Administrative Boards perform the analysis of pressures and impact and status classifications at water body level on behalf of the River Basin District authorities. The impact of human activity on the status of surface waters related to both priority and river basin specific substances was assessed by each county during the first river basin management cycle and will need to be reviewed every 6th year (next time 2013). However, the methodology used has varied even between counties in the same district, some focusing entirely on Geographic Information System (GIS) analysis of land use while others primarily focusing on the location of point sources with release to water and high risk contaminated sites and still others on monitoring data to estimate heavy metal load. The assessment was in some counties not specifically directed towards the assessment of hazardous substances whereas others checked release data on individual priority substances and potential river basin specific substances. Certain types of pressures have not been included, such as point sources mainly releasing hazardous substances into air. Also the susceptibility of the receiving water system (such as the size and turn over time of lakes, river flows etc) was so far not considered. An ongoing national project run jointly by the river basin districts aims at developing an assessment tool and national guidance on how to proceed with the review of the current analysis of pressures and impacts.

Surveillance monitoring programs should generate data to assess overall surface water status within each catchment or subcatchement as well as any long term changes. Operational monitoring programs should rather be able to generate data that can be used to assess the status of those bodies identified as being at risk of failing to meet their environmental objectives; and assess any changes in the status of such bodies resulting from the measures undertaken. In some cases investigative monitoring is needed in order to find out causes for non compliance, if unknown, or to assess the status of water bodies where objectives are not likely to be fulfilled but operational monitoring was not yet performed (Box 3.8.).

Box 3.8. Surveillance, operational and investigative monitoring

The objectives (described in Annex V of the WFD) of <u>surveillance monitoring</u> programmes are to supplement and validate the impact assessment procedure; provide information for the efficient and effective design of future monitoring programmes; provide information for the assessment of long-term changes in natural conditions and resulting from widespread anthropogenic activity respectively.

Surveillance monitoring shall be carried out at sufficient surface water bodies, to provide an assessment of the overall surface water status within each catchment or subcatchments within the river basin district. In particular, large rivers and lakes as well as significant transnational water bodies are pointed out as relevant locations for such monitoring stations, as well as stations that are required to estimate pollutant load that is transferred across boundaries or into the marine environment.

Surveillance monitoring should include all biological quality elements, hydromorphological elements and general physico chemical quality elements. Monitoring of hazardous substances are limited to priority list pollutants which are discharged into the river basin or sub-basin, and other pollutants discharged in significant quantities in the river basin or sub-basin. If the previous surveillance monitoring exercise showed good status and impact of human activity has not changed, it is sufficient to perform surveillance monitoring every third river basin management plans. Otherwise, surveillance monitoring should be performed every water cycle.

As opposed to surveillance monitoring programs, <u>operational monitoring</u> programs should rather be able to generate data that can be used to establish the status of those bodies identified as being at risk of failing to meet their environmental objectives; and assess any changes in the status of such bodies resulting from the programmes of measures.

Operational monitoring programs need to be established for all water bodies which, on the basis of either the impact assessment or data generated during surveillance monitoring, are identified as being at risk of failing to meet their environmental objectives and for those bodies of water into which priority list substances are discharged. Sufficient monitoring stations should be included in each water body that is at risk from significant point source pressures, to be able to assess magnitude and impact of the point source. If there are several significant point sources, monitoring stations can be selected to assess the magnitude and impact of these pressures as a whole. Water bodies at risk from significant pressure from diffuse sources should also be monitored and monitoring stations should be established at selected water bodies, representative of relative risks, in order to assess magnitude and impact from diffuse source pressures. Those quality elements that are of relevance to the pressures should be monitored in an operational monitoring programme.

Finally, <u>investigative monitoring</u> may be needed if the reason for non compliance is unknown; surveillance monitoring indicates that the objectives are not likely to be achieved but operational monitoring was not yet established; and to ascertain the magnitude and impacts of accidental pollution. Investigative monitoring will probably frequently be in the form of a special short term project/campaign and designed in a case specific manner.

As a general rule of thumb, in the water compartment priority substances should be monitored every month whereas river basin specific pollutants every third month, unless greater intervals would be justified on the basis of technical knowledge and expert judgement (Annex V section 1.3.4. of the WFD). Member states can, for compliance checking, instead choose to monitor accumulating priority substances in other compartments (sediment and biota) on an annual basis, unless technical knowledge and expert judgement justify another interval (2008/105/EC). Trend analysis of accumulating priority substances should be based on the monitoring of sediment or biota every third year, unless technical knowledge and expert judgement justify another interval.

3.2.1.5.3 Chemical status classification of surface water

For chemical status classification of surface water, there is a list of substances in Annex I of 2008/105/EC (annex X of WFD) for which there are specified EU wide Environmental Quality Standards (EQS). If the EQS of any of these substances are exceeded, the status of the water body does not comply with the requirements for good chemical status. If the status is not good ("non compliance"), measures need to be implemented in order to improve the status. Also the substances and requirements according to the Fish and shellfish directives described above need to be fulfilled in order to comply with the requirements of good chemical status. The article 3.3. of 2008/105/EC also states that concentrations of accumulating priority substances cannot increase significantly in sediment and/or relevant biota.

At present, there are 33 priority substances but 2008/105/EC also includes other substances with EU wide standards. There are currently also 17 candidates that might become priority substances during the revision (see Appendix, chapter 11), if the proposal by the European Commission (COM) is accepted by the Parliament and Council. The priority substances are divided into two categories: Priority Substances (PS), and Priority Hazardous Substances (PHS). The pollution from PS substances should be progressively reduced, whereas emissions, discharges and losses of PHS substances should cease or be phased out (recognizing that for substances occurring naturally, cessation is impossible).

The current EQS values are expressed for the water compartment only, with the exception of mercury, hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD)

for which there are also biota standards that need to be fulfilled. Member states can however, for compliance checking, instead choose to monitor accumulating priority substances in other compartments (sediment and biota), as long as the national EQS values for such compartments provide at least the same level of protection as the EU wide standards specified in annex I part A (art 3.2.b of 2008/105/EC). National draft marine sediment EQS values for several priority substances were recently (May-June 2011) presented for commenting and with the revision of the list of priority substances, EQS values for other compartments than water are to be expected, in particular for biota.

3.2.1.5.4 Ecological status classification

In annex V of the WFD, quality elements for the classification of ecological status are listed in section 1.1. These include biological, hydromorphological and chemical/physico-chemical elements.

3.2.1.5.4.1 Biological quality elements

The biological quality elements of rivers, lakes, transitional waters and coastal waters all include composition and abundance of aquatic flora and composition and abundance of invertebrate fauna. For lakes, coastal and transitional waters, also biomass of phytoplankton is included. In addition, biological elements for rivers, lakes and transitional waters also include composition and abundance of fish fauna. For rivers and lakes, also age structure of fish fauna is included. Current biological assessment criteria, such as indices to measure composition and abundance, rarely respond to changes induced by the effects from hazardous substances (further described in chapter 9).

3.2.1.5.4.2 River basin specific pollutants

As a precautionary approach, supporting chemicophysical parameters should also be taken into account in the assessment of ecological status. The type of parameters that should be considered are described in Annex VIII of the WFD and is an indicative list of the so called "main pollutants", of which many can be considered to be hazardous substances, (groups in bold in box 3.9.) and in this context referred to as "river basin specific pollutants" (RBSPs)¹¹. Thus, there is no list on international or national level specifying the RBSPs as this has to be determined by the member states (see Naturvårdsverket 2007 for Swedish guidance on procedures). Nevertheless, a supportive document (Naturvårdsverket 2008b) was developed by the Swedish Chemicals Agency on behalf of the Swedish Environmental Protection Agency, with proposed national EQS values for thirty hazardous substances, (listed in the Appendix, chapter 12). However, so far there are no formally decided EQS values for river basin specific pollutants on river basin level (to be determined by the river basin district authorities), although the counties have identified several potential substances that can be considered RBSPs during

¹¹ In Sweden called "Särskilt farliga ämnen", SFÄ (see NFS 2008:1)

the last management cycle. The reason seems to be a lack in either legal implementation or procedures.

Box 3.9. Indicative list of main pollutants according to the WFD (Annex VIII of that directive). Groups of pollutants that could be considered to include hazardous substances are indicated in bold.

Organohalogen compounds and substances which may form such compounds in the aquatic environment.

Organophosphorous compounds. Organotin compounds.

Substances and preparations, or the breakdown products of such, which have been proved to possess: carcinogenic or mutagenic properties or properties which may affect steroidogenic, thyroid, reproduction or other endocrine-related functions in or via the aquatic environment.

Persistent hydrocarbons and persistent and bioaccumulable organic toxic substances. Cyanides.

Metals and their compounds.

Arsenic and its compounds.

Biocides and plant protection products. Materials in suspension.

Substances which contribute to eutrophication (in particular, nitrates and phosphates). Substances which have an unfavourable influence on the oxygen balance (and can be measured using parameters such as BOD, COD, etc.)

In annex V of WFD, it is specified that if levels of "specific pollutants" exceed national EQS values, ecological status should be classified as moderate ("non compliance"). In theory, also priority substances can be considered river basin specific pollutants, but in CIS 13 it is stated that "..once environmental standards have been adopted at Community level for the priority substances listed in Annex X, these substances should only be taken into account in the classification of surface water chemical status and should not be used as supporting elements for the classification of ecological status."

3.2.1.5.5 Reporting requirements related to the WFD

Member States provide data under the WFD to WISE (The Water Information System for Europe). WISE is developed by the DG Environment, JRC (Joint Research Center), Eurostat and EEA (European Environment Agency), and the Reportnet of the EEA is used. The first report on monitoring programmes was made in 2007 and on River basin management plans in 2010.

CIS 21 includes a specification that the following data should be reported for each surveillance and operational monitoring programme and for each surface water category (rivers, lakes, coastal and transitional):

- Intended start date (if it differs from 22 December 2006)
- Total number of monitoring sites and frequency to be (or expected to be) monitored for each quality element. If individual substances are monitored at different frequencies, then the monitoring frequency for each substance/group of substances should be reported

• List of Priority Substances and other substances discharged in significant quantities to be monitored

Only if an incident requiring investigative monitoring has occurred, reporting related to investigative monitoring is required. If so, the following should be reported:

- Type of investigative monitoring programme (e.g. incident response, unknown exceedances, likely failure of objectives)
- Quality elements and parameters monitored
- Number of monitoring stations for each programme
- Number of monitoring occasions

Information about the current compliance with EQS values (for priority substances and river basin specific substances respectively) is also to be reported as maps. A map for each substance is not necessary but rather related to types of substances (pesticides, industrial etc).

In the 2007 WISE reporting, none of the monitoring programs related to priority substances were reported by the River Basin Districts to the Commission. However, screening data on priority substances were reported¹². Besides the more formal requirements, member states (MS) are also requested to provide information such as monitoring data, Level of Detection (LOD) and LOQ for different chemical analyses etc, within different working groups related to the WFD implementation strategy. One such working group is the CMEP (Chemical Monitoring and Emerging Pollutants).

3.2.1.5.6 Current status classifications related to hazardous substances

In order to assess the need for revision of current monitoring programs, knowledge about current compliance is very useful. Also measures to fulfil quality objectives related to hazardous substances are often to a large degree depending on what substances are causing the non compliance, in particular if measures are to be undertaken on a large scale (use restrictions of particular substances).

Because the reporting of status related to hazardous substances is not on a substance specific basis, information on exact number of water bodies considered to exceed EQS values due to individual substances were so far not published (except non compliance related to Hg). However, such information was retrieved by combined searches of the VISS database (performed by the author in 2011).

It should be kept in mind that the status classifications will probably be revised significantly during the current, second River Basin Management water cycle because the community level EQS values were so far primarily expressed for water concentrations, whereas Swedish data, other than metals and pesticides, are primarily available for other compartments. It is also possible that some water bodies were classified based on risk assessment rather than monitoring data and in other cases based on very limited monitoring data. In addition, several EQS values are being revised and new priority substances will be added. The current status

¹² Axel Hullberg, Swedish EPA, pers comm.

classifications are therefore very uncertain. Nevertheless, current status classifications do point out several problematic substances that should be dealt with.

3.2.1.5.6.1 Current chemical status classifications

Sweden has a large data set on mercury in fish and there is also an EU level EQS for Hg in biota within the WFD. A comparison between the available dataset of fish data with this EQS¹³, clearly indicates that all data (even fish from coastal areas) do not comply with the requirements of "good chemical status" (Box 3.10).

Box 3.10 Current red national map: the mercury problem

The mercury problem in limnic carnivore fish, where concentrations frequently exceed food related restriction levels, has been well known for decades in Sweden. The largest Hg emissions on a global scale are related to the burning of coal. The mercury problems in Sweden are primarily related to atmospheric deposition due to long range transport (present and historic), although there may be additional local sources such as foundries and other industrial emissions (including chlor alkali industry), crematoria, and waste treatment. Also agroforestry activities can increase the leaching of Hg into local aquatic systems¹⁴ from Hg rich upper surface layers. Hg and its compunds can therefore still be considered a major threat.

Sweden has very strict legislation related to Hg. In 2009, a national ban on Hg was implemented (SFS 2009:14)¹⁵, so that Hg cannot be used or released on the Swedish market and also not be professionally exported. There are some exceptions to this ban, in particular related to products that are regulated on a European level (light sources, batteries and vehicles). Because atmospheric deposition resulting from European and also other international emissions needs to be reduced significantly to improve the current situation, Sweden works actively on an international scale to improve EU legislation and also promotes a legally binding UN convention related to mercury.

None of the Swedish water bodies reach "good chemical status". The EQS for Hg in biota is substantially lower than the food related restrictions because of risks of secondary poisoning in mammals and birds, and not even the concentrations found in e.g. coastal fish reach these low levels. Several lakes, especially oligotrophic, smaller lakes with limited water circulation, do not comply with the substantially higher food related value. Such lakes were therefore previously "black listed" due to mercury, but today the Swedish National Food Administration instead has issued recommendations on food intake, especially for children and fertile, pregnant and breast feeding women.

These recommendations are also based on the concentrations of Polychlorinated biphenyls (PCBs) and dioxins. The group is recommended not to consume fish that can contain high concentrations of dioxin and PCB more frequently than 2-3 times per year. The species pointed out as being of risk to contain too high concentrations of PCB and dioxins are herring, wild salmon and trout from the Baltic sea and the lakes Vänern and Vättern as well as char from Vättern.

¹³ The natural background concentrations of Hg in biota were not taken into account

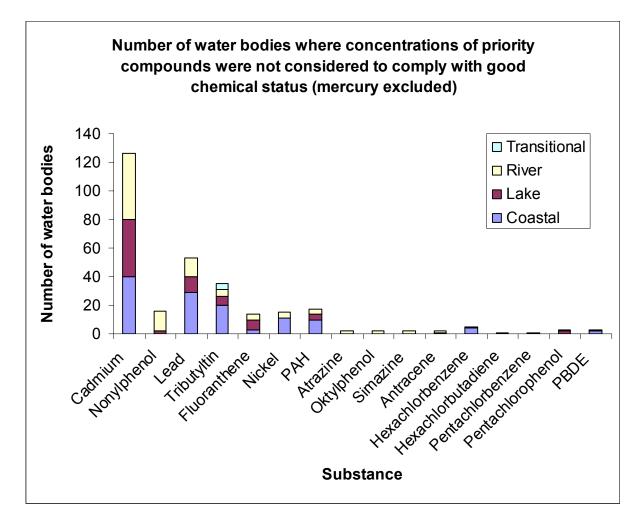
¹⁴ Increased transportation of both Hg and methylated Hg are thought to be caused by elevated ground water in turn increasing transportation pathways through upper soil levels that are rich in Hg from current and historic depositions. In addition, increased methylation can occur due to the creation of oxygen free environments, because of the elevated ground water and creation of stagnant water in tracks from machinery. Such anaerobic environments are optimal conditions for sulphur reducing bacteria, thus methylation is increased (Eklöf & Bishop 2010).

¹⁵ SFS 2009:14. Förordning om ändring i förordningen (1998:944) om förbud m.m. i vissa fall i samband med hantering, införsel och utförsel av kemiska produkter. <u>http://62.95.69.3/SFSdoc/09/090014.PDF</u>

Women that are or try to become pregnant or that breast feed should also avoid fish that contains high concentrations of mercury more than 2-3 times per year. The species pointed out are perch, pike, pike-perch, burbot (Lota lota), and large predatory fish such as fresh tuna, swordfish, shark, ray and large halibut (Hippoglossus hippoglossus). Also people that frequently consume fish that was caught from hobby fishing in lakes are adviced not to consume fish high in PCB, dioxins and mercury more frequently than once a week.

However, if excluding mercury from the status classifications, there are 63 lakes, 73 coastal water bodies, 69 rivers and 4 transitional waters that do not comply with the requirements for good chemical status for other reasons, see fig 3.1.





3.2.1.5.6.2 Current ecological status classification regarding hazardous substances

Figure 3.2. illustrates substances that so far were considered present in high enough concentrations to indicate moderate ecological status. In total 38 coastal, 4 lake and 4 river water bodies were considered to have moderate ecological status due to "synthetic river basin specific substances", but the names of the substances were not always specified in VISS. Correspondingly 39 coastal, 77 lake, 164 river water bodies currently are not considered to comply with good ecological status due to "non synthetic river basin specific substances" (i.e. metals). There are no transitional water bodies where concentrations of river basin specific substances were considered to indicate moderate status. More than one substance may give rise to moderate ecological status of the same water body. The overall ecological status of the water body may also be considered moderate or of even lower quality for other reasons. The criteria used in these assessments are not always the proposed EQS values of Naturvårdsverket (2008b) but can also have been based on other criteria, such as OSPAR ("Oslo Paris Convention") values. This information is currently not easily retrieved from searching the VISS database. Nevertheless, it can be concluded that the metals Zn, Cu and Cr are the primary reason for not considering the concentrations of RBSPs to comply with good ecological status.

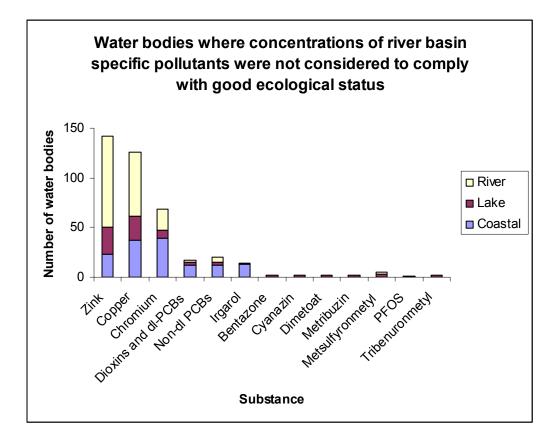


Fig 3.2.

3.2.1.5.7 Monitoring and control measures

An important purpose of the monitoring requirements and subsequent status classifications within the WFD is to identify those water bodies for which control measures are needed to obtain water of good quality within a specified time frame (normally 2015).

The WFD (annex VI part B) lists so called supplementary measures that MSs can adopt as part of the programme of measures (required under Art 11(4) in the WFD), see box 3.11. Several of these measures can be relevant for hazardous substances.

Box 3.11. Supplementary measures included in WFD annex VI part B that can be adopted as part of the programme of measures.

Legislative instruments Administrative instruments Economic or fiscal instruments Negotiated environmental agreements Emission controls Codes of good practice Recreation and restoration of wetland areas Abstraction controls Demand management measures, inter alia, promotion of adapted agricultural production such as low water requiring crops in areas affected by drought Efficiency and reuse measures, inter alia, promotion of water-efficient technologies in industry and water-saving irrigation techniques Construction projects Desalination plants Rehabilitation projects Artificial recharge of aquifers Educational projects Research, development and demonstration projects Other relevant measures

Working with measures related to hazardous substances is already part of the continuous work on both local, regional and national/international scales in Sweden. Several measures are therefore already being and have been undertaken to reduce the impacts from sources of hazardous substances since decades. Nevertheless, the monitoring and analysis of pressures and impact performed within the WFD context are two important constituents in further work and prioritisation of measures related to water quality. The higher the costs of a particular measure, the more important it will become to actually know in advance that it will be efficient and necessary. In order to know which measure will be most efficient on local-regional-national and international scales respectively, different monitoring strategies and tools might be necessary. In order to learn from previously implemented measures it is important with follow up studies, bearing in mind that even if the concentrations of individual substances were analysed primarily for a limited number of parameters before and after, it can be assumed that these measures have made a significant contribution to reducing the pressure on the environment of both analysed and not analysed substances.

Substances that are present in elevated levels on a national scale might also exhibit an important variability on a local scale, implying that both national/international and local measures may be necessary in these cases. However, there may also be natural reasons for this variability, such as different trophical status; oligotrophic lakes e.g. usually having higher Hg levels than eutrophic. Local measures might also be necessary, in addition to national and international restrictions, in order to reduce emissions from past activities. The use of organic tin compounds in anti fouling products for several decades and high concentrations still being found in surface sediment, in spite of bans, suggest that local measures could also be necessary to improve the situation at certain locations.

Depending on the nature of the problem, supplemental measures related to hazardous substances on different geographical scales can be necessary (box

3.12.). Because of the complexity of the problems related to hazardous substances, a significant need for investigative monitoring projects can be anticipated.

Box 3.12. Measures to promote good status related to hazardous substances

Measures to limit negative effects from hazardous substances could be very diverse and of different character. Using different legislative instruments on national and international scales it may be necessary and efficient to implement use restrictions. These can be of different characters, such as "positive" and "negative lists". Annex I substances of the Biocidal Products Directive (98/8/EC) is a typical positive list, i.e. it lists substances that can be used as active substances in biocidal products in the EU, while others cannot be used. Negative lists include the annex XVII in REACH (EC/1907/2006), listing substances for which there are use restrictions. The annex XIV of REACH also lists substances that would need authorisation before they can be used. Although most Swedish regulatory work related to chemicals on an international level are performed on European level, there are also examples of international conventions such as the Stockholm convention related to POPs (Persistent Organic Pollutants, such as PCBs, dioxins and DDT) and IMO (International Maritime Organisation) ban on TBT use as antifouling agent. Such global measures are often necessary for an effective reduction of emissions of hazardous substances that can be subject to long range transport in the atmosphere or by widespread use. There are also several other examples of Swedish initiatives to limit emissions due to chemicals used outside the EU by import restrictions¹⁶

The Commission has developed fact sheets for the current priority substances and the candidates, including an assessment of most significant sources and measures (see also Appendix chapter 17). However, the measures identified are primarily related to EU directives and international conventions, i.e. measures on international level that have been implemented in the national regulatory system. It also distinguishes between more supportive measures such as monitoring and information.

On local and regional scales, environmental inspection and enforcement is performed by both local (municipalities) and regional (county administrative boards) authorities. A major driving force related to point sources is e.g. the establishment of release permits and related monitoring programs of effluents/emissions. In Sweden, WEA (Whole Effluent Assessments) have been performed on certain types of industry effluents since the 80s.

The recently finalised project SOCOPSE¹⁷ identified cost effective measures, including both source control and end of pipe solutions, such as treatment options for individual priority substances (Appendix, chapter 18). The assessment was performed primarily on a European scale and the most efficient measures might differ between countries and local situation. A parallel project, ScorePP¹⁸, has also developed control strategies to reduce emissions from urban areas. Several deliveries from the different work packages are available, such as priority pollutant behaviour in different treatment systems (stormwater, household wastewater, industrial wastewater, sewage sludge), examples of voluntary initiatives and decision support system on identification of measures.

However, the most suitable and cost effective measures on local scale will in the end have to be assessed on a case to case basis, depending on the most significant sources. Measures directed

¹⁶ The Swedish Chemicals Agency e.g. recently declared an interest to ECHA in collecting information on nonylphenol and nonylphenol ethoxilates in order to initiate import restrictions on textiles containing these substances to the EU. <u>http://www.kemi.se/templates/News</u> <u>6656.aspx?epslanguage=sv</u>

The Medical Products Agency also proposed revisions on GMP, Good Manufacturing Practice, in order to reduce emissions during the production of pharmaceuticals. <u>http://www.lakemedelsverket.se/Alla-nyheter/NYHETER-2011/Gron-tillverkning-av-lakemedel-kan-bli-verklighet-genom-svenskt-forslag/</u>

¹⁷ SOCOPSE, Source Control of Priority Substances in Europe, funded by the 6th framework programme, http://www.socopse.se/

¹⁸ ScorePP, Source Control Options for Reducing Emissions of Priority Pollutants, funded by the 6th framework programme <u>http://www.scorepp.eu/</u>.

towards substances that are problematic on a national and international scale also requires the identification of the most significant sources, of which many can be suspected to be located far from the impacted area. For certain types of measures, such as information campaigns, it may be difficult to estimate effectiveness of the measure, and follow up studies were so far scarce. Other measures, such as additional treatment of effluents, most likely reduce emissions of several other substances as well as the target, and the assessment of cost effectiveness should therefore take these synergistic effects into account. Finally, the costs of other measures such as food recommendations and use restrictions are sometimes difficult to estimate.

3.2.1.6 Marine Strategy Framework Directive

The Marine Strategy Framework Directive (MSFD), 2008/56/EC, was implemented in Sweden in 2010 by SFS 2010:1341 ("Havsmiljöförordningen"). The MSFD is similar to the WFD and constitutes one part of the European "Marine Strategy". It requires that Member States develop marine strategies, containing detailed assessments of the state of the environment, including a definition of "good environmental status" at regional level, the establishment of environmental targets and monitoring programmes. By the Commission Decision 2010/477/EU, criteria and methodological standards to evaluate the extent to which Good Environmental Status (GES) is being achieved were laid down.

There are eleven descriptors related to GES (Box 3.13). GES is linked to sustainable use, but this concept is not further defined. The MSFD only recognizes two environmental status classes; GES and subGES (cf "good" and not good chemical status within WFD).

Hazardous substances are primarily related to descriptors no 8 and 9. It is pointed out that proper coordination between the implementation of the WFD and the MSFD should be ensured, also having regards to the information gathered and approaches developed in regional sea conventions.

Box 3.13. The eleven descriptors listed in annex I of the MSFD.

1. Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions.

2. Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems.

3. Populations of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy stock.

4. All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.

5. Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters.

6. Sea-floor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected.

7. Permanent alteration of hydrographical conditions does not adversely affect marine ecosystems.

8. Concentrations of contaminants are at levels not giving rise to pollution effects.

9. Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards.

10. Properties and quantities of marine litter do not cause harm to the coastal and marine environment.

11. Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment.

The MSFD also refers to the EQS values of priority substances and specific substances. As opposed to the WFD however, the MSFD goes one step further in that descriptor 8 includes two criteria related to concentrations and effects of contaminants on ecosystem components in the marine environment.

In april 2010, the JRC (Joint Research Centre of the European Commission) and ICES (International Council for the Exploration of the Sea) published joint reports related to these descriptors. In the report from task group 8 (Law et al 2010), it is stated that monitoring programmes should include

- the assessment of concentrations of contaminants in environmental matrices. More specifically, concentrations should be below "environmental target levels" (such as EQS or EACs) identified on the basis of ecotoxicological data and the occurrence should not be increasing
- 2) the quantification of biological effects of contaminants at different levels of biological organisation. More specifically, levels of pollution effects should be below environmental target levels representing harm to organism, population, community and ecosystem levels and the occurrence of and severity of effects should not be increasing

The 8th concentration descriptor thus comprises several substances and in different compartments, and the EC decision on GES criteria (2010) emphasizes that substances indicators are comparable with the list of priority substances but that also other substances that are considered significant should be considered. The effects related GES criteria were given two indicators in the same decision: one related to effects on ecosystem component level, regarding selected biological processes and taxonomic groups, where a cause and effect relationship has been established. The other related to acute pollution events and physical impact, see table 3.1. The 9th descriptor is also related to two GES indicators in the 2010 decision (detected levels and number of contaminants exceeding maximum regulatory levels related to human food intake as well as frequency of exceedence).

Table 3.1. EC decision 2010/477 regarding descriptor 8 and 9.

Descriptor	GES criteria	GES indicators
8	8.1. Concentrations of contaminants	8.1.1. "Concentration of the contaminants mentioned above ¹⁹ , measured in the relevant matrix (such as biota, sediment and water) in a way that ensures comparability with the assessments under Directive 2000/60/EC"
	8.2. Effects of contaminants	 8.2.1. "Levels of pollution effects on the ecosystem components concerned, having regard to the selected biological processes and taxonomic groups where a cause/effect relationship has been established and needs to be monitored" 8.2.2. "Occurrence, origin (where possible), extent of significant acute pollution events (e.g. slicks from oil and oil products) and their impact on biota physically affected by this pollution"
9	9.1. Levels, number and frequency of contaminants	9.1.1. "Actual levels of contaminants that have been detected and number of contaminants which have exceeded maximum regulatory levels"9.1.2. "Frequency of regulatory levels being exceeded"

Although the selection of contaminants, species and effects to monitor can vary between regions, harmonisation is strongly recommended to allow for the comparison between regions. The annex 17 of the JRC-ICES report e.g. refers also to OSPAR JAMP guidelines on monitoring contaminants in biota, sediment as well as monitoring of effects (contaminant specific and general, as well as integrated monitoring).

The monitoring programmes are described in Annex V of the MSFD. Member States shall establish and implement coordinated monitoring programmes for the ongoing assessment of the environmental status of their marine waters. Reporting

¹⁹ Text referred to: "The Member States have to consider the substances or groups of substances, where relevant for the marine environment, that:

⁽i) exceed the relevant Environmental Quality Standards set out pursuant to Article 2(35) and Annex V to Directive 2000/60/EC in coastal or territorial waters adjacent to the marine region or sub-region, be it in water, sediment and biota; and/or

⁽ii) are listed as priority substances in Annex X to Directive 2000/60/EC and further regulated in Directive 2008/105/EC, which are discharged into the concerned marine region, sub-region or subdivision; and/or (iii) are contaminants and their total releases (including losses, discharges or emissions) may entail significant risks to the marine environment from past and present pollution in the marine region, sub-region or subdivision concerned, including as a consequence of acute pollution events following incidents involving for instance hazardous and noxious substances."

should be made every 72nd month, starting in July 2014. Documents of Working Group on European Marine Monitoring and Assessment (EMMA) workshops and meetings before the entry into force of the MSFD can be found on the Marine strategy CIRCA library²⁰.

By 2012 assessment criteria to estimate GES should be established. The MSs then also need to report their preliminary assessment of current marine status. Reporting related to MSFD will be done in a separate WISE module, the marine-WISE.

3.2.2 Regional conventions

3.2.2.1 Convention for the protection of the marine environment of the north-east Atlantic (OSPAR CONVENTION)

Sweden has signed the convention for the protection of the marine environment of the north-east Atlantic, also called OSPAR (Oslo Paris Convention). OSPAR has developed five thematic strategies related to the main threats identified: the Biodiversity and Ecosystem Strategy, the Eutrophication Strategy, the Hazardous Substances Strategy, the Offshore Industry Strategy and the Radioactive Substances Strategy. Correspondingly there are several main committees, one being HASEC, Hazardous Substances and Eutrophication Committee.

3.2.2.1.1 Monitoring requirements

There is a general obligation to collaborate in the regular monitoring and assessment and therefore OSPAR has also developed a Strategy for the Joint Assessment and Monitoring Programme (JAMP) to assess the status of the marine environment (Annex VI of the OSPAR Convention²¹). The JAMP strategy was renewed in 2010 for the period 2010 to 2014, with particular focus on supporting Contracting Parties that are EU member states to implement the MSFD.

Regular JAMP activities comprise the CEMP (Co-ordinated Environmental Monitoring Programme), CAMP (Comprehensive Atmospheric Monitoring Programme) and RID (Comprehensive Study on Riverine Inputs and Direct Discharges).

3.2.2.1.1.1 CEMP

CEMP comprises technologies for which guidelines and QA (Qualtiy Assessment) procedures are sufficiently developed so that monitoring can take place

²⁰ Documents related to MSFD before its implementation can be found at <u>http://circa.europa.eu/Public/irc/env/marine/library</u>

²¹ <u>http://www.ospar.org/html_documents/ospar/html/ospar_convention_e_updated_text_2007_annex_iv.pdf</u>

convention-wide. Mandatory²² CEMP components (according to 2010 CEMP agreement²³) related to hazardous substances are the monitoring of:

- cadmium, mercury and lead in biota and sediment
- PCB (congeners 28, 52, 101, 118, 138, 153, 180) in biota and sediment
- The PAHs (Polycyclic Aromatic Hydrocarbons) anthracene, benz[a]anthracene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, fluoranthene, ideno[1,2,3-cd]pyrene, pyrene and phenanthrene in biota and sediment
- TBT-specific biological effects and TBT in sediment or biota.
- the brominated flame retardants HBCDD (hexabromocyclododecane) and PBDEs (Polybrominated Diphenyl Ethers) penta no 28, 47, 66, 85, 99, 100, 153, 154 and 183 in biota and sediment, and BDE (brominated diphenyl ether) 209 in sediment

The following components are currently part of the pre-CEMP and are to be monitored on a voluntary basis. Sweden has voluntarily agreed to monitor those methods that are indicated in bold (2010 CEMP agreement).

- the planar PCB congeners CB 77, 126 and 169 in biota. Monitoring of those congeners in sediment should be undertaken only if levels of marker PCBs are e.g. 100 times higher than the Background Assessment Concentration, BAC.
- the alkylated PAHs C1-, C2-, and C3-naphthalenes, C1-, C2- and C3phenanthrenes, and C1-, C2- and C3-dibenzothiophenes and the parent compound dibenzothiophene in biota and sediment
- PFOS (Perfluorooctanesulfonic acid) in sediment, **biota** and water
- Polychlorinated dibenzodioxins and furans in biota and sediment
- PAH- and metal-specific biological effects (PAH specific battery: CYP 1A activity, bulky aromatic-DNA adducts, PAH metabolites in bile, liver histopathology and macroscopic liver neoplasms (= liver nodules). Metal specific battery: ALA-D and MT)
- general biological effects (sediment, water bioassays, CYP1A, lysosomal stability, liver histopathology, externally visible fish diseases, reproductive success in fish)

3.2.2.1.1.2 RID

RID data are also to be reported annually and used to estimate the loads of certain pollutants into the marine areas.

²² It is possible to "opt out" for certain reasons, but Sweden follows the mandatory program according to the CEMP agreement

²³ Updated annually and can be found at

http://www.ospar.org/content/content.asp?menu=00900301400000_000000_000000

Mandatory monitoring and reporting requirements are concentration and load of cadmium, copper, lead, mercury and zinc, lindane, nitrogen and phosphorus species and suspended particulate matter.

In addition PAHs, mineral oil, PCBs and other hazardous substances, especially organohalogens, are recommended for voluntary monitoring.

3.2.2.1.2 JAMP Guidelines

Within JAMP, several guidelines have been published²⁴ that should be used by contracting parties to address specific issues. Guidelines were developed to tackle issues related to general quality assessment, local impact assessment as well as contaminant specific monitoring.

More specifically, the purposes of monitoring contaminants in biota (box 3.14.) are to assess:

- effectiveness of measures (temporal trend monitoring, assuming that changes in loads will be reflected in concentrations over time),
- existing levels (spatial distribution monitoring) to investigate large scale regional differences in contamination),
- harm to marine life (integrated chemical and biological effects monitoring) aiming at the identification of sites where contaminant specific effect programmes should be applied but also to investigate chemical causes of observed effects

Box 3.14. Available JAMP guidelines related to biota monitoring of hazardous substances²⁵

The biota guidelines developed by OSPAR include guidance on species and tissue to analyse. For shellfish the whole soft body should be analysed and for seabird eggs the whole egg content. Technical annexes include specific guidance on the monitoring of Metals, PAHs (Parent and alkylated PAHs), PBDEs, HBCD, Perfluorinated compounds, Organotin compounds, Dioxins/furans and planar CBs.

For time trend and spatial distribution monitoring using fish, muscle is only recommended for Hg monitoring whereas the liver is recommended for all other determinands. An important exception is the monitoring of herring, where muscle is recommended also for organic contaminants but other trace metals should be monitored in liver, if sampling to minimise natural variability. Fish and shellfish species recommended for spatial distribution monitoring include shellfish Mytilus (2nd choice Pacific oyster), the flatfish dab (2nd choice flounder) and the roundfish cod (2nd choice whiting or hake). For temporal trend monitoring it is essential that long time series are obtained with one species. In addition to the species recommended for spatial distribution monitoring within the OSPAR area: eelpout, herring and plaice.

²⁴ Guidelines can be found at

http://www.ospar.org/content/content.asp?menu=00900301400135_000000_000000

²⁵ Biota guidelines (agreement 1999-2): <u>http://www.ospar.org/documents/dbase/decrecs/agreements/99-02e_jamp contaminants biota rev 2010.doc</u>

However, the recommended species and tissue may vary between substance monitored. PAH monitoring is e.g. recommended in shellfish (blue mussels) rather than fish because of rapid metabolism²⁶, whereas PBDE and HBCD concentrations in shellfish are generally low, thus making higher trophic level organisms more suitable. PFCs (perfluorinated compounds) are primarily found in blood, especially serum, and organs such as liver and kidneys because of its affinity to proteins. Therefore liver is recommended for PFCs monitoring of fish. Finally, bivalves are recommended for the monitoring of organotin compounds. Monitoring in gastropod tissues can also be performed in relation to effect monitoring but because gastropods do not feed as continuously and have a higher TBT metabolism capacity, tissue concentration variability increases. Also, there is a time-lag between current TBT levels and imposex induced irreversibly in the early life stages.

The purposes of sediment monitoring (box 3.15.) are to assess:

- temporal changes in sediment quality
- retrospective assessment by examining surface and subsurface sediment, normally including the identification of background and preindustrial conditions
- spatial distribution to identify areas of enhanced concentrations of contaminants and relative degrees of contamination and location of sources

other purposes such as to support studies of effects of contaminants (such as TBT)

Box 3.15. Available JAMP guidelines related to sediment monitoring of hazardous substances²⁷

Technical annexes include specific guidance on the monitoring of chlorobiphenyls, metals, PAHs (Parent and alkylated PAHs), PBDEs, HBCD, Perfluorinated compounds, Organotin compounds.

There is also an annex on normalisation, in order to reduce variances of contaminant concentrations due to differences in bulk sediment composition and increase power, when comparing obtained data to assessment criteria or when assessing trends. Most substances show a higher affinitiv to fine particulate matter compared to coarse fractions. Organic matter and clay minerals contribute to the affinity to contaminants in this fine material. Sieving (e.g. <20 um or <63 um)²⁸ to obtain fine fractions is considered an important first step to reduce different granulometric conditions. Alternatively, or as a second step, normalisation of data using cofactors to consider grain size fraction and organic carbon are recommended, especially when assessing trends or comparing to background criteria and environmental assessment criteria that are derived for a defined sediment composition. An example of the latter is illustrated by the fact that the so called Background Concentrations are expressed as normalised values (to 5% aluminium for metals and 2,5% TOC, Total Organic Concentration), derived from a data set primarily based on conditions in the northern part of the OSPAR area. Such reference values may therefore not be appropriate for all OSPAR areas. TOC normalisation is relevant primarily for organic substances but has also been suggested for metal normalisation of surface sediments. However, for sediment samples containing low organic carbon concentrations (sandy sediments), contaminant concentrations can be close to analytical detection limits and in these cases normalisation to organic carbon is inappropriate as it can magnify the analytical errors. In addition, PAHs can be associated to soot and ash and although PAH concentrations then can be high the biological activity associated to such material is low. If analysing sieved fractions (<63 um), this problem seldom arises.

²⁶ However, it is also stated that the exposure of fish to PAHs can be assessed by analysing PAH bile metabolites and the induction of mixed-function oxygenase enzymes which catalyse the formation of these metabolites.

²⁷ Sediment guidance (agreement 2002-16): <u>http://www.ospar.org/documents/dbase/decrecs/agreements/02-16e_JAMP guidelines contaminants in sediments.doc</u>

²⁸ The 20 um fraction would be preferred for metal analysis but is considerably more time consuming and therefore 63 um fractions are acceptable. It is also the recommended fraction for analysing organic contaminants.

There are also guidelines on water and biological effects monitoring (box 3.16. and 3.17.).

Box 3.16. Available JAMP guidelines related to water monitoring of hazardous substances

At present there is only one guideline related to the monitoring of PFCs (perfluorinated compounds) in seawater²⁹. According to this guideline it is e.g. recommended to analyse not only PFOA (perfluorocctanoic acid) and PFOS but also short-chained PFCs (\leq C8), in particular perfluorobutane sulfonate (PFBS) and perfluorobutanoic acid (PFBA) because of their good water solubility and frequent use as PFOS substitutes.

Box 3.17. Available JAMP guidelines related to biological effects monitoring³⁰

The effects based monitoring guidelines are divided into guidelines for tools that monitor contaminant specific effects (metal-, PAH-, TBT- and oestrogen-specific effects) and those that monitor general biological effects (whole sediment bioassays, sediment pore water bioassays, sediment seawater elutriates, water bioassays, CYP1A, Lysosomal stability, macroscopic liver neoplasms, externally visible fish diseases, reproductive success in fish). There are also guidelines for the integrated monitoring and assessment of contaminants and their effects in preparation. The effect based tools are described in more detail in chapter 9 in this report.

3.2.2.1.3 Reporting

Contracting parties are encouraged to report their monitoring data to the ICES database. In Sweden, it is the intention that all marine national monitoring data related to hazardous substances should be reported to ICES, but there may be some gaps. Although Sweden has e.g. long time series of effects data none were reported to the ICES database according to ICES WGBEC (Working Group on Biological Effects of Contaminants) report 2007³¹.

3.2.2.1.4 Measures related to sources and substances

The objective within the strategy related to hazardous substances is "...preventing pollution of the maritime area by continuously reducing discharges, emissions and losses of hazardous substances, with the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances."

²⁹ Water guidance (agreement 2010-8): <u>http://www.ospar.org/documents/dbase/decrecs/agreements/10-08e_JAMP guideline PFCS in water.doc</u>

³⁰ Contaminant specific effect based monitoring (agreement 2008-9) and general biological effects monitoring guidelines (agreement 1997-7) respectively: <u>http://www.ospar.org/documents/dbase/decrecs/agreements/08-09e_contaminants specific BEF.doc</u> and <u>http://www.ospar.org/documents/dbase/decrecs/agreements/97-07e.doc</u>

³¹ ICES WGBEC Report 2007. ICES Marine Habitat Committee CM 2007/MHC:03. Ref ACME. Report of the working group on biological effects of contaminants (WGBEC). 19-23 March 2007 in Alexandria, Italy.

According to the reports from the contracting parties most of the OSPAR control measures related to hazardous substances recommended/decided/agreed³² have been implemented. Such measures include control measures to promote the use of BAT (Best Available Techniques) and emission limit values for most important point sources (industries) as well as measures related to diffuse sources (including consumer products), such as substitution, use bans/restrictions, best environmental practices.

In 2003 the focus within this strategy shifted from specific sectors and activities to substances and the DYNAMEC (Dynamic Selection and Prioritisation Mechanism for Hazardous Substances) was developed and based on the inherent properties (OSPAR PBT cut off values) of different substances. The DYNAMEC tool was used to establish a list of Substances of Possible Concern and to revise the List of Chemicals for Priority Action (listed in the Appendix, chapter 13). Because of the implementation of the WFD and REACH, the OSPAR decided to instead collaborate with the European Commission on future work related to selection and prioritisation of substances.

3.2.2.1.5 Assessment criteria

OSPAR has developed EAC (Environmental Assessment Criteria) and BAC (Background Assessment Concentration) values for a number of the listed substances, to be used to evaluate CEMP monitoring data. Values below EAC are considered not to give rise to unacceptable effects, analogous to e.g. the WFD EQS values. In 2004, EAC values were proposed for a large set of substances and in 2008 updated values for PAHs and PCBs were proposed. BACs are on the other hand used to determine whether concentrations observed are near background values for naturally occurring substances (such as metals) and close to zero for man made synthetic substances. There are also assessment criteria underway for many of the effect based tools (see chapter 9).

Finally there are EcoQO (Ecological Quality Objective) values developed by OSPAR. These are values that have been set to indicate a desired state of individual aspects of the structure and function of marine ecosystems.

Box 3.18. Established OSPAR EcoQOs related to hazardous substances

"The average level of imposex in a sample of not less than 10 female dogwhelks (Nucella lapillus) should be consistent with exposure to TBT concentrations below the environmental assessment criterion for TBT. Where Nucella lapillus does not occur naturally or where it has become extinct, other species may be used."

"The average concentrations of mercury in the fresh mass of ten eggs from separate clutches of the common tern (Sterna hirundo) and Eurasian oystercatcher (Haematopus ostralegus) breeding adjacent to certain estuaries should not significantly exceed concentrations in the fresh mass of ten eggs from separate clutches of the same species breeding in similar, but not industrial, habitats."

³² Several recommendations/decisions/agreements related to control measures of hazardous substances can be found at http://www.ospar.org/content/dra.asp?menu=01070304570000_000000_000000

"For each site, the average concentrations in fresh mass of the eggs of the common tern (Sterna hirundo) and Eurasian oystercatcher (Haematopus ostralegus) should not exceed: 20 ng/g of PCBs; 10 ng/g of DDT and metabolites; and 2 ng/g of HCB (hexachlorobenzene) and of HCH (hexachlorocyclohexane)."

3.2.2.1.6 Quality Status Report

In 2010, OSPAR published its second Quality Status Report (QSR 2010³³), as a follow up of the previous and first report (QSR 2000). The report is based on monitoring data from 1998-2008. The levels of PAHs, PCBs and metals are decreasing but still the overall status is not good. The percentage of monitored sites with overall unacceptable status within the OSPAR region II ("Greater North Sea"), exceeds 50% for PCBs> PAHs>Pb, and exceed or are equal to 20% for Hg>Cd (OSPAR 2009a and OSPAR 2009b).

The EcoQOs in QSR 2010 also identify TBT specific effects to still be a problem although the situation is improving, whereas Hg is less problematic. The situation related to PCBs and DDT also seem to be improving in Sweden.

Box 3.19. The assessment of EcoQS compliance in the OSPAR QSR 2010

"While the situation is improving, TBT-specific effects are still found over large parts of the OSPAR area and the EcoQO related to imposex is mostly not met in the North Sea. There is a clear relationship with shipping, with high effect levels near some large harbours (e.g. Rotterdam, Clydeport, Vigo) and lower levels in areas with less large vessel traffic. But even in areas of less large vessel traffic, harbours can have a noticeable impact, highlighting the importance of local sources and historic contamination of harbour sediments."

"The EcoQO for mercury is slightly exceeded at most sites, being met at one site in the Ems estuary (Netherlands) and sites in Norway and Sweden."

"The EcoQO for Organochlorines was not met at any of the monitored sites. This was because concentrations of PCBs and DDT exceeded the level of the EcoQO at all sites, approaching concentrations close to the EcoQO only in Norway and Sweden. In contrast, concentrations of HCB and HCHs were below or close to the level of the EcoQO at most sites, but are substantially elevated at sites in the outer Elbe estuary and to a lesser degree in the Ems estuary. A similar spatial pattern can be seen in DDT concentrations, but most sites were above the level of the EcoQO."

3.2.2.2 Convention on the Protection of the Marine Environment of the Baltic Sea Area (HELCOM convention)

Sweden has signed the Convention on the Protection of the Marine Environment of the Baltic Sea Area, also called the Helsinki Convention³⁴. Work related to hazardous substances is one out of four focal areas within HELCOM and was also the main focus of the initial Convention signed in 1974. There are five main working groups of which one is related to monitoring, the MONAS (Monitoring and Assessment) group.

³³ <u>http://qsr2010.ospar.org/en/index.html</u>

³⁴ The 1992 Convention: <u>http://www.helcom.fi/stc/files/Convention/Conv1108.pdf</u>

3.2.2.2.1 Monitoring requirements and the COMBINE manual

The HELCOM monitoring system consists of several programs, one being COMBINE (Cooperative Monitoring in the Baltic Marine Environment), which is related to the aquatic monitoring of hazardous substances (as well as other parameters such as nutrients) to assess both trends and quality/status.

Sweden has agreed to monitor Cd, Cu, Hg, Pb, Zn, DDT compounds, CBs, HCHs, HCB in perch, viviparous blenny, blue mussel from two coastal locations and in herring (5 sites), cod (2 sites) and guillemot egg (1 site) in open sea. Also, dioxins and planar CBs are monitored in herring (3 sites) and brominated compounds in guillemot (1 site). The contaminant monitoring programme is integrated with the ecological (fish species and age composition, catch, growth, gonad weight, fecundity, condition factor, external diesease indication) and physiological fish monitoring programmes (performed at three locations; including the variables gonadosomatic index, liver somatic index, hematocrit value, leucocyte count, plasma ions, cytochrome P-450, EROD activity, blood lactate and tissue glycogen). In addition population status of top predators (white tailed eagle, ringed, common and grey seals) is monitored by annual countings (cf chapter 4).

Box 3.20. HELCOM Manual for Marine Monitoring³⁵

Part D of the manual is related to the monitoring of hazardous substances in biota (including effects)³⁶.

The aims of this program are to:

- compare the level of contaminants in selected species of biota (including different tissues) from different geographical regions of the Baltic Sea in order to detect possible contamination patterns, including areas of special concern

- measure levels of contaminants in selected species of biota at specific locations over time in order to detect whether levels are changing in response to the changes in inputs of contaminants to the Baltic Sea.

- measure levels of contaminants in selected species of biota at different locations within the Baltic Sea, particularly in areas of special concern, in order to assess whether the levels pose a threat to these species and/or to higher trophic levels, including marine mammals and seabirds.

In addition, biological effects measurements are to be carried out particularly at sites of special concern in order to asssess whether the levels of contaminants are causing effects on biota (e.g. change in community structure), and to study the relationships between concentrations and effects.

It should be pointed out that the assessment of quality of seafood with regard to human consumption is not included but rather considered the responsibility of appropriate national authorities.

In the COMBINE manual, monitoring parameters are divided into core variables, main variables and supporting studies. The core variables need to be measured on a regular basis from all regions, whereas main variables are equally important but need not to be monitored by all Contracting Parties. They should however be covered on a work sharing basis. Supportive studies are rather in the form of a campaign or project and performed by individual contracting parties or groups of these. The core variables are therefore studied over the entire area, providing the best available comparable information on time trends and spatial distribution.

³⁵ <u>http://www.helcom.fi/groups/monas/CombineManual/en_GB/Contents/</u>

³⁶ <u>http://www.helcom.fi/groups/monas/CombineManual/PartD/en_GB/main/#top</u>

Core variables in herring and main variables in cod and guillemot eggs from the open sea are Hg. Cu, Cd, Pb, Zn, DDTs, CBs (28, 52, 101, 118, 138, 153, 180), HCB and HCH (alpha and gamma). For all fish species, all metals are monitored in liver except Hg that is monitored in muscle. The organic substances are monitored in muscle except for cods, where they are monitored in liver.

The same variables are to be monitored in Mytilus, Macoma, perch, viviparous blenny, eggs of common tern, guillemot and seal tissue according to a tentative coastal program.

Main variables of suspended particulate matter or dissolved phase in sea water are Cu, Cd, Pb, Zn, but for total concentrations: Hg, DDTs, CBs (same congeners as above), HCB, PAH and HCH (alpha, beta, gamma). However, Sweden has not committed to monitor contaminants in seawater.

ICES was invited to advice on biological effects monitoring methods, primarily related to monitoring effects on reproduction, immunology and metabolism. Also programms should be harmonized with OSPAR recommendations. Recommended species for effect studies in the Baltic areas include bivalves (Macoma balthica, Mytilus edulis), coastal fish such as Perca fluviatilis and Zoarces viviparus (physiological monitorin was carried out on national level since 1988), and top predators (seabirds, seals; continuation of national studies on pathology and seal population size is considered important)

Main and core variables related to hazardous substances in sediment are not specified in the COMBINE manual although there are technical notes for guidance in annex B-14 on the determination of PAHs, chlorinated biphenyls and metals in marine sediment. Sweden has not commited to monitor hazardous substances in sediments.

Annex B-13 are also technical notes on the determination of heavy metals and persistent organic compounds in biota. It includes five appendices related to sampling and handling, as well as determination of PAHs, chlorinated biphenyls and organochlorine pesticides, metals and total mercury. Analogous to the OSPAR JAMP guidelines for PAH monitoring in biota, HELCOM also recommends shellfish (blue mussels) rather than fish because of rapid metabolism³⁷

3.2.2.2.2 Reporting

Monitoring data of hazardous substances should be submitted to ICES in September the year after sampling. Although it is unclear whether this was done for all types of data, sediment data from the HELCOM area was indeed included in evaluation reports³⁸.

3.2.2.2.3 *Measures*

The HELCOM Baltic Sea Action Plan³⁹ (BSAP) is intended to restore good status of the Baltic Sea by 2020, a vision based on ecological and management objectives. More than 30 measures related to hazardous substances are specified, in addition to specific measures related to maritime activitites. The emission of hazardous substances should gradually decrease and be eliminated. Eleven selected substances /group of substances/ are subject to special actions (listed in the Appendix, chapter 14), focusing on the restriction and substitution in important sectors, within an agreed timetable. In this context, the COHIBA project initiated (see Box 3.21). The HELCOM ministerial meeting in May 2010 decided that core

³⁷ HELCOM also states that the exposure of fish to PAHs can be assessed by analysing PAH bile metabolites and the induction of mixed-function oxygenase enzymes which catalyse the formation of these metabolites. ³⁸ Tove Lundeberg, Swedish EPA pers comm..

³⁹ http://www.helcom.fi/BSAP/en_GB/intro/

set indicators with quantitative targets should be developed for each part of the BSAP while also ensuring that the developed indicators can be used for e.g. MSFD GES assessments.

Box 3.21. Control of hazardous substances in the Baltic Sea region (COHIBA)

The aim of the HELCOM COHIBA project⁴⁰ (2009-2012, final conference held in Helsinki in October 2011) was to identify the sources and inputs of the 11 hazardous substances of concern into the Baltic and to develop control measures to reduce these substances. The latter is achieved by the development of a Whole Effluent Assessment (WEA) strategy to harmonize and support the evaluation of effluent ecotoxicity and for the setting of effect based emission limit values. The final report on a recommended HELCOM WEA strategy focuses entirely on ecotoxic effects, but recognizes that some of these can also give an indication about persistence and bioaccumulation (COHIBA 2010). Through a one year screening study of municipal and industrial waste waters as well as landfill effluents and storm waters at selected sites in several case studies, sources of hazardous substances were identified. Although the final version of the report is not yet available, a draft related to Swedish sources can be found⁴¹. Two additional work packages are aimed at providing a guidance on management options and knowledge transfer. The Swedish partners of the project are IVL (Swedish Research Institute), the Swedish Chemicals Agency and the City of Stockholm.

3.2.2.2.4 Ecological Objectives

MONAS has coordinated the development of ecological objectives, EcoOs, forming the core of the HELCOM Baltic Sea Action Plan. The EcoOs will be defined and developed further as parts of the BSAP activity. There are a few initial EcoOs related to hazardous substances (Box 3.21).

Box 3.21. HELCOM BSAP EcoOs related to hazardous substances

Concentrations of hazardous substances in the Baltic Sea near background values for naturally occurring substances and close to zero for man-made substances⁴².

No illegal oil spills

All fish caught in the Baltic Sea should be suitable for human consumption.

Toxic substances shall not cause sub-lethal, intergenerational or transgenic effects to the health of marine organisms (e.g. reproductive disturbances).

Attain pre-Chernobyl concentrations of man-made radioactivity in the Baltic Sea ecosystem causing risk neither to human nor the natural systems.

⁴¹ <u>http://www.cohiba-</u>

⁴⁰ <u>http://www.helcom.fi/projects/on_going/en_GB/cohiba/</u> and <u>http://www.cohiba-project.net/</u>

project.net/sources/results/en_GB/reports/_files/86358964785776507/default/SE%20WP4%20National%20R eport%20DRAFT%2020111003.pdf

⁴² Three different target levels were identified to define "concentrations close to natural levels". The primary target for all substances is that concentrations should decrease in concentrations over time. An intermediate target level for certain substances, Hg, Cd, dioxins, furans and dl-PCBs, is that concentrations in muscle meat of fish should not exceed health related maximum levels. Ultimately, near background concentrations should be reached for naturally occurring substances (Hg, Cd, dioxins, furans and dl-PCBs) and to reach close to zero concentrations for man made synthetic substances (TBT, PFOS). Such ultimate target levels reflect good ecological status.

There are also indicators for healthy wildlife⁴³ (related to predatory bird health, fish health and seal health) but except for seal health, indicators were not yet determined.

3.2.2.2.5 Baltic Sea Assessment Reports

HELCOM assessments of the environmental status of the Baltic are available from 1987, 1990, 1996, 2002 and 2003 and 2010. The most recent assessment for hazardous substances (HELCOM 2010a) included quantitiative indicators and targets reflecting good environmental status, based on an integration of indicators using the HELCOM Hazardous Substances Status Assessment Tool CHASE (described further in annex 1 of HELCOM 2010). It should be kept in mind that there is at present no agreed fixed set of criteria and therefore, differences in the assessment between sites could be due to differences in the set of substances included, threshold values used or matrices monitored. Nevertheless, key substances of concern identified include PCBs, heavy metals, TBT, dioxins, DDT/DDE, PAHs and alkylphenols. The following substances were found to exceed their threshold levels nearly at all sites: PCBs, DDT/DDE, cadmium, lead, TBT and cesium-137. Also PCBs, lead, mercury, cesium-137, DDT/DDE, TBT, benz[a]anthracene and cadmium exhibited the highest Contamination Ratios (CR; concentration/target level), in the assessment units classified as "moderate", "poor" or "bad". Assessments were primariy based on biota data.

Effect based data also show that coastal fish populations suffer more from pollution than at open sea sites. In particular, a five fold increase in EROD and significant time trends for several health variables (such as 20-30% gonad reductions and increased number of white blood cells) in coastal perch have been observed at Kvädöfjärden between the years 1988 and 2010. Also effects on lysosomal membrane stability in flounder indicated significant impacts in coastal and harbour areas of the southern Baltic in the early 2000s. The flounder micronucleus tests also confirm poorer status of coastal sites and reproductive disorders are observed in marine snails (imposex) and eelpout.

It could be concluded that biota and sediments are affected by hazardous substances in all parts of the Baltic Sea. However, it is also pointed out that several management actions have proven to be successful, reducing atmospheric inputs of mercury, lead, and cadmium, and reducing the inputs of certain persistent organic pollutants, such as DDT, PCBs and TBT.

An initial holistic assessment is based on integrated thematic assessments of eutrophication, biodiversity and hazardous substances and provides a baseline for follow ups under the BSAP (HELCOM 2010b).

3.2.2.2.6 CORESET

⁴³ <u>http://www.helcom.fi/stc/files/Krakow2007/HazardousSubstances_MM2007.pdf</u>

There is a recently started HELCOM project, "CORESET" 44, focusing on the development of indicators for hazardous substances and biodiversity according to the BSAP EcoO. Within WP 3 of CORESET, indicators for hazardous substances (Core Set of Indicators for hazardous substances, CSIHS) are being developed. This project started in June 2010 and runs until 2013 but preliminary targets and indicators are being delivered during 2011. The aim is to link core indicators also to MSFD as well as WFD and its daughter directive 2008/105/EC on priority substances. One requirement is that the assessment results should be comparable across the region although target levels can vary among substances or sites depending on natural conditions. As a common principle for status targets for core indicators, targets should be derived through the use of an acceptable deviation from reference conditions. There are also pressure targets related to anthropogenic pressures that should guide towards the achievement of GES.

There are many similarities but also some differences between current HELCOM assessments and those necessary in the MSFD and WFD context. HELCOM thematic assessments e.g. use a five level classification (cf WFD ecological status: high, good, moderate, poor, bad), as opposed to the two MSFD GES classes. A pragmatic approach of the CORESET project was to consider the GES boundary as equivalent to WFD ecological boundary between good and moderate status. The HELCOM EcoO related to concentrations of contaminants aims at reaching "natural levels" for contaminants (e.g. zero concentrations of synthetic pollutants; cf requirements for "high ecological WFD status") whereas the MSFD GES boundary is related to levels that are not causing pollution effects and therefore rather would correspond to HELCOM intermediate targets.

3.2.3 Other international reporting

Data from monitoring stations located within the arctic region are also included in the Arctic Monitoring and Assessment Programme, AMAP, included in the Global Monitoring Plan (GMP) of the Stockholm Convention⁴⁵. Stations and parameters are specified in the Swedish NIP (National Implementation Plan⁴⁶).

The Environment Agency⁴⁷ (EEA) annually collects limnic and marine data on hazardous substances through the WISE-SoE⁴⁸ data collection process. The reported data is used for State of the Environment reports. Next reporting of data is due by the end of October 2011. During the last delivery, Sweden reported data on lakes for metals (As, Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Ni, V and Zn) and PAHs, PCBs and metals in *Mytilus edulis*. Preferred hazardous SoE substances are included in the Appendix, chapter 15.

The Swedish Food Agency annually also reports dioxin data to the European Commission (related to EC 1881/2006 requirements), although this is largely based

⁴⁴ http://www.helcom.fi/projects/on going/en GB/coreset/

⁴⁵ http://chm.pops.int/Implementation/GlobalMonitoringPlan/Overview/tabid/83/Default.aspx

⁴⁶<u>http://amap.no/documents/index.cfm?dirsub=%2FAMAP%20National%20Implementation%20Plans&CFID</u> =8156&CFTOKEN=14B25B66-1865-10BA-AA791140BC37430C&sort=default

⁴⁷ <u>http://www.eea.europa.eu/</u> ⁴⁸ Previously known as EUROWATERNET (EWN) and EIONET-Water

on monitoring campaigns, performed in different areas and on different species each year.

3.3 Conclusions

3.3.1 Swedish environmental quality objectives – indicators needed

Only one of the indicators formally developed to assess the achievement of the environmental quality objective "a non toxic environment" is related to monitoring of the aquatic environment and restricted to plant protection products in surface water. There is therefore a need to develop formal indicators based on monitoring of other types of substances as well as effects that can be linked to the presence of hazardous substances in the aquatic environment. Chemical and ecological status classifications according to the WFD will be revised by 2014 and the current classifications are expected to change significantly. It is therefore recommended that chemical and ecological status related to hazardous substances should not be used as an indicator until after this revision. Indicators being developed within the marine conventions and as part of the MSFD work should also be considered. The results of a recently finalised project could be used to develop an indicator based on concentrations of organic tin compounds in pleasure craft harbours. In addition, an ongoing project investigates the potential to use biomarkers in diatoms as an indicator in this context.

3.3.2 International monitoring requirements

Although there are numerous substances on lists to consider during aquatic monitoring, for only a few of them there are also assessment criteria available to estimate effects or exceedence from background levels. Only a few are also more or less mandatory to monitor, although within the WFD all priority substances that are released should be monitored and also river basin specific substances (for which there are no actual lists specified) that are released "in significant amounts". By reviewing listed substances, mandatory monitoring requirements and available assessment criteria, common monitoring requirements and possibilities to evaluate data of specific hazardous substances in different contexts can be identified, see table 3.2.

Table 3.2. Substances for which there are aquatic monitoring requirements and clear internationally agreed assessment and/or pass/fail criteria for at least one compartment (water, sediment, biota). Additional substances, for which there are no assessment criteria but monitoring is mandatory are also included.

Substance	Fish directive (2006/44/EC)	WFD priority substances and other substances with common European assessment criteria (listed in 2008/105/EC) ⁴⁹	Drinking water directive (audit monitoring requirement, assessment criteria available at tap) ⁵⁰	OSPAR (Mandatory CEMP components and EAC and/or BAC values available) ⁵¹	OSPAR mandatory CEMP component but no assessment criteria available	HELCOM core variables (Herring); no clear assessment criteria available
Acrylic amide			YES			
Alachlor		YES				
Antimone			YES			
Arsenic			YES			
Atrazine		YES				
Benzene		YES	YES			
Boron			YES			
Bromate			YES		1/50	
Brominated diphenylethers		YES (penta BDE 28, 47, 99, 100, 153, 154) ⁵²			YES (PBDE28, 47, 66, 85, 99, 100, 153, 154 and 183 in biota and sediment, and BDE 209 in sediment)	
Cyanide			YES			
Cadmium		YES (including Cd compounds)	YES	YES, mandatory to monitor in sediment and biota		YES
Carbon tetrachloride		YES				
Chloroalkanes , C10-C13		YES				
Chlorfenvinph os		YES				
Chlorpyrifos (- etyl)		YES				
Chromium			YES		1	
Copper	YES		YES		1	YES
Cyclodienes		YES (aldrin, dieldrin,				

 ⁴⁹ There are also 17 candidates that may become priority substances during the 2011 revision.
 ⁵⁰ Also "pesticides" are included in audit monitoring requirement.
 ⁵¹ HBCD is also mandatory to monitor but there are no agreed assessment criteria

⁵² From 2011 also octaBDE 197

Substance	Fish directive (2006/44/EC)	WFD priority substances and other substances with common European assessment criteria (listed in 2008/105/EC) ⁴⁹	Drinking water directive (audit monitoring requirement, assessment criteria available at tap) ⁵⁰	OSPAR (Mandatory CEMP components and EAC and/or BAC values available) ⁵¹	OSPAR mandatory CEMP component but no assessment criteria available	HELCOM core variables (Herring); no clear assessment criteria available
		endrin, isodrin)				
DDT		YES (p,p- DDT)				YES (incl metabolit es)
1,2- dichloretane		YES	YES			,
Dichlorometha		YES				
DEHP		YES				
Diuron	1	YES				
Endosulfan		YES				
Epichlorhydrin e			YES			
Fluoride			YES			
HBCD					YES	
HCB		YES				YES
HCBD (hexachlorobut adiene)		YES				
НСН		YES				YES (alpha and gamma)
Isoproturon		YES				
Lead		YES (incl Pb compounds)	YES	YES, mandatory to monitor in sediment and biota		YES
Mercury		YES (incl Hg compounds)	YES	YES, mandatory to monitor in sediment and biota		YES
Nickel		YES (incl Ni compounds)	YES			
Nonylphenol		YES (4- nonylphenol)				
Octylphenol		YES				
РАН		YES (anthracene, fluoranthene, benzo(b)fluor anthene, benzo(k)fluor	YES (benzo(b) fluoranth ene, benzo(k)f luoranthe	YES ⁵³ mandatory to monitor in sediment and biota (phenanthrene, anthracene,		

 ⁵³ In addition, naphthalene, dibenzothiophene, triphenylene, benzo(k)fluoranthene,
 ^{benzo(b)}fluoranthene, {C1-, C2-, C3-naphthalene/phenanthrene/anthracene/dibenzothiophene} are not mandatory CEMP components, but there are EAS/BAC values available

Substance	Fish directive (2006/44/EC)	WFD priority substances and other substances with common European assessment criteria (listed in 2008/105/EC) ⁴⁹	Drinking water directive (audit monitoring requirement, assessment criteria available at tap) ⁵⁰	OSPAR (Mandatory CEMP components and EAC and/or BAC values available) ⁵¹	OSPAR mandatory CEMP component but no assessment criteria available	HELCOM core variables (Herring); no clear assessment criteria available
		anthene, benzo(ghi)pe rylene, indeno(1,2,3- c,d)pyrene, benzo(a)pyre ne, naphthalene)	ne, benzo(gh i)perylen e, indeno(1, 2,3- c,d)pyren e, benzo(a) pyrene)	fluoranthene, pyrene, benz(a)anthracen e, chrysene, benzo(a)pyrene, benzo(ghi)peryle ne, indeno(123cd)pyr ene, benz(a)anthracen e)		
PCB				YES (28, 52, 101, 118, 138, 153, 180 are mandatory to monitor in sediment and biota)		YES (28, 52, 101, 118, 138, 153, 180)
Pentachlorobe		YES				
nzene PCP		YES				
Radon		163	YES			
Selenium			YES			
Simazine		YES	163			
Tetrachloroeth		YES	YES			
ene		120	120			
Tributyltin compounds		YES (TBT cation)		YES mandatory to monitor in sediment and biota. Also TBT specific effects are mandatory to monitor.		
Trichlorethene		YES	YES			
Trichlorobenze nes		YES				
Trichlorometh		YES				
ane						
Trifluralin		YES				
Trihalomethan es			YES			
Vinylchloride			YES			
Zink	YES					YES

3.3.3 Problematic substances identified from current status classifications

Within WFD the most problematic substance identified during the previous cycle is by far Hg, and the current status map related to this substance is red on a national scale, even including coastal areas. More than 200 water bodies were also considered not to achieve good status based on other priority substances than mercury, primarily TBT, PAH, fluoranthene, Cd, Pb, Ni and nonylphenol. In total about 200 water bodies were not considered to achieve good ecological status regarding hazardous substances, primarily due to Zn, Cu and Cr but also dioxins, PCBs and irgarol (considered to cause moderate ecological status at >10 water bodies).

The OSPAR assessment for PAHs, PCBs and metals in region II concludes that the concentrations of these substances are decreasing but levels are still unacceptably high, in particular for PCBs, PAHs and Hg (unacceptable levels measured at more than 50% of monitoring stations) but also for Pb and Cd.

The latest HELCOM assessment identified PCBs, DDT/DDE, cadmium, lead, TBT and cesium-137 as being problematic at more or less all sites and PCBs, lead, mercury, cesium-137, DDT/DDE, TBT, benz[a]anthracene and cadmium exhibited the highest threshold exceedences.

3.3.4 Effects assessment

The WFD does not require effect based monitoring tools to be implemented whereas these are mandatory components of the HELCOM and OSPAR monitoring programs and therefore available for the marine environment. OSPAR EcoQ on TBT effects is not fulfilled, as TBT specific effects are still found over large parts of the OSPAR area. The HELCOM effect based assessment also identifies coastal fish populations to be more stressed from contaminants than open sea Baltic populations. This was shown for several variables such as reproductive disturbances in eelpout, EROD and several biochemical and physiological variables in perch, lysosomal stability, micronucleus test and imposex.

In particular, Swedish data on coastal perch from one of the Baltic stations (Kvädöfjärden) show a five fold increase in EROD and significant time trends for several health variables (such as 20-30% gonad reductions and increased number of white blood cells) between the years 1988 and 2010.

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4 Current monitoring of hazardous substances in the Swedish aquatic environment

The current monitoring system of hazardous substances and effects on both national and regional/local scales have been developed during several decades, many programs starting in the 70s and 80s. In general, the purpose of national monitoring programs are to identify large scale impacts and time trends, whereas hot spot areas should be monitored by responsible operators and in regional monitoring.

4.1 Regular monitoring programs

In VISS (Water Information System Sweden), there are now more than 46 000 monitoring stations registered, where several different types of parameters (not restricted to hazardous substances) are monitored regularly for different purposes such as national, regional and municipal monitoring as well as drinking water monitoring, monitoring of protected areas, impact monitoring and studies of the effects of liming acidified waters.

4.1.1 National monitoring of hazardous substances (and effects)

Sweden has several ambitious national monitoring programs related to hazardous substances and their effects since several decades. The national regular monitoring programs of hazardous substances are largely focused on investigating time trends of particular hazardous substances or effects on a national or large regional scale. Monitoring stations are therefore normally located at sites far from known local sources. An important exception is the monitoring of imposex along the West and East Coast, where studies in impacted areas are also included. Limnic monitoring of pesticides in so called "type areas" are also located close to sources (agricultural regions). A summary of the regular national monitoring programs is included in table 4.1, wheras details are included in the Appendix chapter 19. There is also a screening programme for hazardous substances.

National monitoring of hazardous substances in sediment is focused on the marine environment and located at sites that are off shore. There is currently no regular national monitoring of freshwater sediments except for the largest lake, Vänern, where monitoring takes place every 10th year and in the agricultural areas (entirely focused on pesticides). However, sediment metal data from the reference lakes are also available⁵⁴. There is also a description on how limnic sediment monitoring shoud be performed⁵⁵.

⁵⁴ These sediment data, from 1998-99 are still to be found at SLU:

⁵⁵http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/sotvatten/ met_sedm.pdf

The monitoring of biota in the marine environment includes species from several trophic levels (different species of fish, guillemot eggs and mussels) although there are also approximately 30 fish monitoring stations located in lakes. The program of specimen banking for biota retrieved within these programs is also well developed, although this program does not provide supportive information to assess the current situation.

One of the marine programs integrates monitoring of the concentrations of hazardous substances in fish and effects observed on population and physiological level (biomarkers). However, fish physiological effects are only monitored at a few stations (one on the West Coast and three on the East Coast) and thus this part of the program is rather focused on time series than to cover large geographical areas. There is also no sediment monitoring at these stations. Furthermore, monitoring of other biota such as mussels is only performed at two out of these 4 stations/areas (Kvädöfjärden and Väderöarna).

Other effect based monitoring is related to embryonal development in benthic invertebrates, but this program is largely performed in two areas, and thus focused also on the monitoring of time series rather than covering larger geographical areas. An extension of the program is being considered though⁵⁶. This program is coordinated with the monitoring of benthic fauna (to enable the analysis of any correlation between effects on reproduction with abundance). To coordinate with the current sediment chemistry monitoring programme is considered limited because such monitoring is performed at sites with high degree of lamination (where benthic fauna is missing). However, other supportive data (O₂ in water and sediment as well as organic carbon in sediment) are collected and the biomarker primarily considered to monitor stress from hazardous substances is "malformed embryos" (Wiklund & Sundelin 2004), see also chapter 9.

Water chemistry related to hazardous substances is entirely focused on metals and pesticides in the limnic environment. Pesticides are monitored in four "type areas" and two larger rivers, whereas there are several programs related to metals, including the monitoring of riverine inputs, lakes and Integrated Studies of the Effects of Liming Acidified Waters (IKEU). The OSPAR RID monitoring is performed within the program for rivers that end at sea and metal data are reported for the following rivers: Enningdalsälven, Örekilsälven, Bäveån, Göta Älv, Viskan, Ätran, Nissan, Lagan, Rönne å. Data is also submitted for Strömsån, Kungsbackaån, Rolfsån, Himleån, Suseån, Fylleån, Genevadsån, Stensån but these have been calculated.

In addition samples are also taken for specimen banking (blue mussels, fish) and there are also monitoring programs rather related to population levels of e.g. seals (*Phoca vitulina, Pusa hispida, Halichoerus grypus*), otter, beaver and mink, benthic organisms, white tailed eagles, other seabirds and fish that could also be of interest in a non toxic environment perspective. Within the monitoring of white tailed eagle populations, unhatchable eggs, shell thickness as well as blood samples are taken for analysis of genetical parameters. Also seal pathology is studied.

⁵⁶ Tove Lundeberg Swedish EPA, pers comm..

Table 4.1. National monitoring programs within which hazardous substances are currently monitored (chemically or by effect based parameters such as biomarkers). Programs entirely related to population monitoring are not included. A more detailed table regarding monitored parameters, stations and frequency is included in the Appendix, chapter 19.

Monitoring program	Marine/ Limnic	Substances/ endpoints	Monitoring stations /and species/	Monitoring frequency	International reporting requirement s (voluntary and mandatory)	National data host; availability
Integrated coastal fish monitoring	Marine	Population related parameters and biomarkers	4 stations, perch and eelpout	Annual	OSPAR HELCOM	Swedish Board of Fisheries ⁵⁷ Data not available on homepage but can be obtained directly on request.
Metals and organic hazardous substances in marine biota	Marine	Metals, PCB, DDT, HCH, HCB, PBDE, HBCD, PCDD/F, d- PCB ⁵⁸ s, Perfluorinate d compounds, PAHs. Supportive data.	22 distinct monitoring stations. Baltic Herring, Herring, cod, perch, eelpout, blue mussel, guillemot egg	Annual	OSPAR HELCOM	IVL biotadatabase Data available on web page ⁵⁹
Pathology in seal	Marine	Macro and microscopic parameters	West and East coast	Annual	HELCOM	SMHI Data available on web page
Biological effect monitoring caused by tinorganic compounds	Marine	Biomarkers and tissue concentratio ns of organic tin compounds	West and East coast	Annual	OSPAR HELCOM	IVL biotadatabase Data available on web page
Embryonal development in Monoporeia	Marine (East coast)	Biomarkers related to reproduction. Supportive	East Coast	Annually (one location twice)	HELCOM	SMHI Data are not available on web

 ⁵⁷ This task will probably be taken over by the new national authority.
 ⁵⁸ CB-28, CB-52, CB-101, CB-118, CB-138, CB-153 and CB-180

⁵⁹ However, the PAH indeno(1,2,3-c,d) are not to be found in the database

Monitoring program	Marine/ Limnic	Substances/ endpoints	Monitoring stations /and species/	Monitoring frequency	International reporting requirement s (voluntary and mandatory)	National data host; availability
affinis and Pontoporeia femorata		parameters				page
Metals and organic substances in marine sediment	Marine	PAH, HCB, PCB, HCH, chlordane, DDTs, PBDE, nonylphenol, octylphenol, DEHP, alachlor, atrazine, diuron, endosulfan, isoproturon, chlorfenvinph os, chlorpyrifos, PCP, simazin, trifluralin, tinorganic compounds, alkylated Pb, Metals. Supportive parameters.	n=16 (sea, non coastal)	Every 5 th year	OSPAR	SGU Data available on web page but only for samples taken in 2003
Fish	Limnic	Metals, perfluorinated substances, PCB, DDTs, HCH, HCB. PCDD/F, PBDE, HBCD. Supportive parameters.	32 lakes. Mainly perch but at some stations pike, arctic char or roach	Annually	ΑΜΑΡ	IVL Data available on web page
Integrated Studies of the Effects of Liming Acidified Waters	Limnic (lakes and rivers)	Metals (Hg also in perch)	26 lakes and 43 rivers are included in the national program	Water chemistry monthly Hg in fish annually		Water: SLU ⁶⁰ Biota: IVL Data available on web page
Pesticides in agricultural areas	Limnic	Pesticide substances (>115	6 monitoring areas/statio	Surface water weekly in 4		SLU ⁶¹ Data available on

 ⁶⁰ The metals are at the moment located in separate databases though and can be found at http://info1.ma.slu.se/max/www_max.acgi\$Project?ID=Intro&pID=-6 but a revision is ongoing. Tobias Vrede, SLU pers comm..
 ⁶¹ http://www.slu.se/sv/fakulteter/nl/om-fakulteten/institutioner/institutionen-mark-och-miljo/miljoanalys/vaxtskyddsmedel-typomraden-aar/

Monitoring program	Marine/ Limnic	Substances/ endpoints	Monitoring stations /and species/	Monitoring frequency	International reporting requirement s (voluntary and mandatory)	National data host; availability
		substances in water and >60 substances in sediment).	ns	type areas, sediment annually. Rivers 1-2 times monthly		web page
Physicoche mical rivers and lakes	Limnic	Metals, supportive parameters.	67 trend rivers, 47 estuaries, 10 trend lakes and 800 randomly picked lakes	Monthly in rivers, twice a year in trend lakes, annually in random lakes	OSPAR (RID)	SLU ⁶² Data available on web page
	Limnic	Vänern	Vänern	Vänern		Fish: IVL
		Fish: Metals, PCBsum7, planar PCB, dioxins,	Fish: Perch: 1, Pike 1	Fish: Perch annually and pike every 5 th		Sediment: SGU ⁶³ Water chemistry: SLU
The great		PBDE, perfluorinated compounds. Sediment: dioxins, dl- PCBs, PCBsum7, PBDE, PAH, DEHP, tinorganic compounds, metals Water: metals <i>Vättern</i> Fish: Hg, PCB, DDT, HCB, HCH, dioxins and	Sediment: 7 stations Water chemistry: 14 stations <i>Vättern</i> Fish: Char: 3 Water chemistry: 9 stations Sediment: 3 stations	year Sediment: every 10 th year Water chemistry: monthly <i>Vättern:</i> Fish: Char every 5 th year Water chemistry: 4 times annually in lake, monthly in		Data available on web pages
lakes monitoring programs in Vänern and Vättern		dibenzofuran s Sediment: metals and		in- and outflow Sediment: every 6 th		

 ⁶² <u>http://info1.ma.slu.se/db.html</u>
 ⁶³ <u>http://info1.ma.slu.se/db.html</u>
 ⁶⁴ However, Vättern data from 2004 are to be found in the IVL screening database. Other sediment data not available on home page but can be retrieved on request from Vätternvårdsförbundet, ; <u>www.vattern.org</u>

Monitoring program	Marine/ Limnic	Substances/ endpoints	Monitoring stations /and species/	Monitoring frequency	International reporting requirement s (voluntary and mandatory)	National data host; availability
		organic substances.		year		
		Water: metals				

4.1.2 Regional monitoring

Regional monitoring is often performed as part of a Coordinated Impact Monitoring (SRK, Samordnad Recipientkontroll) program, financed by the members, primarily representing industries and municipalities in the area. As opposed to the national monitoring programs, these monitoring programs frequently aim at representing the situation not only at reference locations but also at sites close to point sources. The data are not always reported to the national data hosts and seldom used to fulfil international reporting requirements. The purpose of these programs is rather to fulfil Swedish law, requiring that the operators should have sufficient knowledge about their impact on receiving waters. Information from these programs are therefore to be used primarily by the local and regional authorities and may not always be allowed to use for official purposes.

Regional monitoring can also be performed on a County Administrative Board level (RMÖ, Regional Miljöövervakning) or, rarely, on municipality level (KÖ=Kommunal Övervakning). The RMÖ programs are financed by the Swedish EPA but designed by the county administrative boards, in order to provide background information for the evaluation of regional quality objectives. They are also being reviewed in order to better fulfil the requirements of the WFD. The County Administrative Boards also have an obligation to coordinate the different types of monitoring programs (SRK, NMÖ and RMÖ, KÖ) within their county.

Figures 4.1. – 4.3. illustrate the extent of regional monitoring of priority substances and other substances included in 2008/105/EC. The graphs are based on information on monitoring stations registered in the VISS database. It should be pointed out that the registered information has not been subject to quality control. It is also not mandatory to specify which priority substance and in which compartment it is monitored and if this was unclear such stations were not included. In addition, monitoring programs might have been revised since the registration. Nevertheless, the graphs should give a rough estimate of the extent of regional monitoring performed today and substances and compartments monitored in different water body types⁶⁴. Please note that the total number of water bodies of a different type varies significantly (there are 602 coastal water bodies and 7267 lake water bodies, 15 599 river water bodies and 21 transitional water bodies). In addition to the

⁶⁴ Please note that the water body type might deviate from the actual ecosystem that is monitored. The monitoring station might e g be located in a smaller lake that is part of a water body that is formally registered as a river water body type.

information shown as graphs, there are currently about 50 monitorings stations related to the aquatic moss Fontinalis (Cd, Hg, Pb and Ni).

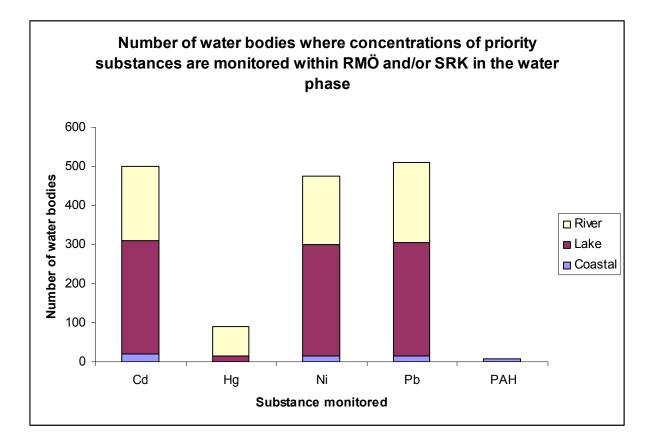
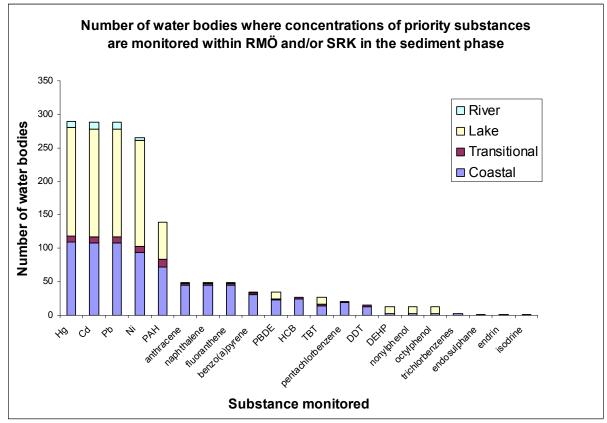
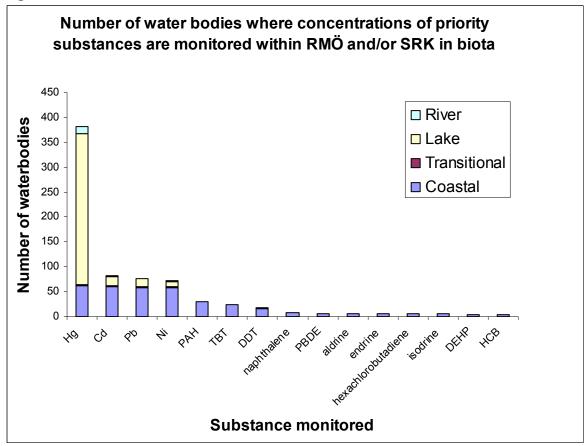


Fig 4.1.









Regular effect based monitoring of hazardous substances has been confirmed to be included in one SRK program (performed along the West coast by the Bohuskustens vattenvårdsförbund) but this information is not registered in the VISS database and the data were so far not reported to the national data hosts. The program is being revised this year (2011) and in addition to fish biomarkers (eelpout) at four sites along the northern West Coast (Göteborg, Stenungsund and Brofjorden and the reference site Fjällbacka), lysosomal stability in blue mussels will also probably be monitored at the same sites. Effect based studies were also included in a campaignwise manner in several additional programs. See also chapter 9.

4.1.2.1 Monitored parameters

Heavy metals (Cd, Hg, Pb and Ni) are by far the most frequently monitored priority substances on a regional level (RMÖ and SRK). In rivers and the biota and water compartment of lakes, these are actually the *only* priority substances monitored.

Organic substances are primarily monitored in the marine environment and to a minor extent also in lake sediment. The most frequently monitored organic parameter is PAH. Other organic parameters that are monitored at more than 10 stations in Sweden (including all water body types) include HCB, TBT, DDT, PBDE and pentachlorinated benzene.

4.1.2.2 Type of water bodies monitored

Besides an extensive regional monitoring of mercury in lake fish (n=269 RMÖ stations and n=34 SRK stations), biota other than *Fontinalis* is primarily monitored in coastal water bodies. In rivers, metals are frequently monitored in Fontinalis, but other biota is only rarely monitored.

Surface water is primarily monitored in lakes and rivers, and only to a small extent in coastal water bodies. However, it is entirely restricted to the metals Cd, Ni and Pb and to a lesser extent Hg.

Sediment is primarily monitored in lakes and coastal water bodies. However, monitoring is primarily focused on metals and PAH and to a much lesser extent to other organic substances, especially in lakes.

4.1.2.3 Compartment monitored in different types of water bodies

In rivers, the water compartment is the most frequently monitored matrix, but Fontinalis monitoring (primarily *Fontinalis antipyretica*) is also important, although in the past few years, Fontinalis monitoring programs are often being phased out or performed to a more limited extent. Other organisms and sediment is rarely monitored in rivers.

In lakes, metals are primarily monitored in water and sediment, but to a much lesser extent in biota, with the major exception being mercury, that is the most frequently monitored substance and also most frequently monitored in biota. Organic substances, primarily PAH, are only monitored in sediment.

In coastal and transitional monitoring programs, sediment is the major compartment monitored although biota is also important. The water compartment is less monitored in coastal water bodies and not at all in transitional waters.

Unfortunately, type of monitored biota (only specified for Fontinalis) is not registered in VISS. To at least obtain information on species and tissue analysed would be desirable to assess the possiblities to use the data generated to evaluate status.

4.1.2.4 Importance of SRK programs vs county level monitoring

The SRK programs play a significant role in Swedish regional monitoring. There are currently 45 SRK programs and 22 RMÖ programs that were registered in the VISS database to monitor at least one of the priority substances in one of the matrices sediment, water or biota. The RMÖ stations exceed the total number of stations monitored by the SRK programs only for the water compartment monitoring of metals in lakes and transitional waters, and for the monitoring of Hg in biota in lakes and rivers. The only organic contaminants monitored by the counties are PAH in sediment (from both coastal, lake and transitional waters), except for PBDE and HCB being monitored in a few biota samples from coastal locations.

It should however be kept in mind that several counties and municipalities do contribute also financially to the SRK programs. To distinguish between what is financed by authorities and by operators is therefore not easily done.

4.2 Drinking water monitoring

In Sweden, surface water is frequently utilized for drinking water purposes (162 lakes and 48 rivers are designated water bodies for drinking water according to registered information in VISS). Although it is not mandatory to take samples from the lakes and rivers this is frequently done. However, reporting of data to the database "vattentäktsarkivet"⁶⁵ is not mandatory. These types of monitoring programmes are also not registered in VISS.

⁶⁵ Previously called DGV, databasen för grundvatten. In spite of its name, the database also includes surface water data and drinking water data from protected drinking water areas.

4.3 Screening

Within the national monitoring program "Toxic substances coordination", there are also annual screening campaigns of hazardous substances, in addition to specimen banking and sludge monitoring.

The purpose of the screening programme is to verify the incidence of primariy emerging hazardous substances in the environment and its impact on humans and environment. Screening results may call for inclusion of new substances in the regular monitoring programs and also in depth investigations and measures to be undertaken at certain sources. The substance directed screening approach to idenfity new substances to monitor within regular monitoring programs primarily focuses on substances that may be of a nationwide (or possibly international) problem, although new knowledge can also be created about important local/regional sources for particular substances.

Reports from screening campaigns are published on the Swedish Environmental Protection Agency homepage⁶⁶. The screening projects are performed either as campaigns towards certain substances or groups of substances or as broad screening of certain sources of hazardous substances. Before deciding what substances and in what compartments to screen, a literature study is generally performed. Decisions on substances to screen are made on national level but the 21 counties are invited to participate in the screening of certain substances or sources by contributing with additional regional samples.

4.3.1 Directed screening of certain compounds

A large number of substances were screened since the start of the screening program (see box 4.1.). The same substance is usually screened in several compartments in parallel, and the choice depends primarily on the inherent properties of the substance but also other factors such as previous monitoring data. Compartments that were screened so far include surface waters (Coast, Sea, Freshwater), sediment (Coast, Sea, Freshwater), fish (Coast, Sea, Freshwater), other biota (Coast, Sea), groundwater, leachate (landfills), food, breast milk, human blood and urine, soil, plants, elks, air (background, urban, precipitation/deposition, indoor), sludge (STP, Sewage Treatment Plant), influents and effluents (STPs), raw and drinking water.

⁶⁶ http://www.naturvardsverket.se/sv/Tillstandet-i-miljon/Miljoovervakning/Rapporter-och-nyhetsbrev/Rapporter---Miljogiftssamordning/

Box 4.1. Substances that were screened between 1996 and 2008				
RTEX (homeone toluone etc.) mosticidae e large number of motole 1000, 1000				
BTEX (benzene, toluene, etc.), pesticides, a large number of metals 1996–1999				
Hexabromocylododecane (HBCDD) 2000 Chlorinated phenols 2001				
Organotin compounds 2001				
Octylphenol 2001				
Phosphorus-based flame retardants 2001–2002				
Perfluorinated substances (PFAS) 2001–2003				
Musk compounds 2002				
Triclosan 2002				
TBBPA 2001-2002				
Antimony compounds 2001				
Phthalates 2002–2003				
Certain pharmaceuticals 2002				
Hexachlorobutadeine (HCBD) and chlorinated benzenes 2002				
Chlorinated paraffins 2002–2003				
Bisphenols and 2,4-chlorophenyl-sulphon 2003–2005				
Antioxidants, methylphenols, alkylphenols 2003–2004				
Measurements of various substances and groups of substances in sludge 2002–				
Adipates, limonene, mirex, isocyanates 2004–2005				
Octachlorostyrene, siloxanes, endosulfan 2004–2006				
Measurements of substances on the Water Directive priority list 2001–2005				
Antibiotics, anti-inflammatory substances, hormones 2005–2007				
Certain perfluorinated substances 2005–2007				
Bronopol, resorcinol 2005–2007				
Organotin compounds 2005–2007				
Benzotriazoles, 4-chloro-3-cresole, n-didecyl dimethyl ammonium chloride, propiconazol, parabenes 2005–2005				
Certain other pharmaceuticals, veterinary medical products, catalysotormetals, certain phthalates, cyclododecatriene, chromium compounds, zinc pyrithione 2006–2007				
Measurements of substances in water prioritised by the Water Directive 2005-2008				
Certain phenolic substances 2006-2008				
Certain amines, esters and amides, certain biocides, certain organic iodine compounds, certain				
organic halogens, pigment dyes, concrete additives, sucralose, musk substances, silver 2007-				
Analysis of certain matrices, certain biocides, unintentionally produced substances, lubricants, nonylphenol 2008-				

In 2006, priority substances were screened in water samples from 92 stations, of which most are located in locally exposed areas. Twentythree of the priority substances were also analysed with passive samplers in parallel to filtered and non filtered water samples. The purpose was to identify which priority substances occur most frequenty in concentrations above or close to EQS, whether there are regional differences and what sources are most important but also to study the usefulness of passive samplers in this context. Concentration variation was also studied in water in 2007, in order to determine a suitable sampling frequency. In the latter study, eight limnic and seven marine stations were included, representing different types of sources, and in addition to water, also sediment samples were included. From these two studies, it could be concluded that nonylphenol, TBT, Cd, Pb, Ni and occasionally DEHP (bis (2-ethylhexyl)phthalate) could be problematic in Swedish surface waters. All of these substances are also found in sediment, but also PAH and octylphenol is frequently detected in sediment (SWECO 2009a). Priority substances have also been analysed within other screening campaigns of certain substance groups, e.g. organic tin compounds, phthalates and brominated flame retardants.

4.3.2 Broad screening

Except for the regular monitoring of sewage sludge (and recently also STP effluents) there have also been screening campaigns of certain sources or compartments. There was e.g. a broad screening of sediment (SWECO 2009b), urine (SWECO 2010), storm water (SWECO 2009c) and landfill leachates (ongoing).

4.4 Conclusions

The monitoring programs related to hazardous substances in the aquatic environment are primarily performed at national level, and with major focus on the marine environment (monitoring of hazardous substances in several types of biota and sediment as well as different types of effect based monitoring programs). In the limnic environment, hazardous subsances are primarily monitored in fish, pesticides in water and sediment in agricultural areas and metals in rivers. There is also a national screening program, including both substance directed screening and broad screening campaigns.

Only a few regional programs monitor hazardous substances other than metals on a regular basis. However, in addition to the regular regional monitoring programs, there are also monitoring campaigns. The counties frequently join the national screening campaigns and special investigations can be performed also within SRK contexts.

In rivers, the water compartment is the most frequently monitored matrix, although some regional programs also include monitoring in aquatic moss (*Fontinalis antipyretica*). Parameters primarily include metals and in some areas also pesticides. In coastal areas, there is more or less no monitoring of water, whereas sediment and biota are monitored within both national and regional programs and including also organic substances. In lakes there is no regional monitoring of organic substances in biota registered, although fish is analysed in several reference lakes in the national program.

In chapter 5, gaps in the current national and regional programs will be further analysed in relation to the requirements described in chapter 3.

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5 Do current regular programs fulfil WFD, OSPAR, HELCOM and MSFD requirements?

The national and regional monitoring programs of priority substances were described in detail in chapter 4. The focus of the current chapter is to identify major gaps related to the monitoring requirements described in chapter 3, regarding substances, water body types and compartments that are monitored. Only the "listed" substances are possible to evaluate, because unlisted substances (relevant in the context of ecological status classification within WFD) are determined on a case to case basis, and so far the River Basin District authorities have not specified which substances should be considered RBSPs and assessment criteria have not yet been formally established. Therefore, this chapter is primarily focused on priority substances (WFD) and substances that are mandatory to monitor within HELCOM and OSPAR. Any gaps related to required effect based monitoring within HELCOM and OSPAR are also assessed.

It should be pointed out that the current chapter should not be considered a comprehensive assessment of the gaps related to the identification of risks of hazardous substances to or via the aquatic environment. Because there is a tendency to monitor primarily regulated, listed compounds, knowledge about the presence and effects from other substances is low. In turn, these unmonitored substances seldom become regulated, although regulation of also so far non monitored compounds can be expected to increase with the implementation of REACH, at least for substances produced in large amounts.

5.1 Gaps in the national monitoring system

As can be seen from the compilation in the previous chapter, the national monitoring stations probably give a good picture of the general state of the aquatic environment regarding the substances that were regulated since decades, in particular in the marine environment, but also in fish from the limnic environment. However, some gaps can still be identified.

5.1.1 Fulfilling regional marine conventions

The current HELCOM core variables required for off shore locations are monitored. However, compared to a tentative coastal program, it can be concluded that current national Swedish East Coast monitoring primarily lacks regular monitoring of bivalves, common tern and seal tissues (although the latter are sampled and kept in specimen banks for retroactive studies).

The Swedish Baltic effect monitoring programs are performed on HELCOM recommended species (eelpout, perch, seabirds, seals) but not on bivalves.

The mandatory OSPAR requirements (CEMP) on substances and compartments are also fulfilled except for the BDE congeners 28, 66, 183 not being monitored in marine sediment nor in biota. HBCDD is also not yet monitored in sediment.

Swedish national monitoring of organic substances in both West and East coast fish is largely performed in muscle (in both herring, perch and eelpout), except for perfluorinated compunds and metals (monitored in liver) and East coast monitoring of cod (liver). OSPAR actually recommends monitoring all determinands in liver, except for Hg (in muscle) and except for monitoring of herring (organics should be monitored in muscle). On the other hand, HELCOM recommends muscle for organic determinands except for monitoring of cods (liver). If following such general recommendations within HELCOM and OSPAR, eelpout liver should therefore rather be monitored than muscle on the West coast but not on the East Coast. Nevertheless, by monitoring the same tissue, geographical comparisons can be made.

Pre CEMP components that were so far NOT included but may be considered in the future are planar PCB 77, 126, and 169 in biota (and sediment if levels are significantly higher than BAC), alkylated PAHs in biota and sediment, PFOS in sediment and water and dioxins/furans in biota and sediment, in order to increase regional harmonization within the OSPAR area.

The only mandatory effect based CEMP tool, imposex, is also already monitored. In addition, Sweden monitors several recommended pre CEMP tools, such as CYP1A, liver histopathology, DNA adducts, externally visible fish diseases, and reproductive success in fish, in the annual integrated fish monitoring program. However there is actually only one monitoring station located in the OSPAR area (along the West Coast), although so far an SRK program has also performed studies every 6th year on three additional stations exposed to local sources (Göteborg, Brofjorden, Stenungsund).

Nevertheless, the following pre CEMP components are not yet monitored in fish by Sweden: PAH metabolites in bile and ALA-D. Also water and sediment bioassays and lysosomal stability are not included in regular programs. Benthic effect monitoring related to hazardous substances in fact lacks entirely on the West Coast but there are mussel monitoring stations of concentrations (not effect). Therefore, there is an important coordination potential with the program monitoring hazardous substance concentrations in blue mussels and this program would probably be a very cost effective way to obtain general effects related data. Only small volumes of blood are necessary for the effects assessment, leaving most of the tissues for chemical analysis. The method is inexpensive and Swedish expertise is available (chapter 9). To investigate the suitability of current mussel monitoring stations to also act as coastal sediment monitoring stations in the future is therefore recommended.

OSPAR RID requirements are fulfilled although lindane is only monitored in 2 major rivers (except for the monitoring taking place at the four type areas). The voluntary RID components such as PAHs, PCBs and oil are not monitored.

5.1.2 Fulfilling WFD surveillance monitoring requirements

Sweden has a long tradition of monitoring organic substances primarily in biota and sediment rather than water but all priority substances have been included in a national screening campaign to investigate which priority substances could be of concern in surface water on a national scale (see chapter 4). Which compartment to prefer for a particular substance in order to fulfil the WFD obligations is discussed further in chapter 8. Nevertheless, all priority substances are included in at least one of the current national monitoring programs. The BDE congener 28 that is missing in relation to the OSPAR CEMP is also one of the priority substances and thus there is a gap that also needs to be filled to fulfil the requirements of the WFD. The other priority substance PBDEs that should be included in the evaluation of the EQS are already monitored. However, with the future probable inclusion of also octa BDE (197), an additional parameter would need to be considered.

Depending on the program, the geographical coverage and WFD relevance varies. The following priority substances are only covered regularly by off shore sediment monitoring (generally not within the WFD regulated area): benzene, C10-13 chloroalkanes, 1,2-dichloroethane, dichloromethane, DEHP, hexachlorobutadiene, nonylphenol, octylphenol, pentachlorbensene, PCP (pentachlorophenol), trichloromethane, trichlorobenzenes. Also the pesticide program being performed in the four type areas usually not include large enough water courses to be considered water bodies. Although such monitoring could be used to assess the large scale trends of the substances, data are less appropriate for compliance checking.

The possibilities to extend the marine sediment monitoring program to also include near coastal stations should be investigated, in order to also monitor WFD relevant areas. Such an extended program could perhaps be coordinated with other programs, in particular of effects and concentrations in biota for an integrated and ecosystem oriented assessment of the aquatic environment. There is also no limnic sediment monitoring program, except for the monitoring being performed in Vänern (largest Swedish lake) every 10th year and Vättern every 6th year (although program not exactly specified).

The marine and limnic biota monitoring program cover WFD relevant areas but not all priority substances that can be suspected to accumulate in biota are included. Some parameters, such as DEHP and alkylphenols could probably be relevant to add at least in monitoring campaigns in order to evaluate future regular monitoring needs in biota (see chapter 7).

5.1.3 Gaps related to the monitoring requirements of the Marine strategy framework directive

Although it is yet too soon to identify gaps related to the MSFD monitoring requirements, one can assume that because OSPAR and HELCOM requirements are largely fulfilled and Sweden has a long tradition of marine monitoring including also effect based tools on different levels of biological organisation and trophic levels, gaps are small related to MSFD. Nevertheless, several of the recommended effect based ICES methods (chapter 9) are not included and could be valuable as MSFD indicators. In general also a limited number of stations are included in the effect based programs.

5.1.4 Implications for the establishment of assessment criteria

Assessment criteria are needed in many contexts. From the description of requirements in chapter 3 it is clear that assessment criteria are needed in status classifications within the WFD and to evaluate indicators within MSFD. They are also needed in the context of control measures and other management related activitities, such as dredging, sediment remediation, effluent evaluations. To support the evaluation of data, baseline levels often need to be established. In order to support the need for sediment remediation, it is e.g. necessary to not only consider whether there are risk of effects, but also to judge whether the site is to be considered a hot spot or the exceedence compared to other sites is actually low.

This identifies the need to have access to several types of monitoring data and some of these are not available due to lack of regular national monitoring programs. There is e.g. a lack of national limnic data on sediments. Such data would be needed to evaluate contaminated limnic sediments because comparisons to off shore data may not be suitable in this context. There are also no limnic effect based programs, although several of the fish biomarkers used in marine environments could also be applicable to limnic conditions and integrated with the current limnic biota program. Such baseline levels would be valuable for comparison, if effect based tools are to be used more regularly. There are also no national monitoring programs of lower trophic level biota, again having implications for the possiblitites to establish assessment criteria.

5.2 Gaps in regional monitoring system vs WFD operational monitoring requirements

5.2.1 Gaps in the number of sites monitored

This chapter aims at identifying gaps related to operational monitoring requirements on a national scale as far as possible. To assess the operational monitoring needs of each river basin district in detail, including which water bodies and substances to monitor would require a far more detailed assessment than what was possible within this project. Nevertheless, a general assessment of the size of the major gaps is made in this chapter.

Taking into account that there are far more limnic water bodies than marine, the monitoring of the limnic environment is currently very limited. Furthermore, limnic regional monitoring of organic hazardous substances is entirely restricted to lake sediment and does not include any biota. Today 6% of the water bodies in Sweden are pointed out as being at risk of not achieveing good chemical status (if excluding mercury). Marine waters dominate on a relative scale, table 5.1. Such a risk classification would in fact justify a higher operational monitoring frequency of coastal water bodies.

	Total number of water bodies	Classified at risk of not achieving good chemical status (excl Hg)	Relative risk (% water bodies of a particular type that is considered to be at risk)
Coastal	602	225	37%
Lake	7267	277	4%
River	15599	854	5%
Transitional	21	5	24%
Total	23489	1361	6%

Table 5.1. Perceived risk of non compliance for different types of water bodies according to information registered in VISS.

By comparing water bodies registered as being "at risk" of not fulfilling good chemical status with those water bodies that are registered to be monitored at least regarding one of the priority substances in one compartment (according to all such monitoring stations registered in VISS), a rough analysis of major monitoring gaps can be made.

More than 70% of the rivers and lakes, and slightly less than 70% of coastal water bodies that are being considered to be at risk of not fulfilling the quality objectives are not being monitored at all (not even metals), see fig 5.1. There is therefore only

minor differences in the relative extent of operational monitoring of different types of water bodies, if related to the identified needs.

These assessments may be somewhat misleading because in some cases the monitoring station may be located in a water body downstreams the one that is registered to be at risk (would be of particular importance and relevance for rivers), or next to the water body being at risk (relevant in particular for coastal systems). The gaps would then be overestimated. However, the percentage of all surface water bodies that are not being monitored in spite of being at risk is 73%. In addition, the number of water bodies indeed being monitored does not take into account whether the substances actually emitted into the water body are being monitored or if the number of monitoring stations and monitoring frequencies would be considered sufficient. Monitoring gaps related to the WFD requirements are therefore probably significantly larger.

In the assessment of monitoring gaps, the "random lakes" program was excluded because these monitoring stations were at the time of the analysis not registered in the VISS database. In addition, monitoring is performed as grab sampling of water taking place once a year and thus fairly limited.

It should also be pointed out that the numbers perceived being at risk of not acheiveing good chemical status will probably be revised significantly within the current management cycle. The risk assessment was performed in different ways in different districts and even different counties within the same district, probably reflecting the type of and number of water bodies that were so far pointed out as being at risk.

A similar assessment comparing the monitoring of potential river basin specific pollutants with water bodies perceived to be at risk of not achieving good ecological status because of hazardous substances was not performed. The risk assessments were mostly not performed on a substance specific level. However, it can be assumed that water bodies pereceived to be at risk due to priority substances (excl Hg) would to a large extent coincide with the water bodies at risk due to hazardous substances other than priority substances as well.

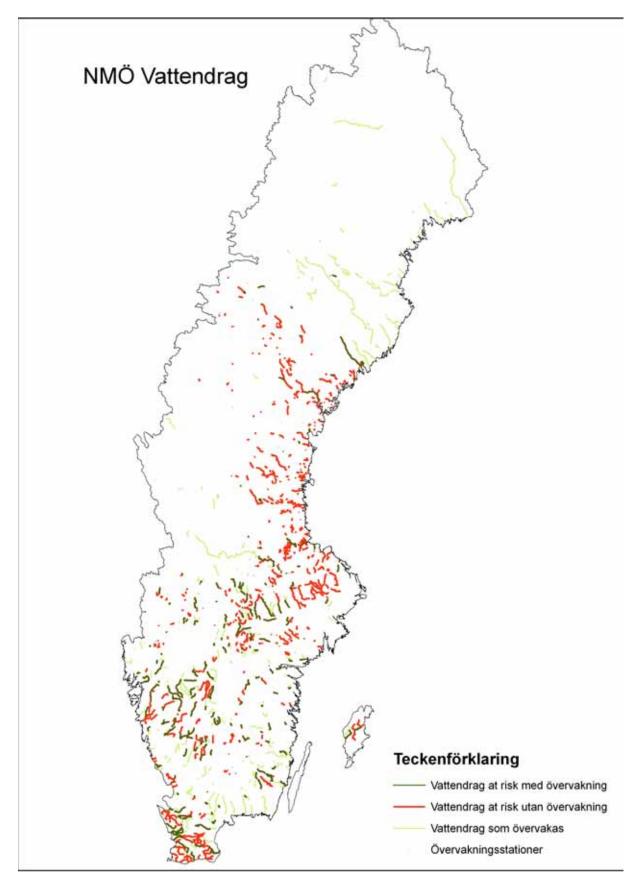


Fig 5.1. River water bodies where no priority substances are monitored (in red) in spite of being at risk of not achieving good chemical status (excl Hg).

To conclude, the extent of the regular operational monitoring programs related to hazardous substances is currently very limited. However, during the last status classifications, the large gaps in regular monitoring could to some extent have been overcome by utilizing results from monitoring campaigns being performed. Such campaigns are not registered in VISS, and data may not even be available from national data hosts. The extent of which such data has been utilized in status classifications is therefore difficult to estimate on a national scale, as well as the suitability of doing so.

5.2.2 Gaps in substances monitored

The possibilities to identify gaps in operational monitoring related to certain substances are limited, as the registered information related to certain local sources and impacts is not on a substance level. However, one can assume that there are major gaps in the number of priority substances and potential river basin specific pollutants being monitored regularly in operational programs. In lakes, other organic parameters than PAH are e.g. only monitored by one single program (SRK Norra Vänern) and thus regional data on organic substances are only covering a very limited geographical area (chapter 4).

The regional monitoring programs, and especially SRK programs, would be expected to focus on substances that could be considered problematic on a local scale and related to point sources. It is therefore somewhat surprising that also the regional programs primarily are focused on monitoring the classical substances, and primarily in areas that were not identified as being at risk of achieving good chemical status. The lack of monitoring of "unlisted substances" could be a result of lack of knowledge about the risk of other substances being present as well (because they are generally not analysed on effluents), but could also be due to the lack of assessment criteria and analytical routines.

Nevertheless, as previously pointed out, monitoring campaigns and screening studies performed are not registered in the VISS database. Several SRK programs perform irregular or low frequency monitoring studies on occasion and such data can also be of value in status classifications and in identifying the needs for local measures.

5.2.3 Implications for estimating load

Because there is an overall lack of monitoring of other substances than metals and pesticides in the water compartment of rivers, there are also difficulties to estimate load. Such data would be useful to evaluate efficiency of control measures related to inland sources but also to estimate relative contributions from inland, off shore and atmospheric sources to the sea. However, to give recommendations on how such monitoring should be performed is not straightforward (see chapter 7).

5.3 Need for WFD investigative monitoring

To evaluate the need for investigative studies on a national/regional level is outside the scope of this report. However, one can assume that because the lack in operational programs, investigative monitoring would need to be performed at the many sites that are considered to be at risk of not acheiveing good status. In addition, although several sources have been investigated within broad screening campaigns (effluents, leachates etc) the actual impact on "water body level" and in different situations would need to be assessed more thoroughly in order to assess local impact in different situations and based on monitoring data. Sewage treatment plants are e.g. frequently being pointed out as important point sources of several priority substances and priority substance candidates (Appendix chapter 16; PAHs, benzene, PBDE, DEHP, Pb, Ni, octylphenol, TBT, EE-2, 17 beta estradiol, diclofenac, cyanide, dioxins, HBCD, ibuprofene, PCBs, Zn, and one can also suspect⁶⁷ nonylphenol and PFOS to be emitted).

5.4 Conclusions

- Some gaps related to the marine conventions could be identified, but they were largely related to components that are not mandatory. OSPAR mandatory requirements (CEMP components) are largely fulfilled with a few exceptions.
- Larger gaps could be identified in comparison to recommended monitoring. The current national Swedish Baltic monitoring program primarily lacks regular chemical monitoring of bivalves and common tern, and seal tissue analysis (although sampled for specimen banking). Planar PCB 77, 126, and 169 in biota (and sediment if levels are significantly higher than BAC), alkylated PAHs in biota and sediment, PFOS in sediment and water and dioxins/furans in biota and sediment are preCEMP components not yet being monitored on the West Coast. None of the voluntary RID components related to hazardous substances are included.
- There is also no regular effect based monitoring on bivalves as recommended by HELCOM and there is only one monitoring station for preCEMP fish effect based monitoring that is located in the OSPAR area (along the West Coast). PAH metabolites in bile and ALA-D are also not monitored preCEMP components. Water and sediment bioassays and lysosomal stability in blue mussels are not included in any regular programs. Benthic effect monitoring related to hazardous substances lacks entirely on the West Coast.
- In the WFD context, all priority substances are included in national monitoring programs. However several priority substances (including e.g. DEHP, nonylphenol, octylphenol and PCP) are actually only monitored at locations that are outside the area covered by WFD (off shore locations). Such coastal monitoring would also be useful for MSFD purposes.

⁶⁷ Indicated by Swedish screening data on STP effluents

- Although the current monitoring programs performed on regional levels are important, very large gaps in operational regular monitoring programs were identified, related to both substances and compartments included and the number of sites covered. In addition, major needs of investigative monitoring projects can be anticipated to make intial assessments on the need for regular operational monitoring programs.
- Lack of national limnic monitoring programs for sediment, effects and lower trophic level biota has implications on the potential to establish baseline levels, and thus to develop assessment criteria.

5.5 Literature cited

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6 Filling the gaps between current and required monitoring – potential developments

There are several ways to fill the gaps between the desired monitoring and current monitoring, of which some are described here.

6.1 Refining the assessment and investigative monitoring needs

Because the operational monitoring requirements are related to the outcome of the analysis of sources and impacts, it is important that the source and impact assessment is performed with care, neither over- or underestimating the sites that are identified as being at risk of not achieving good status and subsequently should be monitored. The aim of an ongoing national project for the river basin districts is to identify a harmonized approach that can be used by all County Administrative Boards, and also include factors that were so far not accounted for, including the variability in sensitivity of the different water bodies due to factors such as dilution effects (see chapter 3). In the end however, the final assessment of risk and monitoring needs related to those water bodies that were identified in this way should probably be performed on a case to case basis on local level in a more refined assessment. Important tools would be local modelling and/or initial investigative studies before regular monitoring programs are established.

6.2 Selecting and deselecting priority substances to monitor in operational and surveillance monitoring programs

To focus monitoring on those substances that can be considered relevant based on risk of non compliance is another way to reduce efforts involved. Substances not detected or found only in low concentrations in spite of sampling close to local sources that are suspected to emit the substances could act as decision support in deselecting substances to monitor in an operational programme at least for a particular compartment.

Unless significant local emissions can be suspected, or the receiving water is pointed out as being particularly sensitive (higher impact than normal can be expected), it would perhaps be possible to limit the monitoring of ubiquitous hazardous substances to river basin and national level, because in these cases more or less all water bodies should be considered to be at risk of not achieving good status. In this way, the operational monitoring programs could be focused on such substances that can be considered primarily local problems (see also Box 6.2.).

An international evaluation of water data on 500 compounds and from the four rivers Elbe, Scheldt, Danube and Llobregat could conclude that the highest risks related to current priority substances were found for the following substances: endosulfan, DEHP, diuron, alachlor, TBT, simazine, 4 nonylphenol (tech and n), aldrin, endrin, fluoranthene, benzo[b]fluoranthene, indeno(1,2,3-c,d)pyrene, benzo[k]fluoranthene, benzo[ghi]perylene, atrazine, isoproturon, t-octylphenol,

lindande, benzo(a)pyrene, trichlormethane, hexachlorbenzene, trifluoraline, PCP, dieldrin, 1,2,4,-trichlorbenzene (von der Ohe et al 2011).

A Swedish evaluation was also performed by the Swedish Environmental Protection Agency (Naturvårdsverket 2008a), concluding that the following current priority substances are the ones that constitute the most significant problem in the water compartment: nonylphenol, cadmium and TBT. For these three, both national and regional monitoring is recommended (although it should be pointed out that only cadmium is currently being monitored in the water phase on a regular basis within the national programs). Additional substances that were suggested to be evaluated further regarding the relevance to monitor them on national level in the water compartment include DEHP, Hg, octylphenol, penta BDE, pentachlorbenzene, endosulfan, fluoranthene and PAH. Whereas the substances alachlor, anthracene, atrazine, benzene, Pb, diuron, dichlorethan, dichlormethan, HCB, HCBD, HCH, isoproturon, chlorinated alkanes, chlorfenvinphos, naphthalene, Ni, PCP, chlorpyrifos, simazine, trichlorbenzene, trichlormethane, diuron and trifluralin were not considered relevant to monitor in the water compartment on national level. On regional level, more or less the same conclusions could be drawn, but PBDE, PAH, octylphenol, Hg, fluoranthene, endosulfan, DEHP, benzene, Pb, and Ni are also considered relevant to monitor (due to expected regional emissions), in addition to TBT, Cd and nonylphenol.

The conclusions made from the evaluations above are related to water monitoring, whereas several priority substances can be assumed to primarily be relevant to monitor in other compartments (sediment and biota) due to persistence and accumulation potential. Therefore, a preliminary evaluation was also performed within the context of this report to also include and evaluate concentrations found in biota and sediment. Based on this preliminary evaluation, current priority substances that can probably be deselected from monitoring of water, sediment and biota for compliance checking unless there are clear local sources, are listed in the 2nd column of table 6.1. below (see Appendix Chapter 21 for comments). One should be aware of the major limitations to this assessment. Candidate priority substances and the other substances regulated in 2008/105/EC were e.g. not included in the evaluation and the assessment. The evaluation of biota compliance is also very limited because it requires information on several supportive parameters that frequently were not available. Several substances were also so far monitored in biota of different types (several fish tissues, invertebrates, plants) only to a minor extent or not at all. Nevertheless, the table below can give an indication about substances to prioritize for certain compartments (see also chapter 7) and program purposes.

Although surveillance monitoring programs should include all priority substances, it can be assumed that in particular those that are ubiquitous (suspected to be causing large scale non compliance) and exhibiting increasing trends should be prioritized and monitored more frequently on a national scale, to be able to follow trends and the effectiveness of control measures (Box 6.1. and table 6.1.).

Box 6.1. Substances exhibiting increasing trends

In the 2008/105/EC it is stated that accumulating priority substances should be monitored in accumulating compartments (sediment/biota) and that concentrations cannot increase significantly. In the river basin management plans measures to reduce any significant emissions of these substances should be included. Although an increasing time trend being observed for a particular substance does not per see infer that the substance in question is causing negative effects, significantly increasing time trends should be a cause of concern also because of the risk of future effects (non compliance), in particular in areas also exposed to significant local emissions.

Several recently published reports on trends of substances monitored in biota are available, such as Bignert et al (2010) and Gustavsson et al (2010). Also, according to official statistics based on national monitoring data, trends can be observered for several hazardous substances in marine fish and/or guillemot eggs. The concentrations of Pb⁶⁸, BDE 47 and PCB can be observed to decrease in biota:, whereas HBCD and PFOS increase⁶⁹. Dioxin concentrations decreased previoiusly but are no longer decreasing. Also Cd concentrations are not decreasing. For Hg the trend is unclear and varies between sites investigated. Except for fluorene at one station, PAH concentrations in blue mussels are not increasing (Bignert et al 2010).

The Cu concentrations in lakes and rivers have slightly decreased. For fish there is no trend observed for Hg but PCB concentrations are decreasing. For PFOS, sufficient data for limnic trend analysis are not available.

⁶⁸ There is a datagap in metal concentrations in marine biota due to analytical problems between 2004-2008. Nevertheless, the concentrations of lead in herring, cod and perch livers are decreasing. The increasing trends of cadmium concentrations in herring liver from the Baltic Proper and from the Bothnian Sea in 1980 to 1997 has levelled out but are not lower than the levels in the beginning of the 80s (Bignert et al 2010).
⁶⁹ <u>http://www.naturvardsverket.se/sv/Start/Statistik/Officiell-statistik/Statistik-efter-amne/Miljotillstandet-i-kust-och-hav/</u>. From bignert et al (2010) it can also be concluded that PCB-153 is decreasing in herring at most sites, perch and guillemot from the Baltic sampling sites and also in herring, perch, cod and blue mussel from Fladen and Väderöarna at the Swedish west coast. Also DDE is decreasing in herring, perch, cod and blue mussel from all sampling sites and also in blue mussel from Väderöarna at the Swedish west coast. Also DDE is decreasing in herring, perch, cod and blue mussel from all sampling sites and also in blue mussel from Väderöarna at the Swedish west coast. Also DDE is decreasing in herring, perch, cod and blue mussel from all sampling sites and also in blue mussel from Väderöarna at the Swedish west coast. However, we functional sampling sites and also in blue mussel from Väderöarna at the Swedish west coast. However, how we functional sampling sites and also for the mussel from Väderöarna at the Swedish west coast. The sum of PAHs is decreasing in blue mussels from Väderöarna at the Swedish west coast.

the significant decrease of TCDD/TCDF observed in guillemot eggs from St Karlsö between 1970 and the middle of the 80-ies has levelled out and in herring there is also no decrease in TCDD-equivalents during the investigated time period 1990-2008. In addition, HBCD is increasing in guillemot eggs from the Baltic Proper, but it is decreasing in herring from Utlängan (Baltic Proper) and from Fladen (cod liver and herring muscle) and Väderöarna at the Swedish west coast. PFOS is increasing in guillemot eggs from the Baltic Proper.

Table 6.1. Preliminary conclusions on monitoring relevance of different priority substances to check for compliance, based on available data retrieved from national data hosts. An assessment of increasing or not decreasing trends on a national scale is also included (see box 6.1.). Priority substances that are not included in any of the 2nd, 3rd or 4th columns from the same row could not be evaluated. For substances in bold, more or less the same conclusions could be drawn as in the report by Naturvårdsverket (2008a) (this comparison was only made for the water compartment).

	Can probably be deselected unless clear source identified	Suspected to be ubiquitous	Non compliance cannot be excluded	Trends are increasing or not decreasing in a national perspective
Water	Alachlor, Anthracene Atrazine, Benzene ⁷⁰ , C10-13 chloroalkanes, Chlorpyrifos, Dichloromethane, Endosulfan ⁷¹ , HCB, HCH, Naphthalene, Pentachlorbenzene ⁷² , PCP, Simazine, Trichlorbenzenes ⁷³ , Trifluralin		Isoproturon ⁷⁴ , TBT Possibly also: PBDE , Cd ⁷⁵ , Chlorfenvinphos ⁷⁶ , 1,2-dichlorethane ⁷⁷ , DEHP , Pb , Hg , Ni , Diuron ⁷⁸ , Fluoranthene , Hexachlorbutadiene ⁷⁹ , Nonylphenol ⁸⁰ , Octylphenol, PAH ⁸¹ , Trichlormethane ⁸²	Pb (limnic)
Sediment	C10-13 chloroalkanes, PCP, 1,2-dichlorethane, alachlor, atrazine, dichlormethane chlorfenvinphos, Hexachlorbutadiene,	TBT (marine) PAH (marine) Octylphenol (marine) Anthracene (marine)	Anthracene, DEHP, Fluoranthene, HCB, Cd, Hg, PAH, Naphthalene Nickel, Nonylphenol, Octylphenol, Pentachlorbenzene,	Not possible to estimate on national scale (off shore sediments

 70 In the report by Naturvårdsverket (2008a), it was concluded that benzene could exceed EQS close to certain point sources such as land fills

^{γ_1} In Naturvårdsverket (2008a) it is pointed out that LOQ levels are insufficient and it is therefore desirable to investigate this substance further.

⁷² In Naturvårdsverket (2008a) it is pointed out that LOQ levels are insufficient and it is therefore desirable to investigate this substance further.

⁷³ In Naturvårdsverket (2008a) it is concluded that EQS exceedences were not identified but limited monitoring could still be relevant due to the risk of PCB formation in aquatic environments

⁷⁴ Not considered prioritized to monitor regularly in water, except for the current pesticide monitoring program (Naturvårdsverket 2008a).

⁷⁵ Cd in water is considered a problematic substance in the report by Naturvårdsverket (2008a) but also mentions the problem in evaluating the substance (different EQS values for different water hardnesses, non filtered samples being analysed etc. That report suggests monitoring in both water and biota and on both national and regional levels.

⁷⁶ Not considered prioritized to monitor regularly in water (Naturvårdsverket 2008a).

⁷⁷ Not considered prioritized to monitor regularly in water (Naturvårdsverket 2008a).

⁷⁸ Not considered prioritized to monitor regularly in water (Naturvårdsverket 2008a).

⁷⁹ Not considered prioritized to monitor regularly in water (Naturvårdsverket 2008a).

⁸⁰ Nonylphenol in water is considered a problematic substance on national scale in water in the report by Naturvårdsverket (2008a)

⁸¹ In Naturvårdsverket (2008a) it is pointed out that LOQ levels are insufficient and it is therefore desirable to investigate this substance group further.

⁸² Not considered prioritized to monitor regularly in water (Naturvårdsverket 2008a).

	Can probably be deselected unless clear source identified	Suspected to be ubiquitous	Non compliance cannot be excluded	Trends are increasing or not decreasing in a national perspective
Biota	simazine, Trichlorobenzenes, trichlormethane Trichlorobenzenes	Possibly also: Fluoranthene (marine) Mercury (fish) Cadmium (blue mussels) Pb (blue mussels) Possibly also: PBDE, TBT (gastropods) ⁸³	TBT, Diuron, endosulfan, isoproturon, HCH Ni (blue mussels) TBT Possibly also: PBDE, HCB, HCH, PAH (blue mussels), Cadmium (fish), Pb (fish)	were only monitored in 2003 and 2008 so far) HBCD (marine) PFOS (marine) Dioxins (marine) Cd (marine) Hg (marine and limnic) Ni, Zn, Cu (marine)
				``´`

"Ubiquitous" substances (such as Hg and TBT) would possibly be sufficiently monitored within surveillance monitoring. By utilising information from surveillance monitoring programs and knowledge about the water body characteristics, there may also be sufficient knowledge to be used for status classifications regarding such ubiquitous substances also in water bodies not monitored. However, substances identified to be of major concern on a large geographical scale could also be suspected to be present in even higher concentrations on a local scale if there are additional significant local emissions or other factors causing elevated impacts locally. In such cases, local measures and both operational and investigative monitoring is justified⁸⁴. The 3rd column in table 6.1. lists priority substances that can be suspected to be causing large scale non compliance (see Appendix chapter 21 for comments).

Box 6.2. What substances are "ubiquitous"?

It is essential to identify substances that should be considered problematic on larger geographical scales to identify needs to implement measures on these scales but also to reduce significant local impact, because the margins of safety are already small or no longer existing. Based on available monitoring data it was in the previous management cycle already concluded that the biota concentrations of Hg exceed EQS values on a national scale (the Swedish map is red).

Other /candidate/ priority substances can also be suspected to give rise to more or less red maps. The following WFD priority substances were considered to be of concern on a national scale in the water compartment (Naturvårdsverket 2008)t: Cadmium, Nonylphenol and TBT. Also DEHP, endosulfan, fluoranthene, mercury, octylphenol, PAHs, PBDE and pentachlorbenzene were considered necessary priority substances to evaluate further from a national perspective.

⁸³ Assessment refers to concentrations, but effects can be considered ubiquitous

⁸⁴ These substances could e.g. be prioritized in investigative monitoring of emissions such as leachates and effluents from potential local sources.

In previous chapters related to the WFD, OSPAR end HELCOM conventions, the most problematic substances were also identified. Based on current WFD classifications, except for Hg, also TBT, PAH, fluoranthene, Cd, Pb, Ni, nonylphenol, Zn, Cr, Cu, dioxins, PCBs and irgarol were considered to cause less than good status at more than 10 water bodies and therefore considered potential ubiquitous compounds. Based on current OSPAR assessments, levels of PCBs, PAH and Hg are still unacceptably high at more than 50% of the monitoring stations and thus confirming that these substances are probably still to be considered "ubiquitous". Also Pb and Cd concentrations are frequently found in unacceptable levels. Based on HELCOM assessments for the Baltic, PCBs, DDT/DDE, cadmium, lead, TBT and cesium-137 are being problematic at more or less all sites and PCBs, lead, mercury, cesium-137, DDT/DDE, TBT, benz[a]anthracene and cadmium exhibited the highest threshold exceedences.

A report from the Swedish limnic monitoring program of biota suggests that of the monitored compounds, only PFOS could be considered ubiquitous in this context (Gustavsson et al 2010).

One can also obtain an indication of problematic substances by comparing effect based sediment standards for PAHs, TBT and HCH with the draft sediment standards based on relative concentrations. Unfortunately, the comparison cannot be made on a 1% organic carbon basis (would correspond to a comparison with site specific standards) because the standards were not based on normalised data. Nevertheless, if comparing 5 percentile values ("limit between class 2 and 3) with QSbent values based on 5% TOC, PAH and HCH effect based standards are above these levels but TBT is not (boundary between class 2 and 3: 0.006 mg/kg and effect based trigger: 0.000011 mg/kg respectively). However, the effect based EAC criteria (OSPAR) for PAHs and metals in sediments recently proposed were also identified to exceed BAC⁸⁵.

Although some substances can be assumed to be problematic on a national (and international) scale, problems may be larger in some areas than others. In Bignert et al 2010, it was e.g. concluded that cadmium concentrations in blue mussels from the Baltic Proper are about 5 times higher than the suggested background levels for the North Sea and 3 times higher than in blue mussels from the Swedish west coast. Also PCB153 and TCDD/F concentrations are significantly higher in herring (generally more than three times) from the Baltic Proper and the Bothnian Sea compared to the Swedish west coast.

Based on this general current picture, the following substances would need to be prioritized in WFD surveillance monitoring programs and within operational monitoring if local sources can be suspected (substances are not listed in order of priority): Hg (in particular for predatory fish, monitored in muscle), Zn, Cu, Cr, Ni, Pb, Cd (also water), Dioxins, PCBs, Irgarol, PAHs, Nonylphenol (water), TBT (also water). The needs to also include other substances such as DEHP, endosulfan, octylphenol, PBDE and pentachlorbenzene would need to be investigated further, as suggested in 2008, but the most relevant compartment may in these cases not be water.

In table 5.2. above, substances that were found to occur in concentrations above /draft/ EQS values (or QS values) and compartments being primarily at risk, based on available data retrieved from data hosts, are indicated in the third column. If not only maximum concentrations are exceeded in e.g. screening studies but also most data recorded from references stations, such substances are considered to be potential ubiquitous WFD substances.

Substances included in the 4th column of table 6.1. refer to the substances for which non compliance can probably be confirmed from data available at least once. Substances not included in any of the columns of the same row could not be evaluated.

⁸⁵ EAC values were therefore not recommended to be used for sediment evaluation in developing the QSR 2010 (Quality Status Report). Instead, ERL values (developed by the US EPA) were recommended for CEMP assessments of PAH in sediment (to indicate "green" assessment, i.e. status is acceptable).

6.3 Program and sampling coordination and specimen banking

To save costs related to the sampling of sediment and biota, coordination of the monitoring of hazardous substances also with other activitites should be considered. The national integrated fish monitoring program e.g. comprises the monitoring of fish population, fish physiology and concentrations of hazardous substances in tissues. There are several test fishing programs that potentially could be utilized also to generate material for the analysis of hazardous substances. Besides the national coastal test fishing programs there are also regional programs (Andersson & Ljunghager 2007)⁸⁶.

Specimen banking could also be a solution to save resources. Biological tissues are already being collected and saved within a national specimen banking program on a routine basis. For small amounts of material, additional banking of regional samples would be free of charge and accepted if there are plans to use the samples in the future.

The current specimen banking program is limited to biological tissues and primarily mussel and fish samples are stored at the Swedish Museum of Natural History⁸⁷. Other biota tissues would be possible to store as well. The possibilities to save other types of samples of relevance to aquatic monitoring and screening, such as freeze dried sediment, should also be investigated to find relevant facilities.

In chapter 5, a promising coordination potential was identified related to integrative monitoring of bivalves, by adding recommended effect based monitoring tools (such as lysosomal stability) to the current chemical monitoring. Other coordination potentials in order to develop integrated monitoring programs should also be investigated and discussed with the performers. Because the monitoring of hazardous substances in general and effect based monitoring in particular is very limited on regional levels, efforts should be made to coordinate national and regional programs to a larger extent. Unexpected concentration peaks and effects observed in certain areas investigated in the national programs should e.g. be followed up by regional monitoring efforts of the same parameters to identify potential local sources. Such an approach would benefit the generation of data that are useful as support for local and regional control measures.

6.4 Funding of regional monitoring programs needs to be solved

Monitoring of hazardous substances is expensive and a major obstacle to fulfill the monitoring requirements is the lack of funding. This is particularly evident for regular operational monitoring. The current funding of regular regional monitoring programs (RMÖ) related to hazardous substances is extremely limited compared to the needs to fulfill gaps evident in chapter 5. Most water bodies considered to be at risk of not achieving good chemical status priority substances are not monitored

⁸⁶ The following additional stations are monitored: Ringhals, Hakefjorden, Kullen, Barsebäck, Vinö, Simpevarp, Mönsterås, Aspöja and Askö.

⁸⁷ <u>http://www.nrm.se/en/frontpage.16_en.html</u>

regularly. In addition, for those water bodies that are monitored, there are major limitations in the number of substances that are monitored. Although the river basin district authorities have the responsibility to make sure that there are adequate operational monitoring programs, long term funding to support such regular programs is not available.

Because Sweden already has an established system with SRK programs, one possible approach would be to improve such programs and revise them according to the needs of the WFD. As could be concluded from chapter 4, the SRK programs do fill an important role in regional scale monitoring. Nevertheless, the possiblities to fill monitoring gaps are limited due to legal and economic reasons.

The most obvious gap that would be difficult to fill with SRK programs would be the monitoring of substances that cannot be related to large point sources, including substances that are subject to long range transport. In addition, individuals cannot be required to perform monitoring although several of the emerging substances may come from private use and diffuse emissions (personal care products, anti fouling paints, sun screens etc). To direct monitoring requirements in the recipient towards smaller operators, where each polluter may not have a significant impact but a clustered situation could cause major influences even on local level, is probably not possible within the current regulatory framework. Furthermore, at contaminated sites due to historical activities, responsible operators may not have been identified and even if so, monitoring efforts often stay within the borders of previous activitites. These more or less diffuse impacts or unclear sources and responsibilities probably would result in the need for authorities to sponsor monitoring of substances released from diffuse and historic activities on both district and local levels.

Nevertheless, even if emissions are well characterised and related to point sources, the potential to rely on SRK programs to fulfil the purpose of operational monitoring is low, with current funding and legal framework (Box 6.3.).

Box 6.3. Monitoring claims towards operators according to polluters pay principle - legal possibilities

The SRK programs are based on the polluter's pay principle, but instead of having each polluter monitoring its impact on the recipient, the programs are coordinated and monitoring stations are therefore not necessarily located close to the points of effluents. Therefore, the established monitoring stations are probably already located at points that would to a large degree fulfill the requirements of operational monitoring within WFD, i.e. located at sites that give a representative picture of the water body. However, from a regulatory perspective it is not possible to require that a polluter should join such a program and not all potential polluters in an area are members of an SRK program. Furthermore, although local and regional authorities frequently join program committees and can recommend parameters to monitor, the authorities cannot strictly require such a coordinated program e.g. to monitor a particular substance at a particular site, not even if it is clear that at least one of the members release this substance in significant amounts.

An alternative, in order to be able to make any monitoring requirements, it would be necessary to direct such claims towards the individual polluter/operator. In such cases there needs to be a clear connection to the activities of that operator. To request monitoring at locations far from point of release is difficult in practice, in particular if other potential sources are located in the same area. Moreover, the emissions of hazardous substances from single operators are currently also not known in detail. If a substance is not monitored even in the effluent (usually only done on

substances for which there are established emission limit values), it is difficult to require monitoring of such substances in the receiving water bodies.

In some cases, authorities also contribute financially to the SRK programs, or co-coordinate with regional/national monitoring programs. In such situations, the possibility to influence the SRK programs would be expected to increase.

There are some Swedish guidance documents (Allmänna råd NFS 2001:2; Naturvårdsverket 2001) on requirements that can be directed towards a single operator and it is clear that there are limitations in these obligations and that these cannot have a "research character", possibly limiting also the use of SRK programs for certain types of investigative monitoring. The possibilities to ask operators to adapt the chosen monitoring compartment or species to also be able to predict effects on human health seems to be limited, because the instructions in the handbook (Naturvårdsverket 2001) clearly states that the obligations of the operator refer to the <u>environment</u> and that any influence from the activities on <u>health</u> of people (specified groups or individuals) are not included. However, whether this only refers to direct human exposure or also exposure through the environment is probably a matter of interpretation. Because the EQS values for priority substances but also river basin specific substances also take effects on human health into account, a situation is possible where monitoring of primarily edible parts of fish that is actually consumed would be recommended in order to be able to evaluate obtained data in status classification. However, it would probably be difficult to request an operator to monitor a particular species or tissue for that reason.

It can therefore be concluded that a significant increase in funding of regular regional monitoring programs of hazardous substances is necessary, in order to fulfil operational monitoring requirements. A change in the legal framework and clarification in national guidance could possibly also enhance potential to make actual claims but still utilize existing SRK organisations and benefit from coordination possibilities⁸⁸. Other options could also be considered, such as the introduction of "monitoring fees", related to the size and type of activities of the "polluter".

6.5 Guidance and Platforms

6.5.1 Guidance needed

To identify, prioritize and design cost effective operational monitoring programs of hazardous substances in the aquatic environment, knowledge about the regional and local environment and sources is necessary. The most prioritized places (*"Where to monitor"*), and the most relevant variables (*"what to look for"*) must be identified. A systematic and harmonized approach is valuable to roughly identify the most prioritized locations on a national scale and taking major sources into account. However, for further prioritizations, it is also necessary to make a local/regional risk assessment of the sources. Therefore, in the design of operational programs and investigative projects, additional communication

⁸⁸ In October 2011, the Swedish EPA expressed the opinion to the Environmental Ministry that such a revision should be initiated. See: "Yttrande om underlag med anledning av frågor om Europaparlamentets och rådets direktiv 2000/60/EG om upprättande av en ram för gemenskapens åtgärder på vattenpolitikens område. 2011-10-20 Ärendenr: NV-09623-11 "

platforms are probably necessary, as monitoring and inspection is frequently divided between different departments and authorities.

Furthermore, the sensitivity and local character of the recipient needs to be assessed in order to identify high risk locations, taking into account that the emission of the same amount of a certain substance to two different water bodies may not pose the same risk, and the area being at highest risk may be located further downstreams. The next steps in designing a monitoring program include also decisions about what compartments to monitor, how frequently and when and with what type of equipment etc (*"how to monitor"*). Another aspect to take into account with both operational monitoring programs and, in particular, investigational proejcts, is to generate data that can support the identification of suitable control measures or to assess progress after measures have been undertaken to reduce emissions.

In complex situations or when the substances released from a particular source is largely unknown, also "unlisted" compounds need to be considered, for which there is little available guidance. Clear "analytical packages" may also not be available and not all substances are possible to analyse even by research laboratories. Such situations make it really tricky to choose the most approapriate variables to monitor. Finally, the evaluation of monitoring data is generally not straightforward, so professional judgement is usually also necessary.

Therefore, in designing programs and data evaluation, knowledge about environmental chemistry, exotoxicology, sedimentology, limnology, ecology, statistics, regulatory frameworks etc is usually required. It is clear that a rather detailed guidance is needed to assist local and regional authorities. Such a guidance document is being developed by some of the counties at the moment and it includes several aspects described above. Nevertheless, also in the future, this document needs to be revised according to new findings and regulatory needs and tools being available. Guidance is also needed on how to evaluate data, whereas at the moment, there is a major lack in assessment criteria for hazardous substances in the aquatic environment.

It is likely that also additional case specific questions arise and it would probably be very valuable to establish a national expert group that includes all of the expertise mentioned above, to support the regional authorities in both the design of programs/campaigns and in the interpretation of data.

6.5.2 How to handle RBSPs and ecological status classification?

A special challenge is how to deal with guidance and assessment criteria related to RBSPs but also issues related to legislation and procedures. RBSPs should be identified on a water body or river basin level (see chapter 3). The Counties are developing/revising regional monitoring programs and also interpreting the data for status classifications. According to the Swedish legislation, the criteria for such RBSPs should be developed by the River Basin District Authorities (see NFS 2008:1 appendix 2 chapter 7). In practice, they are probably better developed on national level, to harmonize the evaluations and classifications (and also because it

is not realistic to assume that each district should have access to all the expertise and background data that is necessary to develop criteria on a large number of substances). However, a site specific⁸⁹ assessment should and can probably better be made by the counties that are actually interpreting the data.

So far, assessment criteria for RBSPs were not established formally and it is unclear from a legal point of view how this should be done. However, criteria were "suggested" by the Swedish Environmental Protection Agency (and actually developed by the Swedish Chemicals Agency, see Naturvårdsverket 2008b). So, substances have been identified, suggested criteria are available for at least some of them, but it is still unclear how the criteria should be "developed" by the River Basin Districts.

To include the assessment criteria in the legislation (as was done for many other variables in NFS 2008:1) has been proposed to be a proper solution, but the consequences need to be analysed. The RBSPs could in the end, at least in theory, constitute a very large number of substances and not all of them are relevant at all sites. So it would need to be very clear that such a potentially long list of substances does not list substances that should be monitored at every site because most of them are probably not relevant at all sites. In fact, if a substance is found to be relevant at all sites, this substance should probably rather be nominated to become a priority substance in future revisions of the WFD and 2008/105/EC.

Because of the huge potential number of RBSPs to be found, the legislation may also need to be revised frequently. Other options to "develop" these criteria by the River Basin District authorities should therefore also be investigated. Or it should be considered to revise the current legislation and find an alternative option on how to handle RBSPs from a legislative point of view.

Either way, this situation asks for good communication and clear procedures between the counties, river basin district authorities, and national authorities on how to deal with the matter of RBSPs once suspected to pose a problem. It also calls for expert support in identifying new such substances. Preliminary criteria either developed before hand for a large number of substances or "on demand" once "new" substances have been found and that are suspected to pose the properties identified in Annex VIII to the WFD could become valuable support. In a more general sense, a strategy on how to deal with the matter of hazardous substances in the context of ecological status should be established. In chapter 9, it was found that also considering effect based tools in this context, could provide some major support in how to deal with these "unlisted" substances.

⁸⁹ In the formal advice to this chapter it also says that the river basin district authorities shoud develop assessment criteria for the compartment to which the most sensitive organism is exposed and also take background concentrations of non synthetic substances and also factors that influence the bioavailability of the RBSP.

6.5.3 Communication platforms for coordination and information exchange related to risk assessment, monitoring, inspection and enforcement work

In Sweden, the Swedish Environmental Protection Agency is responsible for the national monitoring of hazardous substances in the aquatic environment and SGU (Sveriges Geologiska Undersökningar; Geological Survey of Sweden) in groundwater, whereas chemical regulation of substances and products are managed by the Swedish Chemicals Agency (Kemikalieinspektionen) and pharmaceuticals by the Medical Products Agency (Läkemedelsverket). Health risks related to drinking water exposure are generally dealt with by the National Food Administration (Livsmedelsverket), whereas the County Administrative Boards have responsibilities related to primary production of food (e.g. fish for sale caught in Swedish waters). Since June 2011, the responsibilities related to the WFD and MSFD were transferred from the Swedish Environmental Protection Agency to a new national authority, ("Havs och vattenmyndigheten", HaV, Swedish Agency for Marine and Water Management) but the monitoring of hazardous substances at least on national level and within screening remain a responsibility of the Swedish Environmental Protection agency. The exact divisions of different types of issues between HaV and the Environmental Protection Agency was at the time of writing this report not entirely clear. In addition, several experts, from e.g. the Swedish Museum of Natural History (Naturhistoriska Riksmuséet), Department of Applied Environmental Science at the Stockholm University (ITM, Institutet för tillämpad Miljöforskning), Göteborg University, Swedish University of Agricultural Sciences (SLU, Sveriges Lantbruksuniversitet), Umeå University and the SGU are involved in national monitoring and interpretation of data.

It is clear that in addition to the risk of available data not being utilized or similar work being performed twice or unharmonized, there is a great risk that non monitored substances will not be regulated, and non regulated substances will not be monitored. Good communication platforms can facilitate coordination and information exchange about the current state of the environment and needs for additional national, local and regional control measures. Assessment criteria constitute a common interest for most involved. In addition to the European EQS values for priority substances, national EQS values for other compartments than than the ones specified on European level are necessary. Also EQS values for RBSPs should be established. Within chemical regulation, PNEC (Probable No Effect Concentration) and similar values are calculated according to relevant legislation (such as REACH and BPD), to assess the risks of chemicals in the environment. Those values are compared to predicted environmental concentrations (PEC), whereas measured concentrations (monitoring data) could provide valuable information and actually even provide a basis for revising such calculated values, and therefore influence the risks perceived. EQS (Environmental Quality Standards) values related to the WFD are calculated in similar but not identical ways, frequently utilizing the PNEC values available from such risk assessments.

Also local and regional work related to environmental inspection and enforcement as well as regional monitoring performed by the counties and municipalities, would probably benefit from increased communication platforms. This would facilitate the use of local monitoring data to support the implementation of local measures⁹⁰, but also the identification of monitoring needs due to known local emissions. Local and regional authorities also suggest criteria that cannot be exceeded in the context of activities such as remediation and dredging. In addition, the establishment of emission limit values need to be guided also by good knowledge about the local recipient and its current state and sensitivity as well as dilution/accumulation to be expected. National harmonisation of how environmental aspects are taken into consideration by different counties, municipalities and courts when establishing release permits would also increase legal security.

Both local/regional and national authorities would therefore need to be involved in such platforms. Also the participation of other experts such as researchers is important to make use of available knowledge but also to stimulate the generation of new knowledge about the state of the environment (including the monitoring of non regulated, emerging substances and effects), inherent properties of substances, new cost effective tools that can be used etc. The participation of representatives from NGOs and IND could also be considered to be involved for discussing certain policy issues.

Progress is being made in this context. A good example is the reference group related to screening activitites, with participants from County Administrative Boards, National authorities, consultants and researchers. The use of the database "Products Register"⁹¹ has e.g. been suggested to be used in combination with a "dispersal index" as a decision support tool to identify interesting substances for the directed screening campaigns (Naturvårdsverket 2003). The national database contains information about 145 000 chemical products that are imported/produced in Sweden in amounts above 100 kg/year. Statistics based on the data from all similar Nordic databases can also be obtained from the SPIN database⁹², financed by the Nordic Council of Ministers. A similar approach could be discussed for other type of chemicals such as cosmetic ingredients and pharmaceuticals (not included in the Products Register). There are also plans to start a discussion group related to environmental assessment criteria. As stated above, such criteria are much needed in several contexts, such as dredging activities, sediment remediation, food recommendations, status classifications under the WFD and MSFD, risk assessment of chemicals, emission limit values (release permits), and by several authorities.

⁹⁰ One good example of a successful use of monitoring data by regional authorities is the outcome of a national screening campaign on perfluorinated compounds. After observing concentration peaks in fish from a lake outside one of the larger Swedish airports, it was found that there was need for local remediation measures due to continuous use of PFOS containing fire fighting products in the exercise area. It was then discovered that a similar situation had occurred next to another of the largest airports for the same reason. Remediation measures have now been implemented and a research project was initialized to investigate area of impact and follow recovery of the system. http://repath.ivl.se/omrepath.htm

^{2972.}aspx

 ⁹¹ http://www.kemi.se/templates/Page 2972.asp
 ⁹² http://195.215.251.229/DotNetNuke/default.aspx

6.6 Handling of monitoring data

The monitoring of hazardous substances in the environment is often very costly. It is therefore very crucial that data that have already been generated are also available and quality controlled. Easily accessible data facilitates the planning and prioritisation of future monitoring programs and special investigations on national and regional scales as well as the implementation of control measures. The data can also provide background information to develop assessment criteria and to indicate which compartment to monitor. Easy access to monitoring data is a prerequisite within the WFD work for status classifications (compliance checking), that should be revised by 2013, and thus considered in the program of measures. The status classifications are in turn to a large extent the driving force behind requirements to include control measures in the Programme of Measures.

6.6.1 National data

Data related to hazardous substances that are generated within regular national monitoring programs should be and are generally reported to national data hosts⁹³. Data should be available on the internet within 2 months of delivery. However, it can be concluded that data from the following national monitoring programs are currently not yet available on the internet:

- Sediment data from 2008 monitoring of marine sediment
- Effects related data on Monoporeia.
- Integrated fish monitoring data

Moreover, the Vättern sediment data are available in the IVL screening database rather than on the SGU homepage (official data host for sediment data). In addition, previous national sediment data can both be found at the SGU (marine) and SLU (limnic) homepages, although the SLU data should now be transferred to SGU⁹⁴, so that SGU will handle all sediment related data on hazardous substances.

6.6.2 Regional data

There is no obligation to report data that is generated within SRK and RMÖ programs, but reporting is highly desirable. An obligation to manage data from regional monitoring programs is also included in the contracts of the data hosts⁹⁵. The data should however be quality controlled before submission and the data host shall provide a template to simplify data deliveries.

Although there are such RMÖ and SRK data on hazardous substances, the data from several monitoring programs and campaigns are currently not available from

⁹³ Data hosts for different types of data can be found at <u>http://www.naturvardsverket.se/sv/Start/Tillstandet-i-miljon/Miljoovervakning/Miljoovervakningsdata/</u>

⁹⁴ Anna Apler, SGU, pers comm.

⁹⁵ Anders Foreaux, Swedish EPA, pers comm.

the data hosts and are therefore either not reported (most likely) or made available yet. In general, data from several regional monitoring campaigns but also even regular programs could not be found in any of the national databases.

One possible reason is that there does not seem to be a clear policy on where to deliver regional data. If available, the regional data are therefore sometimes divided between different hosts.

In principle, both national and regional data should be sent to the data host that is appointed to receive data from this particular matrix. However, it is less clear if this should also include data from non regular monitoring, such as campaigns. Because national screening campaign data are delivered to the IVL screening database, this data host has to a large extent also acted as a data host for regional monitoring data even in the case of more or less regular programs⁹⁶.

Water related data from both national and regional programs are reported to SLU⁹⁷. The regional sediment data that is possible to retrieve from the SGU home page is very limited⁹⁸. Regional sediment data is instead available at least to some extent in the IVL screeningdatabase but in many cases only in the form of reports.

There is also currently very limited data from metal monitoring in Fontinalis available. Data are generated within several SRK programs but are normally only available as tables in printed reports. Because there is no national monitoring program of metals in Fontinalis, the few reported data are made available in the screening database. Other regional biota data are also available in the screening database but if the species and substance is reported in a national program, regional data can instead be found in the IVL biota database – but only if the data are received from a regular program and not a single campaign.

Effect based data has been reported to a very limited extent to the data hosts. Although such studies are still probably scarce on regional levels, future instructions should also include such data. For biomarkers being analysed in national programs, the corresponding data host would be reasonable, but for other types of assays, such as in vitro assay data, it should be discussed whether these data should rather be reported to the screening data base.

Nevertheless, in order to improve the reporting frequency of regional data, it could e.g. be considered to require such reporting (if suitable data hosts are available) when relevant monitoring programs/campaigns are funded. To facilitate further, a reporting requirement could be part of the contract issued with performers, although the Counties then need to make sure that the data has been quality controlled before submission.

⁹⁶ Regional monitoring data that are so far available in the screening database are listed here: <u>http://www3.ivl.se/miljo/db/IVL_screening_registersida.htm</u> ⁹⁷ Monitoring data from SRK programs are found here:

http://info1.ma.slu.se/max/www_max.acgi\$Project?ID=Intro&pID=-1

⁹⁸ The following data can be retrieved: national monitoring data from Vänern, data from Dalarna, Västerbotten data from 1975, data from 1990 from Norrbotten, RMÖ data from Kalmar.

Non regular monitoring campaigns and projects

Only regular monitoring programs are registered in VISS, although there are additional special projects being performed on occasion by authorities, such as county administrative boards and municipalitites, and SRK associations but also by the National Food Agency. Neither the projects themselves nor the generated data from such investigations are generally reported to the data hosts but can sometimes be available on web pages. Pesticide data from special campaigns are being reported on a voluntary basis to SLU ("regionala databasen"). Monitoring campaigns performed jointly by the National Food Agency and the Swedish Environmental Protection Agency are reported to the IVL databases but other data can be obtained on request⁹⁹. The dioxin data reported to COM are also published as reports on the web page of the National Food Agency¹⁰⁰.

Data from research projects are also less accessible, and even if published in scientific journals, full text documents are usually only accessible through university libraries (unless published in the so called open access journals). Furthermore, raw data are not always published but requires direct communication with the researcher, and even if abstracts frequently can be found by using common search engines, it can be difficult to locate data from a particular region unless this is clear from the title or abstract. Nevertheless, search engines on the internet can be very useful to find abstracts of scientific papers. The NORMAN database EMPODAT¹⁰¹ contains several types of data, including research data and data collected by individual countries and also the JRC (European Commission). Besides chemical monitoring data there are also data from effect based monitoring. However, Swedish data in this database is limited to data from national screening projects and it would be desirable to investigate the possibilities to recommend Swedish researchers to submit relevant raw data to this database.

Data from investigations required by regulators, such as the analysis of metals and tinorganic compounds in dredged sediment, are normally only available in paper format and may be confidential and/or protected information.

6.6.2.2 Improved quality control needed

If RMÖ and SRK data are reported to any of the national data hosts, they can so far primarily be found in the IVL screening database¹⁰² unless biota is monitored and the same species and parameter is monitored in the national biota monitoring program. In the latter case, the regional data can sometimes be found in the IVL biota database.

One should be aware of the different circumstances preceding a screening campaign though. There are e.g. usually no standard analytical protocols for

⁹⁹ Marie Aune, National Food Agency, pers. comm..

http://www.slv.se/sv/grupp1/Risker-med-mat/Kemiska-amnen/Dioxiner-och-PCB/
 http://www.normandata.eu/empodat_index.php

¹⁰² Reported regional data is listed here: <u>http://www3.ivl.se/miljo/db/IVL_screening_registersida.htm</u>

emerging substances available, concentrations to expect or suspected to cause effects may be unknown and therefore it is difficult to make any specific quality claims. For economic reasons, often grab samples are taken rather than repeated composite samples.

The counties have little legislative power to influence what parameters to include and monitoring frequency and other aspects related to the design of an SRK program (Box 6.2.). To make a distinction between what is a single campaign and what is /going to be/ a regular monitoring program is usually difficult. Several SRK organisations do make "special campaigns" but may also repeat such campaigns after some time.

If regional monitoring data, regardless if generated in a campaign or within a regular program, should be delivered to the IVL screening database and used by the counties within status classifications, the routines for guality control need to be greatly improved so that important metadata is always included. Currently, important information is frequently missing, such as information on species analysed, dry weight, sampling date, organic carbon content of sediment, lipid content of biological tissue, sampling coordinates, etc. Erronous information has also been identified (such as wrong units or non realistic sampling coordinates). It is also not possible to sort out surface water samples from effluent samples etc and the latter clearly do not reflect the conditions of a water body. In general, because data from different types of investigations can fill different purposes, it is essential to know whether the data retrieved from the database is from a location exposed to local sources, and if so, what type of sources (if known). Today this is actually not always easily understood from any of the databases and because biota data also from SRK and RMÖ programs are on occasion e.g. included in the biota database it is important to be able to dinstinguish between the different data types (background vs exposed) from each other. Some supportive information that is needed for the evaluation of biota data is also missing in the biota database, such as dw of N nitidus (analysed if sufficient material is available), lipid and dw of blue mussels. Erroneous reported tissue for PFOS data was also discovered¹⁰³.

Status classifications based on available monitoring data would be greatly facilitated if the retrieved data are also possible to sort according to water body identity (EU_CD) and not only according to x and y coordinates of the monitoring station. However, it should then also be clear wether the data reflect the conditions of this water body or an upstreams location. One should also be aware of the fact that the definition of water bodies might change, so there is a need to update such information if changes have been made.

In order to facilitate and ensure that regional monitoring data is reported to the data hosts and made available in a correct format, the structure of the results and information that the contract labs should provide (LOQ, LOD, standard etc) should be specified already from the start.

Although the compounds to monitor in drinking water include several priority substances (chapter 3) these type of data were probably used to a very limited

¹⁰³ reported to be monitored in muscle but should actually be liver

extent so far within the chemical status classifications and risk assessments of surface waters. As was pointed out in chapter 4, SGU has developed a database that includes also data related to surface water used for drinking water purposes ("vattentäktsarkivet"). The reporting of data (performed by the laboratories analysing drinking water) to the database is not mandatory, and the water producer needs to agree to such a submission. Certain information is also confidential (exact coordinates), but other information is available to County Administrative Boards for WFD purposes, although not easily accessible on line¹⁰⁴. By promoting the reporting and enhance the awareness of the database on regional level additional valuable information could become available also for the purposes of surface water assessments.

6.7 Tools needed

The possibilities to increase coordination of current trend monitoring with also monitoring required for compliance checking need to be investigated in order to establish cost effective coordinated programs. An assessment of the most suitable monitoring compartment is made for the current priority substances in chapter 8, taking into account several factors such as the Swedish official position that the compartment to which organisms that are pointed out as being most at risk should be prioritised in status classifications, current national monitoring compartment (providing also baseline data), the possibilities to fulfil the QA QC directive requirements and the compartment that you would expect to respond first (being most critical based on currently available monitoring data).

Of special current international concern is also the possiblities to predict effects from combined exposures to several substances. The WFD takes a "one out – all out" approach in chemical status classifications, i.e. it is sufficient to conclude that one substance exceeds EQS to draw the conclusion that there is no compliance. However, it is possible that substances can interact even at concentrations below such critical values and cause effects. The chemical approaches in use today to predict effects from combined exposures are described in chapter 8 of this report.

With several substances being present in concentrations indicating non compliance, effect based monitoring tools to monitor effects of these compounds could provide additional information that could be valuable in prioritisation between different types of measures and investigations. Effect based tools could also be of particular value in investigative studies on local scales, if the most relevant substances to monitor are largely unknown. There are several other objectives related to the use of effect based tools as a complementary approach to substance-by-substance based monitoring, including the possibilities to assess the effects from several substances combined and bioavailability and physiological interaction, see chapter 9.

¹⁰⁴ Data can be retrieved by sending a request to <u>vattentaktsarkivet@sgu.se</u>

6.8 Conclusions

- To reduce the gaps between current and required operational monitoring. several measures are possible. The monitoring needs can be reassessed, by refining the impact assessment, to prioritize those water bodies being most likely to be at risk of not fulfilling the objectives due to local conditions. To design cost effective operational monitoring programs in future revisions, care should be taken to primarily include those substances that are most likely problematic and deselect substances that are most likely not (unless clear sources can be identified). Substances that are more or less ubiquitous may not be relevant to also include in operational monitoring programs, unless significant additional emissions can be suspected (investigative monitoring could be necessary). By primarily monitoring those compartments that are most relevant for compliance checking for these particular substances (further discussed in chapter 7) the most cost effective approach can probably be achieved. In national/surveillance programs, substances being suspected to be ubiguitous and for which downward trends are not observed, should be prioritized and monitored more frequently.
- By coordination and considering/developing new tools, cost effective programs could also be developed. Nevertheless, a crucial factor is the lack of sufficient funding and means of legal instruments, in particular on regional level. For several priority substances it is at the moment not possible to evaluate whether they could be relevant to deselect or whether non compliance could be confirmed. Such an evaluation would be desirable as guidance in the design of future monitoring programs.
- Data handling also needs to be improved in order to have good quality data available by the 2013 revisions of status (compliance checking). A clear policy and instructions to both the counties, water organisations and data hosts is needed on how to handle regional data. An obligation to also report results to the corresponding data host (if available) could e.g. be considered to be included in the funding of relevant regional projects and in the contract with performers in the future. It should be clearly specified how to handle data from campaigns and regular programs. Not only chemical data but also different types of effects related data should be included in these instructions.
- The Counties should make efforts to make sure that quality controlled data are
 reported to the data hosts and coordinate the deliveries so that data hosts are
 prepared to receive the data. Quality control includes not only checking that the
 correct figures are being reported but also important metadata such as correct
 units, sampling coordinates, sampling date, species, age, sex, tissue, necessary
 and available supportive information (dry weight, lipid weight, organic carbon
 content etc) as well as information required to check compliance with the QAQC
 directive (LOQ, LOD, standard etc) is reported and structured according to the
 template provided by the data host. The needs to further develop and revise
 current templates should also be discussed. To facilitate reporting, agreements
 with laboratories or consultants can be made on how data should be reported (data
 arranged in a suitable way and including necessary analytical information) at an
 early stage.

• In Sweden several stakeholders and regulators are involved in the management and monitoring of hazardous substances in the aquatic environment. To facilitate coordination and generate useful decision support, there is a need to develop platforms related to the monitoring and evaluation of hazardous substances.

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PART II. THE MONITORING TOOLBOX

7 Prioritisation of monitoring compartment for chemical WFD compliance checking

National monitoring of hazardous substances in the aquatic environment was so far largely performed to analyse trends and for geographical comparisons, whereas evaluation against absolute criteria was more limited. With the implementation of the WFD, there is also a need to be able to assess chemical and ecological status based on effect based assessment criteria. Nevertheless, so far there are no national effect based assessment criteria for biota and only draft criteria for marine sediment available.

As could be seen from chapter 4, most organic accumulating hazardous subtances were so far monitored in biota, such as carnivore fish, in lakes and marine environments but also sediment primarily from off shore locations. Monitoring of water is primarily performed in freshwater (both rivers and lakes) and focuses on metals but in some agricultural areas also pesticides.

This chapter is therefore focused on investigating the most prioritised compartment to both monitor and develop criteria for in order to generate and evaluate data for WFD compliance checking. The objective is to illustrate different aspects that need to be considered and to identify needs for research, validation studies and the development of assessment criteria. Preliminary recommended monitoring compartments and compartments for which EQS values should be developed are also presented, based on an evaluation of these aspects. The overall goal was to coordinate the current monitoring system with the new WFD demands as far as possible in a cost saving approach, but also take the precautionary principle, current WFD monitoring guidance and analytical requirements according to the QA/QC directive (2009/90/EC) into account.

7.1 The most sensitive organism (A)

A Swedish position is that the EQS should primarily be set for the compartment in or via which the most sensitive organisms are exposed, although other compartments could be justified due to substance properties¹⁰⁵. This would minimise uncertainties in estimating exposures to the most critical protection objective.

The most critical protection objectives for the priority substances, according to current background documents related to the EQS values of the priority substances

¹⁰⁵ Linderoth M (Swedish EPA) pers comm.; see also e.g. page 13 in Naturvårdsverket 2009 (report 5973).

(SDS, substance data sheets¹⁰⁶), are indicated in table 7.1. (line A) and described in more detail in Appendix chapter 22. Identifying the most critical protection objective is normally estimated by recalculating all QS values into a corresponding water concentration. The data in these tables are based on information in the current published SDSs, in which calculations were based on equations in the manual developed by Lepper (2005).

According to this principle, if pelagic organisms are the most critical objective to protect (QSpelag), water should be monitored (if substance properties do not suggest another compartment). Correspondingly, higher trophic biota if secondary poisoning (QSsec pois), sediment if benthic organisms (QSbenthic) and seafood if human health through seafood intake (QShh)¹⁰⁷. It should be pointed out that substances for which secondary poisoning has been identified as the most critical criterion not necessarily are considered to be subject to biomagnification.

The instructions to calculate EQS values are being revised, but at the time of writing this report, guidance is still not published (draft CIS 27). Moreover, the EQS values of the priority substances are also subject to revision. With the new guidance on how to develop EQS values, QSbenthic will no longer have an impact on the overall EQS. Instead it should be evaluated as a separate parameter.

If human health related to drinking water abstraction is the most critical objective, water should be monitored and evaluated, but because not all water bodies are relevant in this context, this aspect was not included in the general evaluation in this report. It can rather be stated that if drinking water criteria are the lowest, monitoring water should be considered at relevant sites. However, it can be pointed out that the drinking water criteria are actually valid at the tap.

7.2 Other important aspects to consider

The assessment of the most critical compartment is not always clear and might change with the revision of EQS values in the future. To actually determine which organisms are the most sensitive is a difficult task, and in general there is frequently a high level of uncertainty related to the calculation of water concentrations from other compartments (see e.g. Lilja et al 2010). Furthermore, the most critical objective has in some cases been found to depend on whether fresh water or the marine water environment is monitored, primarily due to the reason that an additional assessment factor frequently needs to be used when marine data are not available. Thus, such criteria and identified most sensitive organisms are less certain. Altogether, this suggests that although this principle should be of major importance, other aspects can become crucial as well, for practical, economic and scientific reasons. This chapter therefore describes other

¹⁰⁶ Published at public CIRCA website

http://circa.europa.eu/Public/irc/env/wfd/library?l=/framework_directive/thematic_documents/priority_substa nces/supporting_background/substance_sheets&vm=detailed&sb=Title

¹⁰⁷ This is triggered by the following classifications of substances (or available information about such hazardous properties): known or suspected carcinogen/mutagen/to affect reproduction; possible risk of irreversible effects; the potential to bioaccumulate plus danger of serious damage to health by prolonged exposure or harmful/toxic/fatal when swallowed

factors that could provide additional support in identifying the most critical compartment to monitor for compliance checking. The assessment is entirely based on conditions and circumstances applicable to current priority substances but a similar approach would to a large extent be applicable also to other hazardous substances.

7.2.1 Inherent properties and detection frequency in certain compartments (B)

Substances that neither accumulate in biota nor in sediment are difficult to monitor in these compartments, whereas hydrophobic persistent substances may be difficult to detect in surface water although they may be present in high concentrations in biota and/or sediment because of accumulation processes. An assessment of the accumulation potential of the priority substances in sediment and biota can be made based on their inherent properties such as Koc and BCF. The inherent properties were also of major importance in the assessment of the preferred, optional or not recommended matrix to monitor, made in CIS 25 (Appendix chapter 23). By combining information on inherent properties with detection frequency in different matrixes based on data retrieved from the national data hosts (see e.g. Appendix chapter 20), conclusions were made about relevant monitoring compartment/s (Appendix chapter 23 and table 7.1 (line B).

7.2.2 Analytical requirements (C)

It is also important that analytical requirements can be fulfilled according to the QA/QC directive, including requirements related to the limit of quantification (LOQ). In particular, the LOQ levels for accumulating substances in water is frequently problematic (see box 7.1).

Box 7.1. Difficulties in fulfilling LOQ requirements

From a precautionary principle point of view, the mere presence of hazardous substances is of course reason for concern. However, an environment free of toxic and persistent substances is not only more or less impossible to achieve but also to define. Concentrations "close to zero" are in practice often interpreted as "below detectable levels". If concentrations below detection are to be established as a "goal", such a definition needs to be combined with technical specifications, or it can be either too conservative or under-protective. The more advanced analytical techniques that are available for a particular substance, the more stringent such a criteria will become. And the better analytical tools, the more substances can be detected in any environmental sample, even in pristine areas. The definition of "high ecological status" in Annex V of the WFD is related to LOD but combined with technical requirements: "Concentrations close to zero and at least below the limits of detection of the most advanced analytical techniques in general." So far however, such aspects were probably not taken into account in the ecological status classifications.

Whithin the WFD, the definition of "good ecological status with regard to specific substances" and "good chemical status" is that concentrations should be below the EQS values. At the time of writing this report, the "QA/QC directive" (2009/90/EC), related to chemical monitoring of the aquatic environment, is being implemented in Sweden. One requirement is that the Limit of Quantification (LOQ) should be less than 0.3 times the EQS value of that substance. Nevertheless, even if using the most advanced analytical techniques, LOQ levels may not be sufficiently low. For several substances, current analytical tools are still not sufficiently sensitive to detect the low levels of hazardous substances required. For the following priority substances, there are either no ISO or EN

standards available or the existing standard is not sensitive enough to fulfil the LOQ requirements (CEN 2007) :

Pentabromodiphenyl Ether and C10-13-chloroalkanes No standard available **Endosulfan, Pentachlorobenzene, Benzo(ghi)perylene, Indeno(1,2,3-cd)pyrene, Tributyltin compounds, Aldrin, Endrin, Isodrin, Dieldrin** Existing standard method not sensitive enough

In addition, only well equipped laboratories were able to meet target LOQ criterion in water for **Cadmium and its compounds (**ISO 17294-2:2003; ICP-MS)

Di(2-ethylhexyl)phthalate (DEHP) (ISO 18856:2004; GC/MS). Although the method is applicable to the analysis of DEHP in surface water and allows achieving sufficient low LOQ to conduct compliance checking in principle, many laboratories have serious blank problems and are hence, not able to meet the LOQ performance criterion

DDT (4 Isomers) (EN ISO 6468:1996; GC/ECD). LOQ low enough to allow compliance checking is difficult to achieve, or even impossible, for DDT due to the fact that 4 isomers have to be determined **Hexachlorobenzene (**EN ISO 6468; GC/ECD)

Hexachlorocyclohexane (EN ISO 6468:1996; GC/ECD)

NonyIphenols (ISO 18857-1:2005; GC/MS). Although the method is applicable to the analysis of NP in surface water and allows achieving sufficient low LOQ to conduct compliance checking in principle, many laboratories have serious blank problems and are hence, not able to meet the LoQ performance criterion

Octylphenol (4-(1,1,3,3)-Tetramethylbutylphenol) (ISO 18857-1:2005 GC/MS). LOQ criterion can be met for inland surface waters but not other surface waters. **Benzo(b+k))fluoranthene (**ISO 17993: 2002 HPLC/Fluo)

In practice, the problem of LOQ exceeding EQS can be even larger because of matrix disturbances, low amounts of samples etc. For marine waters, chemical analyses may in some cases be difficult due to disturbance from high salinity and marine EQS values are also often lower than corresponding EQS for freshwaters due to higher assessment factors. Nevertheless, different laboratories have different possibilities to fulfill the requirements and technical progress is also being made¹⁰⁸.

Current detection frequencies observed in monitoring data could give an indication about such problems. The highest LOQ levels registered in the IVL screening database for several priority substances monitored in water would not fulfil the QA QC requirements (see Appendix chapter 20), although the lowest LOQ levels would usually comply. However, for TBT, LOQ was never found to comply with the LOQ requirements. Any observed LOQ problems in the data evaluation being performed in this report are indicated in table 7.1 (line C).

By sampling another compartment (or with alternative techniques) than water for several of the hydrophobic substances, these technical difficulties can sometimes be avoided (see box 7.2.).

¹⁰⁸ During 2011 a questionnaire from a Euoropean Working group, CMEP (Chemical Monitoring and Emerging Pollutants), collects updated information on LOQ values possible to achieve by laboratories within the different Member States in order to assess the potential so fulfil the QAQC requirements.

Box 7.2. Possibilities to overcome LOQ problems in water: sampling aspects

All of the substances for which there are technical analytical difficulties described in box 7.1.are known to accumulate in other matrices than water, such as sediment and biota. Therefore, by monitoring accumulating substances in accumulating matrices instead, these technical difficulties to check compliance could in many cases (but not all) be overcome. Passive sampling of water can also reduce LOQ problems by the addition of more samplers but also due to the fact that larger volumes are generally sampled (thus longer exposures reduce the detection levels).

Sediment and suspended matter provide time integrated monitoring possibilities that would reduce LOQ problems for several accumulating substances. However, there may be uncertainties in the assessment of bioavailability of hazardous substances in sediment (see box 7.3.)

The detection limits of most POPs and PCBs varied between 0,2-10 pg/l sampled with passive samplers in a study by Larsen et al 2009. However, passive samplers only sample the dissolved fraction and therefore concentrations found to be below EQS are not a guarantee for compliance if the EQS is based on total concentration (which is the case for organic substances). Nevertheless the total concentrations in the surrounding water can be estimated if supporting variables are analysed. The combination of passive sampling devices and sediment traps have been suggested to be an alternative to overcome such limitations but there is little experience of such an approach at least on national level.

Different sampling techniques will be described in a guidance document on operational monitoring, being developed on County Administrative Board level in Sweden¹⁰⁹.

7.2.3 Potential to coordinate compliance check with current, required and prioritized trend monitoring (D, E, F)

There are major potential cost savings if choosing the same compartment for both trend and compliance monitoring. In addition, currently available monitoring data act as important background information providing baseline levels for geographical comparisons when also assessing need for local measures (identifying "hot spots"). Trend monitoring should preferably be based on the same compartment (and in the case of biota even the same species, tissue, etc) each time to facilitate evaluation. It is of major importance not to break any time trend monitoring series, in particular for ubiquitous substances and substances that do not exhibit downward trends (chapter 6). By analysing sediment and biota instead of water, analytical costs are also reduced because chemical analysis can be performed less frequently. Therefore, the current choice of monitoring compartment/s (used for trend analyses) on a national scale should be considered.

Detecting substances does not necessarily imply that they are of concern, although an increasing trend is, and the 2008/105/EC on priority substances clearly states that concentrations of priority substances cannot increase significantly for accumulating substances. Trend monitoring requirements in sediment and/or biota are explicitly identified for a number of priority substances in art 3.3.

Current national monitoring compartment, trend monitoring requirements and observed trends are indicated in table 7.1 (line D, E and F).

¹⁰⁹ Usually referred to as "Prioprojektet". Project leader is Fredrik Andreasson County Adm Board of Blekinge, and the estimated finalisation of the guidance document is by spring 2012.

7.2.4 Risk of non compliance, ubiquitous (G)

From a precautionary principle point of view, it is important to make sure that a 1st tier study of non compliance minimizes type II errors (false negatives). As long as the assessment is considered to be of high certainty, further monitoring to make conclusions about the current status can also be minimised and therefore constitutes a cost saving approach, although investigative studies may be necessary in order to identify the reasons for non compliance if not known.

The likeliness to detect an exceedance of EQS for a certain substance can be estimated by comparing observed concentrations with the QS for the monitored compartment(s). If e.g. the highest measured concentration in a screening study or studies performed at locally impacted sites does not exceed EQS, the potential to detect any non compliance can be considered low on a national scale for that compartment. On the other hand, if all or most concentrations analysed, even in reference areas, exceed the QS for a particular compound the likeliness to detect non compliance if monitoring this particular compartment is very likely and the substance can therefore be called "ubiquitous". In table 7.1. (line G), such aspects are indicated, summarizing the findings for priority substances (see also chapter 6).

7.2.5 Other general aspects (H)

In some cases, as an option to monitoring parent compounds, the monitoring of metabolic transformation products would be possible. This would be the case for e.g. PAHs in fish, due to rapid metabolisation.

Sampling and analytical costs may be reduced significantly if choosing the same compartment for all parameters, especially those that are included in a particular analytical package for groups of substances, although the choice may be "second best" (but still acceptable) for some of the individual parameters. PAHs are usually included in the same analytical package and these aspects were therefore taken into account. Other substances, such as several pesticides, frequently also come in "packages". Because this could vary between different laboratories, such aspects were not considered here for the other priority substances but could be an important aspect on a case to case basis.

Sediment is suggested as a suitable 1st tier compartment for several substances because it was found that it has a potential use as worst case compartment for many of the substances for which pelagic organisms are considered the most critical objective (although QSbent is actually unknown). In the case of non compliance, effect based tools could become useful 2nd tier tools (provide supportive information) in estimating the needs for local management measures.

These aspects are mentioned in table 7.1. (line H).

7.2.6 Other site specific aspects

For practical/economical reasons it may sometimes be more suitable to monitor a certain compartment rather than the recommended. Suitable fish species may be absent in sufficient numbers, there may be no appropriate sites for sediment sampling, the amounts necessary would not be possible to collect in a sediment trap, frequent sampling of composite water samples may not be possible for economic and practical reasons etc. In some areas predatory fish may be unavailable or the populations are considered too mobile, or the monitoring can be coordinated to sediment sampling for other reasons¹¹⁰. Such considerations are necessary on a case to case basis but are not included in the overall assessment made below.

In general however, to monitor accumulating substances in rivers is a great challenge at the moment. So far the only hazardous substances monitored in Sweden on a national scale in rivers were metals and pesticides in agricultural areas. However, if practical circumstances imply that it is not possible to follow the recommendations, 1st tier studies could probably be performed in downstreams locations (lakes or other accumulation areas). 2nd tier studies could then primarily be performed to locate sources upstreams for decision support on measures (not for status classifications), and such investigative studies could use complementary methods. For status classifications, data also from less recommended compartments and/or from the use of complementary methods would probably need to be used with expert judgement and on a case to case basis, until firm guidance on a suitable approach can be provided.

7.3 Preliminary conclusions for current priority substances and EQS values

Based on the factors described above, preliminary conclusions on which compartment to monitor can be made for the current priority substances and based primarily on current EQS values. The most critical objective of protection and the substance properties are suggested to be the most relevant aspects to take into account. However, in some cases these two aspects do not point in the same direction and then other aspects are considered important.

For substances for which the most critical objectives are secondary poisoning, and/or human health through consumtion of fish/seafood, biota should primarily be evaluated. However, the type of biota to evaluate also depends on the properties of the substance and metabolisation abilities. Substances for which the most critical protection objective is pelagic organisms but that are also known to accumulate, while benthic toxicity is actually unknown, are more complicated to evaluate. If such substances are not known to accumulate in higher trophic level pelagic biota (such as the fish species normally monitored) but rather at lower trophic levels, one can assume that benthic organisms may be as sensitive as pelagic organisms but exposed to higher concentrations.

¹¹⁰ If the primary management option e.g. is related to sediment remediation (no significant current emissions identified), a first relevant tier assessment would be to analyse surface sediments.

The following subchapters describe these different aspects and suggest ways to consider them for individual priority substances. A summary table (table 7.1) is also provided.

7.3.1 Substances for which QShh or QSsec pois is the most critical objective

QSsecpois is currently the most critical objective for the following substances:

- PBDE (marine)
- C10-13 chloroalkanes
- Hg
- DEHP and Pb, but QShh is almost as low for these compounds

QShh is currently the most critical objective for the following substances:

- PBDE (limnic)
- HCB, hexachlorobutadiene, Trichlorbenzenes (limnic), but QSsec pois is almost as low

The selection of species and tissue to monitor and evaluate might differ depending on the objective that is most critical (QShh or QSsecpois). If QShh is the most critical protection objective, edible species and tissues (usually fish muscle) should be monitored. If QSsecpois is the most critical, the whole organism should preferably be monitored. However, this is today primarily performed on lower trophic levels such as blue mussels (at least all soft tissues), whereas higher concentrations could be expected at higher trophic levels (predatory fish). In fish, either liver or muscle is monitored in current national programs and in order to increase potential for coordination, the current tissue could be considered as long as the concentration on whole fish level can be estimated (by e.g. recalculations based on lipid concentrations of the monitored tissue and the rest of the organism). Nevertheless, validation studies would be recommended in all these cases, especially when e.g. muscle is recommended because it is the most frequently monitored compartment, while liver concentrations are known to generally be substantially higher (frequently due to higher lipid contents).

If QSsec pois<QS hh but concentrations frequently also exceed QShh in relevant species and tissues, it is actually according to the WFD not strictly required to specifically be able to evaluate also the risks for human consumption by monitoring edible species and tissues. However, especially if human health issues pose a major concern at the particular site (fishing lakes), it is recommended that the monitoring program should produce data that can be used both for the purpose of compliance checking according to the WFD but also for the purpose of estimating local human health risks (for regional management and to provide supportive information for the National Food Agency).

7.3.1.1 Mercury

Organic mercury is the major concern related to mercury in the aquatic environment and compliance checking. QSsecpois is the most critical objective and

considered to be exceeded in all predatory fish (even from coastal areas), but also the QShh is frequently exceeded in lake predatory fish (such as pike). Trend monitoring is required, implying that sediment or biota would need to be monitored. By coordinating compliance checking with trend monitoring, costs can be reduced. A general downward trend in biota cannot be established. In 2008/105/EC an EQS is already established for biota.

One of the compartments monitored within the national monitoring program is fish muscle, and on several species that are frequently used for human consumption (perch, herring, pike, cod, char). Such monitoring would therefore provide data that can be used for trend monitoring and for both types of compliance checking. The most prioritized monitoring compartment also for technical reasons¹¹¹ and inherent properties (biomagnification aspects) would be fish rather than blue mussels or sediment and water. However, in the Swedish draft assessment criteria for marine sediment, trigger values are proposed based on parallel data from biota and sediment due to a strong correlation between sediment and biota data found for Hg. Therefore, if proper species is not available, first tier sediment monitoring could be an option.

The recommended compartment for monitoring and compliance checking is therefore predatory fish muscle used for human consumtion (QSsec pois being the most critical protection objective, whereas QShh is also frequently being exceeded, and fish muscle is already included in the national and several regional programs). Preferably species that are already included in the national program should be included to facilitate relative geographical comparisons.

7.3.1.2

PBDE

For PBDE both QShh and QSsec pois are the most critical objective of protection, depending on whether limnic or marine environment is monitored. In the revision of 2008/105/EC an EQS will probably be established for biota. Trend monitoring is required, implying that sediment or biota would need to be monitored. By coordinating compliance checking with trend monitoring, costs can be reduced.

One of the compartments monitored within the national monitoring program is fish muscle, and on several species that are frequently used for human consumption (perch, herring, pike, cod, char). Such monitoring would therefore provide data that can be used for trend monitoring and for both types of compliance checking.

The recommended compartment for monitoring and compliance checking is therefore predatory fish muscle used for human consumtion and preferably species that are already included in the national program to facilitate relative geographical comparisons.

¹¹¹ To monitor methyl mercury in sediment and water is complicated from a technical point of view.

7.3.1.3 C10-13 Chloroalkanes

Because secondary poisoning is the most critical objective, and the substance group is known to be subject to biomagnification, biota on higher trophic levels should be monitored. Also, trend monitoring is required, implying that sediment or biota would need to be monitored. By coordinating compliance checking with trend monitoring, costs can be reduced. Inherent properties would also suggest sediment or biota to be more relevant to monitor than water, and there might be analytical difficulties to monitor these substances in water. In Naturvårdsverket (2008) it is concluded that sediment is a relevant compartment.

A preliminary recommended compartment for compliance checking is therefore predatory fish or other higher trophic levels. However, choice of tissue is unclear. The substance was not detected in fish muscle according to data registered in the screening database, but lack of detection was not assessed regarding analytical requirements, and liver does not seem to have been investigated. Sediment could possibly be used for 1st tier assessments because C10-C13 chloroalkanes are now monitored in off shore sediment (but data not yet available). Nevertheless, any risk of non compliance has not been identified by sediment screening data.

7.3.1.4 HCB

Because QShh is the most critical objective, and an EQS for biota is already established in 2008/105/EC, biota species and tissues used for human consumtion should be used for compliance checking. Trend monitoring is required, implying that sediment or biota would need to be monitored. By coordinating compliance checking with trend monitoring, costs can be reduced. Inherent properties would also suggest sediment or biota to be more relevant to monitor than water, and there might be analytical difficulties to monitor HCB in water. In particular, biomagnification is suspected to occur, and QSsec pois is almost as critical as QShh, thus suggesting higher trophic levels to be relevant.

One of the compartments monitored within the national monitoring program is fish muscle (edible tissue), and on several species that are frequently used for human consumption (perch, herring, pike, cod, char). Such monitoring would therefore provide data that can be used for trend monitoring and for both types of compliance checking.

The recommended compartment for monitoring and compliance checking is therefore predatory fish muscle used for human consumtion and preferably species that are already included in the national program to facilitate relative geographical comparisons. For DEHP, QSsec pois is the most critical objective of protection but QShh is almost as low. Inherent properties also suggest both sediment and biota to be relevant compartments to monitor, but the potential for microbial degradation and metabolization should be taken into account. In Naturvårdsverket (2008) it is concluded that water and sediment are relevant compartments to monitor close to point sources. The substance is monitored in sediment on a national scale, and risk of non compliance has been identified based on sediment data registered in the screening data base. There is also a possible risk of non compliance in water, but there are analytical difficulties if monitoring water. Risk of non compliance in biota was not evaluated. However, the substance was detected in various biota (Fucus vesiculosus, blue mussels, and fish muscle; but liver does not seem to have been analysed).

In spite of the most critical objective being QSsec pois (and QShh), the substance is not considered to be subject to biomagnification in aquatic food chains (rather being metabolized in fish). In addition, bioconcentration factors are higher for amphipods and molluses than fish according to the current SDS. Due to the hormonal activity it can be anticipated that fish and mammals are more sensitive and a crucial pathway would be e.g. exposure to benthic prey organisms. The preliminary recommended compartment for monitoring and compliance checking is therefore biota with low level of metabolisation abilities and that are relevant both as prey species as well as for human consumption (because QShh is almost as low as QSsec pois). However, because there is so far limited experience in monitoring this substance in biota (there are no national programs monitoring DEHP in biota), and in general lack of monitoring of hazardous substances in lower trophic level biota in the limnic environment (see box 7.5), sediment could probably be a suitable 1st tier assessment compartment. This also facilitates the use also of national data for geographical comparisons.

In the case of non compliance being suggested by sediment data but where such an assessment is uncertain (only low levels of exceedence), 2nd tier investigations would be recommended to also investigate bioavailability and risks, before e.g. costly remediation measures are to be undertaken.

7.3.1.6 Hexachlorobutadiene

Because QShh is the most critical objective, and an EQS for biota is already established in 2008/105/EC, biota species and tissues used for human consumtion should be used for compliance checking. Trend monitoring is required, implying that sediment or biota would need to be monitored. By coordinating compliance checking with trend monitoring, costs can be reduced. Inherent properties would suggest biota to be the most relevant compartment, but sediment and water could be considered optional, but there might be analytical difficulties to monitor hexachlorobutadiene in sediment. The substance is only monitored in off shore sediment, but a risk of non compliance is possibly indicated for water but not indicated by sediment data from the screening database, whereas the risk of non compliance in biota based on these data has not been assessed (only 4 records are available). Biomagnification is not suspected to occur, but QSsec pois is almost as critical as QShh.

Because of an established biota EQS, the recommended compartment for monitoring and compliance checking is biota, choosing a species and tissues that are used for human consumption.

7.3.1.7 Lead

Inherent properties suggest all compartments to be relevant to monitor, although in fish preferably liver rather than muscle. QSsec pois is the most critical objective of protection but QShh is almost as low and according to the draft dossier this might change to QSpelag with the revision of current EQS. Trend monitoring is required, implying that sediment or biota would need to be monitored. Sediment is not monitored on a regular basis in the limnic environment whereas water is frequently monitored in several limnic programs but none of the marine. By coordinating compliance checking with such trend monitoring, costs can be reduced. Biomagnification is not suspected. Trends in limnic water are not decreasing. In Naturvårdsverket (2008) it is suggested that Pb should preferably be monitored in both biota and sediment.

One of the compartments monitored within the national marine monitoring program is blue mussels, providing data that can be used for trend monitoring in accumulating matrixes and for both types of compliance checking. Current data and criteria also suggest a risk of large scale non compliance based on blue mussel data (ubiquitous).

Due to difficulties in evaluating metals in sediment, and due to the absence of marine water monitoring, the preliminary recommended compartment for monitoring to check compliance is difficult to determine. Water is relevant in the limnic environment, especially if QSpelag will be the most critical objective of protection after the future revision. With such an approach, trend monitoring in accumulating compartments (biota is already monitored on national level in both limnic and marine environments) and compliance checking would need to be performed in two parallel programs. However, because the current trend of Pb in water is also not decreasing, to study trends in both accumulating compartments and water is relevant.

If QSsec pois and QShh would still be considered the more critical objectives, fish liver data (generated within current trend monitoring programs) could probably be used to evaluate compliance regarding secondary poisoning but the data would need to be recalculated into whole organism level. Because the major amounts could be suspected to be found in the liver, rather than in muscle, liver data could probably also be used as a 1st tier assessment of QShh compliance, only triggering muscle to be analysed if liver data suggest non compliance and human health effects are considered important to assess.

To identify one single compartment for status classifications in the marine environment if QSpelag is the most critical objective is difficult. Marine water sampling is not included in any of the national programs and only to a very limited extent on regional level. Fish would be possible to monitor as well but there are probably difficulties in recalculating fish liver data to water. Effect based tools are also available to investigate physiological responses in fish and could provide additional support in the evaluation of compliance and to support the need for management measures.

To conclude, the most prioritized compartment to monitor is unclear and the results from the future revision of 2008/105/EC should be taken into account. Until then, all three compartments (water, sediment and biota) should preferably be monitored.

7.3.1.8 Trichlorobenzenes

QShh is the most critical objective in the limnic environment, but QSsec pois is almost as low, whereas in the marine environment QSpelag is the most critical objective. For the limnic environment, the calculated QS values for biota are only somewhat more critical than QSpelag. Overall EQS actually also deviates from all calculated QS values.

Inherent properties would suggest that either sediment or water should be monitored rather than biota. Fate modelling suggests about equal distribution between water and sediment. Within the national programs, trichlorobenzenes are only monitored in off shore sediments. However, trend monitoring is not required and risks of non compliance have not been identified for any of the compartments (sediment, water, biota).

The preliminary recommended compartments for monitoring and compliance checking are therefore either sediment or water.

7.3.2 Substances for which QSpelag is the most critical objective but trend monitoring is required

Although QSpelag is being the most critical objective for most priority substances, it can also be concluded that accumulation in sediment and/or biota can occur for several of these substances.

For some of the priority substances in this category, there is already a clear requirement to also monitor trends in sediment and/or biota. By coordinating compliance checking with such trend monitoring, costs can be reduced.

The following substances for which QSpelag is considered to be the most critical objective should also be subject to trend monitoring in sediment and/or biota:

- PAHs
- Cadmium
- HCH
- Pentachlorbenzene
- TBT

7.3.2.1 PAHs, fluoranthene, naphthalene, anthracene

For accumulating substances that do not biomagnify but rather reach the highest concentrations in organisms that have less developed metabolic systems, benthic organisms may be the most sensitive subjects and would thus trigger sediment to be evaluated. This is currently observed primarily for fluoranthene. For the other PAHs, QSpelag is considered to be the most sensitive objective of protection, but QSbenthic is actually unknown (being calculated by EqP) and it is therefore not possible to state that QSpelag would be more sensitive than QSbenthic.

However, the draft dossier (Feb 2011) indicates that for fluoranthene and benzo(a)pyrene, actually QShh would be the most critical objective and for benzo(a)pyrene+benzo(b+k)fluoranthene+indeno(1,2,3-c,d)pyrene a separate biota EQS might be suggested in parallel to EQS for water, whereas for fluoranthene the overall water EQS is suggested to be based on QShh. The future overall EQS values will not take benthic effects into account. Therefore a separate assessment for potential effects on benthic organisms should be done in parallel.

PAHs and including anthracene and naphthalene are analysed in the same analytical packages as fluoranthene. Thus it would be cost effective to analyse all these compounds in the same compartment. Trend monitoring is also required for most of the PAHs (except naphthalene), implying that sediment or biota would need to be monitored. By coordinating compliance checking with trend monitoring, costs can be reduced. Inherent properties of the compounds and analytical requirements would also support such an approach.

Sediment and blue mussels are already being monitored within the national programs, providing baseline data for relative geographical comparisons. Both sediment and blue mussels are probably also relevant to monitor from previous indications of non compliance related to some PAHs but sediment data would probably indicate non compliance more frequently. Blue mussels are also primarily monitored on the west coast.

Due to rapid metabolisation in fish it is generally not recommended to analyse PAHs in fish and it is not included in the national fish monitoring programs. PAH metabolites in bile can be analysed to assess the exposure of PAHs to fish. If combined with other biomarkers, such as EROD, that would respond to PAHs (as well as dioxins, dibenzofurans and planar PCBs, see chapter 9), such studies could provide suitable options to estimate impact of PAHs on fish but would probably rather be performed as 2nd tier or as part of a weight of evidence approach.

If sediment PAH concentrations found are substantially higher than QSbent sediment, data would probably be sufficient to conclude that there is non compliance. If concentrations are below, compliance is confirmed, whereas concentrations just above would trigger 2nd tier assessments, such as pore water analysis and effect based studies of pelagic and benthic community.

On the other hand, if blue mussel concentrations are above QShh or QSsec pois, this indicates non compliance, but if below, there might still be non compliance regarding risks to benthic organisms. A "trigger value" for blue mussels based on

the revised PAH EQS values once determined should therefore be developed to facilitate such evaluations and identify potential risks.

To conclude, the compartment/s to monitor and data to be used for compliance checking of PAHs, fluoranthene, anthracene and naphthalene would depend on the specific situations (expected concentration range) and purpose of monitoring (surveillance, operational) and on the revision of the current EQS, but sediment and blue mussels are generally preferred rather than water¹¹² or fish.

To conclude, the most prioritized compartment for compliance checking is unclear and would depend on the type of water body monitored. Preferably more than one compartment should be monitored.

7.3.2.2 Cadmium

According to inherent properties, water, sediment and biota could all be relevant compartments to monitor but in fish only liver (not muscle). There may be analytical problems in water. Trend monitoring is required, implying that sediment or biota would need to be monitored, and current trend monitoring does not suggest that cadmium concentrations are decreasing in marine biota. Naturvårdsverket (2008) recommends both water and biota monitoring. Biomagnification is not suspected. Due to difficulties in evaluating metals in sediment, and due to the absence of marine water monitoring, the preliminary recommended compartment for monitoring to check compliance is difficult to determine. Risk of non compliance has been identified for sediment and blue mussels (ubiquitous), and possibly also water and fish. Sediment is not monitored on a regular basis in the limnic environment, whereas water is frequently monitored in several limnic programs but not in marine.

The preliminary recommended compartments for monitoring and compliance checking is water in the limnic environment. In the limnic environment therefore, trend monitoring in accumulating compartments (biota is already monitored on national level) and compliance checking would need to be performed in two parallel programs. To identify one single compartment for status classifications in the marine environment is difficult, because marine water sampling is today not included in any national program and very scarce on regional level. Current blue mussel data suggest that there is a risk of large scale non compliance.

Cadmium concentrations in biota can be suspected to depend at least in part on concentrations and redox conditions of sediment (cadmium being released to a larger extent in oxygen rich environments). Monitoring sediment may help the interpretation of possible concentration changes in biota with time. Effect based tools are also available to investigate impact on pelagic organisms. By monitoring several compartments and include biomarkers the basis for management control measures will be considerably improved.

¹¹² Although water should also be considered in water bodies used for drinking water extraction

7.3.2.3 HCH

Inherent properties suggest sediment and biota to be most relevant but also water. Trend monitoring is required, implying that sediment or biota would need to be monitored. By coordinating compliance checking with such trend monitoring, costs can be reduced. Currently HCH is monitored in both sediment, water and biota, but risk of non compliance was primarily identified for sediment and possibly for biota. There may be analytical difficulties in both water and sediment, but possibly also in biota.

The preliminary recommended compartment for 1st tier compliance checking is therefore sediment and/or biota rather than water.

7.3.2.4 Pentachlorbenzene

Inherent properties suggest sediment and biota to be relevant to monitor. In addition, there may be analytical problems in water. Trend monitoring is required, implying that sediment or biota would need to be monitored. By coordinating compliance checking with such trend monitoring, costs can be reduced. Currently, pentachlorbenzene is only included in the national monitoring of off shore sediments. Sediment data suggest risk of non compliance, whereas risks of non compliance in water has not been identified and biota has not been evaluated.

The preliminary recommended compartment for monitoring and compliance checking is therefore sediment, at least for 1st tier assessments.

7.3.2.5 TBT

Inherent properties of TBT would suggest sediment and biota to be the most relevant compartments to monitor, but water could also be relevant. However, there are substantial analytical problems in water, and not even the best LOQ identified so far fulfills the requirements of the QAQC directive. Trend monitoring of TBT is required, implying that sediment or biota would need to be monitored. By coordinating compliance checking with such trend monitoring, costs can be reduced.

TBT is regularly monitored in the marine environment only, and tissue analysis of gastropods is only performed on the West coast (due to the small size on the East Coast), but imposex is monitored on both Coasts. Off shore and Vänern sediments are also monitored in national programs.

Large scale non compliance is particularly suggested by sediment data and effect based tools (specific biomarker imposex), by which effects can still be observed even at reference stations.

For chemical monitoring of biota, blue mussels are considered to be more appropriate because they are less prone to metabolize the compounds, but TBT is currently not being monitored in the national blue mussel program. Blue mussels and the more sensitive gastropods also generally not exposed to TBT by the same pathways. The gastropods monitored along the west coast primarily inhabit fine grained sediments and are exposed to pore waters, whereas blue mussels are more frequently found in sandy sediments or on hard surfaces being exposed to overlying water. Sediment concentrations are highly variable between exposed sites and more pristine areas and a strong correlation has been found between imposex and sediment concentrations (see box 3.6.).

The recommended compartment for monitoring and compliance checking is therefore sediment, at least for 1st tier assessments. If the ratio between EqP based sediment criteria and analysed concentration is large, it would be possible to assume that there is non compliance related to TBT, as this is also suggested by field effect data.

Furthermore, as decision support for local management purposes, also the ratio between TBT and its degradation products DBT and MBT in sediment can provide valuable information to estimate whether the input of TBT is greater than its degradation rate, unless there are other potential DBT or MBT sources (Box 3.6.). Subrecent sediment can also be investigated to obtain information on time trends at a particular location. It can be assumed that the effects still observed in gastropods to a large extent is due to TBT from pore waters rather than surface water. If current emissions to the overlying water phase can be suspected¹¹³, blue mussels could indicate such exposure from the water phase, in particular if employed (caged) above the sea floor.

Studies using the biomarker imposex in gastropods could provide additional support in the evaluation of compliance and to further support the need for management measures, but it should be kept in mind that imposex is an irreversible effect and that the actual exposure could have occurred during earlier life stages and does not necessarily reflect current conditions, although this is also the case for sediment monitoring. In addition, if concentrations are high enough, one should be aware of the risk that the species may have become locally extinct or significantly reduced. Imposex monitoring in harbours have sometimes failed because no individuals of *N nitidus* could be found although suitable habitats were being investigated.

If non compliance is uncertain due to a lower conc/QSbenthic ratio, and where effect based tools are not yet possible to employ (such as in limnic environments, but also in some areas which represent habitats unsuitable for gastropods), an alternative to confirm non compliance would be pore water extraction, by which obtained data can directly be compared to water-EQS.

¹¹³ Such as during boat uptake in harbours and during dredging operations

7.3.3 Substances for which QSpelag is the most critical objective and trend monitoring is not required

The substances for which QSpelag is the most critical objective and trend monitoring is not required according to art 3.3. in 2008/105/EC comprise the rest of the priority substances:

- Alachlor
- Atrazine
- Benzene
- Chlorfenvinphos
- Chlorpyrifos
- 1.2-dichlorethane
- Dichlormethane
- Diuron
- Endosulfan
- Isoproturon
- Nickel
- Nonylphenol
- Octylphenol
- PCP
- Simazine
- Trichlormethane
- Trifluralin

In most cases, this would suggest that water is the most relevant compartment to monitor and also to use for compliance checking. However, again some substances could be assumed also to accumulate in either sediment and/or biota at least on a local scale, even if trend monitoring is not strictly required according to art 3.3.. For substances that could accumulate, it can be assumed that monitoring of either sediment or biota could probably be considered suitable 1st tier compartments because they are more likely to represent worst case situations, at least in situations with continuous releases (chronic exposures).

7.3.3.1 PCP

Although data available so far did not indicate a situation with non compliance related to water nor sediment, and trend monitoring is not identified in article 3.3. (2008/105/EC), the situation on a local scale could support the need for trend monitoring as the substance can accumulate¹¹⁴ in sediment and biota. In such a situation, the monitoring of sediment and/or biota also for compliance checking could be relevant.

¹¹⁴ The behaviour of the substance is strongly influenced by the pH of the environment.

7.3.3.2 Trifluralin

Substance properties suggest that sediment is the most relevant compartment to monitor but this was not confirmed by available monitoring data (not detected), and art 3.3. does not specify trend monitoring to be required for this substance. According to CIS 25, biota is optional but no biota data are available. Therefore, as a preliminary assessment, it can be assumed that water would be the most relevant compartment to evaluate for compliance checking, but in the event of a contaminated area, local scale trend monitoring should be considered, as the substance can accumulate¹¹⁵ in sediment and biota. In such a situation, the monitoring of sediment and/or biota also for compliance checking could be relevant.

7.3.3.3 Chlorpyrifos

For chlorpyrifos, although data available so far did not indicate a situation with non compliance related to water nor sediment (never detected), and trend monitoring is not identified in article 3.3. (2008/105/EC), the situation on a local scale could support the need for trend monitoring as the substance can accumulate in sediment and biota. In such a situation, the monitoring of sediment and/or biota also for compliance checking could be relevant.

7.3.3.4 Nonylphenol and octylphenol

Based on substance properties, for nonylphenol and octylphenol all compartments could be relevant to monitor. In Naturvårdsverket (2008), water and biota are suggested for investigations. There may however be analytical difficulties for water and the only compartment being monitored within regular programs is off shore sediment. Furthermore, risk of non compliance can be confirmed primarily for sediment (octylphenol being even potentially ubiquitous), although non compliance could possibly also be confirmed for water (based on data registerered in the screening database). Although trend monitoring is not strictly required, accumulation in sediment and biota can be suspected to occur and could be relevant to monitor. Biomagnification is not suspected. As a 1st tier assessment, sediment data could be useful for compliance checking. If concentrations are just above trigger values or QSbent based on EqP calculations, non compliance could be confirmed by monitoring additional compartments (water and biota). There are also effect based tools that would be valuable in estimating effects in pelagic organisms (fish) due to oestrogenic substances, such as nonylphenol and octylphenol. However, oestrogenic effects due to other more potent substances (EE-2) cannot always be excluded.

¹¹⁵ The behaviour of the substance is strongly influenced by the pH of the environment.

7.3.3.5Diuron and endosulfan

Based on substance properties, both diuron and endosulfan could be relevant to monitor in water and sediment, endosulfan possibly also in biota. Analytical difficulties were not identified for diuron but for endosulfan in both water and sediment. Both are monitored in the national pesticide program in water and sediment, and data so far indicate that incidences of non compliance primarily can be confirmed for sediment. Therefore, although not strictly required, trend monitoring in sediment could be relevant due to sediment accumulation and sediment could also be used as 1st tier compliance checking compartment in locally exposed areas.

Table 7.1. Summarizing conclusions on preliminary recommended compartments to monitor for compliance checking according to an integrated assessment of the aspects discussed for current priority substances. See text for more details.

Substance	Conclusion: most prioritised compartment for compliance checking	A: most critical objective/s B: relevant compartment according to inherent properties and available data C: LOQ fulfilling QA QC requirements? D: national monitoring compartment/s E: trend monitoring required (art 3.3.)? F: Downward trends not observed? G: Risk of non compliance, ubiquitous? H: other aspects (including specific biomarkers available)
Alachlor	Water	A: QSpelag B: Water C: Lowest recorded LOQ for water in the screening database fulfils QAQC criteria but not the highest D: Water and sediment in agricultural areas, off shore sediment. E: Trend monitoring not required F: G: Risk of non compliance not identified for water or sediment, biota not evaluated H:
Anthracene	Sediment and/or blue mussels, depending on purpose and situation (see text)	 A: QS pelag but QS benthic calculated byEqP B: biota and sediment most relevant, but also water C: Lowest recorded LOQ for water in the screening database fulfils QAQC criteria but not the highest D: Blue mussels, off shore and Vänern sediment E: Trend monitoring required F: G: Risk of non compliance identified for sediment (ubiquitous), but not water and probably also not biota H: Specific Specific PAH biomarkers available, substance normally included in analytical package of PAHs
Atrazine	Water	 A: QS pelag but QS benthic calculated by EqP B: water C: Lowest recorded LOQ for water in the screening database fulfils QAQC criteria but not the highest D: Water and sediment in agricultural areas, off shore sediment. E: Trend monitoring not required F:

Substance	Conclusion: most prioritised compartment for compliance checking	A: most critical objective/s B: relevant compartment according to inherent properties and available data C: LOQ fulfilling QA QC requirements? D: national monitoring compartment/s E: trend monitoring required (art 3.3.)? F: Downward trends not observed? G: Risk of non compliance, ubiquitous? H: other aspects (including specific biomarkers available) G: Risk of non compliance not identified for water or sediment, biota not evaluated H:
benzene	Water	 A: QS pelag B: water (but can also be found in sediment and biota¹¹⁶) C: D: Off shore sediment E: Trend monitoring not required F: G: Risk of non compliance not identified for water. Risk of non compliance in sediment not possible to assess¹¹⁷. Biota not evaluated. H:
PBDE	Predatory fish muscle used for human consumption	 A: QShh for freshwater and sec pois in marine env; draft suggested for QShh¹¹⁸ B: sediment and biota most relevant but can also be found in water C: D: Off shore and Vänern sediment, marine and limnic fish muscle (incl Vänern), blue mussel, guillemot egg. E: Trend monitoring required F: G: Risk of non compliance possibly identified for water and biota (possibly even ubiquitous but the assessment depends on criteria used), whereas sediment was not evaluated H:
Cadmium	Unclear, several compartment s strongly recommende d (see text)	 A: QS pelag B: all compartments, but in fish only liver (not muscle) C: CEN (2007) states that only well equipped laboratories can fulfill target LOQ criterion for water D: Water (IKEU, Vänern, Vättern, rivers that end at sea, trend rivers and lakes), marine and limnic fish liver (incl Vänern), blue mussels, guillemot egg, off shore and Vänern sediment E: Trend monitoring required F: Not decreasing in marine biota G: Risk of non compliance identified for sediment and blue mussels (ubiquitous), and possibly water and fish. H: Specific biomarkers available
C10-13 chloroalkane s	Higher trophic level biota, but tissue unclear and sediment possible as 1 st tier (see also text)	 A: QSsec pois; biomagnifies B: sediment and biota C: Lowest recorded LOQ for water in the screening database fulfils QAQC criteria but not the highest D: Off shore sediment E: Trend monitoring required F: G: Risk of non compliance not identified for water or sediment, biota not evaluated (not detected in muscle, not investigated in liver) H:

 ¹¹⁶ Fucus vesiculosus
 ¹¹⁷ Detected but trigger to derive QSbent not met
 ¹¹⁸ According to the draft dossier on PBDE, QShh would be considered much more sensitive than QS sec pois, in both marine and fresh water environments.

Substance	Conclusion: most prioritised compartment for compliance checking	A: most critical objective/s B: relevant compartment according to inherent properties and available data C: LOQ fulfilling QA QC requirements? D: national monitoring compartment/s E: trend monitoring required (art 3.3.)? F: Downward trends not observed? G: Risk of non compliance, ubiquitous? H: other aspects (including specific biomarkers available)
chlorfenvinph os	Water	 A: QS pelag B: water and biota C: D: Water and sediment in agricultural areas, off shore sediment. E: Trend monitoring not required F: G: Risk of non compliance not identified for sediment but possibly for water H:
Chlorpyrifos	Water, but if accumulation is suspected, biota and/or sediment could be relevant (see text)	 A: QS pelag B: biota and sediment C: Analytical difficulties in sediment (based on empirical data); Lowest recorded LOQ for water in the screening database fulfils QAQC criteria but not the highest D: Water and sediment in agricultural areas, off shore sediment. E: Trend monitoring not required F: G: Risk of non compliance not identified for water; sediment and biota not possible to evaluate H:
1,2- dichloroethan e	Water	A: QS pelag B: water C: D: Off shore sediment E: Trend monitoring not required F: G: Risk of non compliance not identified for sediment but possibly for water H:
Dichlorometh ane	Water	A: QS pelag B: water C: D: Off shore sediment E: Trend monitoring not required F: G: Risk of non compliance not identified for water or sediment, biota not evaluated

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Substance	Conclusion:	A: most critical objective/s
	most prioritised	B: relevant compartment according to inherent properties and available data
	compartment	C: LOQ fulfilling QA QC requirements?
	for	D: national monitoring compartment/s
	compliance	E: trend monitoring required (art 3.3.)?
	checking	F: Downward trends not observed?
	checking	G: Risk of non compliance, ubiquitous?
		H: other aspects (including specific biomarkers available)
		H:
DEHP	Sediment as	A: QS secpois but QShh almost as low, not considered to be subject to
	first tier	biomagnification
	assessment;	B: sediment and biota ¹¹⁹
	most suitable	C: CEN (2007) states that only well equipped laboratories can fulfill target
	choice of	LOQ criterion for water ¹²⁰
	biota unclear	D: Off shore and Vänern sediment
	(see text)	E: Trend monitoring required
		F:
		G. Risk of non compliance identified for sediment and possibly for water,
		but not evaluated for biota
		H:
diuron	Sediment as	A: QS pelag
	first tier	B: water and sediment
	assessment	C: D: Weter and a dimension arise to an a finance of the second in a state
	(see text)	D: Water and sediment in agricultural areas, off shore sediment.
		E: Trend monitoring not required F:
		G: Risk of non compliance identified for sediment and possibly also water,
		biota not evaluated
		H:
endosulfan	Sediment as	A: QS pelag
	first tier	B: water and sediment, perhaps also biota
	assessment	C: Technical difficulties in sediment (based on empirical data); lowest
	(see text)	recorded LOQ for water in the screening database fulfils QAQC criteria but
		not the highest, water analysis not considered sufficiently sensitive by
		CEN (2007)
		D: Water and sediment in agricultural areas, off shore sediment.
		E: Trend monitoring not required
		F:
		G: Risk of non compliance not identified for water but for sediment
a		
fluoranthene	Sediment	A: QS bent (but overall EQS is under revision)
	and/or blue	B: sediment and biota
	mussels,	C: D: Off abore and Vänern codiment, blue mussele
	depending on	D: Off shore and Vänern sediment, blue mussels
	purpose and	E: Trend monitoring required F:
	situation (see text)	G: Risk of non compliance identified for sediment (possibly ubiquitous),
	lex()	possibly also for water but not confirmed for blue mussels
		H: Specific PAH biomarkers available, substance normally included in
		analytical package of PAHs
	Predatory	A: QShh but QSsec pois is only slightly higher; EQS developed for biota
	fish muscle	B: sediment and biota ¹²¹
	used for	C: Lowest recorded LOQ for water in the screening database fulfils QAQC
НСВ	human	criteria but not the highest; CEN (2007) states that only well equipped

 ¹¹⁹ but taking into account an expected aerobic microbial degradation in sediment and metabolization in higher trophic levels (fish)
 ¹²⁰ For DEHP there are serious blank problems
 ¹²¹ Because of biomagnification, higher trophic levels should be considered

Substance	Conclusion:	A: most critical objective/s
ousstance	most	B: relevant compartment according to inherent properties and
	prioritised	available data
	compartment	C: LOQ fulfilling QA QC requirements?
	for	D: national monitoring compartment/s
	compliance	E: trend monitoring required (art 3.3.)?
	checking	F: Downward trends not observed?
		G: Risk of non compliance, ubiquitous?
	consumption	H: other aspects (including specific biomarkers available) laboratories can fulfill target LOQ criterion for water
	consumption	D: Off shore sediment, marine and limnic fish muscle (incl Vättern), blue
		mussel, guillemot egg.
		E: Trend monitoring required
		F:
		G: Risk of non compliance not identified for water but for sediment and
		possibly also for biota
	Sediment/	H: A: QS pelag
	biota rather	B: sediment and biota most relevant, but also water
	than water	C: Analytical difficulties in sediment; lowest recorded LOQ for water in the
		screening database fulfils QAQC criteria but not the highest; CEN (2007)
		states that only well equipped laboratories can fulfill target LOQ criterion
		for water
		D: Water and sediment in agricultural areas, off shore sediment, marine
		and limnic fish muscle (incl Vättern), blue mussel, guillemot egg.
		E: Trend monitoring required F:
		G: Risk of non compliance not identified for water but for sediment and
		possibly for biota
НСН		H:
	Biota that is	A: QShh, but QSsec pois is only slightly higher; EQS developed for biota
	relevant for	B: biota most relevant but also water and sediment
	human	C: Technical difficulties in sediment (based on empirical data), lowest LOD
	consumption	registered in the screening database fulfils criteria D: Off shore sediment
		E: Trend monitoring required
		F:
		G: Risk of non compliance possibly identified for water but not for
Hexachlorob		sediment and not evaluated for biota
utadiene		H:
	Water	A: QS pelag
		B: water C:
		D: Water and sediment in agricultural areas, off shore sediment.
		E: Trend monitoring not required
		F:
		G: Risk of non compliance identified for water and sediment
Isoproturon		H:
	For now, all	A: QS sec pois but QS hh nearly as low, and in draft dossier Qspelag
	three	could be the most critical objective
	compartment s should be	B: all compartments, but in fish only liver (not muscle) C:
	monitored	D: Water (IKEU, Vänern, Vättern, rivers that end at sea, trend rivers and
	(see text).	lakes), marine and limnic fish liver, blue mussels, guillemot egg, off shore
		and Vänern sediment
		E: Trend monitoring required
		F: Not decreasing in water (limnic)
		G: Risk of non compliance identified for blue mussels (ubiquitous), and
Lood		possibly water and fish. Sediment was not assessed.
Lead		H: Specific biomarkers available

Substance	Conclusion:	A: most critical objective/s
Substance	most	B: relevant compartment according to inherent properties and
	prioritised	available data
	compartment	C: LOQ fulfilling QA QC requirements?
	for	D: national monitoring compartment/s
	compliance	E: trend monitoring required (art 3.3.)?
	checking	F: Downward trends not observed?
		G: Risk of non compliance, ubiquitous?
		H: other aspects (including specific biomarkers available)
	Predatory	A: QS sec pois, biomagnifies; EQS developed for biota
	fish muscle	B: for inorganic mercury sediment and water, for organic mercury biota
	used for	most relevant
	human	
	consumption	D: Water (IKEU, Vänern, Vättern, rivers that end at sea, trend rivers and
		lakes), marine and limnic fish liver (incl Vänern, Vättern and IKEU), blue
		mussels, guillemot egg, off shore and Vänern sediment
		E: Trend monitoring required F: Not decreasing in biota (marine and limnic)
		G: Risk of non compliance identified for biota (ubiquitous), sediment and
		possibly also water
Mercury		H:
	Sediment	A: QS pelag; Drinking water standard is actually lower than overall EQS.
	and/or blue	B: all compartments
	mussels,	C:
	depending on	D: Off shore and Vänern sediment, blue mussels
	purpose and	E: Trend monitoring not required
	situation (see	F:
	text), also	G: Risk of non compliance identified for sediment but not for water. Not
	water if used	evaluated for biota.
	for drinking	H: Specific PAH biomarkers available, substance normally included in
	water	analytical package of PAHs
	extraction	
Naphthalene		
Napitilalene	Water	A: QS pelag ¹²²
	Water	B: all compartments but in fish only liver (not muscle)
		C:
		D: Water (IKEU, Vänern, Vättern, rivers that end at sea, trend rivers and
		lakes), marine and limnic fish liver (incl Vänern), blue mussels, guillemot
		egg, off shore and Vänern sediment
		E: Trend monitoring not required
		F: Not decreasing in biota (marine)
		G: Risk of non compliance identified for sediment and blue mussels,
		possibly also water
Nickel		H:
	Sediment as	A: QS pelag
	first tier	B: all compartments
	assessment	C: CEN (2007) states that only well equipped laboratories can fulfill target
		LOQ criterion for water ¹²³
		D: Off shore sediment
		E: Trend monitoring not required F:
		G: Risk of non compliance identified for sediment and possibly also water,
		but not evaluated for biota
Nonylphenol		H: Specific biomarkers available
Nonyiphenol	Sediment as	A: QS pelag
Octylphenol	first tier	B: all compartments
		Brail compartmonto

¹²² For Nickel there is also a draft dossier but the most critical objective is not reported to change. ¹²³ For nonylphenol many laboratories have blank problems

Substance	Conclusion:	A: most critical objective/s
Cubstance	most	B: relevant compartment according to inherent properties and
	prioritised	available data
	compartment	C: LOQ fulfilling QA QC requirements?
	for	D: national monitoring compartment/s
	compliance	E: trend monitoring required (art 3.3.)?
	checking	F: Downward trends not observed?
		G: Risk of non compliance, ubiquitous?
		H: other aspects (including specific biomarkers available)
	assessment	C: CEN (2007) states that only well equipped laboratories can fulfill target LOQ criterion for water ¹²⁴
		D: Off shore sediment
		E: Trend monitoring not required
		F:
		G: Risk of non compliance identified for sediment (ubiquitous) and possibly
		also water, but not evaluated for biota
		H: Specific biomarkers available
	Sediment	A: QS pelag; Drinking water standard is actually lower for BaP than overall
	and/or blue	EQS. In draft dossier, QShh is suggested as more critical and separate
	mussels,	biota EQS is suggested for the sum of the 5-6 ring PAHs.
	depending on	B: sediment and biota ¹²⁵
	purpose and	C: water analysis of benzo(g,h,i)perylene and indeno (1,2,3-c,d) pyrene
	situation (see	not considered sufficiently sensitive, and only well equipped laboratories
	text), also water if used	can fulfill target LOQ criterion for benzo(b/k)fluoranthene in water (CEN,
	for drinking	2007) D: Off shore and Vänern sediment, blue mussels
	water	E: Trend monitoring required
	extraction	F:
	oxtraotion	G: Risk of non compliance identified for sediment (ubiquitous), possibly
		also for blue mussels and water
		H: Specific PAH biomarkers available, substance normally included in
PAH		analytical package of PAHs
	Sediment as	A: QS pelag
	first tier	B: sediment and biota
	assessment	C: Lowest recorded LOQ for water in the screening database fulfils QAQC
		criteria but not the highest; water analysis not considered sufficiently sensitive by CEN (2007)
		D: Off shore sediment
		E: Trend monitoring required
		F:
		G: Risk of non compliance not identified for water but for sediment, and
Pentachlorob		not evaluated for biota
enzene		H:
	Water, but if	A: QS pelag (drinking water standard is actually lower)
	accumulation	B: all compartments
	is suspected,	C: D: Off above acdiment
	biota and/or	D: Off shore sediment
	sediment could be	E: Trend monitoring not required F:
	relevant (see	G: Risk of non compliance not identified for water or sediment, biota not
	text)	evaluated
PCP	,	H:
	Water	A: QS pelag
		B: water
		C: Lowest recorded LOQ for water in the screening database fulfils QAQC
		criteria but not the highest
Simazine		D: Water and sediment in agricultural areas, off shore sediment.

¹²⁴ LOQ can be met for inland waters but not other waters
 ¹²⁵ In biota, preferably organisms with low level of metabolisation (e.g. blue mussel)

Substance	Conclusion:	A: most critical objective/s
	most	B: relevant compartment according to inherent properties and
	prioritised	available data
	compartment	C: LOQ fulfilling QA QC requirements?
	for	D: national monitoring compartment/s
	compliance	E: trend monitoring required (art 3.3.)?
	checking	F: Downward trends not observed?
	Ŭ	G: Risk of non compliance, ubiquitous?
		H: other aspects (including specific biomarkers available)
		E: Trend monitoring not required
		F:
		G: Risk of non compliance not identified for water or sediment, biota not
		evaluated
		H:
	Water	A: QS pelag (QSbenthic is actually lower, but trigger not met)
		B: water most relevant, but also detected in sediment and biota
		C:
		D: Off shore sediment
		E: Trend monitoring not required
-		F:
Trichlorometh		G: Risk of non compliance not identified for sediment but possibly for
ane		water
(chloroform)	o	H:
	Sediment as	A: QS pelag
	first tier	B: biota and sediment most relevant but also water
	assessment,	C: Water analysis not considered sufficiently sensitive by CEN (2007), not
	combined	even the lowest LOQ recorded fulfills requirements
	with imposex studies in	D: Gastropods (tissue analysis only on West Coast), off shore and Vänern sediment
	marine	E: Trend monitoring required
	environments	F:
	(see text)	G: Risk of non compliance identified for sediment (ubiquitous) and biota
		(possibly ubiquitous in gastropods), possibly also risk of non compliance
		for water. Large scale effects still observed in the field
ТВТ		H: Specific biomarkers available in the marine environment
	Sediment or	A: QSpelag in marine env, QShh in freshwater, but QSsec pois almost as
	water	low
		B: sediment and water
		C:
		D: Off shore sediment
		E: Trend monitoring not required
		F:
Trichlorobenz		G: Risk of non compliance not identified for water, sediment or biota
enes		H:
	Water, but if	A: QS pelag
	accumulation	B: sediment and biota (?)
	is suspected,	C: Technical difficulties in sediment (based on empirical data)
	biota and/or	D: Water and sediment in agricultural areas, off shore sediment.
	sediment	E: Trend monitoring not required
	could be	F:
	relevant (see	G: Risk of non compliance not identified for water, not possible to assess
- .a	text)	for sediment (never detected) and biota (no data available)
Trifluralin		H:

7.4 Evaluating compliance

Certain aspects that should be kept in mind when evaluating compliance related to data from a certain compartment are described in this chapter, in particular

recalculations and supportive data that are necessary in using sediment and biota data for compliance checking. However, it should also be pointed out that it is often recommended to analyse not only one compartment to assess status and needs for management measures, due to the uncertainties involved in both estimating assessment criteria and the calculation of the most sensitive organism. This is particularly recommended in situations where measures to reduce exposure are costly.

7.4.1 Evaluating water compliance

Because most EQS values for priority substances are already expressed for water, the comparison to analysed concentrations is rather straightforward. However, in the event of non compliance for metals, the evaluation can take bioavailability and natural background concentrations into account. The EQS values for metals are already expressed for filtered samples and for cadmium there are different EQS values depending on water hardness. The BLM (Biotic Ligand Model) approach, has also been suggested to be used to assess metal compliance, but there is yet no consensus on how to use such models for Swedish conditions¹²⁶. There is also no European consensus on how to compare analysed data to MAC-EQS (i.e. whether a single value exceeding MAC would justify non compliance or rather 95% percentile values should be used for comparison).

For simplicity, in this report, the assessments performed previously (chapter 6) are based on comparing maximum concentrations reported with AA-EQS, without taking background concentrations (for Cd also not water hardness) into account and without distinguishing between filtered and non filtered¹²⁷ samples for compliance checking. The assessment in this report of substances that potentially could cause non compliance is therefore most likely over estimating the number of relevant substances and extent of non compliance.

Of special concern when evaluating compliance based on water concentrations is the sampling procedure. Because of the large variability in water concentrations to be expected, grab sampling could both over and underestimate compliance depending on when the sample was taken. The WFD recommends grab sampling for compliance checking to take place 12 times a year, increasing the costs for analyses. In addition, for accumulating substances, checking compliance against water samples taken from surface layers are likely to underestimate non compliance. In such cases, pore water sampling or sampling from lower layers could provide a more realistic picture of the status in the most exposed areas of a water body, in particular for stratified lakes. Automatic time integrated sampling procedures are also available, as well as passive sampling, although in the latter case there is not yet consensus on the use of such devices for WFD compliance checking.

¹²⁶ Maria Linderoth, pers. comm.. See e.g. Palm-Cousins et al 2009 and Hoppe et al 2009.

¹²⁷ Chemical analyses of metals in Sweden are often performed not on filtered samples but rather on samples after a period of sedimentation or after decantation. A report comparing filtered and non filtered samples was recently published (see Köhler 2010), including supportive parameters such as TOC, pH and alkalinity. Other potentially supportive parameters in recalculations such as DOC, POC, colour and suspended matter were not included though.

Regardless of the character of the substance, if QShh drinking water is the most sensitive standard and the water is intended to be used for drinking water production, it is recommended that compliance related to QShh drinking water should be evaluated. However, it should be kept in mind that drinking water criteria according to the drinking water directive are actually related to quality "at the tap".

7.4.2 Evaluating sediment compliance

Sediment is traditionally monitored for trend analyses by repeated sampling of surface layers but also through analysing sediment layers of different depths to assess historic trends. A major advantage with sediment monitoring in the context of compliance checking is that the determination of natural background values for metals and other compounds that occur also naturally in the environment, is rather straightforward, compared to this type of assessments in water or biota. Baseline levels can be determined from analyses in pristine areas but also by analysing deeper undisturbed sediments. For certain substances such as PAHs and dioxins, analysing sediment rather than biota also enables the possibility for fingerprinting analysis to identify type of source, see e.g. studies on dioxins by Sundqvist (2009)¹²⁸.

For compliance checking using sediment (as well as for trend analyses), it is important to monitor concentrations at accumulation areas (indicated by particles having a size of <63 um). The sediment depth that should be sampled and sampling frequency depends on the sedimentation rate (see also CIS25). In 2008/105/EC annual sampling of sediment is recommended if data are to be used for compliance checking, but such a high sampling rate for sediment could be questioned regarding relevance and practical possibilites¹²⁹. However, it is reasonable to assume that the sample analysed should represent the conditions during the river basin management plan period.

If a substance does not primarily accumulate in higher trophic level pelagic biota but rather at lower trophic levels, one can assume that benthic organisms are frequently anticipated to be as sensitive as pelagic organisms, but they will be exposed to higher concentrations. Therefore, sediment would in this case be a suitable 1st tier screening compartment rather than water (from surface layers).

The use of surface sediment data alone for compliance checking may not be appropriate if there are large uncertainties involved in the estimation of QSbenthic and the concentrations found are within the limits of this uncertainty¹³⁰ but also if

¹²⁸ Fingerprinting would necessitate analysis of more congeners than are normally analysed by commercial laboratories though (i.e. include also other congeners than the seventeen 2,3,7,8-substituted congeners). A corresponding fingerprinting analysis in biota would not be possible because these other congeners are metabolized, Kristina Sundqvist Umeå University pers comm.

¹²⁹ At least for whole sediment sampling. It would rather be relevant if using sediment traps to sample the most recent material in situations with fluctuating concentrations in the suspended material (due to e.g. fluctuations also in the water phase).

¹³⁰ There may e.g. be uncertainties in estimating the pelagic EQS based on lack of toxicological data (high assessment factors) but also additional uncertainties in the EqP calculation into QSbent because of e.g. large

data are checked against trigger values. Nevertheless, in draft CIS 27, a tiered approach is recommended in case of large uncertainties, in particular if costly remedial measures are to be considered. In the 1st tier, concentrations are compared to a "worst case" sediment EQS value. If concentrations are failing this criteria, bioavailability and/or biological information should be considered to assess whether the benthic community is really impaired.

The bioavailability of organic substances in sediment generally decreases with higher organic carbon contents, although also the quality of the organic matter can influence the availability¹³¹. If the QSbenthic is based on EqP calculations using default values for organic carbon (5% is used in CIS 27, but 1% would rather be relevant for worst case assessments for Swedish conditions¹³²), a first step could be to recalculate the QSbenthic based on an actual value of organic carbon fraction¹³³ instead. This is easily done by multiplying such a 1% worst case criteria with the actual orcanic carbon fraction (expressed as a percentage) before comparison with the concentration of the particular sample. In the evaluations performed in chapter 6 in this report, site specific criteria were calculated if possible before comparison to concentrations found. Such studies can also include the study of pore waters or using different types of extractions to estimate exposure of benthic organisms and water sampled from deeper layers to estimate direct exposure to pelagic organisms. Pore waters are frequently considered to represent the bioavailable fraction, and concentrations of accumulating substances can be expected to be higher than in the water at the surface. In Sweden there is little experience from analysing hazardous substances in pore waters and suspended matter though¹³⁴, and the applicability of the methods could be limited due to difficulties in obtaining sufficient material. The draft CIS 27 guidance (pp 145-148) mentions several analytical methods to assess the bioavailability of hazardous substances in sediment. Data retrieved by using SPME (Solid Phase Microextraction) or POM (poly-oxy-methylene) can be compared to water-EQS, whereas data from Tenax ® extraction can be compared to sediment-EQS. In this report, sediment was not recommended to be used as the only data for compliance checking of metals. Nevertheless, the draft guidance also mentions methods under development to measure bioavailability of metals, such as DGT (Diffusive

to 5 times if organic matter was of favourable quality, such as directly after algal blooms. 132 TO C

range in Koc values identified. In addition, organic fraction and redox potential can influence the equilibrium between particles and pore water.

¹³¹ Strong soot sorption reduces bioavailability of e.g. PAHs (see e.g. Sundelin et al 2004). Granberg ME (2004) also concludes that the concentrations of organic substances inside benthic organisms increased by up

¹³² TOC concentrations of the off shore sediment samples vary e.g. between 1.8 and 17%

¹³³ For limnic sediments it could usually be estimated from LOI, Loss on Ignition, but in marine sediments it should be analysed.

¹³⁴ There are however some examples that could be utilized for capacity building. In the Interreg project DiPol some partners used e.g. continous centrifuges to obtain suspended material, whereas others (including the Swedish partner SGI) used novel types of sediment traps in order to obtain sufficient material for chemical analysis (Gunnel Göransson SGI pers comm..; http://www.northsearegion.eu/ivb/projects/details/&tid=91). In the 70s equipment for flow proportional sampling of suspended matter was constructed at the Uppsala University (although 10 m3 of water is necessary to extract 30 g); Håkan A Johansson County Adm Board in Stockholm pers. comm. Pore waters were extracted and analysed in the investigations performed in Viskan. In this case vacuum extraction was used (http://www.lansstyrelsen.se/vastragotaland/Sv/miljo-och-klimat/verksamheter-med-miljopaverkan/fororenade-

<u>omraden/efterbehandlingsprojekt/viskan/Pages/index.aspx</u>). Other ways to extract pore water include e.g. centrifugation (normally recommended for sediment pore water bioassays). Care should be taken not to alter the redox potential before centrifugation.

Gradients in Thin-films) and in particular the SEM-AVS (Simultaneously Extracted Metals – Acid Volatile Sulphides) concept¹³⁵ (see box 7.3).

Draft CIS 27 also mentions the monitoring of effects on benthic communities. Nevertheless, where pelagic organisms are the most sensitive protection objective, in combination with the fact that substances may not primarily accumulate in e.g. fish because of metabolisation, but still provoke a response (be bioavailable), fish biomarkers that respond to the substances in question could provide useful tools to investigate effects on pelagic organisms. Such tools could primarily be used where the implementation of local measures are expensive and there is thus a need for additional decision support (a weight of evidence approach; chapter 9).

The choice to monitor sediment even for substances that may actually be more relevant to monitor in higher trophic level biota could be necessary because of practical circumstances. Sediment can also act as both a sink and source of such substances due to e.g. bioturbation (see e.g. Josefsson et al 2010). There is therefore a need to develop not only QSbenthic values to assess the risk of impact to benthic organisms and pelagic organisms, but also to provide so called "trigger values" that can be used to make at least a 1st tier assessment of overall compliance based on sediment data. In particular, in draft CIS 27 (p 141) it is also pointed out that in a situation where sediment is the primary source of exposure for target species, data should be compared to sediment-EQS values based on QSbiota. However, it is also declared that the methodology to be used needs to be site or regional specific, because simple models (based on BSAFs) are not recommended due to published values being highly variable. Nevertheless, in the draft national marine sediment assessment criteria, there are two values available for several substances. The "trigger value" is based on recalculating the overall EQS into a sediment concentration using EqP theory, and the aim is to use it for such purposes. The "riktvärde" would rather correspond to the QSbenthic. The lowest of these was used in the assessment of non compliance in chapter 6.

Comparison with baseline sediment data should preferably be done, in order to find out whether the site is actually a "hot spot" or if concentrations rather reflect general conditions and the substance should be considered to be ubiquitous (chapter 6). Measures on a wider geographic scale should then be considered.

Tier II biological investigations could involve studies of the benthos and/or bioassays¹³⁶ (chapter 9). If an impact cannot be demonstrated after considering bioavailability and/or biological responses, further monitoring can be considered sufficient further action.

Box 7.3. SEM/AVS ratio to assess metal bioavailability also in Sweden?

¹³⁵ AVS (Acid Volatile Sulphides): molar concentration of sulphide released from sediment with cold weak acid treatment. SEM (Simultaneously Extracted Metals): concentration of metals that are simultaneously extracted with the AVS. Affinity decreases in the order Ag>Cu>Pb>Cd>Cd>Zn>Ni (Morse et al 1987 and Morse 1995).

¹³⁶ Both in situ and ex situ bioassays are mentioned, although it is also hinted that both benthos assessment and bioassays could be difficult to use and therefore needs to be considered on a case to case basis (p 144-145 in draft CIS 27).

In anoxic sediments, bioavailability of metals can be limited due to the formation of stable complexes with sulphide. The SEM/AVS ratio is therefore suggested as a normalising factor for certain metals and has been used in the voluntary REACH risk assessment reports by the industry to estimate effects from copper and lead related to sediment contamination. According to the SEM/AVS concept, the lower the SEM/AVS ratio, the lower metal toxicity, because of lower metal bioavailability.

In Sweden and Swedish oligotrophic lakes in particular however, sediments generally do not contain high sulphid concentrations. In particular environments such as parts of the Baltic Sea and West Coast fiords, with oxygen depletion and higher sulphur concentrations, the sediments are more prone to form sulphides. In areas that have been more or less completely oxygen depleted, benthic organisms are lacking due to toxic conditions from hydrogen sulphide, making conclusions about metal bioavailability of less importance to assess the overall sediment quality. Furthermore, the analysis of AVS and SEM requires special sampling procedures, limiting the use of this concept to estimate metal bioavailability in sediments¹³⁷.

The SEM/AVS concept is based on the assumption that pore water constitutes the bioavailable fraction and major pathway for metal uptake by sediment invertebrates. This assumption has however also been questioned for invertebrates that feed on particles, in which case also the particle adsorbed material can be available for uptake (Byeong-Gweon et al 2000; Ahlf et al 2009). Wiklund & Sundelin (2002) also stated that the concentrations of Cd, Pb, and Zn in sediment were more important for body burdens in amphipods than metals in porewater in a study performed on Swedish urban brackish and fresh water sediments. Sulphide-metal complexes could therefore probably become dissolved in the guts and become bioavailable. The bioavailability of Cd was found to be primarily related to salinity (higher bioavailability in freshwater environments. Other metal ligands, in addition to AVS, were also found to be important for metal bioavailability and toxicity in anoxic and suboxic environments in sulfidic coastal sediments (Sundelin & Eriksson 2001).

It can therefore be concluded that the use of SEM/AVS to assess bioavailability of metals in sediment is not a tool that can be universally applicable for compliance checking in Sweden.

By analysing the sediment pore water and the overlying water with passive samplers it is also possible to make conclusions about the extent of equilibrium between sediment and surface waters, equilibrium being indicated by a ratio of 1:1. If, on the other hand, the pore water concentrations are higher than those in the overlying column, the sediment probably can act as a source of contaminants. If the opposite ratio is observed, the sediment can be assumed to act as a sink. In a recently performed Nordic study, equilibrium was found for most stations but at one station the sediment acted as a source of benzo(a)pyrene (Larsen *et al* 2009). A Swedish report related to Baltic sea sediments was also recently published (Wiberg et al 2009) and an additional paper is to be published¹³⁸.

7.4.3 Evaluating biota compliance

To evaluate biota compliance one needs to be aware of the type of protection objective that is the most critical and assess whether available data can be used for the purpose. For the evaluation of QShh, concentrations found in relevant food items such as edible aquatic fish, mollusc, crustacean and cephalopod species are to be used, but only tissue that is normally consumed (primarily muscle of fish but sometimes also skin) should be used for comparison. Before comparing biota data

¹³⁷ Hans Borg, ITM, pers comm.

¹³⁸ Anna Sobek, pers comm

to QShh food it is therefore necessary to consider if the analysed species and tissue could be considered to fulfil these criteria or at least be possible to consider as an acceptable option to assess realistic worst case situations for the particular substance.

The QSsec pois and QShh values (as well as overall biota-EQS) are expressed on a wet weight basis, whereas in most cases, data generated within the biota monitoring programmes are frequently expressed on lipid basis (such as ng/g lipid) or on dry weight basis. In most cases, but not always, a recalculation can be done, if necessary supportive information is available. Within the national monitoring programs, the lipid content of the tissue analysed is generally monitored and reported as well. This facilitates the possibilities to recalculate monitoring data into wet weight for the tissue analysed by simply multiplying the reported concentration with the lipid weight (divided by 100 if expressed as percentage). If data was expressed on a dry weight basis, a similar recalculation can easily be done by multiplying with the dry weight of the sample.

If evaluating QShh based on data from fish muscle or blue mussels, the "correct" tissue has been monitored so the evaluation is rather straightforward. However, to evaluate liver data from a human health perspective is more complicated. If the substance can be assumed to be found primarily in the liver because of its high lipid content, lipid normalisation can again be done, but taking into account that the lipid content of the liver is higher than in the muscle. Instead of multiplying the concentration with the lipid concentration of the liver, it should rather be multiplied with the lipid concentration of the muscle, which is generally much lower. Otherwise there is a risk of being too conservative in estimating non compliance (creating false positives).

However, for metals and perfluorinated compounds, such a recalculation is not straightforward, because the reason for finding these substances in higher amounts in the liver is not primarily due to its lipid content¹³⁹. In these cases, liver data could probably rather be used as worst case data as a 1st tier compliance check. If data indicate compliance, there is probably no need for further studies, but if data suggest non compliance, further studies should be performed and data rather used to indicate risk related to QShh.

Box 7.4. Which fish species etc should be monitored (and evaluated)?

As with all biota monitoring, one prerequisite in fish monitoring is e.g. that there are sufficient individuals available both in the area of investigation and in reference areas. The mobility needs to be considered so that analysed concentrations reflect local exposure, especially in operational monitoring. If data should reflect the situation in a larger geographical area, more mobile species can suit the purpose. The life cycle and environmental requirements need to be well known, and if short term changes should be possible to detect, the species should respond accordingly. The availability of assessment criteria and baseline data facilitates evaluation.

The marine fish species that are recommended in the national program are perch, cod, herring, eelpout and flounder. Along the Swedish West Coast, both the eelpout and flounder (and eel before migration, although eel fishing is restricted) can be considered to be less mobile. On the East Coast, eelpout and perch are the less mobile of these species. A major advantage in choosing eelpout for monitoring is that reproduction disturbances can also be studied in an integrated program, as

¹³⁹ Perfluorinated compounds can primarily be found in protein rich tissues, including liver and blood

eelpouts are viviparous organisms (chapter 9). However, eelpout is not usually used for consumtion, limiting its relevance in the evaluation of health related criteria. In addition, the species is no longer abundant in all areas¹⁴⁰. A suggested alternative species for such integrated effect and chemical studies is flounder. For limnic programs, perch, pike, roach and char are recommended.

In biota monitoring, also other aspects need to be considered than picking a suitable species. One needs to consider e.g. size (related to age/trophic level) and tissue to analyse.

For QS sec pois, results should be expressed on whole organism wet weight concentration before comparison, and not only for a specific tissue or organ. In certain biota, such as blue mussels, concentrations are already monitored on whole organisms, but for fish either the liver or muscle (or other tissues) are usually monitored and seldom the whole organism. In addition, the lipid content of the whole organism is also seldom monitored. In the current report, a rough estimate has been done by multiplying muscle data¹⁴¹ with lipid content of the muscle, but being aware of the fact that such a recalculation probably underestimates non compliance (creating false negatives) in fish species for which the overall lipid content is higher than in the muscle. In the future therefore, such supportive data (lipid concentration of the whole organism) should be generated to facilitate QSsec pois evaluations. The costs for such additional analysis are considered low. For fish liver data and where the liver is the target organ, again these data recalculated into wet weight for the particular tissue could probably be used as a 1st tier compliance check of QSsec pois, but could lead to an over estimation of non compliance, if it can be assumed that the average concentrations of the rest of the organism is lower.

A recently started project at the Swedish Museum of Natural History will include parallel measurements of metals in muscle, liver and whole fish for herring and perch, in order to provide supportive data that can be used for recalculation before comparisons to biota criteria for wet weight whole organism and muscle respectively¹⁴².

The recalculations described above were also done before comparisons made in chapter 6. If dry weight was not available for blue mussels or gastropods, it was assumed that these were approximately 1%.

Furthermore, according to draft CIS 27, toxicity studies included are to be recalculated into 5% lipid content¹⁴³, if the substance is known to accumulate primarily in lipids (the case for most hydrophobic organic substances¹⁴⁴). However, such an instruction was not included in the former "Lepper manual" on how to calculate QSsec pois and overall EQS values. Therefore, the current QSsec pois

¹⁴⁰ Rising water temperature could cause decreasing population levels (see e.g. Larsson et al 2011)

¹⁴¹ In the Swedish biota program, most lipophilic compounds are monitored in fish muscle, with the exception of cod liver. In this report, cod liver data were not recalculated into whole organism but rather used for inter species comparison, based on lipid weight data. If used for compliance checking a similar recalculation based on whole organism lipid weight should be done, or at least muscle lipid weight, but again being aware of the fact that this might be underestimating the risks of non compliance.

¹⁴² Elin Boalt, NRM pers comm..

¹⁴³ Lipid normalisation is recommended according to draft EQS manual if possible and scientifically justified; i.e. the substance primarily accumulates in body lipids.

¹⁴⁴ Perfluorinated compounds such as PFOS can also be found in the lipid rich liver but these substances are rather to be found in protein rich tissues, including liver, blood and eggs.

values are probably not lipid normalised and could indicate a need to revise current QSsec pois values. In this report, a recalculation into a corresponding 5% value was not performed before comparisons were made in chapter 6.

For trend analysis or for the comparison of non compliance between species, it might not be necessary or even recommended to recalculate data into whole organism level if a certain tissue has been used, as this could introduce additional variability. Instead, only lipid normalisation is performed if relevant.

Box 7.5. Monitoring accumulating substances in other biota than fish – marine and limnic options

In the marine environment, there is national and international experience from monitoring concentrations of hazardous accumulating substances in bivalves such as blue mussels (Mytilus edulis) and in the Baltic also Macoma Baltica¹⁴⁵. Blue mussels are recommended in many contexts, including the CIS guidance 25 and by ICES. The concentrations in water can be estimated by dividing the lipid normalized concentration of the substance in the mussel by BCF (or Kow). If the mussels are not defacated before chemical analysis, the amounts in the gut may need to be estimated¹⁴⁶. Also effect based analyses are possible to perform on the mussels (chapter 9) on the same individuals that are analysed chemically (because of only small amounts of blood are necessary), thus facilitating truly integrated monitoring. Previously, assessment criteria were also available for both Mytilus edulis and Macoma baltica¹

In Sweden, in addition to the more traditional choice of biota, fish and mussels, metals have been monitored in Fontinalis within several regional programs since decades, to monitor trends and to compare downstreams locations to upstreamds locations. However, there is no such national program and it is unclear if EQS values to be used for compliance checking within the WFD could be possible to derive. With the introduction of passive samplers, and need to check compliance, several of the Fontinalis programs are now being phased out.

In the limnic environment, bivalves are also regularly being monitored but generally not to analyse the concentrations of hazardous substances. Nevertheless, the CIS 25 recommends the following invertebrate species to be used for biota monitoring in rivers: the bivalves zebra mussel (Dreissena polymorpha), Anodonta cygnea, Unio pictorum and Corbicula fluminea as well as the macroinvertebrates Gammarus pulex and Chironomus spp. In addition to the alien bivalve species D polymorpha, the two native species Unio tumidus ("spetsig målarmussla" in Swedish), swollen river mussel and Anodonta anatina (= A. piscinalis, "allmän dammussla" in Swedish), duck mussel, have both been suggested as promising Swedish candidates¹⁴⁸. U tumidus is 5-8 cm and can be found in Skåne to Värmland and Medelpad, whereas A anatina usually reaches 7-10 cm and can be found in the whole country (although less frequently in the north), whereas the D polymorpha was so far primarily found in Lake Mälaren and Lake Hjälmaren and rivers and streams connected with ⁹. However, so far there is rather limited national experience from monitoring hazardous them¹ substances in freshwater bivalves and baseline data would need to be developed. Nevertheless, U tumidus was e.g. used in a screening study of phenols, phthalates and tinorganic compounds¹⁵⁰ and

¹⁴⁵ See e.g. "Förekomst av krom i Östersjömussla (Macoma baltica) utanför Expancel i Sundsvallsbukten" 2007; metals and dioxins were analysed in (Macoma baltica in Nätrafjärden (near industrial area) and in reference area (Gaviksfjärden) in 2004.

¹⁴⁶ This amount can be estimated from the product of water concentration, Koc and mass of organic carbon in the gut per weight of mussel normally found.

¹⁴⁷ Bedömningsgrunder för miljökvalitet. Naturvårdsverket. Report 4914. Under revision.

¹⁴⁸ Per Ingvarsson, Naturcentrum; pers comm. See also e.g. Lundberg & Bergengren 2008 regarding the potential to include analyses of hazardous substances in national programs of limnic bivalves (A anatina). ¹⁴⁹ Natural History Museum;

http://www.nrm.se/en/menu/researchandcollections/departments/invertebratezoology/research/factsoninvertebrates/thelarg efreshwatermussels.734 en.html ¹⁵⁰ Only Dibutylphthalate was detected in the screening study using U tumidus in one out of two locations

whereas the arsenic concentrations were considered high in Lymnaea stagnalis (dammsnäcka in Swedish)

arsenic concentrations in Lymnaea stagnalis ("Stor dammsnäcka" in Swedish) were recently monitored at two locations. Metals and organic substances were also analysed in Lymnaea peregra (Oval dammussla) (Larsson 2005).

Chironomids are also monitored on occasion (see effect based tools, both regarding species composition and mouth deformation) and could be coordinated with a chemical tissue analysis¹⁵¹ although again baseline data would need to be established. The concentrations of metals and organic hazardous substances have also been studied in limnic crayfish (Pacifastacus leniusculus)¹⁵² and zooplankton¹⁵³. The three species Dreissena polymorpha, Asellus aquaticus and Chironomus plumosus were analysed regarding PAH, DDT, HCB and PBDE in a gradient study in lake Mälaren (Lithner et al 2001).

For metals, aquatic plants could be a suitable alternative. Baseline data and method descriptions¹⁵⁴ do exist for using Fontinalis in metal monitoring, which is also included as a candidate species in CIS 25. On the West Coast there are some experience and baseline data available also for Fucus vesiculosus through regular monitoring within SRK programs of the "Bohuskustens vattenvårdsförbund". There are also data from investigations of irgarol, copper and zink in the Stockholm archipelago (Kylin 2006) and previous assessment criteria for the marine environment included metal concentrations in Fucus vesiculosus¹⁵⁵. In addition, plants were included in monitoring activitites of metals in the Sundsvall area¹⁵⁶.

There are therefore several potentially suitable invertebrates and aquatic plants that could be used for the monitoring of substances that are rather prone to accumulate in lower trophic level organisms than fish. However, in most cases there would be a need to create baseline data and to investigate advantages and drawbacks related to their use within WFD monitoring. For macroalgae, the adaptation abilities of certain natural populations to e.g. metals need to be taken into account¹⁵⁷, and certain bivalves are known to migrate and depend on fish as reproductive hosts.

The potential to replace trend monitoring in biota with passive sampling techniques is limited for substances for which biomagnification would need to be taken into account (because food intake has a major impact on concentrations in biota). Nevertheless, the use of passive sampling instead of mussels, especially at sites where mussels are difficult to find or have difficulties to survive was e.g. suggested to be a promising approach within OSPAR (Larsen et al 2009).

7.5 The purpose of using effect based tools also in chemical compliance checking

compared to other investigated biota (crayfish and fish) from the same locations. Gunnel Hedberg, County Adm Board Jönköping, pers comm. (see also Eriksson 2011)

¹⁵¹ Karin Jönsson County Adm Board Västernorrland, pers comm

¹⁵² Metals have been monitored in crayfish within a RMÖ program performed by the County Adm Board in Jönköping in 1995, 1996, 2001 and 2002 (Gunnel Hedberg, County Adm Board Jönköping, pers comm.; see also Carlsson 2004). A more recent study of both metals and organic substances was also performed by Lindeström et al 2009.

¹⁵³ Juha Salonsaari County Adm Board Västernorrland, pers. comm.

¹⁵⁴ Method description:

http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/sotvatten/me t_vamo.pdf

¹⁵⁵ Bedömningsgrunder för miljökvalitet. Naturvårdsverket. Report 4914. Withdrawn due to updates.

¹⁵⁶ Karin Jönsson County Adm Board Västernorrland, pers comm

¹⁵⁷ See e.g. Nielsen et al 2003 A review paper also draws the conclusion that both Fucus and Ulvae species might be less suitable as quantitative biomonitors of metal pollution, because there is evidence for increased resistance in natural populations growing in metal contaminated sites, the mechanism for which is, in part, due to metal exclusion (see Brown MT et al in press).

As opposed to the MSFD, ecotoxicological effect based tools are not strictly required for compliance checking within the WFD even for ecological status assessments. It is also clear that chemical status should be based on chemical analyses.

Tools that measure specific effects from the substances in question are valuable, but could be used primarily as 2nd tier assessments to investigate effects on pelagic organisms. Some of the more specific and sensitive tools are the biomarkers imposex (for TBT) and ALA-D (for lead), but also other biomarker tools could provide important information (such as EROD, that responds to PAHs, dioxins, dibenzofurans; and VTG, being very sensitive to estrogenic substances). Although ecological relevance has been guestioned, also MT would respond to Cd, Zn and Cu exposure.

However, for some priority substance candidates, other types of effect based tools (in vitro assays) could also become useful tools even for chemical compliance checking if they are accepted from a legal point of view. In particular, dioxins, diobenzofurans and planar PCBs are all expensive chemical analyses, whereas in vitro bioassays can be used as less expensive screening tools and are already acceptable for the purposes to estimate health risks from fish and other seafood according to current EU legislation¹⁵⁸. Because the most critical objective in the draft dossier on dioxins and dioxinlike compounds is human health, one can assume that a similar approach would be accepted for WFD compliance checking purposes¹⁵⁹.

Another candidate priority substance is 17-alpha ethinyl oestradiol (EE-2) with a draft limnic EQS of 35 pg/l, which will cause chemical analytical problems. In the recently performed national screening study of pharmaceuticals in the environment, the substance could not be detected in any samples. However, LOD was 10 ng/l which is almost 300 times higher than the draft EQS and clearly not fulfilling the QA QC requirements. In the case of EE-2, this is not a substance that is accumulated in biota or sediment, and therefore analysing accumulating compartments is not considered a possible approach to reduce LOQ problems (as could be the case for several of the current priority substances). In this respect, sensitive effect based tools could become useful for the screening of oestrogenic substances. This is a matter currently being discussed in one of the working groups related to chemical monitoring within WFD¹⁶⁰ (see also chapter 9).

¹⁵⁸ Community regulation 1883/2006; <u>http://eur-</u> lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0032:0043:SV:PDF

¹⁵⁹ However, for the purpose of temporal trend monitoring, DR CALUX data can be difficult to interpret because the response is rather related to effect monitoring and the observed effects could be due to a range of compounds, including brominated or mixed halogenated dioxin analogues, polychlorinated naphthalenes, PAHs etc. or compounds antagonising the AhR (e.g. di-ortho-substituted PCBs). The relative contribution can vary between congeners and years. Therefore, it may not be recommended to replace current time trend monitoring based on individual congeners with a parameter such as DR CALUX entirely. However, in operational programs, where the main focus is related to compliance checking, such a method, integrating several chemicophysical parameters could be an option that can be considered for both economic and scientific reasons to obtain information that can be used to estimate effects, whereas trend data can be generated with lower sampling intervals than data for compliance checking. ¹⁶⁰ CMEP, Chemical Monitoring and Emerging Pollutants. Activity 3.2.C. Effect based tools.

Effect based tools can also be considered for additional purposes within the WFD but also for compliance checking related to GES within the MSFD. Less specific effect based tools could e.g. be valuable as an indication of the general health conditions or used as screening tools in a situation where it is less clear what substances to analyse, which is the case with River Basin Specific Pollutants. In particular, some biomarker tools would actually not add substantial costs, if biota is monitored for other purposes (because the analyses themselves are frequently rather inexpensive and costs are primarily related to the sampling of organisms). Other effect based tools are rather useful as complementary methods in sediment and water monitoring. In addition there are promising effect based tools that would probably fulfil the requirements of biological indicators, because they monitor effects on ecological levels. Effect based tools are therefore described in more detail in chapter 9.

7.6 *Research, development and validation studies needed*

For certain substances the most prioritized compartment to monitor is difficult to determine due to lack of sufficient good quality data. This is particularly the case for substances that were so far not included in any national monitoring program than off shore sediments. In particular, there is currently lack of available data to asses the most suitable species and tissue for the monitoring of DEHP, nonylphenol and octylphenol in 2nd tier studies. Investigations (practical and/or literature searches) to make recommendations would be desirable because sediment data indicate risks of non compliance (in particular for octylphenol, for which currently available sediment data suggest large scale impact).

In general, for some substances lower trophic level biota data rather than fish would be relevant for compliance checking and trend analyses. However, there are no such national regular monitoring programs in the limnic environment.

Because of the limited experience in lower trophic level biota monitoring, it is at the moment difficult to recommend certain species to be used for such purposes. Lack of standardized monitoring organisms could cause non harmonized status classifications and a continued lack of baseline data to facilitate geographical comparisons in the limnic environment. There is therefore a need for validation studies on different species of invertebrates and aquatic plants that could be used for such compounds, but also to evaluate the use of passive sampling devices in this context.

For rivers, the monitoring of accumulating substances is frequently a great challenge and there is limited national experience. Strategies also for the Swedish environment would need to be developed and could include the need for validation studies using complementary sampling tools and approaches such as passive sampling devices, sediment traps, and the analysis of suspended matter. Several member states are primarily monitoring rivers, also regarding the concentrations of organic accumulating substances¹⁶¹ and the experience gained should be considered. There are also several ongoing projects to validate the use of passive sampling techniques within e.g. the NORMAN network¹⁶².

Sediment is frequently suggested as a 1st tier approach in this report (because of the limited experience in appropriate biota monitoring but also because of observations that sediment rather than water frequently can be considered the most critical compartment based on available data). A national limnic sediment monitoring program or campaign could provide useful baseline data for smaller lakes (current limnic sediment monitoring is primarily performed in the largest lakes Vänern and Vättern). Such baseline levels are e.g. valuable in identifying local hot spots, thus providing supportive information for the needs of local measures. Limnic sediment data could also provide indications on sources of substances for which there is non compliance in biota from higher trophic levels.

There is also a need to develop/evaluate specific effect based tools that could be used in 2nd tier assessments, in particular for substances that primarily are suspected to cause effects in pelagic and/or benthic organisms (rather than mammals, birds and humans). Such substances are likely to cause local effects and additional investigations could provide important decision support in the prioritisation of local measures. In particular there is no established biomarker to assess the prevalence of negative effects from TBT in the limnic environment, in spite of laboratory studies showing that also limnic organisms can be as sensitive¹⁶³. Such a tool would be very useful because of high TBT concentrations also can be found in e.g. limnic sediments.

In order to utilize biota data, sufficient supportive parameters need to be analysed and recalculation methods developed based on parallel analyses of several tissues and species as well as whole organisms. Such data could be used to develop more firm guidance on the use of fish data for compliance checking. Studies are currently being performed for metals, but would be needed for other substances that are relevant to monitor in fish. To utilize also e.g. data generated within the national monitoring program of perfluorinated compounds in fish liver for status classifications there is a need to assess QShh compliance, as this is suggested to be the most critical protection objective in the draft dossier¹⁶⁴.

7.7 Conclusions

• Recommendations on the most prioritized compartment to monitor and evaluate in chemical status compliance checking should, for economic, practical

¹⁶¹ A British project includes e.g. parallel measurements on water, biota and passive samplers (Paul Whitehouse, pers. comm.). There are also recommendations on national criteria for suspended matter (as well as sediment and biota) in Austria (Rüdel et al 2007)

¹⁶² http://www.norman-network.net/index_php.php

¹⁶³ Current SDS lists effect data also for freshwater organisms and the lowest NOEC found was 1 ng/l (significantly reduced egg laying in snails, *Biomphalaria glabrata*), which is the same NOEC value as has been observed for the marine gastropod *Nucella lapillus*.

¹⁶⁴ PFOS is included as one of the priority substance candidates

and scientific reasons, take several aspects into account. The current Swedish position, that the EQS should be set for the compartment in or via which the most sensitive organisms are exposed, is one such important factor. Additional aspects include inherent substance properties, current analytical possibilities to fulfil QA QC requirements, trend monitoring requirements, current national monitoring compartment, and an assessment of worst case exposure.

- With such a policy, most of the data that are being generated within regular trend monitoring programs could be used also for compliance checking, which would be a very cost effective approach. However, national assessment criteria to be used for chemical status compliance checking are urgently needed for sediment and biota data from both limnic and marine environments for the most prioritized compartments to evaluate. The biota criteria should be species and tissue specific. Additional, indicative values (so far called "trigger values") would also be needed in cases where another compartment than the most prioritized was monitored for case specific reasons. Such indicative values could be used to assess the need for 2nd tier investigations.
- Supportive data required for the evaluation of data for compliance checking includes some parameters that are usually not included in the current national monitoring programs. In particular, for biota, lipid concentrations of both the tissue analysed and the whole organism and the tissue consumed by humans should be included in future monitoring programs. In sediment, organic carbon fraction is important to analyse and take into account.
- The marine and limnic biota monitoring program cover WFD relevant areas but not all priority substances that can be suspected to accumulate in biota are included. Also, screening data are not always sufficiently available. This makes an assessment of the most prioritized monitoring compartment difficult. In particular, there is a need to generate data from different species of biota for the compounds DEHP, nonylphenol and octylphenol because sediment data suggest non compliance could occur and for octylphenol this substance can even be considered ubiquitous based on marine sediment data. Additional screening in order to evaluate future regular monitoring needs in biota (and the most suitable species) would be recommended.
- In general there is a need to generate additional limnic data for several accumulating substances in sediment and lower trophic level biota, to be used as baseline values in geographical comparisons (to identify hot spots for which local measures are necessary) but also to identify suitable species to monitor.
- Recommendations on the use of suitable lower trophic level biota, passive sampling devices, sediment traps and the sampling of suspended matter in the context of compliance checking should be developed. In particular, there is currently very limited national experience from monitoring accumulating substances in rivers, and many of the recommandations on the most prioritized compartment to monitor may not be applicable for practical reasons. Developing a strategy for this monitoring could include such tools. There are several ongoing projects and international experience that could be consulted.

 For decision support in the prioritisation of measures, specific effect based tools would need to be developed/evaluated especially for substances that are suspected to cause effects primarily in pelagic and benthic organisms, based on currently available chemical data. In particular, a tool to assess effects from TBT also in limnic environments is needed. Furthermore, the use of sensitive in vitro assays as bioanalytical tools to monitor some types of compounds should be evaluated from a scientific and legal point of view in the context of chemical status classifications.

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8 Is it possible to predict field effects from combined exposures to chemical cocktails?

The EQS values and other criteria developed to evaluate concentrations of hazardous substances in the environment (described in chapter 9) are generally based on data from laboratory studies of one substance at a time. Within the WFD, the basis for chemical status classification is "one out – all out", *i.e.* if the concentration of one substance exceeds its EQS value (either AA, annual average or MAC, Maximum allowed Concentration), the chemical status is "not good". The same procedure is performed for river basin specific pollutants when assessing ecological status and comparing to national EQS values.

However, organisms in the environment are exposed to a large number of hazardous substances. Therefore, the concept of "one out – all out" may not be sufficiently protective from an ecosystem perspective. The issue of combination effects of chemicals is currently being highlighted in e.g. a report by Bengtsson & Holmqvist (2008), a recent special issue of "Science for Environment Policy" (DG Environment News Alert Service 2010)¹⁶⁵ and by a call for research applications by the Swedish Research Council (FORMAS).¹⁶⁶

As stated in the State of the Art Report on Mixture Toxicity (Kortenkamp et al 2009), there is strong evidence that mixture effects can arise when combining several chemicals at doses/concentrations around or below their respective zero effect levels. Also Baas & Kooijman (2010) found that even when individual contaminant concentrations do not exceed their MPC (Maximum Permissible Concentration) values, combined exposure can cause effects. However, whether there is a cut off concentration-effect level ratio value below which the contribution to the overall effect is negligible is unclear. Syberg et al (2009) suggests that the assessment could be limited to chemicals present in individual ratios of PEC/PNEC>0.1, whereas a review by Kortenkamp et al (2007) illustrates that individual substances present at low concentrations still contribute to the overall mixture toxicity.

The term "Mixture or Cocktail effect" can be interpreted in different ways depending on the context. In some contexts, the main focus is on understanding synergistic/antagonistic effects from a combined exposure of several defined substances. An example of where synergistic effects can be observed is the combined exposure to one substance that may not be very toxic itself but it prohibits degradation of another substance.

This chapter is focused on possible ways to predict additive effects in the natural /polluted/ environment, where organisms are clearly exposed to several hazardous substances, based on chemical data. Thus, focus will rather be on effects that are due to cumulative exposure of several stressors (additive effects can be assumed), and not primarily the prediction of effects from synergistic and/or antagonistic action.

¹⁶⁵ Issue 21. June 2010.

¹⁶⁶ http://www.formas.se/formas_templates/Page____7131.aspx

8.1.1 Concentration addition and Independent Action

For evaluating well defined mixtures, mainly two different concepts have been developed: Concentration Addition (CA) and Independent Action (IA).

CA: $ECx_{MIX} = 1/[i=1\Sigma^n (p_i/ECx_i)]$

Where n: the number of mixture components, p_i : relative fraction of chemical i in the mixture; x: common effect level provoked by an exposure to a single substance or mixture concentration ECx_{MIX} resp ECx_i .

IA¹⁶⁷: E(c_{MIX})=1- _{i=1} Πⁿ [1-E(c_i)]

Where $E(c_{MIX})$: effect provoked by total mixture at a concentration $c_{MIX} = {}_{i=1}\Sigma^n c_i$. $E(c_i)$: effects that the individual components would cause if applied singly at that concentration at which they are present in the mixture.

From studies using well defined mixtures, the concentration addition concept has been found to be the most conservative although the predictions differed by a factor 5 at the most (Kortenkamp et al 2009). SCHER¹⁶⁸ also concludes that the CA concept is more appropriate if mode of action is unknown.

Box 8.1. Application of concentration addition to monitoring data

The CA concept is limited to mixtures of known chemical composition and this is never the case for environmental samples. To evaluate the applicability of the same concept validation studies are needed.

Part 3 of the report by Kortenkamp et al (2009) includes a survey on approaches used by different member states to assess mixture toxicity of such complex samples, including waste waters and environmental samples. Among the approaches chosen by the 14 participating Member States, it can be noted that the majority either use direct whole mixture toxicity testing or a combination of direct toxicity testing and component based modelling approaches.

Effect based methods of both complex environmental and effluent samples (whole effluent assessment) are described in more detail in chapter 9. Component based approaches were included in the assessment of several different types of compartments, in particular waste water, soil, sediment and surface water.

Among the states that did use component based approaches, the most common approach to use was the TEF (Belgium, France, Spain, Estonia, Denmark, and the Netherlands), described below. Other approaches also exist (TUS, Toxic Unit Summation; RPF, Relative Potency Factor; PODI, Point of Departure Index and HI, Hazard Index). Denmark, Spain and the Netherlands also used component based approach based on the IA concept.

Although not included in the Swedish response to the questionnaire, pesticide monitoring data are actually summarised into a PTI (Pesticide Toxicity Index) which is based on the CA concept and described below. Also, the TEF concept has been used to evaluate dioxins and dioxinlike

¹⁶⁷ This equation is valid only if effects increase with increasing exposure, such as is the case for the endpoint mortality. For other endpoints, a slightly different equation is used.

¹⁶⁸ http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_150.pdf

compounds and the concept is included in the proposed national EQS values also for nonylphenol ethoxilates.

8.1.1.1 TEF concept based on CA

In evaluating monitoring data, the most familiar evaluation tool is probably the TEF (Toxicity Equivalent Factor), applied to dioxins/dibenzofurans and dioxinlike PCBs. A single value is obtained that can be used in absolute terms to evaluate the combined exposure of several substances measured in an individual environmental sample.

The TEF calculation is based on the CA concept and thus on the assumption that the substances evaluated have approximately the same mode of action. Which individual substances that should be included in the calculations are specified.

$TEQ = {}_{i=1}\Sigma^{n} (c_{i} * TEF_{i})$

Where c_i is the concentration measured of compound i, and TEFi is the relative toxicity of compound i compared to an index compound (the most toxic compound of the group).

In Sweden, the suggested EQS values for potential river basin specific pollutants include two groups of substances for which the TEF concept has been used: dioxins, dibensofurans and dioxinlike PCBs and nonylphenol ethoxilates respectively.

It is important to note that there are different TEF systems developed for dioxins, and the proposed EQS values are based on the WHO system. For the evaluation of sediment data, the WHO TEF values for fish should be used, but for the evaluation of biota concentrations, such as fish tissues, the WHO TEF values for mammals should be used. Applying the wrong TEF could significicantly influence the results. For WHO TEF values, see Appendix chapter 24.

Using the TEF concept also for nonylphenol ethoxilates is based on the assumption that they have the same mode of action (binding to the oestrogen receptor), and the overall effect being present at the same time can be calculated in terms of nonylphenol equivalents. The TEF values for nonylphenolethoxilates in the Swedish report are based on the Canadian system (Appendix chapter 24).

The TEQ is actually a special case of the concept of Relative Potency Factors (RPF), (Appendix Chapter 24) having the general formula

$Cm = {}_{i=1}\Sigma^{n} (c_{i}*RPF_{i})$

Where Cm is the mixture concentration expressed in terms of an index compound for which i=1.

In Sweden, also the PTI, to evaluate pesticides in the national monitoring program, is based on this concept (see Box 8.2).

Box 8.2. Evaluation of pesticides in Sweden considers cumulative exposure

The Swedish Environmental Quality Objective indicator "Plant protection products in surface water" is based on a so called Pesticide Toxicity Index (PTI), originally developed by U.S. Geological Survey within the American National Water-Quality Assessment (NAWQA) monitoring programme. The PTI index is based on the following equation:

PTI= $_{i=1}\Sigma^{n}$ [(Conc of substance i)/(criteria for substance i)] for i=1, 2, 3,n substances measured.

The criteria for individual substances are equal to "guideline values for surface water quality" ("riktvärde för ytvattenkvalitet" in Swedish) developed for active substances in plant protection products by the Swedish Chemicals Agency¹⁶⁹. The index is used to detect trends in cumulative exposure to about 60 substances¹⁷⁰ measured at the same site. However, because the same substances are included at all sites, the higher the index value, the higher the risk of negative effects in aquatic organisms. When used as an "Environmental Quality Objective Indicator", the values are normalised to the year 2002 (the index of this year is defined as 100). However, in the annual monitoring report the index is used without normalisation.

The index is based on summing all values from one year of sampling. Both individual values that are found at concentrations above and below their individual guideline values are included in the calculation. However, those pesticides (ca 10) included in the monitoring program having an analytical limit of quantification (LOQ) above its guideline value are excluded.

Other evaluation systems were evaluated before finaly choosing the PTI (Asp & Krueger 2005) . These include the Canadian "Water Quality Index". This index does not only evaluate the concentration of individual substances that the organisms are exposed to but also the number of substances for which the guideline value is exceeded as well as with how much and how frequently these values are exceeded. The index is originally based on effect based Canadian Water Quality Guidelines (WQG). Thus the following factors are included in the mathematic calculations: F1. Number of substances whose guideline values are exceeded

F2. Frequency of exceedences

F3. Amplitude, based on adding all exceedances

The reason this index was not used as an environmental quality objective indicator is that the index is more sensitive to the number of substances analysed but also less straightforward to explain to a general public than the American index.

Nevertheless, also the PTI will be sensitive to the number of substances analysed and also what substances are analysed. In the case of national monitoring sites in Sweden the substances that are actually used in these areas are well known and therefore relevant substances are analysed. However, it is possible that in the future new substances will be used in these areas. The LOQ for certain substances might also improve, meaning that substances that were so far not included due to their LOQ values being above their guideline values will eventually be included. This could of course then influence the values of the PTI¹⁷¹.

The PTI index was so far used mainly for trend analyses – as an indicator on overall situation on a national scale. If implementing the PTI index on other sites than the four national monitoring sites, for comparison between sites, it is important to analyse the same package, or at least to calculate PTI indices only based on substances that were analysed at all sites.

¹⁶⁹ These values can be found here: http://www.kemi.se/templates/Page.aspx?id=3294

¹⁷⁰ The PTI index is based on about 75% of the measured substances (approximately 80 substances).

¹⁷¹ Pyrethroids historically had LOQ problems, now the LOQ values have improved meaning that these data also could be included in the assessment. So far, they were excluded from the indicator related to the env qual objective but included in the annual national monitoring report (Krueger J, SLU, pers comm.)

8.1.2 Discussion, conclusions and suggestions

PTI and other chemically based indexes based on CA calculations are sensitive to /the number of/ substances monitored. Simply by analysing more substances, the index value will increase. In fact, the calculated PTI will always be "too low" because not all potential substances were analysed¹⁷².

Normalising to the number of substances included in the analysis would not facilitate the interpretation. The development of absolute assessment criteria based on CA concepts for complex mixtures are therefore difficult. The usefulness of the PTI is primarily in trend monitoring and to compare sites.

Because of the "one out-all out" approach used for evaluating chemical data within the WFD context, the development of such absolute evaluation criteria would from a strict regulatory point of view (compliance checking) only be useful for data where individual substances do not exceed their respective EQS values.

A simple approach in complex environments and analogous to the way other factors of uncertainty are handled in the calculation of EQS values within the WFD, the uncertainty related to mixture effects could possibly be handled in the form of an additional assessment factor, that gradually increases with the number of substances that are below but still close to their respective EQS values, but also decreases with the number of relevant substances that are analysed. In the report by Kortenkamp et al (2009), a tiered approach is discussed for the application of mixture assessment concepts, depending on the amount of data available, also including the application of assessment factors at lower tiers.

For regulatory purposes the concept of dose addition is a sufficiently accurate model to predict combination effects of groups of e.g. endocrine disruptors with similar effects (Kortenkamp, 2007), but in order to do so, an extensive data set of analytically measured EDCs has to be available. In most cases this analytical data set is not available and concentrations below detection limits would need to be considered¹⁷³, whereas different types of effect based tools could respond to such low concentration levels.

It can therefore be concluded that at the moment, the only way to truly investigate effects in the environment is by monitoring the effects directly (chapter 9). Nevertheless, a CA based index could be useful also as a trigger for such investigations. In any case, there is a need to validate a variety of approaches by comparing different types of draft absolute criteria with parallel data of effects observed in complex, not entirely defined samples, or even from field observations to also determine the effects from pulsed exposures. Evaluation studies would also provide valuable information about a potential cut off concentration-effect level ratio value, below which the contribution of a substance to the overall effect can be considered to be negligible, at least for regulatory purposes.

¹⁷² However, data evaluated include about 90% of the pesticide substances sold in Sweden.

¹⁷³ See e.g. previous chapter on draft EQS for EE-2, being 300 times lower than LOD

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9 Effect based monitoring tools of hazardous substances

The current chapter describes the usefulness of certain effect based tools to solve some of the limitations identified with an exclusively chemical monitoring approach.

Advantages and disadvantages of different types of effect based tools in a WFD context are described and need for research and validation studies are identified. The purpose of this chapter is not to provide a comprehensive compilation on available individual tests but some tools are described in more detail because of their routine use today. In particular, the report includes conclusions gained during a national workshop organized jointly by the County Administrative Board/Skagerak and Kategat River Basin District Authority and Swedish Environmental Protection Agency within this project¹⁷⁴.

9.1 *Limitations with an exclusively chemical approach*

9.1.1 Unlimited number of substances – unlimited costs

Analysing hazardous substances is generally expensive compared to analysing e.g. nutrients and other physicochemical elements. Not only are the costs per analysis generally much higher, but the lists of substances to monitor tend to get longer. However, as long as there at least are lists of substances to monitor and criteria to compare with, the approach is rather straightforward. In addition, with knowledge about potential sources, non relevant substances from the lists can be deselected from the monitoring programs. Finding local point sources of individual substances in the event of non compliance is also rather straightforward in less complex exposure situations (limited number of sources), at least if the substances are emitted to water and not transformed in the environment.

Within the WFD, also the concentrations of River Basin Specific Pollutants (RBSPs) are to be accounted for in ecological status compliance checking. The RBSPs are not specified on a European level but should be identified at water body/river basin level, and there are no EU wide assessment criteria. Because there are many potential substances that could fulfill the specifications in the nine first substance groups of Annex VIII in 2000/60/EC, to identify a limited list of relevant compounds to monitor is complicated, especially for the 4th and 5th groups (see box 9.1.). Even for "well known" compounds such as PAHs, the chemical anaysis is generally focusing on a very limited number of parent compounds whereas there are actually a very large number of substituted and alkylated PAHs that could cause similar effects¹⁷⁵. There may also not be sufficient toxicological data to evaluate the results. The costs for analysing a large number of potential

¹⁷⁴ "Effect based monitoring tools and assessment criteria". Göteborg, 25th–26th January 2011. National expertise, including primarily researchers but also regulators, consultants and representatives from commercial laboratories, was invited. In order to share experience within some areas less covered by national experts, also international experts were invited. Upon registration, participants were asked to "nominate" at least one monitoring tool that they thought should be considered for evaluation regarding monitoring purposes in the Swedish environment. The workshop is further described in Appendix chapter 25.

¹⁷⁵ Petroleum products such as crude oil generally do not contain primarily parent PAHs but rather e.g. alkylated PAHs (as opposed to creosote, conataining more significant fractions of parent PAHs).

RBSPs and developing a large number of good quality criteria for each individual substance can reach unacceptable levels and workload¹⁷⁶.

Box 9.1. Line 4 and 5 in annex VIII (2000/60/EC)

Substances and preparations, or the breakdown products of such, which have been proved to possess: carcinogenic or mutagenic properties or properties which may affect steroidogenic, thyroid, reproduction or other endocrine-related functions in or via the aquatic environment.

Persistent hydrocarbons and persistent and bioaccumulable organic toxic substances.

9.1.2 Technical analytical difficulties – insufficient LOQ, availability of analytical methods

As was pointed out in chapter 7, for some substances it is difficult to fulfil the QA/QC requirements, in particular for e.g. endocrine disruptive substances due to their comparatively low EQS values.

In the fourth substance group listed in Annex VIII 2000/60, it is pointed out that also breakdown products should be included but in general it would be difficult to know which transformation products to analyse, and there is frequently limited access to analytical tools for such substances¹⁷⁷.

9.1.3 Uncertainties in predicting effects based on chemical data alone – for individual substances and environmental cocktails

Effect based standards are largely based on effects observed in a highly standardized laboratory environment (Box 9.2.). Within chemical testing, other parameters than the chemical concentration are held on an optimal level in order to be able to compare the toxicity of one chemical with another and to be able to use the data within chemical regulation. The co-occurence of other stressors (such as starvation, low/hot temperatures, low oxygen levels etc) are therefore not taken into account. The results also do not take other important parameters, such as pH and organic matter, into account. Such factors can significantly influence bioavailability (and thus effects) of substances in the environment (cf chapter 7 on the assessment of sediment and water bioavailability). The bioavailability aspects can be overcome by analysing contaminant residuals in the tissues of sampled biota. However, this approach would be limited to substances that actually accumulate in the biota analysed and excludes substances that may have an effect without being accumulated.

Concentration levels in toxicity tests are also less variable than exerienced in the field. The timescale of the tests may both underestimate and overestimate the effects observed in reality. Even in chronic tests, the timeline of exposure is usually significantly shorter than what is experienced from substances that are persistent in

¹⁷⁶ Cf with e.g. costs to generate data to fulfill data requirements within the REACH and pesticide legislation.
¹⁷⁷ Broad chemical screening analyses have been useful in this context, to identify potential problematic substances, but concentrations are at best semi quantitative and the sensitivity is often low. In the recently performed screening of sediment, only a few substances other than already well known compounds were found, see chapter 4 for references.

nature (or released continuosly), thus both potential adaptation and effects observed only after several generations of exposure of the organisms are excluded from the assessment.

The standards were also developed for one single chemical substance at a time and therefore do not take into account the cooccurrence of other chemical substances, which is normally the situation. As could be concluded in chapter 8, there are substantial difficulties in predicting effects from combined exposures to the chemical cocktail that aquatic organisms are exposed to in the environment based on chemical data only.

Box 9.2. Traditional approach: substance by substance

A traditional monitoring approach is to analyse concentrations of individual substances in water, sediment and biota, primarily to evaluate trends, but in come cases also to estimate impact.

The first question then is usually what substances to analyse. The sources are seldom well characterised and even for point sources there is very limited information about what substances are actually emitted. One can assume that many of the control measures that have been implemented in the past decades have been very effective in reducing emissions of both measured and non measured substances. Nevertheless, the emission control programs seldom measure single substance parameters other than nutrients etc, but is rather focused on other types of parameters, such as AOX, BOD, COD, TOC, etc¹⁷⁸. The major reason for the operators to measure these parameters is to check compliance related to limit values and to evaluate control measures and not to keep track on the amounts of individual substances released to the receiving environment, although environmental and economic aspects are also considered in the establishment of release permits and emission limit values.

Although the number of current point sources, emitting hazardous subtances into the Swedish environment in significant amounts, can probably be considered to be low in an international perspective, there are a large number of known or potentially contaminated sites from historic activities identified to impose significant additional risks. In addition there are several other local diffuse sources that are less characterised but could release a complex mixture of substances. The Swedish environment is, due to geographic location and climatic conditions, also highly exposed to hazardous substances subject to long distance transport.

When deciding what to analyse, it is important to be aware of the fact that the substances that were emitted may be more or less transformed into other substances. The extent of these transformation processes depend on many factors such as redox potential, available microorganisms, nutrients and trace elements and even the presence of other hazardous substances. Thus, there is seldom just one or a few dominating hazardous substances in the environment but rather a complex mixture of substances that were emitted, along with their transformation products. It is expensive to analyse all these potential substances, and the final choice usually is to select relevant substances that are "listed", in particular those substances for which there is a reporting requirement and quality criteria available. However, this approach tends to create a vicious circle, where unlisted substances are not monitored and therefore are not regulated (stay unlisted). For many of the potential substances present, there is also a lack of analytical methods. It may be possible to identify the substance but in order to quantify it, it may be necessary to first synthetize a standard, which is very costly. Even for well known substances, the analytical techniques used are frequently not sufficiently sensitive

¹⁷⁸ Besides its primary use, to evaluate improvement before and after control measures, such parameters could possibly be used also as part of a more extended assessment for screening purposes, to identify effluents or sites that should be prioritised for further investigations. AOX and EGOM are e.g analysed within WEA programs. There are several advantages with such parameters, primarily by measuring many substances at the same time in a cost effective way. The major disadvantage is that the estimates are rough and the absolute values depend also on the types of substances present. It is not certain that an AOX value from a particular source that is lower than another AOX value from another source actually should be interpreted as the former source emitting less hazardous effluent from an environmental perspective.

compared to the available assessment criteria, further limiting the possibilities to monitor and evaluate the concentrations of these substances in absolute terms.

Assessment criteria can be calculated in different ways, but two main categories can be identified. Criteria based on relative comparisons between sites, identify different concentration levels that could be considered "high", "moderate", "low" etc in a national relative perspective. The highest level could e.g. be based on the 95 percentile of all available relevant data. National assessment criteria in Sweden were so far largely based on this approach, thus not actually estimating effects but rather relative contamination on a geographical scale. Effect based criteria on the other hand aim at identifying "safe levels" in the environment (absolute assessment criteria). Concentrations above these levels indicate that there is a risk of negative effects on the ecosystem, while concentrations below are considered low enough to not cause any harm. Effect based criteria are generally based on laboratory studies performed on standardised test organisms in an optimal environment for the organisms. An assessment factor (usually between 10-1000) is added to the lowest concentration causing effects in the tested organisms to compensate for the differences between the laboratory and real environment, differences in species sensitivity and exposure time. The assessment factor is usually higher for substances where there is little data available (such as lack of chronic effects data and/or data from different trophic levels). In the field, the bioavailability of hazardous substances varies depending on e.g. surrounding pH, hardness, organic carbon content and quality etc. Standardized test organisms have usually been picked because they are easy to culture and to use in a test but they may not be present in the environment that is actually investigated. The effects studied are mainly focused on survival and mobility in acute tests but other endpoints, such as growth and reproduction may be studied in the chronic tests. The longer the tests, the higher the costs will usually be. Thus, there is a major lack of effect data for most substances emitted to the environment, although progress is made with the implementation of REACH to fill some of these data gaps, at least for high volume chemicals. Preliminary effect-based criteria for sediment and biota for potential river basin specific pollutants (RBSPs) in Sweden were previously presented in Naturvårdsverket (2008b). Draft effect-based criteria for sediment for the existing and draft priority substances were also distributed for comments (May 2011)¹⁷⁹.

Sweden has several vulnerable ecosystems, such as the Baltic Sea, further complicating the task to predict effects based on concentration only. The large uncertainties in the present assessment criteria are also reflected in the large span between criteria developed by different countries, even if using the same methodology. Finally, there are more or less no national chemical criteria developed to evaluate absolute levels of chemical mixtures, to at least take additive effects into account (although the PTI index has been developed for pesticides monitored within the national program, chapter 8). Instead each substance is usually tested one by one, monitored one by one and compared to assessment criteria one by one.

9.2 Effect based approaches to encounter some of the limitations of an exclusively chemical approach

9.2.1 Monitoring "unlisted substances"

The group of substances referred to in line 4 in Annex VIII 2000/60/EC would in part be possible to approach using effect based tools, such as tools measuring mutagenicity, endocrine disruption and reproductive disturbances. By monitoring the mode of action itself, instead of all potential compounds possessing this mode of action, the number of parameters to monitor can be significantly reduced, thus saving costs. In particular, there are certain in vitro assays that could be used to monitor the occurrence of genotoxic substances and endocrine disruptors in environmental samples, whereas biomarkers could be used to estimate occurrence

¹⁷⁹ Contact person Maria Linderoth Naturvårdsverket

of substances causing reproductive impairment as well as endocrine disruption and effects from mutagenic substances. The same assay as was suggested to consider for chemical status compliance checking for dioxins and dioxinlike compounds, but with another extraction procedure, could e.g. also be used also to detect less persistent compounds with the same mode of action¹⁸⁰ and with a potential use in identifying RBSPs.

Furthermore, the endpoints specified in draft CIS 27 and considered relevant to assess in developing EQS values for both priority substances and RBSPs, would also be possible to monitor using ecotoxicological tools, either directly (biomarkers) or on environmental samples such as sediment and surface water (bioassays) (box 9.3.).

Box 9.3. Endpoints being considered relevant to take into account in the evaluation of toxicity data for EQS setting (according to CIS 27)

Growth (weight, length, growth rate, biomass) Number (cells, population) Mortality Immobilisation Reproduction Hatching (rate, time, percentage) Sex ratio Development (egg, embryo, life stage) Malformations (teratogenicity) Proliferation (cells) Filtration rate Carbon uptake (algae) Reburial (of e.g. Certain crustacean species)

At least from a scientifically point of view, one can assume that if significant responses to these endpoints in the environment (biomarkers) or on samples (bioassays) are observed, such effects would be just as relevant (or even more relevant) to consider in the assessment of effects to the ecology. Whether this approach would be possible to adopt from a legal point of view (the exact identity of causing substances may be unknown and it is therefore not possible to refer to specific RBSPs) in ecological status classifications should therefore be investigated. If certain effect based data would be possible to use in expert judgement assessments in the future, guidance is necessary to facilitate harmonisation.

However, there are also limitations in analysing at least some of these latter endpoints. In particular, lethality in short term bioassays would normally not be

¹⁸⁰ This assay is often referred to as PAH CALUX, and the response is expressed in the unit Benzo(a)pyrene equivalents. However, the suitability of comparing this value to the current EQS for benzo(a)pyrene would need to be discussed and would depend on the most critical mode of action for setting the EQS criteria. The EQS for benzo(s)pyrene is at the moment being revised and it could probably be more relevant to compare PAH CALUX results to the future EQS for the sum of the five specified PAHs in a screening approach. The costs of PAH CALUX is normally approximately the same as the chemical analysis of these five individual PAHs (along with other PAHs), so its not recommended for chemical status classification for PAHs. However, if both DR and PAH CALUX are analysed on the same sample, cost reductions are usually possible. If the effect based response is significantly higher than PAH EQS for the five parent PAHs, one can assume that other PAHs could also be present in significant amounts (RBSPs).

expected in surface water samples, unless the water is severely contaminated. Nevertheless, the concentrations of hazardous substances are generally higher in effluents than in receiving waters. Several somewhat less sensitive effect based tools can therefore be useful (and are being used) in the context of whole effluent assessments (WEA) rather than in environmental monitoring. If effects are observed and predicted to cause a risk to the receiving environment, such data would be useful in establishing toxicity based release permits (also suggested in the COHIBA project, see chapter 3). WEA tools can also be useful in retrospective studies, to follow up on effects observed in the field to idenfity sources without actually knowing exactly what substances are emitted.

9.2.2 Analysing rather than predicting effects

It is important to detect effects from hazardous substances at an early stage. If observing effects from hazardous substances already on population level of higher trophic levels (mammals, birds, predatory fish), damages can take a long time to repair, due to the persistency and wide geographical distribution of many hazardous substances. In addition, recolonisation of certain species that have become extinct can take a very long time, much longer than the time frames (6 year management cycles) to consider in the WFD. An alternative to an exclusively chemical approach to estimate the effects from hazardous substances, would be the measurement of effects directly in the field. Such a strategy is tempting for many reasons. Fractions that are not bioavailable will be excluded, whereas the combined exposure of the full range of substances available will be accounted for. Effects that only occur after several generations can also be identified and including combined effects from exposure to other stressors (such as lack of feed) at a particular site. However, although the WFD is already focused on ecological effects, the biological quality elements included so far do not specifically monitor the effects from hazardous substances (Box 9.4.).

As an alternative, biomarkers and/or other relevant tools could be used, at least from a scientific point of view. Several ecological relevant endpoints can be monitored by using biomarkers, such as imposex and reproductive success in eelpouts. Also other biomarkers monitoring general or specific effects (rather than primarily exposure), such as lysosomal stability and ALA-D, can be useful in this context to indicate risks of effects. These tools are also useful as 2nd tier studies to provide additional lines of evidence in situations where there are large uncertainties in the chemical status classification. Such evidence is valuable decision support before e.g. costly remediation is considered (see chapter 7). Again, from a scientifically point of view, it can be assumed that if ecologically relevant responses are observed, ecological status should be moderate due to RBSPs using expert judgement, even if the individual RBSPs cannot be specified.

There are also several biomarkers, such as VTG, for which a significant response does not necessarily suggest negative effects. Such tools are rather valuable as early warning tools or as part of a weight of evidence approach. This suggests that if a significant response is not observed, it is probably safe to conclude that further studies of the compounds known to elicit this type of response do not need to be prioritized. For these purposes, assessment criteria based on baseline level response (background levels) would be useful.

If effect based tools are to be used, with expert judgement, within ecological status classifications, it is imperative that such use is harmonised, to prevent a situation where one river basin district considers even single exposure biomarkers sufficient proof of "non compliance" whereas another district would consider such response as only relevant for at risk/impact assessment purposes. From a scientific point of view, batteries are always preferred rather than single biomarker analyses and can be used in a weight of evidence approach, and an assessment scheme such as the proposal recently presented fish biomarkers (see appendix 29) would be a valuable guidance tool.

Box 9.4. Current biological assessment criteria do not respond to stress from toxic substances

According to art 2 of the WFD, the definition of "Ecological status" is an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters, classified in accordance with Annex V (cf chapter 3 of this report). With these biological quality elements, the classification will largely be based on community structure rather than function, although the definition of ecological status in the WFD clearly also includes function.

The current national assessment criterira for biological quality elements to be used in ecological status classifications are generally not considered to measure stress from toxic substances but rather focus on detecting effects from eutrophication and acidic conditions (Naturvårdsverket 2007). The BQI (Benthic Quality Index) is e.g. sensitive to the proportion between tolerant and sensitive species, number of species and abundance (Appendix chapter 26) but primarily would be expected to respond to eutrophication stressors. Also the fish indices developed (VIX, "Vattendragsindex" for rivers and EQR8 for lakes) rather would be expected to respond primarily to other factors than hazardous substances.

The possibilities to develop new formal biological quality elements for ecological status classifications needs to be considered, bearing in mind that they should, according to the definitions in the WFD, be based on effects on higher organisational levels.

9.3 Different types of effect based tools for different purposes

In order to illustrate advantages and disadvantages related to different effect based tools for certain purposes, a categorization of the types of effect based tools is necessary. The usefulness of different types of tools and interpretation of data depend on e.g. the organisational level and species investigated, exposure time, endpoint studied, whether effects are studied in the field ("in situ") or in the laboratory and choice of compartment studied (box 9.5). Effect based tools are in this report divided into four main categories: in vivo and in vitro bioassays, biomarkers, and tools that directly measure effects on community level. Tools that investigate effects at population and community levels have much similarity to current biological quality elements, whereas biomarkers and bioassays are ecotoxicological tools.

Box 9.5. There are many ways to monitor effects and many types of effects to monitor

A dutch report on the use of "ecoassays", having a more general definition¹⁸¹ than what is considered "effect based tools" in this context, identified such assays to be promising tools to identify causes for non ecological status compliance and to investigate suitable measures to improve status (Rijkswaterstaat 2005). Within ecotoxicology there are a multitude of species to study and effects can be studied on different organisational levels (subcellular – cellular – organ/tissue – individual - population – community – ecosystem level) and with different time frames of exposure and exposure paths.

However, the different type of effect based monitoring approaches can roughly be divided into 3-4 such categories: tools measuring effects on higher organisational levels such as populations and communities, biomarkers that measure effects on individuals (and suborganismal levels) in the field, and finally bioassays, measuring effects on environmental samples (as opposed to toxicity tests measuring effects in a chemical concentration series). The last category can be subdivided into two categories, being either based on in vivo whole organism bioassays or on in vitro assays based on responses observed when exposing cell lines.

Bioassays are defined as tools that measure effects when living organisms (in vivo) or cells (in vitro) are exposed to samples from the environment. In vivo bioassays have much in common with toxicity test protocols, that were developed for chemical regulation purposes (such as the 24h Daphnia magna test). In many cases the same test protocols are actually used although for the purposes of measuring effects from environmental samples, dilution series are frequently not used. Many of the in vitro assays originate from human toxicity screening tests also used for chemical regulation purposes. Some in vitro assays can also be considered bioanalytical tools and results can more or less be treated in the same way as chemical analytical data, in a way that the response can be expressed in chemical equivalents and therefore evaluated in the same way. A prerequisite is that the assay is sufficiently sensitive and specific and that the criteria to be used for comparison is actually primarily based on the mode of action being studied in criteria setting.

Biomarkers and community level tools only respond to the substances that are bioavailable in the real field situation and have caused at least some physiological alteration. For simplicity in this report, also endpoints monitored on single field exposed organisms (organism level) are considered biomarkers. Thus, also e.g. frequency of malformed and dead embryos of the viviparous eelpout are considered biomarker tools as well as Monoporeia embryo deformations. A more common definition would be to only include tissue, cellular and subcellular effects.

Thus the categorization into in vivo, in vitro and biomarker is rather related to type of monitoring approach rather than type of effect or mode of action (endpoint). To illustrate, if analysis of EROD induction is performed on liver samples from wild caught fish, EROD would be a biomarker. Whereas the study of EROD induction in liver cells cultured in the laboratory, and exposing them to samples collected in the field or from effluents, EROD would be an in vitro bioassay. Finally, if studying EROD induction on liver samples from fish kept in the laboratory and being exposed to field collected samples, would be called an in vitro analysis but within the context of an in vivo bioassay. The borders are not always clear. Analysing EROD induction in liver samples from caged fish in the field, would still be called a biomarker in this report but the study is performed as an in situ bioassay. Other combinations exist, such as the monitoring of organisms in the laboratory but exposing them to a flow through system based on continuous field sampling (e.g. the commercial toximeters that exist for fish, daphnia and algae and that are used as alarm systems to protect drinking water).

¹⁸¹ Ecoassays do not only refer to tests to investigate effects related to toxic stress but also other factors such as those related to hydromorphology and ecology.

9.3.1 In vitro bioassays

The so called *in vitro* bioassays focus on measuring effects on lower organisational levels (such as receptor induction, DNA damage etc). However, instead of investigating cells from tissues of organisms that were exposed in the field or from caged whole organisms (as is the case with biomarkers), the effects are studied on cell lines after exposure to environmental samples.

Therefore, an advantage is that, in vitro bioassays can frequently be performed on any¹⁸² matrix (such as /extracts of/ surface water, sediment and pore water, biological tissues, passive samplers and effluents). Only low amounts of sample (grams) is generally needed and the exposure time is generally short compared to the time needed in an in vivo assay to detect a response from the same mode of action. Many in vitro assays are therefore suitable for high throughput and automated applications. In vitro assays are therefore suitable to follow up biomarker results from field, for screening purposes and can easily be used to track local pollution sources by analysing water, sediment, effluent etc in a gradient (investigative monitoring). In vitro assays are also very valuable in EDA/TIE approaches to identify toxic fractions and guide in identifying causative agents.

In most cases, a response in an in vitro assay is sensitive and specific, because it measures effects on a low organisational level and as opposed to chemical analysis it responds to all substances that have the same mode of action. They can therefore be used as "early warning" tools, and be used for screening purposes. As opposed to in vivo bioassays and biomarkers, the system studied is highly simplified compared to the complexity of whole organisms. Thus the interaction between different receptors, cells and organs is not studied and can only be accounted for by studying effects on whole organism level. As in chemical analysis and as opposed to biomarkers measured on field exposed organisms providing more integrated responses, one can only detect effects from substances that are present in the sample and bioavailablity is difficult to assess unless analysing biological tissues¹⁸³.

Today, there are very large numbers of in vitro bioassays available. To cover all or evaluate available individual assays is out of the scope of this report. Nevertheless, assays that have been initially selected for toxicity characterisation and EDA in the MODELKEY¹⁸⁴ project (Thomas 2006), as well as in vitro assays that were recommended by COHIBA (2010)¹⁸⁵ for whole effluent assessment and

¹⁸² Although in vitro bioassays can be used on any matrix /extract/, some are more suited for the assessment of certain matrixes than others, in part because they were so far only validated for certain uses, but also because relevant substances that elicit certain types of responses primarily are found in certain compartments.

¹⁸³ On the other hand, in these cases, if measuring a hormonal response such as "endocrine disruption" care should be taken in the interpretation of in vitro assay data because of the possibilities to detect effects from endogenous hormones rather than xenobiotics. Furthermore, effects from substances that are available but do not accumulate, are difficult to detect by chemical analysis as well as in vitro assays (as opposed to biomarkers measured on field exposed organisms).

¹⁸⁴ http://www.modelkey.org/

¹⁸⁵ COHIBA. 2010. Whole Effluent Asssessment (WEA). Proposed recommendations for the use of toxicity limits. COHIBA (Control of Hazardous Substances in the Baltic Sea Region) WP 3. Coordinator Finnish Environment Institute (SYKE). September 2010.

genotoxicity assays recommended by OSPAR (2002)¹⁸⁶ (both for whole effluent assessment and surface water monitoring), recommended and promising tools according to ICES and assays that were nominated for monitoring purposes during the Göteborg workshop are included in table 9.1.

Table 9.1. In vitro assays that were nominated for monitoring purposes during the Göteborg Workshop (W), recommended for WEA assessments by COHIBA (C) or OSPAR (O), and initially selected for evaluation regarding high throughput screening and EDA purposes in the MODELKEY project (M¹⁸⁷) as well as recommended (I-R) or considered promising (I-P) by ICES

	Workshop/CO HIBA/OSPAR/	Mode of action/endpoint	Standard, if available
	Modelkey/ICE		
Name/s of assay	S		
	W, M , I-P	Androgen receptor	
AR CALUX (anti-)		(activation or blocking)	
DR CALUX	W, M , I-R	AH receptor binding	
ER CALUX ¹⁸⁸	W, M , I-P	/Alpha and beta/ estrogen	
(anti-)		receptors	
GR CALUX (anti-)	W	Glucocorticoid receptor	
PAH CALUX	W, M ¹⁸⁹	AH receptor binding	
PR CALUX	W	Progesterone receptor	
Acetylcholinestera	W	Inhibition of	
se inhibition assay		acetylcholinesterase activity	
Carboxylesterase	W	Inhibition of	
inhibition assay		carboxylesterase activity	
Ames	W, M , O	Genotoxicity: Mutations ¹⁹⁰	ISO 16240, 2005; DIN 38415- 3, 1999 (T98 and T100 strains)
	W, M , C	SOS response to DNA	ISO13829, 2000.
umuC	,, e	damage ¹⁹¹	
	W, M	Competition with thyroid hormone for binding to TTR	
TTR-binding		(transport protein)	
TRb CALUX	W	Thyroid receptor beta	
EROD	С	EROD induction	ISO/TS 23893-2, 2007
YES	C, M , I-R	ER receptor	
YAS	C, M , I-R	AR receptor	
P-53 accumulation	$(M)^{192}$	Genotoxicity	
Green screen	(M) ¹⁹³	Genotoxicity	

¹⁸⁶ The OSPAR Commission (2002) recommends a test battery of bacterial assays (umu C or SOS chromo assay and Ames) and eukaryotic cells (micronucleus or Comet assay) for WEA.

¹⁸⁷ If "M" is typed in bold, the assay was considered to be useful for both water, sediment and tissue bioassays.

 ¹⁸⁸ There are actually two different types; ER and ERalpha, depending on the receptor activated/inhibited.
 ¹⁸⁹ Considered suitable for sediment and tissues

¹⁹⁰ Responds to reactivation of bacteria (Salmonella typhimurium) that can grow without histidine. Frequently used within WEA, german standard for this purpose. Microplate tests are available. TA 98 measures frame shift mutations; TA 100 point mutations.

¹⁹¹ The umuC assay measures the induction of the bacterial DNA reparation system (SOS) and is based on the reporter gene lac Z (beta galactosidase is formed). The assay is routinely used within WEA in Germany and there is also a German standard available (DIN 38415-4;1996). The test variant described by Grummt et al 2000 on surface water samples could also detect genotoxicity of surface water samples from four locations in the Elbe and Rhine.

¹⁹² Considered suitable for sediment assays only

Name/s of assay	Workshop/CO HIBA/OSPAR/ Modelkey/ICE S	Mode of action/endpoint	Standard, if available
RYA	М	ER receptor	
ABC assay	М	Antibiotic activity	
Micronucleus test (V79)	С	Genotoxicity: Damage to chromosomes or mitotic apparatus	ISO 12427-2
Vitellogenin induction test	С	Vitellogenin production	ISO/WD 23893-3, 2009 (under development)
PPARy2 CALUX (anti-)	(W) ¹⁹⁴	Peroxisome proliferator activated receptors (PPARs)	
Comet Assay	I-P, O	Genotoxicity: DNA damage monitored directly ¹⁹⁵	

The frequently used commercial CALUX (Chemical Activated Luciferase Gene Expression) panel is based on a reporter gene approach and the assays produce light when exposed to substances that induce certain pathways, such as Ah or estrogen receptor (ER) binding. The molecule-receptor complex binds to specific DNA sequences (called "responsive elements"), triggering the expression of certain genes, in turn giving rise to the toxicological response. Also yeast cell based assays, such as YES and YAS (recombinant) are being used more frequently within monitoring.

Several literature reviews are available, that include also other assays than the ones mentioned above. Kinnberg (2003) evaluated several in vitro assays, including the YES/YAS, ER CALUX¹⁹⁶ and E screen assays, for determination of estrogenic activity in the environment. Leusch et al (2010) evaluated five oestrogenic assays regarding their usefulness in monitoring. The use of genotoxicity assays for environmental monitoring purposes (surface waters) was evaluated by Grummt et al 2000, and positive water samples were most frequently identified using the Comet assay although genotoxicity was also identified by new test variants of Ames and UmuC. A validation study of tools to determine estrogens in sewage treatment effluents, including E-screen, were also performed by the NORMAN network (NORMAN 2008). Behnisch et al (2001) reviewed different areas of applying in vitro assays in screening studies of dioxin and dioxinlike compounds. Lilja et al (2010) recently reviewed some of the available in vitro bioassays related to genetic effects and endocrine disruption regarding their usefulness in STP water monitoring.

Within the context of contaminated sites, national assessment criteria have been proposed for Microtox¹⁹⁷, UMU C and EROD (see Appendix, chapter 28.1). Norwegian national guidelines on risk assessment of contaminated sediment include also assessment criteria for DR CALUX (SFT 2008).

¹⁹³ Considered suitable for water assays only

¹⁹⁴ Not evaluated during the workshop but included in the discussion.

¹⁹⁵ By staining DNA from eucaryotic cells, exposed either in vivo or in vitro (permanent cell lines, frequently human hepatoma Hep GS) (OSPAR 2002).

¹⁹⁶ The initial ER CALUX assay, not alpha version which was developed at a later stage.

¹⁹⁷ Microtox is a bacterial assay, and could actually be considered both in vitro and in vivo assay

As was pointed out in chapter 7, certain in vitro assays could also become useful tools for chemical compliance checking. In this respect, such in vitro bioassays could be considered bioanalytical tools and the results (expressed in terms of a chemical equivalent) in principle be directly compared to the EQS of the substance /group/, at least for screening purposes. One should however be aware of the fact that a chemical analysis only includes those substances that are actually measured, whereas in vitro bioassays may respond to other substances with the same cellular mode of action. Thus, the effect based signal can be expected to be higher than the chemical signal. Furthermore, the possibilities to compare the results directly with the corresponding EQS other than for screening purposes varies depending on the basis for the EQS value (whether the EQS value was developed for the same mode of action).

In particular, two in vitro assays, YES and ER CALUX (or the non commercial test system T47KBlueC) are frequently being considered suitable options to chemical monitoring of EE-2, E-2 and E-1 (for which current analytical technologies do not fulfil QA QC requiements for acceptable LOQ levels, see chapter 7). Unlike the chemical analysis, the in vitro assays would detect these compounds at sufficiently low concentrations¹⁹⁸. However, care should be taken in the interpretation, because EE-2 and and E-2 are equally potent in the in vitro assay, whereas EE-2 has been shown to be up to 27 times more potent in vivo (due to differences in biological half time, binding to transport proteins etc) (Thorpe et al 2003). This makes the assays less precise in ranking the "expected real effects" at different locations, based on such data alone because a different relative composition at different sites would cause different levels of response in vivo but not necessarily in vitro. Also, final check of compliance against EQS (quantitative analyses) for a certain substance (e.g. EE-2) based on in vitro test data is only possible if aware of the relative conconcentrations of other constitutents present in the sample and with the same mode of action. Nevertheless, if adopting a "worst case" interpretation of the results, the in vitro approach could probably be used on screening level. An alternative would be to develop EQS values for estrogenic substances that are based on the sum and relative contribution of such substances (cf TEF concept for dioxinlike compounds). Tier 2 studies using other tools could be considered, if data indicate that concentrations are above EQS, to confirm non compliance based on a weight of evidence approach, in particular if costly control measures would be considered necessary.

Chapter 7 identified also the potential to utilize AH receptor binding *in vitro* bioassays to detect dioxins and dioxinlike compounds at substantially lower costs (approximately 1/4th) than chemical analyses, and such an approach is already accepted within regulation related to dioxinlike compounds in food.

¹⁹⁸ The ER -Calux system and the non commercial T47KBlueC provide a high sensitivity and are able to detect estrogenic potentials directly in surface water with a LOD/LOEC range of 80-130pg/l for E2. The YES assay is a bit less sensitive with a LOD /LOEC of 490pg/l E2 but has its advantages in a high practicability and high robustness also for waste water asseessements. The estrogenic potential could be backcalculated with the dilution factor in surface water for indicative monitoring purposes. Robert Kase, Swiss Centre for Applied Ecotoxicology pers comm

9.3.2 In vivo bioassays

Most test protocols for in vivo bioassays were originally developed for the toxicity testing of individual substances for chemical regulatory purposes. The most common endpoint in short term tests is probably lethality and immobility. With chemicals toxicity testing the concentration in water is usually increased until a full response is observed, only restricted by the solubility limit of the chemical. Therefore concentrations tested are usually far above environmentally relevant concentrations. Sublethal endpoints such as growth and fecundity can occur at lower, more realistic concentration levels but require long term testing to be performed, which is far more costly. Many organic substances will accumulate in sediments and there are also test protocols developed for sediment toxicity testing after spiking the chemical. Some of these were also used for sediment bioassays, to analyse environmental samples. Organisms are being exposed either through the gills from the pore water (often considered the most important pathway) or from direct gut uptake. Therefore, sediment in vivo bioassays are performed by either exposing the test organisms to whole sediment, pore water or elutriates.

Most in vivo bioassays are performed in the laboratory on cultured organisms but there are also examples of in situ testing, e.g. using caged fish. OSPAR guidelines for in vivo assays¹⁹⁹ include the following in vivo bioassays: whole sediment *Corophium* acute bioassay (intercalibrated), as well as pore water bioassays using either oyster embryos (abnormal development), harpactoids *Tisbe* or *Nitocra* (mortality, reproduction) and polychaete *Dinophilus* (life cycle test). Assessment criteria suggested for certain in vivo assays in the context of evaluation of contaminated sites can be found in the the Appendix chapter 28. Norwegian national guidelines on risk assessment of contaminated sediment include also assessment criteria for algal toxicity (*Skeletonema costatum*) (SFT 2008). A review of primarily in vivo bioassays for the assessment of sediment toxicity was performed by Nendza (2002).

There are normally parallel test series using reference substances. However, the mode of action of a particular reference substance²⁰⁰ does not necessary exhibit the same mode of action as the chemical being tested, because in vivo bioassays generally do not respond specifically to a single mode of action. Therefore the results cannot be expressed in terms of a chemical equivalent as for many in vitro assays. For in vivo bioassays it therefore has to be agreed what would be an acceptable response, and this would depend on the endpoint being monitored and the purpose of the analysis.

¹⁹⁹ <u>http://www.ospar.org/content/content.asp?menu=00900301090135_000000_000000</u>

²⁰⁰ The purpose to test a concentration series of a reference substance in parallel to a concentration series of the substance to be tested is to check the general health of test organisms. Many test organisms are sensitive to changes in culture conditions and handling. Such a parallel test series is often called a "positive control" and the absolute value should lie within a specific range. In Daphnia manga toxicity tests there is e.g. normally a parallel test of sodium dichromate toxicity to make sure that the sensitivity of the individuals are within the normal range. This facilitates relative comparisons between different chemicals even if tests were performed by different laboratories. There are also negative controls, where organisms are only exposed to dilution water. Some response in the control can usually be accepted. In acute in vivo bioassays, 10% response is usually accepted but for chronic assays this criteria is usually higher. However, if larger responses are observed, the test is generally considered invalid.

In vivo bioassays have also been used within Swedish whole effluent assessments (WEA) since the early 80s. In vivo assays were so far not included in any regular Swedish monitoring programs, but several were used in research projects. A test battery based on Nitocra spinipes and fish embryo bioassays was suggested to be used as a prioritisation tool in assessing the needs for sediment remediation in Naturvårdsverket (2006)²⁰¹. There are also assessment criteria available for algal growth and the mussel bioassay for the assessment of contaminated sites (Appendix chapter 28.1). The in vivo assays that were nominated and for which there are available questionnaire responses during the Göteborg workshop are bioassays performed on Nitocra spinipes, Ceramium tenuicorue, Fish embryo toxicity, Daphnia magna, Bacterial luminescence - Vibrio fischeri and Fish sexual development test.

For in vivo bioassays, samples can frequently not be tested in dilution series²⁰²... Instead samples are taken from reference locations (upstreams) in order to evaluate statistically significant differences between these locations, keeping in mind that what may be a statistically significant effect may not necessarily be an ecologically relevant effect²⁰³. This is a matter for expert judgement, but depending on the endpoint being analysed it would be possible to recalculate the effects observed into effects that can be expected to occur with time on population levels. Results e.g. expressed as "It is likely (80% confidence) that the fish population at location A will be reduced by 20% within X generations, compared to location B because of a difference in water quality" would facilitate interpretations from a management perspective. However, results are generally not expressed in this way. One needs to be aware of the fact that in vivo bioassays are normally being performed on cultured organisms (and in the case of Daphnia magna, special clones are used, i.e. the individual variation is very limited) and under optimal conditions. Also, if translating results from bioassays being performed on organisms having different life traits, one also needs to be aware of the different consequences from an observed effect. R and k strategist populations would e.g. respond differently on population level from the same percentage inhibition.

9.3.3 Biomarkers

Biomarkers are in this report defined as tools that investigate effects on suborganism levels, such as tissues, cells and subcellular levels, in organisms that were exposed in the field. As with in vitro assays, there are large numbers of biomarkers available and not all can be described in this report.

Biomarkers can be divided into different categories according to the ecological relevance of the endpoint monitored. Examples of highly relevant endpoints include

²⁰¹ The battery was evaluated on three contaminated areas (Frierfjorden, Örserumsviken, Riddarfjärden) and two reference areas (Björkskär och Slingsviken).

²⁰² Preconcentrataion (XAD extraction) of water samples is possible to increase sensitiivity of the analysis. The rivers Meuse, Scheldt and Rhein are sampled every second month and the test battery includes Microtox, PAM algae test, Thamnotoxkit, Daphnia IQ and Rotoxkit assays. (Durand et al 2009). Potential drawbacks with the preconcentration of samples include the risk of losing some substances, such as metals and some insoluble substances reach saturation sooner than others.

²⁰³ In addition, significant differences between the responses observed at two locations depend on the number of replicates that were analysed. With a large number of replicates, it is easier to detect differences.

reproductive success in the viviparous eelpout and imposex. Others also can be considered to monitor effects (such as lysosomal stability) but the results can be more difficult to interpret in terms of population level effects. Finally, some endpoints rather analyse e.g. the induction of detoxification mechanisms (such as EROD) and would rather be expected to be useful as early warning tools of reduced fitness.

To clearly distinguish and categorize different biomarkers into those that should be considered to be effect biomarkers and exposure biomarkers is not always easy and with new knowledge, the view can change. Appendix chapter 28.2. contains proposed assessment criteria for some individual biomarkers, although most are based on relative comparisons between baseline levels "background response" and levels observed at impacted sites and they are therefore difficult to interpret in absolute ecological terms, at least on an individual basis. Biomarker batteries are therefore preferred and all biomarkers are valuable in a weight of evidence approach. Appendix chapter 29 also includes a proposed (unpublished) approach to integrate the response from fish biomarker batteries used in the Swedish marine fish monitoring program by a weight of evidence approach. Based on the suggested weights of different markers as well as their categorisation into different functions (and the weights of these functions), a preliminary interpretation on how to interpret the degree of ecological relevance of different biomarker tools can be made. The assessment scheme proposed is to be used for biomarker batteries but does not necessarily imply that all biomarkers need to included, but could vary between locations.

Biomarkers can also be divided into specific and general biomarkers, depending on the number of substances/groups of substances the tool would expect to respond to. Imposex (Box 9.6.) e.g. is a very specific biomarker, whereas lysosomal stability is a more general biomarker. Both types of biomarkers can be useful within monitoring depending on the purpose and how much is known about the type of contaminants.

Box 9.6. Imposex – both specific, sensitive and ecologically relevant biomarker

Imposex is the imposition of male sex characteristics on females (whereas intersex can involve organisms with both male and female characteristics). TBT exposure displays a dose-response relationship with imposex physiological progress (Gibbs, P. E. & Bryan, G. W. (1994)²⁰⁴. Common endpoinds are VDSI (an index for penis and vas deference development) and Relative penis size index (RPSI). Several molluscs are highly sensitive and in Sweden, Nassarius nitidus (West coast) and Hydrobia ulvae (Baltic) are included in regular monitoring programs²⁰⁵. RPSI could be influenced by season, but VDSI is not considered season sensitive. However, they could be difficult to find during cold winter conditions. The biomarker is not only specific and sensitive, it also provides indications of effects on individual level which can be used to make inferences on populational level.

²⁰⁴ Gibbs, P. E. & Bryan, G. W. (1994). "Biomonitoring of tributyltin (TBT) pollution using the imposex response of neogastropods molluscs". *In* Biomonitoring of Coastal Waters and Estuaries. Kramer, K.J. (Ed), 1994. CRC Press Inc. Boca Raton, p: 205-226

²⁰⁵ Swedish contact persons are Marina Magnusson and Åke Granmo, Marine Monitoring

For certain substances it is important to consider which species is the most appropriate. To study three trophic levels is generally the preferred approach in chemical risk assessments and could certainly be considered also in a biomarker battery. However, one needs to be aware of the differences in sensitivity between species also from the same trophic level, in particular for substances with specific modes of actions. The specific effects from EE-2 shown in fish do not seem to be present in crayfish but was observed in molluscs and frogs also at environmentally relevant concentrations (based on laboratory data) (Jobling et al 2003; Petterson & Berg 2007).

In Appendix, chapter 27, "fact sheets" are included for several biomarkers that are used on a routine basis in the marine programs and for which there are responses to the workshop questionnaire available. By listing important aspects that need to be assessed before considering to include them in a monitoring program these fact sheets can hopefully provide some guidance on their usefulness and limitations. Although the biomarkers described have previously been used primarily in the marine environment, many would be possible to apply also in the limnic environment (Sternbeck et al 2008).

A limnic biomarker tool that is gaining in popularity on regional level is the monitoring of malformed diatoms. One contributing factor is that the additional costs to also include the assessment of malformations are low if samples are anyway collected and analysed to investigate effects from eutrophication. Another limnic biomarker that would be possible to asses in a coordinated program of both eutrophication and effects from hazardous substances is mentum deformations in chironomids. This analysis has been in use for several decades but some validation studies would be necessary (box 9.7.). Monoporeia malformations, found to respond to hazardous substances, are already part of a national marine monitoring program that includes also other parameters that would respond to other stressors such as low oxygen levels and the program is coordinated with benthic community effects investigations. It was so far primarily used in the marine environment (Baltic).

Box 9.7. Coordinating the monitoring efforts to investigate effects from nutrients and hazardous substances in a cost effective way.

Diatom malformations

As opposed to fish and invertebrate biomarkers, there are no biomarkers for plants being used on a regular basis. However, in e.g. France, UK and Italy there is ongoing research on malformations in diatoms. There is also an ongoing project in Sweden to develop an index to be used for Swedish conditions for the monitoring of effects from hazardous substances²⁰⁶. A major advantage using these organisms for the study of effects from hazardous substances is that diatoms are frequently sampled to assess nutrient²⁰⁷ impacts and acidity in streams and partly lakes, so adding an analysis of deformed shells does not significantly add to the costs²⁰⁸. Also, not only the deformed shells can be analysed but also community changes seem to be influenced by the presence of metals and other pesticides (Falasco et al 2009 and Rimet et al 2011). Because Sweden has a different climate and different diatom flora than central Europe where the other studies about toxic impacts on diatoms are performed, there is a need to develop an index for Nordic conditions. The analysis

²⁰⁶ Project coordinator: Maria Kahlert, SLU. See also Jan-Ers, L. (2009).

²⁰⁷ Indices to assess nutrient impact are based on species abundances and specific affinities for organic/nutrient concentrations

²⁰⁸ Approximately 100 Euro per sample in additional costs

should be performed in autumn. The analysis of diatom shell malformations also on existing material is possible and the analysis can be performed on a commercial basis. Although malformations has been shown to be linked to metals and pesticides (see e.g.Cattaneo et al 2008 and Falasco et al 2009) the sensitivity to different types of hazardous substances needs to be investigated. Several Counties have recently included the analysis in parallel to analysing metals and pesticides (primarily using passive samplers), within a project to develop indicators (for work with environmental quality objectives).

Mentum malformations in chironomids

Mentum (mouthpart) deformations in larvae of chironomids have been found to correlate with the degree of pollution of lakes (Wiederholm 1984) both in a geographical context and investigated in a historical perspective by analysing subfossil material, 100 years or more of age, and comparing results to more recent situations. Deformities were found in species with different mode of living, including sedentary, filter or surface feeding larvae. The results of these studies are expressed as % malformed chironomid larvae and was found to vary between 0,8% (from subfossil material) to 25% (from strongly polluted lakes) in the study by Wiederholm (1984).

Although there are no known test protocols and assessment criteria available, the publication by Wiederholm (1984) provides illustrations of several types of malformations and the data compiled suggest that the baseline level would probably be just a few malformed chironomids per one thousand sampled individuals. The data also suggest that incidences of up to a few % of malformed larvae would indicate reference conditions or low pollution levels. Up to about 10% malformations could be interpreted as moderate pollution and higher incidence suggests high pollution. Mouthpart deformations of chironomids can be studied in lakes but will be limited to areas with relatively high abundances, because a relatively large sample size (hundreds of individuals) need to be analysed. These areas can primarily be found in soft sediments with high carbon content, and the best season to find large larvae is during early spring or late autumn²⁰⁹. Retroactive trend studies of mouth parts are possible by using sediment cores. In Sweden, mentum deformations in chironomids were also studied at contaminated sites such as the Viskan area (Ericsson 2002) and Kolbäcksån. Although the exact frequences were not analysed, mouth deformations were also detected at Bengtsbrohöljen in Stictochironomus sp., Tanytarsus sp. and Polypedilum sp. (Ericsson & Vaught 2000)

In the study by Wiederholm, deformations were mainly suspected to be due to heavy metal contamination, but also occurred in areas that were influenced by other types of contaminants. So far, primarily metals were measured at sites where mentum deformations were studied, and it is possible that other contaminants that were not measured also contributed to the effects. Thus, it would be useful to evaluate whether areas contaminated by primarily organic contaminants also respond in the same way or if this biomarker can primarily be used for metal contaminated environmments. The ecological relevance is unclear. The deformed individuals could be suspected to have increased difficulties in cracking the shells of diatoms. A study measuring e.g. turnover time in the guts could confirm such a hypothesis. The impact of such effects could prolong the generation time and thus population level effects. In the study by Wiederholm, population effects were also observed at the same sites where high deformation frequencies occurred. The analysis is not considered complex but specialist knowledge in taxonomy is required.

The mentum deformations could be investigated in a coordinated program to also determine BQI of lakes (with soft sediment).

Monoporeia malformations

In the national marine program on Monoporeia, several parameters are included, such as fecundity, malformations, and embryo lethality. In particular the parameter malformation has been shown to be very sensitive to slight increase in metal and organic hazardous substance levels (Sundelin & Eriksson 1998), whereas dead eggs and undeveloped embryos are correlated to secondary eutrophication effects (lack of oxygen and increased water temperature). Although primarily studied in the Baltic so far, studies were also performed in the lakes Vänern, Vättern and Mälaren. The marine program is coordinated with studies of benthic community abundance. Both Monoporeia and eelpout reproductive disturbances are suggested to become HELCOM core indicators and

²⁰⁹ Willem Goedkoop SLU pers comm..

assessment criteria have been suggested, and EAC levels are under development to identify levels of malformations that are related to reduced population levels. The current program is largely performed in two areas (focused on trend monitoring rather than to cover large geographical areas) but a revision of the program is ongoing, to include more stations and possibly to coordinate with the current coordinated fish monitoring programme.²¹⁰

9.3.4 Community level effects

Although the methodology to investigate effects on population and community levels is sparse regarding the effects from hazardous substances, a recently developed index, called SPEAR, is based on relative sensitivity of invertebrates to hazardous substances. The SPEAR index measures the proportion between sensitive (SPEAR) and less sensitive (SPEnotAR, "SPEcies not At Risk") species, and is expressed as a percentage.

Community function could also be investigated by the use of PICT (Pollution Induced Community Tolerance). In situ PICT assays using transplanted communities has been suggested as a promising tool that can link ecological and chemical status in the WFD context (Pesce et al 2010a; Pesce et al 2010b; Tlili et al 2010; Tlili et al 2011). The PICT approach has been used for more than two decades now but would need some systematic development to fit the current chemicals of concern under the WFD, and this is needed to study effects also when potential chemical stressors are largely unknown.

Microbial communities provide the basis of the aquatic food web and changes in community function (such as nutrient turnover) could have a large impact on the ecosystem level. However, the biological indicators in the WFD do not include responses in microbial community level. Nevertheless, by the use of metagenomics, it is now possible to study species composition and community functionality as well as identify previous exposure to contaminants.

SPEAR, PICT and metagenomic tools are described in more detail in Appendix chapter 30 of this report.

9.4 Regulatory requirements and national experience

In the MSFD context, it is required to also monitor effects from hazardous substances (see chapter 3) although the specific tools (indicators) still need to be specified. The MSFD emphasizes harmonisation between regions and to utilize the work already ongoing within the regional conventions (such as OSPAR and HELCOM), and some of the effect based tools are already part of the CEMP (OSPAR mandatory) and HELCOM variables (see chapter 3). There is no obligation to apply any of the effect based tools described in this chapter within WFD monitoring, but there are several potential uses. Although there is yet no separate CIS guidance document available on effect based monitoring tools of hazardous substances, such tools are mentioned in particular in CIS 19 (chapter 7

²¹⁰ Brita Sundelin, Stockholm University and Tove Lundeberg Swedish EPA, pers. comm.

on Complementary Methods) and CIS 25 (chapter 7 on Complementary Methods) but also shortly in draft CIS 27 related to tier II sediment monitoring in cases where the exceedence of QSbenthic is uncertain.

Because effect based tools will have a more central role in the future MSFD GES status classifications, there is a risk of non harmonized assessments between the WFD and MSFD in particular in coastal areas. Therefore the possibilities to at least utilise effect based data in the WFD context for in particular ecological status classifications based on expert judgement should be investigated from a legal point of view.

On a national level, effect based tools are also frequenty required in Whole Effluent Assessments (WEA) (Box 9.8.), and used on occasion within certain SRK/RMÖ programs and investigations at contaminated sites. Fish biomarker studies have e.g. been performed close to paper mills with chlorine bleaching (Naturvårdsverket 2008)²¹¹. Along the West coast, biomarkers are monitored within an SRK program²¹² every 5th year at three sites exposed to complex exposures (Göteborg, Brofjorden and Stenungsund). In order to assess the needs for sediment remediation due to previous emissions from e.g. textile industry, effect based studies have been performed in the Viskan area close to the city of Borås²¹³. Reutgard et al (2009) reviewed the results of nine regional programs that used Monoporeia malformations in an SRK context.

The availability of national commercial performers is still limited for many of the effect based tools. However, on research level, effect based tools have frequently been used and national expertise is available (see also Appendix chapter 25).

Box 9.8. Whole Effluent Assessment procedures in Sweden

Sweden has a long tradition of using whole effluent assessments to provide additional decision support in permit applications and supervision of larger industries. The first national guidance in this area came in 1989²¹⁴. The guidance was based on a research project²¹⁵ performed in the early 80s, to identify methods that could be used to assess the environmental hazards of effluents.

The national test program suggested is focused on tools that should identify effluents that are toxic (acute and chronic), persistent and have the potential to bioaccumulate. The national guidance was recently revised (Naturvårdsverket, 2011).

²¹¹ Common effects observed include enlarged livers, EROD induction, suppressed gonadal growth, reduced sex hormone levels, fin damage, skeletal alterations and deformed jaws, as well as reduced number of adult fish and reproduction. Effects have been reduced since then but some changes can still be observed. ²¹² Program <u>http://www.bvvf.se/pdf/2007-2009.pdf</u> and results:

http://www.bvvf.se/rapporter/w3filer/Miljögifter%20hos%20fisk.doc183.doc. In the Göteborg area, significant effects on EROD, LSI and DNA adducts have been observed, whereas reproductive disturbances are primarily observed in the Stenungsund area (with heavy petrochemical industry but also other potential sources) and EROD induction is also observed in the Brofjorden area. Lysosomal stability in blue mussels is also being considered (program is currently being revised).

²¹³ These studies include biomarker studies in perch and caged rainbow trout as well as studies of mentum deformations in chironomids. EROD induction and significant effects on GSI and VTG was observed in both fish species. Some in vitro studies were also performed (SOS chromotest on pore water and sediment methanol extracts). Reports to be found at http://www.lansstyrelsen.se/vastragotaland/Sv/miljo-ochklimat/verksamheter-med-miljopaverkan/fororenade-omraden/efterbehandlingsprojekt/viskan/Pages/Viskanrapporter_bilagor.aspx 214 Allmänna råd 89:5 Biologisk-kemisk karaktärisering av industriavloppsvatten

²¹⁵ "Karaktärisering av industriella avloppsvatten"

The effect based tools in the handbook include acute and chronic bioassays on algae, invertebrates and fish in addition to microorganisms (inhibition in *Vibrio fischeri*)²¹⁶. Species recommended to be used for freshwater are e.g. the algae *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*, higher plants *Lemna minor* and *Allium cepa*, the crustaceans *Daphnia magna*, *Ceriodaphnia dubia*, and the fish species zebrafish, rainbow, stickleback. In salt and brackish waters the algal species *Phaeodactylum tricornutum*, *Skeletonema costatum*, *Ceramium tenuicorne* and invertebrate *Nitocra spinipes* and *Acartia tonsa* can be used as well as blue mussels (larval survival). Perch (only in brackish), eelpout and sticklebacks are recommended fish species. *In vitro* assays recommended include yeast based assays to detect oestrogenic effects (YES, YAS), genotoxicity tests (Ames, Umu-C) and EROD. On higher tier levels, in vitro analyses are also recommended on whole organisms used in bioassays (such as endocrine disruption, liver function, hemathology and immunotoxicity responses).

The national Swedish approach developed in the early 80s was inspired by the test strategy suggested in the United States, where the regulators had identified that a chemical approach to characterise complex effluents has several shortcomings. Effluents contain a great number of toxic chemicals and there are many limitations with a chemical-specific approach from an analytical perspective, but also because the toxic effects of a chemical can change due to reactions with the matrix, and because it would be impossible to predict the effects from combined exposure to the chemicals in the effluent. USEPA therefore issued a new national policy in 1984: "Development of Water Quality-Based Permit Limitations for Toxic Pollutants" (US Federal Register, 49:9016, 1984). With the new American policy, acceptable discharges of individual toxic chemicals would be based on maintaining safe concentrations in the receiving waters. In addition, biological methods (including toxicity tests) were introduced in addition to chemical specifications in release permits.

There are a few major differences between Sweden and the USA in the way the results are used from a regulatory perspective. In Sweden, the limit values are still largely based on average/maximum concentrations/total amounts of either specific substances such as mercury or nonyl phenol, or – more frequently – on other parameters such as TOC, AOX, oil index. As opposed to the situation in the US, WEA was so far primarily required for larger industries, not e.g. effluents from municipal sewage treatment plants. However, screening projects on sewage effluents, waste leachates and storm waters have been performed.

Although WEA data in Sweden have been very useful as decision support, the todays focus on short term in vivo bioassays has its shortcomings. Acute toxicity tests can be used to prevent sudden and severe effects, such as major fish kills, in receiving water where there is limited dilution. Acute tests are also useful to identify appropriate dilution series for /sub/chronic tests to be performed. However, in practice full chronic tests on higher trophic levels are less frequently required. It can be assumed that this is primarily due to the high costs of performing chronic tests but there may also be ethical reasons (to limit the use of tests on vertebrates). It can therefore be concluded that from a WFD perspective, a lack of chronic testing of both vertebrates and invertebrates does limit the possibilites to detect several substances that could be of a high concern to the status of the receiving water (see also box 9.10). There is therefore a need to develop the current test strategy, in order to cover relevant modes of actions in a cost effective manner and toxicity based limit values could be considered also in Sweden; see also e.g. COHIBA (2010).

Other Member States also use effect based tools for certain purposes within their regulatory framework. The Netherlands has included bioassays in the test requirements of dredged material (Microtox solid phase and DR CALUX)²¹⁷. Corophium bioassays are also required for the assessment of dredged material that is to be deposited at sea. Germany has included Microtox assays to tier I and

²¹⁶ If effluent is supposed to be treated by waste water treatment plants: additional tests on microorganisms (effects on nitrification (4 h test, ISO 9509), screening test (NV4424), 16h Pseudomonas putida (ISO 10712), 3h Respiration test (ISO 8192)

²¹⁷ Management of disposal of dredged material at Sea – the CTT approach (chemistry-toxicity test). If the DR CALUX value exceeds 50 ng TEQ/kg dw or 1/EC50 in Microtox exceeds 100% ("signal values"), the cause of exceedence must be investigated.

is considering adding AMES and UMU C assays to tier II testing of dredged sediment for inland disposal (Manz et al 2007). Two in vivo bioassays are also required at tier I assessment of dredged material that is to be deposited in inland waters (Daphnia magna acute immobility and algal growth inhibition). Tier II assays are under development and the following assays are considered : Lemna minor plant growth inhibition, Myriophyllum aquaticum plant sediment contact test, Nematode reproduction, Zebra fish (fish-egg). For marine waters, besides algal assays, also 10 d Corophium bioassays are required.

In France, bioassays are performed on algae, Ceriodaphinia dubia, and the rotifer Brachionus calyciflorus for the assessment of dredged material to be disposed in water. In the UK, CEFAS (Centre for Environment, Fisheries and Aquaculture Science) is developing a weight of evidence approach for evaluating sediments for sea disposal, based on physical, chemical and bioassay data in parallel.

In the report by Kortenkamp et al (2009), conclusions from a survey directed towards the member states included questions related to the use of direct toxicity measurements of complex samples, such as effluents. The YES assay is used for STP effluents in DK whereas the DR and ER CALUX assays, fish cell lines, Comet assays and antibiotic tests are reported to be used in the Netherlands for WEA. Spain reports the use of fish cell lines for all sample types (presumably including effluents). France reports the use of receptor binding assays, in house nuclear and Ah receptor bioluminicsent cell lines for also all purposes.

In the United States, the "Inland Testing Manual" provides a strategy to evaluate dredged material to be deposited in coastal and inland waters. Bioassays are included in tier III and IV (USEPA 1998). Also for off shore deposition, there is a similar manual, called the "Green book" (USEPA 1991). In general the results are assessed in an integrated way.

9.5 Usefulness within a WFD context

9.5.1 Complementary tools

In existing CIS guidance, effect based tools were primarily mentioned in the context of complementary tools. They could e.g. aid in the design of monitoring programs but also in 2nd tier assessments to support the need for control measures (e.g. sediment remediation). There are also many other useful purposes that were described primarily in the previous sections about the different types of tools available (see also box 9.9). It is clear that not all tools are suitable for all approaches.

Certain effect based tools (primarily in vitro assays) are e.g. useful in screening of complex sources and in a WEA context to aid in the prioritization of further studies of effluents and other types of sources and thus aid in the risk assessment of sources (and regulatory work on emission limits), in WFD context relevant during the "Analysis of Pressures and Impacts". Broad chemical screening of sources using chemical tools can identify a large number of compounds but the results are

often difficult to evaluate in absolute terms because there are usually only very limited available knowledge about the effects of the substances identifiyed at such an early stage. In vitro assays would rather identify the presence of certain types of modes of action responding and therefore give a rough estimate about potential effects and importance of different sources. In the context of WEA, in vitro assays could also aid in the prioritisation of further studies on whole organisms using chronic assays. Such a prioritization tool is also valuable in an ethical perspective to limit the use of vertebrate bioassays. In Sweden however, in vitro assays were used very sparingly in both screening and WEA.

Box 9.9. Why use them if not required?

A main argument against the use of effect based tools would be that if they are not required from a legal point of view, they would only add to the costs. However, some advantages with the use of effect based tools have already been illustrated in relation to an exclusively chemical monitoring approach.

Effect based tools are in this context especially valuable when it is not clear what individual substances to monitor, i.e. in complex situations with many potential sources that are insufficiently characterized. Sensitive (early warning) but also general (responding to several substances or groups of substances) biomarkers can e.g. help selecting and deselecting areas or sources from being monitored further.

Other uses would be to investigate reduction efficiency of e.g. effluent control measures or remediation measures to make sure that not only concentrations are reduced but also effects (to ascertain that bioavailability was not increased or other more toxic transformation products were produced as a result)²¹⁸.

As was pointed out in chapter 7, an approach is needed for the monitoring of hazardous substances (in particular accumulating substances) in rivers. In Sweden, there are 47 monitoring stations to monitor metals monthly in the larger rivers that end at sea, primarily to observe trends and to estimate loads into the marine environment. By adding suitable effect based tools at some of these locations where a heavier impact from complex emissions can be suspected could be considered as a first step not only to estimate status along the coast but also to identify where follow up studies are needed to identify local sources upstreams.

Furthermore, if only chemical tools are used to assess WFD status within the coastal water bodies, whereas also effect based tools are used to assess environmental status in areas covered by MSFD, status maps could become very confusing. Strategically placed effect monitoring stations could help harmonize the different assessments.

Effect based tools could also be used in a gradient from potential sources to identify most significant emissions (current or historical) without actually knowing which substances that are emitted. Specific biomarkers can be used to identify a group of probable causing agents that should be monitored. EROD induction combined with LSI response would e.g. suggest that one should suspect effects from AH receptor inducers such as PAHs, planar PCBs and PCDD/Fs and potential sources of such substances should be identified.

²¹⁸ See also American guidance document on Toxicity Reduction Evaluation (TRE), "a site-specific study conducted in a step-wise process to identify the causative agents of effluent toxicity, isolate the source of toxicity, evaluate the effectiveness of toxicity control options, and then confirm the reduction in effluent toxicity after the control measures are put in place." <u>http://www.epa.gov/npdes/pubs/wet_draft_guidance.pdf</u> and <u>http://www.epa.gov/waterscience/methods/wet/</u>

Both in vitro assays and several biomarkers are focused on the study of a particular mode of action. If certain biomarkers are induced, in vitro assays measuring the same mode of action involved could be very useful as a second tier approach or vice versa, or – preferably – in an integrated approach. By combining biomarker studies on caged fish with in vivo and in vitro assays as well as chemical analyses, Veethak et al (2005) could e.g. conclude that EE-2 and possibly nonylphenol/ethoxilates/ were the main responsible contaminants for the feminization of male fish being observed in dutch inland waters. If it is essential to find out which group of substances that are causing effects in order to identify probable sources (such as in an environment where source tracking is difficult by studying gradients), it would in many cases be possible in investigative studies to follow up the effects observed on biomarker level (especially rather specific biomarkers) with the study of corresponding in vitro assays (responding to the same mode of action) by the use of TIE/EDA methodology (Box 9.10). EDA technologies could also be useful in identifying new emerging substances that should be subject to screening campaigns on a broader geographical scale (possibly become future priority substances).

Both in vitro assays and biomarkers are useful in the context of identifying most prioritized water bodies for further investigations (also aid in the analysis of pressures and impacts). In addition, because it is often possible to study the same mode of action with these different approaches, effects found in the field (biomarkers) could be further studied in the laboratory with high throughput in vitro assays and in a controlled environment to at least roughly identify the main causing substances (see Box 9.10).

By the use of certain tools it is also possible to rather focus on identifying the source/s of emissions based on effects assessments rather than identifying the causing substances and still provide important support for local management (identify measures).

Box 9.10. Toxicity Identification Evaluation (TIE) and Effects Directed Analysis (EDA)

As was pointed out earlier, although the Swedish WEA approach partly originates from the approach developed by USEPA, there are some major differences. Because there are more explicit limitations on acceptable toxicity of effluents (toxicity based emission values), the USEPA also developed guidance documents with technical instructions on how to proceed to identify the reasons for any observed toxicity of a sample (USEPA 1991, 1992, 1993a, 1993b)²¹⁹. This approach is called Toxicity Identification Evaluation. The TIE approach was also further developed to be used for the investigation of contaminated sediments (both pore waters and whole sediment), and a revised manual was recently published (USEPA 2007). The TIE approach is divided into three tiers. The first tier provides a rough characterisation of the toxic sample, to determine whether toxicity would primarily be expected to be related to e.g. metal ions, hydrophobic substances, volatile substances or acids/bases. This is done by treating the samples in different ways (such as the addition of EDTA, aeration of the sample or by SPE extraction) and repeat bioassays on the treated samples to detect induced or reduced toxicity. If e.g. toxicity is reduced after SPE extraction, the toxicity can be suspected to be related to hydrophobic compounds, whereas if it is reduced after EDTA addition, it was most likely due to certain metal ions. In the second tier tentative substances causing the effects are identified (by fractionations and elutions after SPE procedures e.g.). Finally, as a third tier confirmative analyses are performed.

A related tool is Effects Directed Analysis and in a sense, EDA could be considered a special case of TIE although EDA was developed with the purpose to identify individual compounds of toxicological relevancs. The focus is on the fractionation step (corresponding to the second tier TIE). TIE (and EDA) are e.g. described in Bakker et al (2007) and Thomas et al (2010).

²¹⁹ <u>http://www.epa.gov/region8/water/wet/documents.html</u>

The success rates and applicability of TIE and EDA approaches are restricted by costs and the complexity of the sample. One can estimate the costs by the number of treatments that are necessary and followed by repeated bioassays. Thus, by prior knowledge about the most likely compounds to suspect, the number of treatments could be reduced, and if using less expensive (high throughput) bioassays²²⁰, further cost reductions can be made. It is not surprising that in a very complex sample, several treatments could elicit a response, and to identify just one substance that is causing the toxicity of the sample is not possible (and probably also is not the case in reality). It is also necessary to have access to both chemical and ecotoxicological expertise for the bioassays involved could probably also perform at least the basic tier I TIE treatments and in many cases it could perhaps be sufficient to know only roughly what type of compounds are causing the toxicity in order to implement efficient control measures. For more complex chemical analyses, research laboratories are probably required, in particular if analytical tools are not yet available for a direct chemical analysis in order to quantify the concentrations²²¹.

Some successful use of TIE/EDA approaches include the studies by Desbrow et al (1998), identifying EE2 and natural oestrogen as the major cause for endocrine activity in YES in sewage treatment effluents; Grung et al (2011) identified toxicity from PAH (acting as AhR agonists) in sediments from the Oslo harbour and nitrogen/oxygen-containing polyaromatic compounds in the Grenland area and Brack et al (2005) identified several PAHs²²² as the cause for genotoxicity of Neckar sediments. Houtman et al (2006) could conclude that the major contribution to estrogenic activity observed in a dutch harbour sediment was related to 17-b estradiol and estrone and PAHs were the major reason for dioxin activity observed. A review article on identified compounds include several well known contaminants but also non priority substances such as substituted phenols. natural or synthetic estrogens and androgens, dinaphthofurans, 2-(2-naphthalenyl)benzothiophene. and N-phenyl-2-naphthylamine (Brack et al 2007). Although the number of scientific publications on TIE or EDA increased substantially since the last two decades (Hecker & Hollert, 2009), studies using TIE and EDA to identify substances that cause effects are very scarce in Sweden. However, by the combination of TIE tier I treatment steps with chemical analysis, cobalt and manganese were e.g. suggested to be the most probable reasons for observed acute sediment toxicity found in the lake Molnbyggen (Dave & Nilsson 2004). However, in a sediment quality triad study including TIE tier I on sediment from road runoff detention systems it was not possible to identify causative single substances (Wik et al., 2008).

In 2001, an American workshop was held to identify success stories and learn from mistakes from a decade of using the TIE approach in WEA ("Workshop on Toxicity Identification Evaluation: What works, what doesn't, and developments for effluents, ambient waters, and other aqueous media"; proceedings published in Norberg King et al 2005). In general, the European experience from using TIE within WEA is limited, although there are a few case studies from the Netherlands described in Norberg King (2005). Nevertheless, summarizing the findings from 84 American TIE studies performed between 1988 and 1993, it can be concluded that the major toxicants that could be identified are related to primarily ammonia and pesticides for municipal STPs, whereas the identification of organic hazardous substances were primarily found to be the cause of toxicity in effluents from refineries and chemical industries²²³.

9.5.2 Used also to assess ecological status?

By monitoring effects that can be considered relevant in a WFD perspective, data can be generated that could be considered useful as part of an expert judgement of ecological status (at least from a scientific point of view). It should be kept in mind

²²⁰ Thus the frequent use of in vitro bioassays in EDA studies

²²¹ Some European contact persons for TIE and EDA studies are e.g. Göran Dave, Göteborg University, Kevin Thomas NIVA Norway, Timo Hamers IVM Netherlands and Werner Brack Leipzig University

²²² Benzo[a]pyrene, benzo[a]fluoranthene, perylene, 11H-indeno[2,1,7-cde]pyrene, methylbenzo[e]pyrene, methylperylene

²²³ Dave Mount, USEPA pers. comm..

that the purpose of taking river basin specific pollutants into account in ecological status classification is not actually to identify the substances ("listing them"), but as a precautionary approach to also take into account elements that can influence the ecological status but without yet being able to observe any changes on the current biological quality elements.

9.5.3 Effect based tools cannot replace chemical monitoring

A few in vitro tools were found to be valuable more or less instead of chemical analyses, and could then be considered "bioanalytical tools" (at least on screening levels or in order to be able to analyse larger number of samples/more frequently). Certain very specific biomarkers (e.g. imposex) could also be used to actually measure the very same effects that a chemical analysis is trying to predict and would thus provide a more immediate assessment (although it should be kept in mind that the damages are irreversible and could have occurred at an earlier stage).

Generally however, it is important to stress that effect based tools such as biomarkers cannot replace chemical tools but should rather be considered useful additional tools for substances with certain modes of actions and in an integrated assessment. Such a conclusion is also supported by HELCOM and OSPAR documents (box 9.11).

Other factors such as high nutrient loads, can disguise potential effects in the future, and with climate change (heavy rain events) the risk of increased leaching of contaminants require predictive analyses rather than retroactive studies. A chemical monitoring approach is also necessary, indicated by the fact that the EQS values of river basin specific pollutants should also take into account effects on predators (birds and mammals) and humans. Such effects cannot easily be detected or predicted by monitoring exclusively effects observed in the aquatic environment itself.²²⁴

Box 9.11. Effect based tools cannot replace chemical analyses but rather be used in a complementary manner

"For many of the HELCOM priority substances, which are defined as so called PBT substances (Persistent, Bioaccumulative, Toxic), biota is considered to be the most relevant matrix. For other types of substances (e.g. endocrine disrupters), biological effect monitoring can be considered to be more practical and of more importance. For substances which are not PBT substances, but give

²²⁴ Effects on birds and mammals are possible, but it is of major interest to detect the risks of such effects at a much earlier stage and it can sometimes be difficult to establish cause effect relationships. Nevertheless, the monitoring of successful breeding in white tailed eagles indicate that current levels are back to the same levels as the 1950s (before DDT and PCB are suspected to have caused serious population declines). Also Baltic grey seal populations are increasing but are still smaller than before population declines occurred. In addition the incidences of intestinal wounds have increased and the average layers of blubber are decreasing (although there is no correlation observed inbetween these two effects). Bignert et al (2010) also concludes that there is a general decreasing trend in condition and fat content of fish at most sites, with the exception of herring and cod at Fladen, exhibiting increases in fat contents as well as increase in condition factor for herring at one station in spring.

reason for concern due to their widespread and extensive use, monitoring of concentrations in water is regarded a more valid strategy. In conclusion, the selection of representative substances and the most relevant matrices to monitor in the Baltic Sea is based on the substance properties, the extent of use and potential effects of the substance."²²⁵

Some type of effects, such as some mode of actions, but also the effects from substances that are subject to biomagnification, would probably not be possible to identify with currently available effect based tools. Chemical monitoring cannot be excluded. Instead the best way forward is usually to include both chemical and effect based approaches in an integrated approach. Both the HELCOM core program and OSPAR WKIMON emphasizes the needs for holistic assessments.

9.6 Weight of evidence approach and integrated monitoring

Several response variables, pointing in the same direction provides a better decision support than a single variable. As was pointed out earlier, an exclusively chemical monitoring approach to predict the environmental impact has some major disadvantages, primarily because concentration levels alone cannot predict effects on an individual or population level and it is not possible to analyse all potential contaminants. Biomarkers and other effect based tools, especially if tested in a battery, respond to a large spectrum of substances and can provide information about health effects on several vital physiological functions. However, there are often difficulties to identify causing substances and to predict population and ecosystem level effects. Monitoring impacts on the structure and functionality on community levels would be a way to detect effects on such higher organisational levels. However, the current Swedish biological assessment indices to be used within the assessment of ecological status (related to the WFD) were not developed to respond to the effects from hazardous substances. Effects on lower trophic levels (benthic community structure) also tell very little about the risk of human impact or secondary poisoning of birds and mammals.

Nevertheless, these three types of tools together provide good decision support on the need for action. In the 80s, the TRIAD concept was established (Long & Chapman 1985) in order to identify sediment hot spots for remediation purposes, based on bioassays, benthic community investigations and chemical analyses performed in parallell. In draft CIS 27, effect based tools are also mentioned as potential tier II studies in the case where large uncertainties are related to QSsediment exceedance, thus suggesting the usefulness of effect based tools in a step wise process.

In a broader sense, effect based tools can be useful as part of a weight of evidence approach to make conclusions about the needs of e.g. costly remedial actions but also as early warning signals and indicators of long term environmental changes in complex exposure situations and on large geographical scales.

To make conclusions about the presence and risk of future effects from hazardous substances in the aquatic environment and on the severity of the effects and their causes, usually several effect based tools and from all four types of monitoring

²²⁵ <u>http://www.helcom.fi/stc/files/Krakow2007/HazardousSubstances_MM2007.pdf</u>

strategies as well as chemical analyses are necessary. Batteries of biomarkers and other tools responding to several types of modes of action are necessary to monitor the impact from the cocktail of substances that the organisms are exposed to. The tools used should preferably be based on several trophic levels and matrices rather than the investigation of a few single modes of actions (such as EROD induction, genotoxicity and effects on reproduction) and endpoints and analysis of single matrices in complex exposure situations.

Effects observed on biomarkers in the field can be followed up by studies of the same endpoint in controlled laboratory environments (bioassays) and in situ biomarker set ups but also by analysing corresponding modes of action using in vitro assays applied to relevant matrices, to identify sources and suitable control strategies. The severity of effects can be estimated by analysing structure and function on population and community levels as well as effects on higher trophic levels (birds and mammals). Chemical analyses provide information about causes (possibly involving EDA approaches) but also risk of secondary poisoning that are not yet detectable.

By truly integrated monitoring strategies, involving measurements of chemical concentrations, ecotoxicological effects and population/community responses in the same area and time of the year and on the same populations and individuals, a holistic picture can be obtained. Such an approach can tell whether the chemicals analysed are bioavailable and giving rise to negative health effects in aquatic organisms, and whether population effects are observed. The WKIMON strategy on integrated ecosystem assessment (see e.g. ICES WGBEC 2007) is based on sediment monitoring (chemistry, characteristics, bioassays, benthic ecology), water monitoring (passive samplers, bioassays, hydrography, bioassays, water chemistry etc) and biota monitoring (tissue chemistry, fish biological effects, mussel biological effects, gastropod biological effects). Sweden has since several decades an integrated fish monitoring program at four reference locations and the results obtained justify such an approach, detecting large scale and long term alterations (see e.g. Hansson et al 2006, Hanson et al 2009 and Sandström et al 2005). At the station Kvädöfjärden, several biomarkers now indicate negative impacts on the immune and reproductive system, on ion regulation and also metabolic changes (Larsson et al 2011). Also other countries have an integrated monitoring approach. In Belgium e.g., integrated monitoring based on ecological, ecotoxicological and chemical parameters is performed at 160 out of 600 monitoring stations (Rijkswaterstaat 2005).

In general, integrated chemical monitoring approaches (where samples taken for chemical analyses are e.g. also analysed regarding effects) would not only facilitate evaluation of data, but also reduce the sampling efforts, although one needs to be aware of practical circumstances in the design of such programs (Box 9.11).

Box 9.11. Integrated monitoring approaches, some practical issues related to hazardous substances

In order to obtain a better decision support for the implementation of measures, and also facilitate cost effective monitoring programs, it would be desirable to utilize the same samples that are used for chemical biota and/or sediment monitoring. In some cases also the sampling to investigate biological quality elements to assess other effects (nutritional status) could be utilised. This is e.g. the case for the study of diatom malformations or mentum deformities in chironomids.

However, the possibilities to use the same material (same species and tissue sampled) for both traditional chemical analyses and the analyses of biomarkers could be restricted if both analyses require large amounts of material. Several biomarkers are e.g. studied on liver tissues, as is the case for several chemical parameters. Some biomarkers also need to be analysed immediately after sampling, suggesting that this should be performed in the field and tissues frozen until transported to chemicoanalytical facilities. In some cases, different treatments of sampled individuals before chemical and ecotoxicological analyses take place are necessary, prohibiting the use of the use of the same individual for both purposes.

Therefore, it is necessary to be aware of these aspects before deciding which tools to include and Appendix chapter 27 of this report includes some important practical aspects that could be useful. Guidance is needed on how to best perform such coordinated monitoring approaches. Annex 21 of the ICES SGIMC (Study Group on Integrated Monitoring of Contaminants and Biological Effects) group can provide some guidance on integrated monitoring approaches as well as assessment of combined data for the marine environment (ICES SGIMC 2011).

There are several suggestions on how to evaluate data on biomarkers and bioassays, community and population effects and chemical concentrations in a systematic way using different types of indices, scoring systems, best professional judgement or statistical tools (see e.g. Chapman et al 2002 in the context of contaminated sediment). To give a thorough description on different alternatives that are suitable in different situations is out of the scope of this report. Nevertheless, an assessment scheme to evaluate the current variables that are included in the national marine fish program (including population effects, health and chemistry) has recently been proposed (see also appendix chapter 29).

9.7 Reliability

In order to include a new method in regular monitoring programs, it is important to be able to refer to an established method (agreed protocol), and to be aware of the variability in results to expect. Among the effect based tools, primarily those that are also used within chemical regulation have been subject to international standardisation. These include the in vitro tools AMES, UmuC, EROD, VTG and micronucleus test and several whole organism tests. All ISO methods have been subject to intercalibration studies (but not necessarily on environmental samples). However, the standardization of new methods is generally a time consuming process and intercalibration studies performed can probably provide sufficient information on the reliability of a particular method to be able to interpret the results.

Within ICES, the Working Group on Biological Effects on Contaminants (WGBEC) regularly categorises effect based monitoring tools according to their current status. Table 9.3. lists tools that are either recommended at national/international levels, considered promising (requiring further research before they can be recommended for monitoring), and tools that would need further development/application before being considered promising to use. To include a method in the category of recommended methods, it needs to be established and available as a published method in the TIMES series²²⁶ or elsewhere. It should also have been shown to

²²⁶ <u>http://www.ices.dk/products/techniques.asp</u>.

respond to contaminant exposure in the field and be able to differentiate between effects of contaminants from background variability. The assessment therefore does not include aspects such as availability of assessment criteria or whether endpoints can be considered of ecological relevance. All recommended ICES methods also have not been subject to intercalibration studies but those that have²²⁷ are indicated in bold in table 9.3. (ICES WGBEC 2007).

Table 9.3. Recommended and promising marine effect based monitoring tools to be used within the ICES area, as well as tools that would need further development or application before being considered promising (ICES WGBEC 2007). Methods that were subject to intercalibration studies are typed in italic.

Recommended tools		Promising tools	Tools that	
Biomarkers	Bioassays	Biomarkers	Bioassays etc	need further
	etc			development
Bulky DNA adduct	Whole	Pre/neoplastic lesions	Whole	Oncogenes in
formation (fish)	sediment	by NADPH producing	sediment	fish
	bioassays on	enzymes (fish)	chronic	
AChE (fish, molluscs,	<u>Corophium,</u>		bioassays on	ELISA for DNA
crustaceans)	<u>Arenicola,</u>	DNA strand breaks	invertebrates	adducts
	<u>Ampelisca</u>	(incl Comet assay)		
Metallothionein (fish,	<u>brevicornis</u>	(fish, mussels)	PICT water	Apoptosis of
<u>Mytilus</u>)			bioassay on	fish cells
	/Pore/	BaP hydroxylase like	microalgae	
EROD/P4501A	water/elutriat	enzymes	and bacteria	AChE for other
induction (fish)	e bioassays	(invertebrates)	Ocetronen	invertebrates
ALA D (fich)	on bivalve	Induction link hitigan of	Oestrogen	Deleved
ALA-D (fish)	embryo	Induction/inhibition of	receptor CALUX assay	Delayed
PAH bile metabolites	<u>Acartia</u>	multidrug/multixenobi otic resistance	CALUX assay	reproduction/go nadal
(fish)	CALUX	(MDR/MXR) in fish	Androgen	maturation in
(IISH)	assays of AH	and invertebrates	receptor	fish
Lysosomal stability (fish,	receptor	other than Mytilus	CALUX assay	Aromatase in
Mytilus, oyster)	active	GST in fish and	CALOA assay	fish
<u>Mythus</u> , Cyster)	compounds	molluscs	DNA strand	11011
Externally visible	compoundo		breaks (incl	
diseases (fish: dab,	YES	Oxidative stress in	Comet assay)	
flounder, cod)		fish and invertebrates	(cell lines)	
· · · · · · · · · · · · · · · · · · ·	YAS			
Macroscopic liver		Immunocompetence		
neoplasms (fish: dab,		in fish and		
flounder)		invertebrates		
Histopathology (Fish		On line monitoring		
liver: of dab, flounder;		with remote biosensor		
blue mussels)		measuring heart rate		
		in mussels and crabs		
Vitellogenin induction				
(male & juvenile fish)		Abnormalities in wild		
listere en la flerie de		fish, embryos and		
Intersex (male flounder,		larvae		
<u>Littorina littorea</u>)		Pulky DNA odduct		
		Bulky DNA adduct		

²²⁷ Laboratory performance studies arranged by either BEQUALM (Biological Effects Quality Assurance in Monitoring Programmes; <u>http://www.bequalm.org/</u>), QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring; <u>http://www.quasimeme.org/</u>) or UNEP MEDPOL (marine pollution assessment and control component of MAP, Mediterranean Action Plan for the Barcelona convention)

Reproductive success in eelpout	formation in mussels and invertebrates	
Scope for growth (bivalve molluscs)	Gene arrays in fish and mussels	
Imposex (neogastropod molluscs)	Histopathology in invertebrates other than <u>Mytilus</u>	
Induction/inhibition of multidrug/multixenobioti c resistance (MDR/MXR) in <u>Mytilys</u>	Spiggin (three spined stickleback)	
edulis Embryo aberrations in	Micronuclei in fish, bivalve, molluscs	
field collected amphipod crustaceans	Peroxisomal proliferation in fish and invertebrates	
	Alkylphenol bile metabolites in fish	
	Cellular energy allocation in invertebrates and small fish	

Intercalibration and validation studies have been performed also in other contexts. Several AH receptor assays used for the analysis of biological tissues were e.g. subject to interlaboratory comparisons by the Örebro University (Engwall et al 2003 and Engwall & Bavel 2004).

9.8 Need for research, validation and guidance

As was already pointed out earlier in this chapter, current tools to be used for ecological status classifications based on biological indicators cannot generally be considered to be sensitive to the effects of hazardous substances on community level. An extensive research program financed by the Swedish Environmental Protection Agency (WATERS) has started to develop the biological assessment criteria and will be finalised in 2015. However, the program is mainly focusing on eutrofication and hydromorphology and will not develop effect based tools for hazardous substances. There are no higher organisational level effect based tools for hazardous substances that are ready to be used on a national regular monitoring basis for regulatory purposes today in Sweden. However, both the SPEAR and PICT approach are promising tools but would need either validation studies (SPEAR) or development (PICT)²²⁸ (see also descriptions of both tools in

²²⁸ The NICE project recently received research funding to develop an approach to assess effects from hazardous substances in the marine environment and that includes biomarkers, PICT and OMICS technology.

Appendix chapter 30). In later years, also different types of OMICS approaches have been suggested for a number of purposes. Metagenomics can e.g. be considered a promising tool also to investigate the effects on community/ecosystem level that in a few years time would be valuable for several applications (Appendix chapter 30).

A diatom malformation index tool that is also under development could be used in lakes and rivers, and has received much attention on regional level. It is considered a promising approach and has the potential to provide also community relevant information in addition to the use of deformed shells as biomarkers. Validation studies are ongoing to develop an index that can be used for Swedish conditions and the potential to include this tool in future monitoring programs is facilitated by the low additional costs and potential to coordinate with other monitoring activitites²²⁹. Nevertheless, its sensitivity to different types of hazardous substances need to be evaluated further (see also box 9.4.).

There are several biomarkers in regular use and even more available, in particular for the marine environment and fish. Several fish biomarkers could be used also under limnic conditions, but baseline levels and assessment criteria are needed.

For invertebrates the possibilites are more limited. No biomarker tools that could be used to investigate effects on invertebrates in rivers were identified to be used in Sweden (at least no such tools were nominated during the workshop). It can also be concluded that there are e.g. no biomarker tool to investigate the presence of TBT specific effects in limnic environments (see also chapter 7).

For chironomid mentum deformations, it can be concluded that it is not clear whether the endpoint responds exclusively to metal concentrations or also other types of contaminants. There is thus a need to investigate the sensitivity towards different types of hazardous substances (would it be considered a specific or general biomarker?). Normal malformation frequencies are low. The deformities could also cause negative effects on gut uptake and generation time but a confirmatory study would be needed to make conclusions about the ecological relevance of the deformations (could it primarily be used as an early warning tool?). Finally, if this tool should be considered to be used on a more routine basis, formal standard protocol would need to be developed, although today there are available scientific papers that include illustrations on deformities and estimated baseline levels, based on historic data and relative comparisons between lakes. As with the diatom malformation tool, a learning period is needed but national expertise is available and the analysis can be performed on a commercial basis. It is also possible to coordinate the sampling with the study of community level effects for cost reduction.

Because the aim of WEA in Sweden is to identify effluents that are toxic, persistent and bioaccumulable, this type of approach is very promising also from a WFD

Project coordinator: Thomas Backhaus, Göteborg University. Part of the development needed for PICT will be performed within this project (Hans Blanck, Göteborg University, pers. comm.).

²²⁹ If used in combination with an assessment of IPS and ACID, indexes that respond to organic/nutrient load and aciditiy respectively in rivers, additional costs are very limited.

perspective in terms of identifying emissions of potential RBSPs²³⁰. If WEA tools can be proven to sufficiently detect the substances that fulfil the properties described in line 4 and 5 of annex VIII in the WFD, the introduction of toxicity based limit values would be a way to control the amounts of potential RBSPs emitted without actually knowing the identity of these substances. A monitoring step would be needed to check compliance on recipient level, and could be considered to either monitor the response by the same tools (if applicable), but also other tools based on the same mode of action²³¹. The potential to identify PBT substances and substances with other relevant inherent properties in WEA by the use of less expensive screening tools should therefore be investigated. To develop WEA tools is part of the BSAP (and included in the COHIBA project) but at the moment in vitro tools are not mentioned in the relevant BREF document and only shortly in the Swedish Handbook (Naturvårdsverket 2011).

There is no single universal effect based tool that can be used for all purposes, but lack of guidance to regulators on both how to choose a suitable tool/approach for a certain purpose, and how to evaluate the data further limits the implementation of these complementary techniques. The development of guidance and assessment criteria for different purposes to be used in the context of the WFD is therefore recommended. Such guidance is being developed on European level but additional guidance will probably be necessary for the use in Swedish contexts.

9.9 Conclusions

- Within the WFD, current biological quality elements to be used in ecological status classifications are of little use to the assessment of effects from hazardous substances. Potential tools are available that could be used to assess effects of hazardous substances on communities but these would need validation studies and further development before being used on a routine basis in Sweden.
- Some in vitro bioassays could be valuable tools also in the context of chemical status compliance checking, due to higher sensitivity and/or lower costs.
- By monitoring "mode of action" instead of individual RBSPs possessing these inherent properties, the number of monitoring parameters could be reduced. The use of effect based data (biomarkers and bioassays) as part of an expert judgement should therefore be considered from a legal perspective. However, in order to utilize effect based data in a harmonized way within status classification, it would probably be necessary to specify which tools can be used individually for this purpose and which should rather be used within a weight of evidence approach.
- Because effect based tools are to be used as indicators in the MSFD GES classification there will be risks of non harmonized assessments between the environmental status in MSFD and ecological status in the WFD regarding hazardous substances. It is therefore strongly recommended that relevant results

²³⁰ Although complementary approaches to identify sources of RBSPs from other local emission sources (air, diffuse sources) are also necessary. Actually emissions from long range transports should also be considered (see e.g. CIS 3, interpreting "being released" to correspond to "occur") in identifying RBSPs but the possibilities to implement necessary measures on a local level are limited.

²³¹ To follow up EROD observed in field (biomarker fish), both EROD and DR CALUX assays would be suitable in vitro assays and vice versa. Behnisch et al (2002)

from using effect based tools should be used within ecological status classifications, in particular for coastal areas to ensure harmonization between MSFD and WFD.

- Effect based tools are valuable in several WFD relevant contexts, such as screening, early warning, and to identify new emerging substances.
- If the Swedish WEA strategy is further developed, this could provide a cost effective approach to limit emissions of potential RBSPs, without actually identifying individual compounds.
- Certain promising biomarkers can be used in a very cost effective way by coordination with monitoring programs to investigate impact on biological quality elements used for ecological status classification regarding eutrophication and acidification.
- Although there are currently only a few standardized effect based tools, several have been subject to intercalibration studies

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APPENDIX

10 European reporting requirements related to hazardous substances

Legislative instruments related to chemicals, that include reporting requirements to the DG environment, European marine conventions, Eurostat, OECD, UN, UNECE and EEA²³². All reporting requirements are not necessarily related to the aquatic environment and some reporting requirements may have terminated. The reporting obligations related to the mentioned legislative instruments are of variable character, such as emission data, monitoring data etc.

Commission decision 2000/479/EC of 17 July 2000 on the implementation of a European Pollutant Emission Register (EPER) according to Article 15 of Council Directive 96/61/EC concerning integrated pollution prevention and control (IPPC)

Convention for the protection of the marine environment of the north-east Atlantic

Convention on Long-range Transboundary Air Pollution

Convention on the Protection of the Marine Environment of the Baltic Sea Area

Conventions establishing the International Maritime Organisation

Council Decision 77/795/EEC of 12 December 1977 establishing a common procedure for the exchange of information on the quality of surface fresh water in the Community. As amended by Decision 81/856/EEC, 84/422/EEC, 86/574/EEC, 90/2/EEC and Council Regulation 807/2003/EC.

Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances / Amended by the Council Directive 92/32/EEC of 30 April 1992

Council Directive 75/440/EEC of 16 June 1975 concerning the quality required of surface water intended for the abstraction of drinking water in the Member States as amended by Council Directive 79/869/EEC (further amended by Council Directive 81/855/EEC and Council Regulation 807/2003/EC) and both amended by Council Directive 91/692/EEC (further amended by Regulation 1882/2003/EC)

Council Directive 76/160/EEC of 8 December 1975 concerning the quality of bathing water as amended by Council Directive 91/692/EEC (further amended by Council Regulation 1882/2003/EC), and Council Regulation 807/2003/EC.

Council Directive 76/464/EEC of 4 May 1976 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community as amended by Council Directive 91/692/EEC (further amended by Council Regulation 1882/2003/EC), and Directive 2000/60/EC (further amended by Decision 2455/2001/EC).

Council Directive 78/176/EEC of 20 February 1978 on waste from the titanium dioxide industry as amended by Council Directive 82/883/EEC(further amended by Council Regulation 807/2003/EC),

²³² Based on searches in <u>http://rod.eionet.europa.eu</u> performed in December 2010.

83/29/EEC and 91/692/EEC (further amended by Council Regulation 1882/2003/EC).

Council Directive 78/659/EEC of 18 July 1978 on the quality of fresh waters needing protection or improvement in order to support fish life as amended by Council Directive 91/692/EEC (further amended by Council Regulation 1882/2003/EC), and Council Regulation 807/2003/EC.

Council Directive 79/869/EEC of 9 October 1979 concerning the methods of measurement and frequencies of sampling and analysis of surface water intended for the abstraction of drinking water in the Member States (Daughter to 75/440/EEC) as amended by Council Directive 81/855/EEC, 91/692/EEC (further amended by Council Regulation 1882/2003/EC) and Council Regulation 807/2003/EC.

Council Directive 79/923/EEC of 30 October 1979 on the quality required of shellfish waters as amended by Council Directive 91/692/EEC (further amended by Council Regulation 1882/2003/EC).

Council Directive 80/68/EEC of 17 December 1979 on the protection of groundwater against pollution caused by certain dangerous substances as amended by Council Directive 91/692/EEC (further amended by Council Regulation 1882/2003/EC)

Council Directive 82/176/EEC of 22 March 1982 on limit values and quality objectives for mercury discharges by the chlor-alkali electrolysis industry (Daughter to 2006/11/EC) as amended by Council Directive 91/692/EEC (further amended by Council Regulation 1882/2003/EC) and Directive 2008/105/EC

Council Directive 82/883/EEC of 3 December 1982 on procedures for the surveillance and monitoring of environments concerned by waste from the titanium dioxide industry

Council Directive 83/513/EEC of 26 September 1983 on limit values and quality objectives for cadmium discharges (Daughter to 2006/11/EC) as amended by Council Directive 91/692/EEC (further amended by Council Regulation 1882/2003/EC) and Directive 2008/105/EC

Council Directive 84/156/EEC of 8 March 1984 on limit values and quality objectives for mercury discharges by sectors other than the chlor-alkali electrolysis industry (Daughter to 2006/11/EC) as amended by Council Directive 91/692/EEC (further amended by Council Regulation 1882/2003/EC) and Directive 2008/105/EC

Council Directive 84/491/EEC of 9 October 1984 on limit values and quality objectives for discharges of hexachlorocyclohexane (Daughter to 2006/11/EC) as amended by Council Directive 91/692/EEC (further amended by Council Regulation 1882/2003/EC) and Directive 2008/105/EC

Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture.

Council Directive 86/280/EEC of 12 June 1986 on limit values and quality objectives for discharges of certain dangerous substances included in List I of the Annex to Directive 76/464/EEC (Daughter to 2006/11/EC) as amended by Council Directive 88/347/EEC, 90/415/EEC and 91/692/EEC (further amended by Council Regulation 1882/2003/EC) and Directive 2008/105/EC

Council Directive 87/217/EEC of 19 March 1987 on the prevention and reduction of environmental pollution by asbestos as amended by Council Directive 91/692/EEC (further amended by Council Regulation 1882/2003/EC), and Council Regulation 807/2003/EC.

Council Directive 91/271/EEC of 21 May 1991 concerning urban waste water treatment as amended by Commission Directive 98/15/EC and Regulations 1882/2003/EC and 1137/2008/EC

Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources as amended by Regulations 1882/2003/EC and 1137/2008/EC.

Council Directive 91/692/EEC of 23 December 1991 standardizing and rationalizing reports on the implementation of certain Directives relating to the environment as amended by Regulation 1882/2003/EC

Council Directive 96/59/EC of 16 September 1996 on the disposal of polychlorinated biphenyls and polychlorinated terphenyls (PCB/PCT)

Council Directive 96/82/EC of 9 December 1996 on the control of major-accident hazards involving dangerous substances

Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption as amended by Regulations 1882/2003/EC and 596/2009/EC

Decision No. 2367/2002/EC of the European Parliament and of the Council of 16 December 2002 on the Community Statistical Programme 2003 to 2007 as amended by Decision No 787/2004/EC

Decision No 1578/2007/EC of the European Parliament and of the Council of 11 December 2007 on the Community Statistical Programme 2008 to 2012

Directive 2000/53/EC of the European Parliament and of the Council of 18 September 2000 on endof life vehicles - Commission Statements

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy as amended by Decision 2455/2001/EC and Directives 2008/32/EC and 2008/105/EC.

Directive 2000/76/EC of the European Parliament and of the Council of 4 December 2000 on the incineration of waste

Directive 2002/96/EC of the European Parliament and of the Council of 27 January 2003 on waste electrical and electronic equipment (WEEE) - Joint declaration of the European Parliament, the Council and the Commission relating to Article 9

Directive 2006/11/EC of the European Parliament and of the Council of 15 february 2006 on pollution caused by certain dangerous substances discharged into the aquatic environment of the community

Directive 2006/113/EC of the European Parliament and of the Council of 12 December 2006 on the quality required of shellfish waters

Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration (Daughter to 2000/60/EC)

Directive 2006/44/EC of the European Parliament and of the Council of 6 September 2006 on the quality of fresh waters needing protection or improvement in order to support fish life

Directive 2006/66/EC of the European Parliament and of the Council of 6 September 2006 on batteries and accumulators and waste batteries and accumulators and repealing Directive 91/157/EEC

Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council

Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy

Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market as amended by Council Regulation 1882/2003/EC)

and Commission Directives 2006/50/EC, 2006/140/EC and 2007/20/EC

EEA Annual Management Plan

European Parliament and Council Directive 94/62/EC of 20 December 1994 on packaging and packaging waste

Regulation (EC) No 166/2006 of the European Parliament and of the Council of 18 January 2006 concerning the establishment of a European Pollutant Release and Transfer Register and amending Council Directives 91/689/EEC and 96/61/EC (Text with EEA relevance)

UNECE Convention on the Transboundary Effects of Industrial Accidents

11 Priority and "other" substances as well as candidate priority substances.

Priority Substances in bold should in particular be considered for trend analysis (as required by art 3.3 of 2008/105/EC). Please refer to the most current version of the original document before use.

Priority and "other" substances (2008/105/EC and annex X of 2000/60/EC)	Candidate priority substances or hazardous priority substances, 2011
Alachlor	17 alpha-ethinylestradiol
Anthracene (PHS)	17 beta-estradiol
Atrazine	Aclonifen
Benzene	Cyanides (free)
Brominated diphenylethers: penta BDE (congener 28, 47, 99, 100, 153, 154); from 2011 also octaBDE (congener 197) (PHS)	Cybutryne (Irgarol®)
Cadmium and its compounds (PHS)	Cypermethrin
Chloroalkanes, C10-13 (PHS)	Dichlorvos (PHS)
Chlorfenvinphos	Diclofenac
Chlorpyrifos (-etyl)	Dicofol (PHS)
4. O diablana ath an a	Dioxin (2,3,7,8 - Tetrachlorodibenzo-p
1,2-dichloroethane	dioxin,TCDD) (PHS)
	HBCDD (1,2,5,6,9,10-Hexabromo-
Dichloromethane	Cyclododecane / 1,3,5,7,9,11-Hexabromo- cyclododecane) (PHS)
di-(2-ethylhexyl)phthalate (DEHP)	Heptachlor/Heptachlor epoxide (PHS)
Diuron	Ibuprofen
Endosulfan (PHS)	Methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate (Bifenox) Perfluorooctane sulfonic acid and its salts
Fluoranthene	(PFOS) and perfluorooctane sulfonyl fluoride (PHS)
hexachlorobenzene (HCB) (PHS)	Quinoxyfen
hexachlorobutadiene (HCBD) (PHS)	Terbutryn
hexachlorocyclohexane (HCH) (PHS)	
Isoproturon	
Lead and its compounds	
Mercury and its compounds (PHS)	
Naphthalene	
Nickel and its compounds	
Nonylphenol: 4-nonylphenol (PHS)	
Octylphenol: 4-(1,1',3,3'-tetramethylbutyl)phenol	
Pentachlorobenzene (PHS)	
Pentachlorophenol	
PAH: benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, Indeno(1,2,3-cd)pyrene) (PHS)	
Simazine	
Tributyltin compounds: TBT cation (PHS)	
Trichlorobenzenes	

Trichloromethane (chloroform)	
Trifluralin	
DDT: p,p-DDT ("Other")	
Cyclodienes: aldrin, dieldrin, endrin, isodrin ("Other")	
Tetrachloro ethylene ("Other")	
Carbon tetrachloride ("Other")	
Trichloro etylene ("Other")	

12 Potential specific pollutants for which there are proposed draft Swedish EQS values, developed by using the previous "EQS manual" (NV 5799)

Please refer to the original document before use.

Chromium Zink Copper Aklonifen Bentazon Cyanazin Diklorprop Diflufenikan Dimetoat Fenpropimorf Glyfosat Kloridazon **MCPA** Mekoprop & Mekoprop-p Metamitron Metribuzin Metsulfuronmetyl Pirimikarb Tifensulfuronmetyl Sulfosulfuron Tribenuronmetyl Bronopol Irgarol 1051 Triclosan C14-17-chlorinated alkanes, MCCP PCBs, dioxins and dibenzofurans Perfluorooktansulfonat Hexabromcyklododekan **Bisfenol-A** Nonylphenol ethoxilates

13 OSPAR List of Chemicals for Priority Action (revised 2009)

Part A of the OSPAR list of chemicals for priority action²³³ contains substances for which there are background documents and for which OSPAR action should be focused. Please refer to the most current version of the original document before use.

The following substances are included in part A:

- cadmium
- lead and organic lead compounds
- mercury and organic mercury compounds
- organic tin compounds
- neodecanoic acid, ethenyl ester
- perfluorooctanyl sulphonic acid and its salts (PFOS)
- tetrabromobisphenol A (TBBP-A)
- 1,2,3-trichlorobenzene
- 1,2,4-trichlorobenzene
- 1,3,5-trichlorobenzene
- brominated flame retardants
- polychlorinated biphenyls (PCBs)
- polychlorinated dibenzodioxins (PCDDs)
- polychlorinated dibenzofurans (PCDFs)
- short chained chlorinated paraffins (SCCP)
- 4-(dimethylbutylamino)diphenylamin (6PPD)
- dicofol
- endosulfan
- hexachlorocyclohexane isomers (HCH)
- methoxychlor
- pentachlorophenol (PCP)
- trifluralin
- clotrimazole
- 2,4,6-tri-tert-butylphenol
- nonylphenol/ethoxylates (NP/NPEs) and related substances
- octylphenol
- certain phthalates: dibutylphthalate (DBP), diethylhexylphthalate (DEHP)
- polyaromatic hydrocarbons (PAHs)
- musk xylene

There are also additional substances on the list, for which there are no background documents because they are either intermediates in closed systems or no current production or use interest:

²³³ To be found at <u>http://www.ospar.org/content/content.asp?menu=00940304440000_000000_000000</u>

- 1,5,9 cyclododecatriene
- Cyclododecane
- 2-propenoic acid, (pentabromo)methyl ester
- 2,4,6-bromophenyl 1-2(2,3-dibromo-2-methylpropyl)
- pentabromoethylbenzene
- heptachloronorbornene
- pentachloroanisole
- polychlorinated naphthalenes (trichloronaphthalene, tetrachloronaphthalene, pentachloronaphthalene, hexachloronaphthalene, heptachloronaphthalene, octachloronaphthalene, naphthalene, chloro derivs.)
- 3,3'-(ureylenedimethylene)bis(3,5,5-trimethylcyclohexyl) diisocyanate
- ethyl O-(p-nitrophenyl) phenyl phosphonothionate (EPN)
- flucythrinate
- isodrin
- tetrasul
- diosgenin

Finally, there is a list of about 300 substances that are considered to be of Possible concern.

14 HELCOM Substances of "specific concern" in the Baltic sea

HELCOM substances of specific concern²³⁴. Please refer to the most current version of the original document before use.

- Dioxins (PCDD), furans (PCDF) & dioxin-like polychlorinated biphenyls
- Tributyltin compounds (TBT) and Triphenyltin compounds (TPhT)
- Pentabromodiphenyl ether (pentaBDE), Octabromodiphenyl ether (octaBDE), Decabromodiphenyl ether (decaBDE)
- Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA)
- Hexabromocyclododecane (HBCDD)
- Nonylphenols (NP) and Nonylphenol ethoxylates (NPE)
- Octylphenols (OP) and Octylphenol ethoxylates (OPE)
- Short-chain chlorinated paraffins (SCCP or chloroalkanes, C10-13) and Medium-chain chlorinated paraffins (MCCP or chloroalkanes, C14-17)
- Endosulfan
- Mercury (Hg)
- Cadmium (Cd)

²³⁴ <u>http://www.helcom.fi/stc/files/Krakow2007/HazardousSubstances_MM2007.pdf</u>

15 EEA Preferred hazardous SoE substances

Please refer to the most current version of the original document before use.

		Marine, coast,
Preferred hazardous SoE substance	Rivers and lakes	transitional
Alachlor	X	X
Atrazine	x	x
Chlorfenvinphos	x	x
Chlorpyrifos	x	x
Diuron	X	x
Gamma-HCH (Lindane)	x	x
Hexachlorobenzene (HCB)	x	x
Isoproturon	x	x
Simazine	X	x
Trifluralin	x	x
Arsenic	x	x
Copper	X	x
Zinc	X	x
Cadmium	x	x
Chromium	x	x
Nickel	x	x
Lead	x	x
Mercury	X	x
Pentachlorophenol	X	x
Aldrin	X	x
DDT, o,p'	X	x
DDT, p,p'	X	x
DDE, p,p'	X	x
DDD, p,p'	x	x
Dieldrin	X	x
Endrin	X	x
Alpha-Endosulfan	x	
МСРА	X	
Месоргор	x	
Anthracene	x	x
Benzene	X	X
Chloroalkanes C10-13	X	x
1,2-Dichloroethane	X	x
Dichloromethane	X	X
Di (2-ethylhexyl) phthalate (DEHP)	x	x
Hexachlorobutadiene (HCBD)	X	x
Naphthalene	X	x
4-nonylphenol	X	x
Para-tert-octylphenol	X	x
Pentachlorobenzene	X	x
Benzo(a)pyrene	x	x
Benzo(b)fluoranthene	X	x
Benzo(g,h,i)perylene	x	x
שבוובט(ג,וו,ו/אבו אובווב	^	^

Benzo(k)fluoranthene	x	x
Fluoranthene	x	x
Indeno(1,2,3-cd)pyrene	x	x
Tributyltin compounds	x	x
Tributyltin cation	x	x
Trichloromethane	x	x
Isodrin	x	x
Linuron	x	x
PCB118		x
PCB101 (2,2',4,5,5'-pentachlorobiphenyl)		x
PCB138 (2,2',3,4,4',5'-hexachlorobiphenyl)		x
PCB153 (2,2',4,4',5,5'-hexachlorobiphenyl)		x
PCB180 (2,2',3,4,4',5,5'-heptachlorobiphenyl)		x
PCB194		x
PCB28 (2,4,4'-trichlorobiphenyl)		x
PCB52 (2,2',5,5'-tetrachlorobiphenyl)		x
PBDE100 (2,2',4,4',6-pentabromodiphenyl ether)		x
PBDE153 (2,2',4,4',5,5'-hexabromodiphenyl ether)		x
PBDE154 (2,2',4,4',5,6'-hexabromodiphenyl ether)		x
PBDE28		x
PBDE47 (2,2',4,4'-tetrabromodiphenyl ether)		x
PBDE99		x
1,1,2-trichloroethene	х	x
1,1,2,2-tetrachloroethene	х	x
Tetrachloromethane	х	x
Toluene	x	x
Alpha-HCH		x
Beta-HCH		x
Alpha-Endosulfan		x

16 Sources of hazardous substances to the aquatic environment

To aid in the selection of substances to monitor knowledge about the most significant sources is important. The tables below summarises information from fact sheets presented by COM (draft stage, non published) on significant types of sources to the priority substances and the 2010 candidate priority substances.

U=Unintentionally produced; PEST=Pesticide (including biocide); MED: Pharmaceutical use; IND: inudstrial use. (PEST): previous use as pesticide (no longer permitted). X: Significant source of substance into aquatic environment (European scale). The original documents also identify sources that are of less importance and sources for which the importance is unknown; this information is not included below. In addition several sources indicated in the original documents (such as different atmospheric pathways) have been integrated in this summary. The assessment should be considered very preliminary. Please note e.g. that the assessment was made on European level and does not necessary should be considered to be relevant also in Sweden. For some substances, industrial emissions are e.g. primarily relevant in the manufacturing of the substance. Some additional sources so far not identified could also be considered relevant (such as STPs and nonylphenol emissions)

Priority substance								s
	Main use area	Atmospheric deposition	Other diffuse sources	Storm water	Sewage treatment plants	X Industry	Waste	Contaminated sites
alaklor	(PEST)					Х		
antracen	U		Х	Х	Х	Х		Х
atrazin	(PEST)		Х					
bensen	IND		X		Х	Х		Х
PBDE polybromerade difenyletrar	IND	X		Х	Х	X		
Cd	IND	Х	X	Х		Х	Х	
kloralkaner c10-1 (klorparaffiner)	IND					X		X
klorfenvinfos	(PEST)							
klorpyrifos	(PEST)		Х	Х		Х		
1,2-dikloretan	IND					Х	Х	
diklormetan	IND							
di-(2-etylhexyl)ftalat (DEHP)	IND	X	Х	Х	Х	X		
diuron	(PEST)	Х	X	Х				
endosulfan	(PEST)	Х						
fluoranten	U	Х	X	Х	Х	Х	Х	Х

Priority substance								S
	Main use area	Atmospheric deposition	Other diffuse sources	Storm water	Sewage treatment plants	Industry	Waste	Contaminated sites
hexaklorbensen (HCB)	(PEST), U							
hexaklorbutadien (HCBD)	IND							
hexaklorcyklohexan (HCH)	(PEST)							
isoproturon	(PEST)		Х	Х		Х		
Pb	IND	Х	Х	Х	X	Х	Х	Х
Hg	IND	Х				X X	Х	
naftalen	IND, PEST, U		X	X	Х	Х	X	
Ni	IND	Х	Х	Х	X	X	Х	Х
4-nonylfenol	IND					X X	Х	
4-tert-oktylfenol	IND		Х	Х	X	Х		
pentaklorbensen	IND, U							Х
pentaklorfenol	(PEST)							
benso(a)pyren	U, PEST	Х	Х	Х	X	Х	Х	Х
benso(b)fluoranten	U, PEST	Х	Х	Х	Х	Х	Х	Х
benso(k)fluoranten	U, PEST	Х	Х	Х	Х	Х	Х	Х
benso(g,h,i)perylen	U, PEST	Х	Х	Х	Х	Х	Х	Х
indeno(1,2,-cd)pyren)	U, PEST	Х	Х	Х	Х	Х	Х	Х
simazin	(PEST)							
tributyltenn föreningar	(PEST), IND				Х	Х		Х
triklorbensen (1,2,4-)	IND					Х		
triklormetan (kloroform)	IND					Х		
trifluralin	(PEST)							

Priority substance candidates in 2010	Main use area	Atmospheric deposition	Other diffuse sources	Storm water	Sewage treatment plants	Industry	Waste	Contaminated sites
17 alfa etinylestradiol (EE-2)	MED				Х	Х		
17 beta estradiol	MED, U		Х		Х	Х		
Aklonifen	PEST	Х	Х	Х		Х		
Bifenox el methyl 5-	PEST	Х	Х			Х		

Priority substance candidates in 2010					ent			sites
	Main use area	Atmospheric deposition	Other diffuse sources	Storm water	Sewage treatment plants	Industry	Waste	Contaminated sites
(2,4-dichlorophenoxy)- 2-nitrobenzoate								
Cyanid	IND		Х	Х	X	X		
Cybutryne (Irgarol)	PEST		X			Х		
Cypermethrin	PEST	Х	Х			Х		
Dichlorvos	PEST		Х			X X X X X		
Diklofenak	MED				X	Х		
Dicofol	(PEST)		Х			Х		
Dioxin (2,,7,8 - Tetrachlorodibenzo-p dioxin,TCDD)	U				X			Х
HBCD; 1,2,5,6,9,10- Hexabromocyclododeca ne resp 1,,5,7,9,11- Hexabromocyclododeca ne	IND			X	X	X		
Heptachlor	(PEST)							Х
Ibuprofen	MED				Х	Х		
Polychlorinated biphenyls (PCBs)	IND				X X			Х
Perfluorooctane sulfonic acid and its salts (PFOS) and perfluorooctane sulfonyl fluoride	IND		X?	?		?		
Quinoxyfen	PEST	Х	Х			Х		
Terbutryn	(PEST)		Х			X X		
Zink	IND		Х	Х	Х	Х		Х

17 Legislation identified to be of relevance to the reduction of emissions of candidate priority substances

In order to control the emissions of hazardous substances into the environment, different types of control measures are possible, including supportive legislation and restrictions. The following legislative instruments were found to be of relevance to the reduction of emissions of 2010 candidate priority substances to the aquatic environment (directly or indirectly). Many are probably relevant also for the current priority substances. Based on preliminary draft information included in several non published fact sheets and the list may therefore not be comprehensive and entirely up to date. The implementation of the different directives could be different in different member states.

Supportive legislation

- 91/271/EEC (urban waste water treatment)
- 2001/83/EC and 2004/27/EC (human medicine)
- 2004/35/EC (prevention and remedying of environmental damage)
- Soil Thematic Strategy (COM(2006) 231) & Proposal for Soil Framework Directive (COM(2006) 232)
- Community Strategy for Dioxins, Furans and Polychlorinated Biphenyls COM(2001) 593
- Directive 75/442/EEC on Waste; Directive 91/689/EEC on hazardous waste; Decision 2000/532 establishing a list of wastes; and decision 2001/118/EC Waste framework Directive 2006/12/EC (which is the codified version of Directive 75/442/EEC as amended).
- Directive 2006/118/EC on the protection of groundwater against pollution and deterioration
- Regulation (EC) No 1185/2009 concerning statistics on pesticides
- Regulation (EC) No 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin; and amendment Regulation (EC) No 149/2008, setting maximum residue levels for products in Annexes II, III and IV
- Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)
- Regulation (EC) No 166/2006 concerning the establishment of a European Pollutant Release and Transfer Register and amending Directives 91/689/EEC and 96/61/EC
- Council of the European Communities. Directive relating to the quality of water intended for human consumption, 3 November 1998 (98/83/EC). Official Journal (1998), L330/32, 5 December 1998.
- Dangerous Substances Directive (76/464/EEC)
- Existing Substances Reg. (793/93/EC).

Control

- 2008/1/EC (IPPC) concerning integrated pollution prevention and control (codified version) as amended by Directive 2009/31/EC.
- Regulation 470/2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin & Regulation (EU) No 37/2010, on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin
- Seveso II Directive 96/82/EC
- Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)
- Council Directive 1999/31/EC of 26 April 1999 on the landfill of waste
- Waste Incineration Directive 2000/76/EC
- Regulation 1107/2009 (replacing Directive 91/414/EC) (plant protection products)
- Directive 98/8/EC (biocidal products)

- Directive 2009/128/EC establishing a framework for Community action to achieve the sustainable use of pesticides
- Directive 2009/128/EC establishing a framework for Community action to achieve the sustainable use of pesticides
- Commission Directive 2008/116/EC of 15th Dec 2008 amending Council Directive 91/414/EEC
- Stockholm Convention and Regulation 2004/850/EC
- Directive 2006/125/EC on processed cereal-based foods and baby foods for infants and young children
- Council Directive 96/59/EC of 16 September 1996 on the disposal of polychlorinated biphenyls and polychlorinated terphenyls (PCB/PCT)

18 Cost effective measures related to hazardous substances (source control and end of pipe)

The table below summaries potential available measures to improve water quality according to fact sheets developed during the SOCOPSE project for a selected number of priority substances²³⁵. The measures are not listed in order of relevance, although some were considered less cost effective than others, please refer to original reports. The original reports also list emerging techniques, not included below. In some cases, chemical regulations have changed since the publication of the reports; TBT is e.g. no longer allowed as biocide (including the use in cooling water and wood pretreatment).

Substance	Source control	End of pipe	Other types of
	measures	control measures	measures
Cadmium	Recycling and reuse Pre-treatment of wastewater	Optimisation of basic wastewater treatment	
	from technological processes	lon exchange	
	Run off management	Sorbtion active carbon	
	Low Cd phosphate rock	Membrane filtration	
	Cd removal from phosphate	Nanofiltration	
	Cd substitution in electroplating	Electrochemical techniques	
	Battery and cells substitution		
	Curbing emissions to air		
Mercury	Recycling and reuse	Air deposition reduction	
	Pre-treatment of wastewater from technological processes	Crematoria – emission reduction techniques	
	Run off management Chlor alkali substitution	Optimisation of basic wastewater treatment	
	Dentistry material	lon exchange	
	substitution	Membrane filtration	
	Electric products substitution	Adsorption techniques	
	Separated collection,	Biological remediation	
	recycling and save disposal	Nanofiltration	
	Good management practices	Reverse osmosis	
		Electrochemical	

 $^{^{235} \ \}underline{http://www.socopse.se/projectoutput/substance reports and finalersr.4.3d9 ff 17111 f6 fef 70e 9800054023.html}{}$

Substance	Source control measures	End of pipe control measures	Other types of measures	
		techniques		
Nonylphenols (incl	Substitutes for NPE	Separation zone NPE pesticides	Stormwater runoff options	
Nonylphenol- ethoxilates, NPE)		Coal adsorption (incl municipal waste water treatment plants, landfill leachate and groundwater) Chemical oxidation (incl municipal waste water treatment plants, landfill leachate and groundwater)	Ban the use of NP/E containing sludge as soil improver Ban the import of NP/E containing textiles	
		Nanofiltration/reverse osmosis		
ТВТ	Avoid disposal of TBT during wood tretreatment (waste water) and TBT coatings (shipyard waste water)	Activated sludge system MBR	Use of environmentally friendly dredging method	
	Substitution of TBT in antifouling paint, in wood preservatives, as fungicide in cooling towers, and of TBT containing stabilisers in PVC	Oxidation	Remediation of sediment	
		Sedimentation Sand filtration	Treatment of TBT containing sewage sludge	
		Micro/ultrafiltration	Prohibit dumping at	
		Nanofiltration/reverse osmosis	sea of TBT containing sediment and/or sludge	
		Coagulation/flocculation + sand filtration (incl municipal waste water treatment plants)	Ban the use of TBT contaminated sludge as soil improver	
		Coal adsorption ²³⁶ (incl municipal waste water treatment plants)	Ban the use of chemicals	
		Coagulation/flocculation + clarification (DAF)	containing TBT (>1% mass)	
		Solvent extraction		
		Moving bed adsorption		
РАН	Improved transport, storage and process modification at	Activated sludge, tar removal and/or gas	Enhancing user awareness and	

²³⁶ Best end of pipe technique was considered to be a combination of coagulation/flocculation + filtration and coal adsorption to reduce both particle bound and dissolved TBT from effluents

Substance	Source control	End of pipe	Other types of
	measures	control measures	measures
	measures wood impregnation plants Use of wood preservation products with lower PAH content Use of alternative construction materials and wood preservation techniques Combustion optimisation and fuel replacement at residential combustion appliances and power stations	control measurestight operation of gas treatment plant at coke oven plantsFlue gas incineration, wet flue gas scrubbing and/or dry flue gas scrubbing at primary aluminium productionDry flue gas scrubbing and/or use of condensation at electrostatic precipitators at production of carbon and graphite and power stationsSour water stripping, flue gas incineration and/or wet flue gas scrubbing at bitumen production/refineriesWet flue gas scrubbing and/or dry flue gas scrubbing at bitumen production/refineriesWet flue gas scrubbing and/or dry flue gas scrubbing at waste incinerators and power stationsWaste water treatment: activated sludge, MBR, GAC-FBR, chemical oxidation/advanced oxidation and/or constructed wet landsConstructed wetlands and/or bioretention of urban runoffTreatment of sewage sludge: aerobic/anaerobic digestion, composting	measures application of product standards related to residential combustion
		and/or incineration	
DEHP	Substitution of DEHP Substitution of PVC	Optimisation of main waste water treatment Advanced waste water treatment (UV, membranes, oxidation)	
		Secondary sludge	

Substance	Source control	End of pipe	Other types of
	measures	control measures	measures
		treatment (incineration, digestion, agricultural use)	
PBDE	Improving raw material handling, compounding process and/or conversion/backcoating in industrial manufacturing Chemical substitution Changing product material	Recycling in industrial manufacturing and waste treatment Landfilling safely	
HCB	Choice of oil- and chlorine free feeds and/or combustion control in secondary aluminium processing and combustion Pretreatment of feed material and/or closure of small scale facilities in secondary aluminium processing and chemical manufacturing Limitation of demagging impacts in secondary aluminium processing Implementing green chemistry, process modification, purification of products by distillation and/or recycling unintentional HCB generation in chemical manufacturing Careful operations and rigorous maintenance Reducing pesticide application rate/frequency, shifting date, controlling sprayers, conservation tillage and/or ground cover	Activated carbon adsorption Sedimentation Gas filtration Vitrification and open burning of waste in combustion Afterburner in in secondary aluminium processing Land management measures in pesticide applications: grass strips, hedges, riparian zones, constructed wetlands	
Isoproturon	Application: rate reduction, shifting date	Ozone Activated carbon	Information campaign
	Conservation tillage	Nanofiltration	
	Ground cover	Reverse osmosis	
	Sprayer inspection Good farming practices	Land management measures: grass strips, hedges, riparian zones,	
	Substitution	constructed wetlands	

Substance	Source control measures	End of pipe control measures	Other types of measures
	Mechanical weed control		
	False seedbed		
	Organic farming		
	On farm/in field filling and cleaning		
	Sharing equipment		
	No farmyard pesticide application		
Atrazine		Powdered/granular Activated Carbon	
		Ozone	
		AOP (Ozone/H2O2)	
		UV	
		AOP (UV/H2O2)	
		Nanofiltration (NF)	
		Reverse Osmosis (RO)	

19 Regular national monitoring programs related to hazardous substances

National monitoring programs within which hazardous substances are currently monitored (chemically or by effect based parameters such as biomarkers). Programs entirely related to population monitoring are not included.

Monitoring program	Marine/ Limnic	Substances/ endpoints and compartment/species monitored	Monitoring stations and species	Monitori ng frequenc y	National data host; availability
"Integrated coastal fish monitoring" 237	Marine	Population structure, sex, age (based on gill lids and otholites), embryo disturbances (only in eelpout), pathology (external damage, parasite damage in liver, histopathology, necrosis/degenerated liver cells, macrofague centers), hematocrite, Hb, blood lactate, liver weight, LSI, GSI, blood cell ratios (lymphocytes, granulocytes, trombocytes), glycogen (liver&muscle), blood glucose, EROD, glutation reductase activity in liver, VTG, DNA adducts, MT, ions (Na, K and Ca) in blood plasma, liver protein conc.	Holmöarna (perch), Kvädöfjärden (perch and eelpout), Torhamn (perch), Fjällbacka (eelpout)	Annual	Swedish Board of Fisheries ²³⁸ Data not available on homepage but can be obtained directly on request.
Metals and organic hazardous substances in marine biota ²³⁹	Marine	Metals (Hg, Pb, Cd, Cu, Zn, Ni, Cr (n=15 fish, n=3 blue mussel, n=1 egg). In fish all metals are analysed in liver except Hg (muscle).	In total 22 distinct monitoring stations for fish (but all substances not analysed at all	Annual	IVL biotadatab ase Data available

²³⁷

http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/programomraden/kust_och hav/beskrivning_kustfisk_halsa_080525_v2_0.pdf and

http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/hav/fiskhals o.pdf

 $^{^{238}}_{239}$ This task will probably be taken over by the new national authority.

http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/programomraden/kust_och_ hav/beskrivning_metaller_organiska_miljogifter_marin_biota_2.pdf and for blue mussels:

http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/hav/gift_bla mussla.pdf fish:

<u>http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/hav/gift_fisk</u> .pdf and for guillemot eggs:

Monitoring program	Marine/ Limnic	Substances/ endpoints and compartment/species monitored	Monitoring stations and species	Monitori ng frequenc y	National data host; availability
		 PCB, DDT²⁴⁰, HCH²⁴¹, HCB (n=15 fish muscle, n=3 blue mussel, n=1 egg) PBDE²⁴², HBCD (n=9 fish muscle, n=3 blue mussel, n=1 egg) PCDD/F, d-PCB²⁴³s (n=9 fish muscle, n=1 egg) Perfluorinated compounds (n=8 fish liver) PAHs²⁴⁴ (n=3; blue mussels) Also supportive data such as Blue mussels: lipid weight of soft tissues and age; lipid weight and shell thickness of guillemot eggs, In fish: age, sex, length, total weight, liver weight (fresh and dry), gonadal weight, muscle lipid content. 	stations): Baltic Herring: Harufjärden Rånefjärden Kinnbäcksfjärden Örefjärden Gaviksfjärden Långvindsfjärden Ängskärsklubb Lagnö Landsort, Västra Hanöbukten, Abbekås, Byxelkrok, Baltic proper off shore Herring: Karlskrona, Utlängan, Fladen, Väderöarna, Kullen Cod: SO Gotland, Fladen Perch: Holmöarna, Långvindsfjärden, Kvädöfjärden, Åvikslandet, Örefjärden Eelpout: Holmöarna, Kvädöfjärden, Väderöarna Blue mussels: Fladen		on web page

http://www.swedishepa.com/upload/02 tillstandet i miljon/Miljoovervakning/undersokn typ/hav/gift agg.pd f

For the monitoring of Baltic herring, three additional stations were added according to report by Bignert et al 2010.

²⁴⁰ **p,p-DDE**, **p,p-DDD**, **p,p-DDT** ²⁴¹ Alpha, beta, gamma HCH

²⁴² BDE-47, -99, -100 If checking against monitoring data that were retrieved from data host, also BDE-153 and 154 were monitored at least until 2008.

²⁴³ CB-28, CB-52, CB-101, CB-118, CB-138, CB-153 and CB-180

²⁴⁴ (Naftalen, Acenaften, Fluoren, Fenantren, Antracen, Fluoranten, Pyren, Benso(a)antracen, Krysen, Benso(b)fluoranten, Benso(k)fluoranten, Benso(a)pyren, Dibenso(a,h)antracen, Benso(g,h,i)perylen, Indeno(1,2,3-cd)pyren)

Monitoring program	Marine/ Limnic	Substances/ endpoints and compartment/species monitored	Monitoring stations and species	Monitori ng frequenc y	National data host; availability
			Kvädöfjärden, Väderöarna Guillemot eggs: St Karlsö		
Pathology in seal ²⁴⁵	Marine	Macro and microscopic (histopathology, immunoshistochemistry) and by indications, investigations on microbial level (parasites, bacteriology, virology) Baltic seals: claw regions lesions, wounded intestines, Arteriosclerosis, Kidney alterations (glomeruli changes, tubular cell proliferations), osteoporousis, adrenocortical hyperplasia. For females also uterus stenosis, - occlusions and tumors	West and East coast	Annual	SMHI Data available on web page
Biological effect monitoring caused by tinorganic compounds in Nassarius nitidus ("Nätsnäcka") and Peringia ulvae ("Tusensnäc ka") ²⁴⁶	Marine (<i>N nitidus</i> on west coast, <i>H</i> <i>ulvae</i> on East coast)	Shell height, VDSI (based on degree of imposex in females), RPLI (based on penis lengths of females and males respectively), Tissue concentrations of TBT, DBT, MBT, TPhT, DPhT, MPhT. Only analysed in West Coast samples (on N nitidus).	West coast: (tot n=11) Heavily exposed areas: Brofjorden stn 2 and 3 ("Råoljekajen" and "Produkthamnen") and Glommens fiskehamn Less exposed areas: Brofjorden stn 1 ("Holmsundsbåda n") and stn 4	Annual	IVL biotadatab ase Data available on web page

²⁴⁵<u>http://www.swedishepa.com/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/hav/salpatologi.pdf</u> and

http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/programomraden/kust_och_hav/beskrivn-sal-havsorn-v3.pdf.

 $\underline{http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/hav/org_ten_nforening.pdf$ and

<u>http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/programomraden/ku</u> <u>st_och_hav/beskrivn_biol_effektovervakn_org_tennforen_v4_0.pdf</u> but stations monitored have been adjusted according to the latest changes; Marina Magnusson, Marine Monitoring pers comm... Hydrobia ulvae has changed name into Peringia ulvae

²⁴⁶ See

Monitoring program	Marine/ Limnic	Substances/ endpoints and compartment/species monitored	Monitoring stations and species	Monitori ng frequenc y	National data host; availability
			("Slätholmen") and in Göteborg: Långholmen and St Varholmen, Ersdalsviken (less exposed in a gradient).	y	
			Reference areas: Gåsenabbe, Burholmarna (=Tjärnö) and Kalvhagefjorden (="Gullmarfjorden s mynning").		
			East coast:		
			(tot n=16)		
			Heavily exposed areas: Bullandö Marina, Oxelösund Marina, Blankaholm Kaj, Hälleviks Hamn, Trelleborgs hamn, Råå hamn		
			Reference locations: Toseboviken, Sydöstra Kittelön, Öster om Öre, Stora Bäckskär, "Trelleborgs hamn referens", Salvikens strandängar.		
			Natural harbours: Lökaö, Ringsöfladen, Vippholmen, Tjärö		
Embryonal development in Monoporeia affinis and Pontoporeia femorata (benthic	Marine (East coast)	Fecundity (egg per female), females with dead eggs in marsupium (%), malformed embryos ²⁴⁸ (%), dead embryos (%), undifferentiated embryos (%), Parasite damage (%), somite damage (%), biomass, production	Baltic proper (n=9) and Bothnian Sea (n=5)	Twice annually (sept & feb) in Baltic proper; annually Bottenha	SMHI Data are not available on web page

Monitoring program	Marine/ Limnic	Substances/ endpoints and compartment/species monitored	Monitoring stations and species	Monitori ng frequenc y	National data host; availability
invertebrate) 247		Supportive data include O2 in water and org C and O2 in sediment. Coordinated with monitoring programme of benthic fauna		vet (feb)	
Metals and organic substances in marine	Marine	 PAH (Naphtalene, Acenaphtylene, Acenaphtene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benso(a)anthrascene, Chrysene, Benso(b)flouranthene, Benso(b)flouranthene, Benso(a)pyrene, Dibenso(ah)anthracene, Benso(ghi)perylene, Indeno(1,2,3-cd)pyrene), HCB, PCB (28, 52, 101, 118, 153, 138, 180), HCH (a, b, g), chlordane (a, g, trans- nonachlor), DDT/D/E (o,p'; p,p'), PBDE (47, 100, 99, 85, 209:deca), HCBD, nonylphenol (4-n; 4-iso), octylphenol (4-n; 4-iso), octylphenol (4-r, 4-t-), DEHP, alachlor, atrazine, diuron, endosulfan, isoproturon, chlorfenvinphos, chlorpyrifos, PCP, simazin, trifluralin, tinorganic compounds (TBT, DBT, MBT, TeBT, TPhT, DPhT, MPhT, MOctT, DOcT), alkylated Pb (TetrametylPb, TetraetylPb, TrietylPb, DietylPb, TricyHexT). Metals: Ag, As, Ba, Be, Ce, 	n=16 (sea, non coastal)	Aproxima tely every 5 th year (perform ed in 2003 and 2008)	SGU Data available on web page but only for samples taken in 2003
sediment ²⁴⁹		Cs, Cd, Co, Cr, Cu, Fe, Hg,			

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http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/hav/monopo reia.pdf and

http://www.naturvardsverket.se/upload/02 tillstandet i miljon/Miljoovervakning/programomraden/kust och hav/kvalitetsdeklaration embryonal vitmarla.pdf

²⁴⁸ This biomarker has been found to be primarily linked to the contamination of hazardous substances (Wiklund & Sundelin 2004). See also chapter 9. ²⁴⁹ A full description of programme has not been possible to obtain but information on metals to analyse in

marine sediment was used

http://www.naturvardsverket.se/upload/02 tillstandet i miljon/Miljoovervakning/undersokn typ/sotvatten/me t sedm.pdf, and information on organic substances analysed according to Jonas Rodhe, Swedish EPA, pers comm. Number of stations based on reported results. From reported results it is also clear that additional parameters were monitored.

Monitoring program	Marine/ Limnic	Substances/ endpoints and compartment/species monitored	Monitoring stations and species	Monitori ng frequenc V	National data host; availability
		Mn, Ni, Pb, Sn, V Zn (and several others) as well as supportive parameters such as LOI, dw, Fe and Mn nodules, Al, Al2O3, C, Li, metal oxides etc; salinity and O2 of water phase,			
Fish ²⁵⁰	Limnic	Metals ²⁵¹ : Hg, Pb, Cd, Ni, Cr, Cu, Zn. All metals are analysed in liver except Hg (in muscle) Perfluorinated substances: PF carboxylates (PFCAs); perfluorheptanoat (PFHxA), perfluorheptanoat (PFHpA), perfluoroctanoat (PFOA), perfluoroctanoate (PFDcA), perfluoroundecanoate (PFUnA), perfluordodecanoat (PFDoA), perfluortridecanoat (PFTriA), perfluortetradecanoate (PFTeA), perfluorinated sulphonates (PFSs): perfluorbutan sulfonate (PFBS), perfluorhexane sulfonat (PFHxS), PFOS, perfluordecane sulfonat (PFDcS), perfluorotan sulfonamid (PFOSA) and 6:2 fluortelomer sulfonat (6:2 FTS), analysed in liver Other organic substances: PCBs (28, 52, 101, 118, 138, 153, 180), p,p-DDE/D/T, HCH (a, β , γ), HCB. PCDD/F, PBDE, HBCD in muscle Supportive parameters: age, sex, length, total weight, liver weight (fresh and dry), gonadal weight, muscle lipid content.	32 lakes. If not specified differently, perch is monitored: Abiskojaure (arctic char), Allgjuttern, Bolmen (pike), Brännträsket, Bysjön, Bästeträsk, Degervattnet, Fiolen, Fräcksjön, Fysingen, Gipsjön, Horsan (perch or roach), Krageholmsjön, Krankesjön (perch or roach), Lilla Öresjön, Limmingsjön, Remmarsjön, Skärgölen, Spjutsjön, St Envättern, Stensjön, Stora Skärsjön, Stor- backsjön, Stor- Björsjön, Storvindeln (pike), Svartsjön (perch or roach), Sännen, Tärnan, Älgsjön, Övre Skärsjön	Annually, but not for all substanc es	IVL Data available on web page
Integrated Studies of the Effects	Limnic (lakes and	The metals Fe, Mn, Al (tot, org and inorg), Cd, Cu, Zn, Pb are included in the water	26 lakes and 43 rivers are included in the	Water chemistry in lakes:	Water: SLU ²⁵⁴

²⁵⁰ Program is described in

http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/rapporter/sotvatten/kvaldekl provbankn2007.pdf ²⁵¹ There is a parallell Nordic program (Sweden, Norway and Finland) analysing mercury in perch

Monitoring program	Marine/ Limnic	Substances/ endpoints and compartment/species monitored	Monitoring stations and species	Monitori ng frequenc V	National data host; availability
of Liming Acidified Waters (IKEU: Integrerad Kalknings- Effekt- Uppföljning) ²⁵²	rivers)	chemistry program Hg is also analysed in perch	national program ²⁵³ , in addition to 10 of the trend lakes that are also financed within this program (see program on physiochemical monitoring of lakes and rivers) 13 of the lakes are also montored regarding Hg in fish in addition to the Hg monitoring performed in trend lakes but financed within this program (see limnic biota	monthly in feb, april- october (8 samples annually) and in rivers: monthly but 8 additional samples during expexted high flow events in 23 of these rivers. Hg in fish annually	Biota: IVL Data available on web page
Pesticides in agricultural areas ²⁵⁵	Limnic	Pesticide substances (>115 substances in water and >60 substances in sediment). The following priority substances are included and analysed in both sediment and water: alachlor, atrazine, chlorfenvinphos, chlorpyrifos, diuron, endosulfan, HCH, isoproturon, simazine, trifluralin	6 monitoring areas/stations (4 "type areas" located in Skåne, Halland, Västergötland, Östergötland, and two larger rivers in Skåne)	Surface water in the type areas every week from May to Novembe r, sediment once annually, The rivers are sampled 1-2 times per month from May to Novembe	SLU ²⁵⁶ Data available on web page

²⁵² http://info1.ma.slu.se/IKEU/ and and also http://www.slu.se/sv/centrumbildningar-och-projekt/ikeu/
 ²⁵³ There are additional stations within RMÖ
 ²⁵⁴ The metals are at the moment located in separate databases though and can be found at http://info1.ma.slu.se/max/www_max.acgisProject?ID=Intro&pID=-6 but a revision is ongoing. Tobias

<u>http://inio1.ma.std.se/max/www_max.acgtsProject/1D=intro&p1D=-6</u> but a revision is ongoing. Tobias Vrede, SLU pers comm..
²⁵⁵<u>http://www.swedishepa.com/upload/02_tillstandet_i_miljon/Miljoovervakning/programomraden/Jordbruks</u>
<u>mark/kvalitetsdekl_jordbruk_pestic.pdf</u> the priority substances monitored: according to reported data.
²⁵⁶ <u>http://www.slu.se/sv/fakulteter/nl/om-fakulteten/institutioner/institutionen-mark-och-miljo/miljoanalys/vaxtskyddsmedel-typomraden-aar/</u>

Monitoring program	Marine/ Limnic	Substances/ endpoints and compartment/species monitored	Monitoring stations and species	Monitori ng frequenc v	National data host; availability
Physicoche	Limnic	Al, As, Cd, Pb, Zn, Hg, Cr, Cu, Ni, Fe, Mn, V, Co Supportive parameters such as TOC, pH, Suspended matter, Ca, Mg, conductivity etc as well as nutrients are also included. Samples normally taken at 0.5 m depth	There are in total 67 trend monitoring stations of rivers, of which Hg is analysed at 20 sites and the other metals at 30 sites ²⁵⁹ There are also monitoring stations of 47 rivers that end at sea (estuaries) ²⁶⁰ There are in total 106 trend monitoring stations of lakes of which metals is analysed at 10 sites, from surface ²⁶¹ . There are also 800 "omdrevssjöar" picked randomly each year out of	y r, and one sediment sample per year Monthly (12 times a year) in rivers At the 10 lake trend stations: twice a year. From the 800 "omdrevs sjöarna": annually (e.g. sept-nov in 2010) and 6 years in a row)	SLU ²⁶³ Data available on web page
Physicoche mical rivers ²⁵⁷ and lakes ²⁵⁸			picked randomly		

 $^{^{257} \}underline{http://www.swedishepa.com/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/sotvatten/vation/willowervakning/willower$

²⁵⁹<u>http://www.swedishepa.com/upload/02_tillstandet_i_miljon/Miljoovervakning/programomraden/sotvatten/</u> KvalDekl trendvattendrag2007.pdf

²⁶⁰http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/rapporter/sotvatten/Kval Dekl flodmynning2008.pdf

²⁶¹http://www.swedishepa.com/upload/02 tillstandet i miljon/Miljoovervakning/programomraden/sotvatten/ KvalDekl trendsjoar2007.pdf

²⁶²http://www.swedishepa.com/upload/02 tillstandet i miljon/Miljoovervakning/programomraden/sotvatten/ KvalDekl omdrevsjoar2007.pdf

tenk_v.pdf ²⁵⁸<u>http://www.swedishepa.com/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/sotvatten/vat</u> tenkemi sjo.pdf also described at http://www.slu.se/sv/centrumbildningar-och-projekt/kompetenscentrumfor-kemiska-bekampningsmedel/verksamhetsomraden/nationell-miljoovervakning/

Monitoring program	Marine/ Limnic	Substances/ endpoints and compartment/species monitored	Monitoring stations and species	Monitori ng frequenc y	National data host; availability
	Limnic	Vänern	Vänern	Vänern	Fish: IVL
		Fish:	Fish ²⁶⁶ : Perch: 1, Pike 1	Fish: Perch	Sediment: SGU ²⁶⁹
		Perch, muscle: Hg, PCBsum7, planar PCB, dioxins, PBDE; every 5 th year also Cd, Pb, Ni. In liver: Cr,	Sediment ²⁶⁷ : 7 stations	annually and pike every 5 th year	Water chemistry: SLU
		Zn, Cd, Cu, Ni, Pb, As, perfluorinated compounds. Pike, muscle: Hg	Water chemistry: 1 station from outflowing water and 13 stations	Sediment : every 10 th year	Data available on web
		In both species also: age, sex, weight, length, liver weight, LSI, gonadal weight, lipid weight. In perch also gut contents (weight).	from outflowing water (none in lake)	Water chemistry : monthly	pages
		Sediment chemistry ²⁶⁵ : dioxins, dl-PCBs, PCB7, PBDE, PAH16, DEHP, tinorganic compounds, Pb, Cd, Hg, Ni, Cu, Zn, As, Cr, Co, Fe, Mn, Ag. Water chemistry: Si, Fe, Mn, Cu, Zn, Cd, Pb, Hg, Cr, Ni, As, Co, Al, V <i>Vättern</i> Fish:	Vättern Fish: Char: 3 Water chemistry: 2 in the lake, 6 from inflowing rivers plus outflow (1 stn). Sediment: 3 stations	Vättern: Fish: Char every 5 th year Water chemistry : 4 times annually in lake, monthly in in- and outflow;	
The great lakes monitoring programs in Vänern and Vättern ²⁶⁴		Char (Salmo salvelinus): Hg, PCB, DDT, HCB, HCH, dioxins and dibenzofurans Supporting parameters: age, sex, weight, length, liver		analysed at different water depths in lakes. Sediment	

²⁶³ http://info1.ma.slu.se/db.html

²⁶⁴http://www.swedishepa.com/upload/02 tillstandet i miljon/Miljoovervakning/programomraden/sotvatten/ KvalDekl storasjoar2008.pdf; the monitored parameters have been updated according to new revised program for Vänern (Agneta Christensen, County Adm Board Västra Götaland, pers comm) and according to the latest program for Vättern (Måns Lindell Adm Board Jönköping, pers comm.) for 2006:

http://projektwebbar.lansstyrelsen.se/vattern/SiteCollectionDocuments/sv/vatternvardsforbundet/publikationer /Rapporter/rapp697.pdf

The program is currently being revised. Hazardous substances are not monitored in Mälaren. ²⁶⁵ Minimum program, other sediment parameters may also be included. Next time sediments will be analysed are in 2019.

²⁶⁶ There are also additional SRK stations for pike and perch.
²⁶⁷ There are also 12 additional ERK stations for sediment

Monitoring program	Marine/ Limnic	Substances/ endpoints and compartment/species monitored	Monitoring stations and species	Monitori ng frequenc y	National data host; availability
		weight, LSI, gonadal weight, lipid weight, gut contents (weight).		: every 6 th year ²⁶⁸	
		Sediment chemistry: selected substances varies but includes metals and organic substances. Larger study also performed in 1972.			
		Water chemistry: Fe, Mn, Cu, Zn, Cd,, Pb, Cr, Ni, As, Co, Va, Al			

 ²⁶⁸ Next time in 2012, performed in parallel with benthic fauna monitoring
 ²⁶⁹ However, Vättern data from 2004 are to be found in the IVL screening database. Other sediment data not available on home page but can be retrieved on request from Vätternvårdsförbundet, ; <u>www.vattern.org</u>

20 Summary statistics for screening data used in this report

20.1 Water samples

Detected substances that are included in 2008/105/EC in water samples in the Swedish screeningdatabase. Please note that the database includes both data from surface water samples, passive samplers and effluents etc. Conc values are in ug/l. Maximum concentrations that are above current AA-EQS values are indicated in bold.

Substance	Maximu m conc detected	Minimum conc detected	Highest LOQ	Lowest LOQ	Number of samples where substan ce was detected	Total number of samples analysed	Detecti on freque ncy (%)
1,2- dichlorethane	36	0,034	1	0,2	8	306	2,6
Alachlor	0,0008	0,0008	1	0,0004	1	382	0,26
Anthracene	0,015	0,000004	0,1	0,00000 9	91	412	22
Atrazin	0,06	0,001	2	0,0006	5	381	1,3
Benzene	0,39	0,0011	0,2	0,001	22	327	6,7
Lead	33,93	0,0007	0,6	0,001	410	442	93
PBDE	0,001	6,9E-08	0,0005	1,9E-08	506	1681	30
C10-13 Chlorinated alkanes	0,2	0,2	0,2	0,1	1	289	0,35
DDT total	0,00002 2	0,0000038	0,00001 2	0,00000 24	28	80	35
Di(2- etylhexyl)ftala t (DEHP)	33	0,06	1	0,07	62	346	18
Dichlormetan	Not detected	Not detected	0,2	0,006	0	125	0
Diuron	32	0,02	5	0,003	10	369	2,7
Endosulfan	0,0027	0,000094	0,03	0,00003 8	12	742	1,6
Fluoranthene	1	0,000035	0,1	0,01	123	412	30
Hexachlorbenz ene	0,0041	0,0000015	0,01	0,0001	118	414	29
Hexachorbuta diene	0,19	0,012	0,01	0,0002	7	296	2,4
Hexachlorocyc lohexane	0,0019	0,0000014	0,01	0,00000 059	268	1339	20
Isoproturon	0,002	0,002	0,05	0,01	1	339	0,29

Cadmium	0,688	0,0002	0,08	0,0003	270	389	69
Chlorfenvinfo	0,004	0,000001	0,05	0,00000	41	417	9,8
S	-)	- ,	-)	69			
Chlorpyrifos	Not	Not	0,05	0,02	0	288	0
17	detected	detected					
Carbontetraclor	0,0024	0,0016	0,2	0,2	7	16	44
ide							
Mercury	1,4	0,00039	0,02	0,002	94	315	30
Naphtalene	0,04	0,00028	0,1	0,00085	117	411	28
Nickel	793	0,0061	0,8	0,2	353	369	96
Nonylphenol	4,59	0,000442	0,13788	0,00053	21	182	12
				8			
Oktylphenol	53	0,002	0,035	0,002	169	509	33
Pentachlorbenz	0,0034	0,0000012	0,01	0,00000	103	414	25
ene				056			
Pentachlorfeno	0,01881	0,0000013	0,1	8,9E-08	47	419	11
1							
РАН	0,29	0,000006	0,1	0,00000	456	2062	22
				4			
Simazin	0,04	0,04	1	0,0007	1	381	0,26
Tetrachloretyle	58	1,1	0,3	0,2	7	10	70
n							
Tributyltin	0,014	0,0001	0,001	0,0003	78	404	19
compounds							
Trifluralin	0,00003	0,000008	0,01	0,00000	49	367	13
	6	6		069			
Trichlorbensen	0,0035	0,0000059	0,01	0,00000	56	1230	4,5
es				2			
Trichoretylen	71	0,00082	0,2	0,0003	6	17	35
Trichlormetan	6,6	0,014	0,2	0,1	29	305	9,5

20.2 Biota samples

Detected priority substances in biota registered in the IVL screening database. The type of biota analysed varies (different species and tissues). An assessment of concentrations in different types of biota and tissues has not been performed due to difficulties in the interpretation and lack of supporting information.

	Number of	Total	Detection
	data above	number of	frequency
Substans	LOD	data	
Anthracene	84	141	60
Benzene	3	12	25
Lead	315	571	55
Bromerade difenyletrar	1139	1320	86
C10-13 Kloralkaner	0	14	0
Cyklodiena bekämpningsmedel	0	32	0
DDT total	116	261	44

Di(2-etylhexyl)ftalat (DEHP)	16	113	14
Diklormetan	1	12	8
Endosulfan	0	15	0
Fluoranten	88	161	55
Hexaklorbensen	140	241	58
Hexaklorbutadien	0	4	0
Hexaklorcyklohexan	203	260	78
Cadmium	468	569	82
Koltetraklorid	2	12	17
Mercury	176	225	78
Naftalen	107	151	71
Nickel	398	546	73
Nonylfenol	22	43	51
Oktylfenol	38	70	54
Pentaklorbensen	18	99	18
Pentaklorfenol	10	16	63
РАН	99	640 ²⁷⁰	15
Tetrakloretylen	0	12	0
ТВТ	93	120	78
Triklorbensener	0	222	0
Trikloretylen	0	12	0
Triklormetan	5	12	42

20.3 Sediment samples

Concentrations exceeding lowest calculated Qsbenthic (or similar values) are indicated in bold.

					Numbe r of		
					values		Upper
	Maximum	Minimum	Highest	Lowest	above	Numb	detecti
	concentrati	concentrati	detecio	detecti	detecti	er of	on limit
Substans	on	on	n limit	on limit	on limit	data	(%)
Pentaklorfenol	0,027828	0,001977	0,01	0,001	7	21	33
				0,00026			
C10-13 Kloralkaner	0,012729	0,008072	0,2	3	2	23	9
Antracen	0,67	0,055	0,6	0,05	9	32	28
Bromerade							
difenyletrar	0,0005	0,0004	0,0002	0,0002	3	24	13
Hexaklorbensen	0,038	0,0015	1	0,003	4	26	15
Naftalen	0,27	0,092	0,6	0,05	5	32	16
Nonylfenol	0,562	0,0032	1	0,3	7	33	21
Pentaklorbensen	0,004	0,0003	1	0,0005	4	23	17
Triklorbensener	0,006	0,0002	0,05	0,0002	6	54	11

²⁷⁰ The figure refers to the sum of samples where Benzo(a)pyrene (analysed in 144 samples), benzo(b)fluoranthene (127 samples), benzo(ghi)perylene (136 samples), benzo(k)fluoranthene (90 samples), indeno(1,2,3-cd)perylene (143 samples) analysed

Di(2-							
etylhexyl)ftalat							
(DEHP)	87	0,0081	0,1	0,003	29	39	74
			0,0873				
Diuron	0,086	0,00006	98	0,00005	84	124	68
Fluoranten	6,4	0,05	0,6	0,05	68	111	61
Kadmium	130	0,0151	2	0,01	637	643	99
Mercury	16	0,01	0,12	0,04	587	605	97
Nickel	3330	1,23			601	601	100
Oktylfenol	0,088	0,00017	10	0,001	42	56	75
ТВТ	370	0,001	3	0,0009	113	131	86
1,2-dikloretan			0,2	0,05	0	15	0
Alaklor			1	0,01	0	28	0
Atrasin			0,05	0,01	0	25	0
Bensen			0,2	0,02	0	15	0
Diklormetan			0,05	0,05	0	3	0
Endosulfan			0,007	0,0005	0	48	0
Hexaklorbutadien			0,05	0,0002	0	18	0
Hexaklorcyklohexa							
n			0,003	0,003	0	36	0
Isoproturon			0,01	0,01	0	12	0
Klorfenvinfos			0,01	0,01	0	12	0
Klorpyrifos			0,01	0,01	0	12	0
Simazin			1	0,01	0	28	0
Trifluralin			0,05	0,05	0	12	0
Triklormetan			0,2	0,05	0	15	0
Lead	6600	0,187			675	675	100
BaP	2,3	0,09	0,6	0,05	38	105	36
Bbfluoranthene	3,5	0,05	0,3	0,05	65	97	67
Benzo(ghi)perylen							
е	1,7	0,05	0,6	0,05	52	105	50
benzo(k)fluoranth							
ene	1,3	0,05	0,6	0,05	37	73	51
indeno(123cd)pery							
lene	1,8	0,06	0,6	0,05	61	115	53

21 Preliminary conclusions on major risk of non compliance

To aid in the selection of monitoring compartment but also as an indication about current major problematic substances (and probably less problematic substances for a certain compartment), preliminary conclusions are made about major risk of non compliance for the three compartments (water, biota, sediment) for the current priority substances. The conclusions are based on available data primarily retrieved from national data hosts. Candidate priority substances and the other substances regulated in 2008/105/EC were not included in the evaluation. The assessment of risk of biota compliance was frequently difficult to make because it requires information on several supportive parameters that frequently were not available²⁷¹. An assessment of increasing or not decreasing trends on a national scale is also included. Such substances should be included in surveillance monitoring and prioritized in operational monitoring if significant local sources can be suspected. If a priority substance is not included in any of the cells of the same row, it could not be evaluated even on a preliminary basis.

	Can probably be deselected for compliance checking, unless clear local source/s/ can be identified ²⁷²	Suspected to be ubiquitous substances ²⁷³ .	Non compliance cannot be excluded ²⁷⁴	Trends are increasing or not decreasing in a national perspective
Water	Alachlor, Anthracene Atrazine, Benzene, C10-13 chloroalkanes, Dichloromethane, Endosulfan, HCB, HCH, Naphthalene, Pentachlorbenzene, PCP, Simazine, Trichlorbenzenes, Trifluralin 275		PBDE, Cd, Chlorfenvinphos, 1,2-dichlorethane, DEHP, Diuron, Fluoranthene, Hexachlorbutadiene, Pb, Hg, Ni, Nonylphenol, Octylphenol, PAH, Trichlormethane, TBT (?) ²⁷⁶	Pb (limnic) ²⁷⁸

²⁷¹ If monitored species and tissue was not specified, such data were not included in the assessment. For data on blue mussels and gastropods, wet weight was assumed to be about 10% and lipid weight about 1% if not specified but necessary for the assessment, in order to make at least a rough estimate of compliance. Normalisation to 5% lipids was not made but rather recalculated into wet weight basis because it was not clear if this had been done in calculating the draft EQS values.

²⁷² Indicated by the fact that none of the data in the screening data base seems to exceed QS values.

²⁷³ Indicated by the fact that also data from reference areas may exceed EQS (or QS for a particular compartment)

²⁷⁴ Indicated by the fact that maximum concentrations registered exceed QS or, if data are from reference locations, only slightly below QS.

²⁷⁵ Based on data from the screening database. The deselection of these priority substances from water monitoring may need to be revised if the EQS of any of these substances becomes substantially lower in future revisions. Furthermore, from the above listed substances, the highest LOQ values do not fulfil the requirements of the QA QC directive: alachlor, anthracene, atrazine, C10-C13 Chloralkanes, chlorpyrifos, endosulfan, HCB, HCH, pentachlorbenzene, simazine. Nevertheless, the lowest recorded LOQ does fulfil the criteria. In addition, alachlor and trifluralin was never detected and simazine and HCH concentrations found in

Sediment	C10-13 chloroalkanes, PCP ²⁷⁹ 1,2-dichlorethane, alachlor, atrazine, dichlormethane chlorfenvinphos, Hexachlorbutadiene, simazine ²⁸⁰ Trichlorobenzenes, trichlormethane ²⁸¹	TBT (marine) PAH (marine) Octylphenol (marine) Anthracene (marine) Fluoranthene (?)(marine) ²⁸²	Anthracene, DEHP, Fluoranthene, HCB, Cd, Hg, Naphthalene Nickel, Nonylphenol, Octylphenol, PAH Pentachlorbenzene, TBT ²⁸³ Diuron, endosulfan, isoproturon ²⁸⁴ HCH ²⁸⁵	Not possible to estimate on national scale (off shore sediments were only monitored in 2003 and 2008 so far)
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a few water samples were always below AA-EQS, in water samples from the four type areas and rivers monitored within the national pesticide programme. Endosulfan and atrazine were detected within the national pesticide program but below AA-EQS. 276.

²⁷⁶ Based on the data contained in the screeningdatabase (registered by March 2011), these substances occurred at least once at or above the current EQS values in water and non compliance can therefore not be excluded. However, because the screening database includes results from both screening studies and regional monitoring campaigns as well as data from effluents and surface water, the interpretation should be made with care, probably over estimating the substances that could cause non compliance based on water related data. Type of sample (effluent or surface water) is frequently not reported in a clear way. Diuron was e.g. never found in concentrations above AA-EQS in the pesticide monitoring program and chlorfenvinphos was never detected. Cd data from rivers that end at sea exceeded worst case EQS (assuming low water hardness and without taking background into account) for 1.4% of all registered data (but none in 2010).

²⁷⁷ Isoproturon was never found in concentrations above AA-EQS according to data from the screening database but within the national pesticide program, this substance is frequently detected in water and maximum concentrations found are above AA-EQS

²⁷⁸ In the limnic environment, official statistics are available on Pb in lakes and rivers, indicating that the concentrations are still not decreasing in spite of the ban of Pb in gasoline. This is thought to be due to Pb being accumulated from previous atmospheric deposition in surface soil.

²⁷⁹ The detection frequency of C10-13 chloroalkanes data registered in the IVL screening database is only 9% (n=23 total number of data) and the highest concentration ever detected is 1/5th of the lowest estimated QSbenthic. In addition, the highest LOD registered is also below the lowest EQS. Approximately the same situation is valid for PCP, for which the detection frequency is somewhat higher, 33% (n=21), but the highest concentration and LOD is below lowest estimated QSbenthic.

²⁸⁰ These substances were not detected in sediment according to data registered in the IVL screening database and inherent properties would not suggest significant sediment accumulation. Hexachlorbutadiene was also not detected but the highest LOD is above lowest estimated QSbenthic but the lowest LOD is below. Alachlor, atrazine, chlorfenvinphos, trifluralin and simazine were also not detected in sediment in any samples from the four type areas within the national pesticide program. Also benzene was never detected according to IVL screening database but in the national monitoring program of off shore sediments, benzene was detected in all samples in 2008; compliance cannot be evaluated because trigger to derive QSbent not met. HCH, chlorpyrifos and trifluralin were also not detected but even the lowest LOD values registered in the database are higher than the lowest estimated QSbenthic. It is therefore not yet possible to conclude that such substances may not indicate non compliance based on sediment data.

²⁸¹ Trichlormethane was detected in all off shore sediment samples in 2008 but at concentrations lower than lowest estimated QSbenthic and never detected according to registered data in the screening database; 1,2,4trichlorbenzene was detected in half of the off shore sediment samples in 2008 but at concentrations lower than a tentative QSbent on ww basis in the current SDS. Moderate detection according to screening database but also lower than tentative QSbent.

 282 TBT concentrations are expected to exceed lowest estimated QSbenthic at all monitored off shore sites (n=16 and TOC values vary between 1.8 and 17% dry wt) with up to 4000 times. The concentration of PAHs are also high in off shore and west coast sediments, but the QSbenthic for fluoranthene might change into a higher value with the revision of current EQS. Octylphenol concentrations in off shore data exceed lowest estimated QSbenthic at all sites where detected (n=10) and where not detected the LOD does not fulfil QA QC

Biota	Trichlorobenzenes	Mercury ²⁸⁷ PBDE (?) ²⁸⁸ Cadmium blue mussels ²⁸⁹ Pb blue mussels ²⁹⁰ TBT gastropods(?) ²⁹¹	PBDE (?) HCB (?) HCH (?) PAH in blue mussels (?) ²⁹² Cadmium, marine limnic fish (?) ²⁹³ Pb, marine, limnic fish (?) ²⁹⁴ Ni blue mussels ²⁹⁵	HBCD (marine) PFOS (marine) Dioxins (marine) Cd (marine) Hg (marine and limnic) ²⁹⁷ Ni, Zn, Cu ²⁹⁸ (marine)
			Ni blue mussels ²⁹⁵ TBT ²⁹⁶	

requirements. The same data set compared to draft EU values for benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene also indicate non compliance at all sites. TBT concentrations in a dataset from the West coast (monitored at n=18 sites) are up to 62 000 times above lowest estimated QSbenthic and for octylphenol with up to 39 times.

²⁸³ Because these substances occur at least once in concentrations above lowest estimated QSbenthic according to sediment data registered in the IVL screening database although the Cd background concentrations were then not subtracted. In addition, Kalmar sediment data for Cd retrieved from the SGU database indicate that QSbent values are exceeded at a few sites with up to 14 times but there are generally less exceedences in the later years (data available from 1985, 1990, 1998, 2003 and 2008). Nonylphenol (n+iso) concentrations in off shore data also exceed lowest estimated QSbenthic at two sites (out of n=16), naphthalene and fluoranthene at seven sites, and octylphenol at all sites where detected (n=10). PAHs, TBT and nonylphenol and octylphenol concentrations along the Swedish West coast also exceed lowest estimated QSbenthic but only once for iso-nonylphenol (although only monitored at n=3 sites).
²⁸⁴ Diuron was detected in 3 out of 7 sediment samples in one of the type areas within the national pesticide

²⁸⁴ Diuron was detected in 3 out of 7 sediment samples in one of the type areas within the national pesticide monitoring program and although concentrations were below quantification limits, these are estimated to be between 3 and 35 mg/kg, whereas the trigger value is 0.007 mg/kg. Isoproturon was also detected in one of the sediment samples and again although not possible to quantify, the concentration is estimated to be 2-10 mg/kg, i.e. far above trigger (0.03 mg/kg). The same situation for endosulfan. In off shore sediments from national monitoring, endosulfan was detected once (out of 16 samples) but again LODs did not fulfil QA QC requirements and exceeded lowest estimated QSbenthic

²⁸⁵ Maximum concentration found in off shore sediments (0.003 mg/kg) exceeds draft trigger value of 0.55 ug/kg. In several cases not detected, therefore not possible to assess potential of being an ubiquitous substance in sediment as well.

²⁸⁶ from >200 samples the substance was not detected in any sample. Also other substances were never detected, but sampled on limited occasions. Hexachlorbutadiene was only analysed in four samples, and C10-C13 chlorinated alkanes and endosulfan in less than 20 samples. In addition, because the LOQ values were not compared to QS values (supportive data were not available), it is not possible to state whether the LOQ levels in these studies fulfill the requirements of the QA QC directive.

²⁸⁷ Exceeds biota-EQS based on available fish data from reference locations, although background concentration has not been taken into account. Frequently also exceeds OShh in lakes (if monitored in pike; highest conc found was 5.6 mg/kg ww in muscle). Marine perch muscle concentrations from reference sites varied between 0.07-0.17 mg/kg (if including all years). Concentrations in blue mussels at reference sites are generally lower (maximum found concentration was 0.05 mg/kg ww). Lipid normalisation was not performed. ²⁸⁸ The assessment of whether PBDE should be considered ubiquitous based on biota data to a large extent depends on which criteria is used for comparison. The new EQS has not been decided yet but in the March 2011 dossier the draft value 0.0085 ug/kg wet weight is suggested (based on QShh). Such a low EQS would justify PBDE to be considered ubiquitous in biota. However, if instead using current PNEC (0.27 mg/kg) from current SDS there is no indication of non compliance. Limnic perch muscle (data only available from 2002 and not for all congeners; LOQ highly variable - if not detected usually high LOQ compared to concentrations in samples where detected): conc of sum detected PBDE congeners: 0.01 - 0.7 ug/kg ww; Marine perch muscle: congeners 47, 99, 100, 153 and 154 detected in all samples, 0.04 - 0.2 ug/kg ww; Herring muscle: 0.04 - 2.8 ug/kg ww; Eelpout muscle: 0.01 - 2 ug/kg ww; Vättern char muscle: 0.006 - 0.074 ug/kg ww (data only available for 7 samples); Cod liver: 7 - 130 ng/g lipid, which is in the same range as concentration on lipid basis as for other species; blue mussel: 16 - 154 ng/g lipid (also in the same range as fish on lipid basis). (Cf perch: 0.9 - 127 and herring: 1.9 - 120 ng/g lipid).

²⁸⁹ QSsec pois according to current SDS is 0.16 mg/kg ww. Fish data are primarily available for liver (in herring concentrations range between 0.03-1.8 mg/kg, in limnic perch 0.006-14 mg/kg, in pike 0.002-0.4

mg/kg ww, in char 0.01-1.0 mg/kg ww and in roach 0.001-0.02 mg/kg ww at reference sites, if including data from all years registered but excluding data for which dw has not been registered), but it is difficult to estimate whole organism concentrations based on data from liver because the liver weight was not registered (and Cd was never detected in fish muscle based on data from reference sites). However, from the latest registered mussel data (2003) concentrations vary between 0.12 - 0.97 mg/kg ww at reference sites, i.e. below or just below QShh (1.0 mg/kg) but more or less always above QS sec pois. Background was not taken into account. ²⁹⁰ In blue mussels, Pb concentrations vary between 0.02 - 5.8 mg/kg ww and most data are above the Swedish Pb trigger value (0.04 mg/kg ww) and some even above limit value for food consumption (1.5 mg/kg ww) (based on all years and where dw was available, but the highest concentrations were found in 2003, the latest year for which data was been registered). Primarily mussel data suggest large scale non compliance in marine environment, whereas the situation in limnic environment and for fish is unclear, see footnote related to Pb in next column

²⁹¹ The Swedish trigger value (0.0002 mg/kg) is exceeded in gastropods also from reference areas (Burholmen 5 concentrations are e.g. estimated to be varying between 0.0002-0.0003 mg/kg ww if assuming 1% lipid and 10% ww, based on data from the last 5 years). From effect based tools it can also be concluded that the imposex frequency is still high in reference areas (30-40% at most sites but 4.5% at new reference site Gåsenabbe).

²⁹² Because PBDE is suspected to be ubiquitous also local non compliance cannot be excluded but the assessment depends on criteria used. For anthracene, maximum concentration found in blue mussels at reference areas are 3.3 ng/g dw (estimated to correspond to about 0.3 ug/kg ww if assuming 10% wet weight). Current trigger of 20 ug/kg ww (based on overall current EQS for water) is not expected to be exceeded at reference locations but also not along northern west coast, where the maximum concentrations found in 2006 was 0.93 ug/kg ww. PAH data from reference areas are just below limits of non compliance if using the draft EQS but not current national trigger and PNEC values: the sum of Benzo(b+k)fluoranthene maximum concentration is estimated to be about 3.6 ug/kg ww (current trigger 300 ug/kg), benzo(a)pyrene maximum concentration is 170 ng/kg ww (trigger 7000 ng/kg), sum of benzo(ghi)perylene + indeno(123 cd)perylene maximum concentration is diffucult to estimate because the concentrations of latter compound not reported, but the maxium conc of former compound is 180 ng/kg ww and trigger for both compounds is 60 ug/kg; if instead checking against the draft EOS of the sum of all five PAHs (0.01 mg/kg ww for molluscs): the maximum concentration (5 ug/kg ww) is just below (but keeping in mind that data for one of the compounds was not available) and it can be assumed that the draft PAH criteria for molluscs could be exceeded in cases of additional local sources. Non compliance cannot be confiremed for fluoranthene in blue mussels, because even if detected in all mussel samples, the maximum concentration found along the Swedish west coast (5 ug/kg ww) is below draft EQS for biota (30 ug/kg ww). For naphthalene, estimated maximum concentration in mussels from reference areas is 0.7 ug/kg ww while current trigger is 60 ug/kg and PNEC 12 mg/kg. Also for HCB, although frequently detected in fish muscle there is low risk of it being a ubiquitous substance, although maximum concentrations are just below the EQS for biota indicating that with local sources there could be a situation with non compliance (e.g. maximum herring concentrations are estimated to be 6 ug/kg ww whereas the EQS is 10 ug/kg. In pike the maximum concentrations are lower (0.1 ug/kg ww) and also limnic perch maximum concentrations are lower than marine (0.09 vs 0.2 ug/kg ww). Again for HCH only data from reference areas have been evaluated and because maximum concentrations (4 ug/kg ww in herring muscle and perch 0.2 ug/kg) are just below the trigger (5 ug/kg), non compliance in areas with local sources cannot be excluded. Corresponding blue musseldata vary between about 0.1-1.6 ug/kg for the sum of HCH congeners and those samples where all congeners were detected.

²⁹³ See footnote fore Cd in ubiquitous column

²⁹⁴ A Swedish Pb trigger value of 0.04 mg/kg ww in biota is not exceeded in the few perch muscle samples available in the biota database (if detected, concentrations are about 0.01 mg/kg ww) but one can assume that there could be a risk of non compliance in locally exposed areas. Fish liver data from reference lakes (perch: 0.0016-1.41 mg/kg ww, pike 0.0016-0.04 mg/kg, roach 0.003-0.08 mg/kg ww, char 0.008-0.18 mg/kg ww) cannot be used to evaluate compliance because it was not possible to convert into whole organism level concentrations. It is therefore unclear if limnic fish data concentrations indicate non compliance at reference sites. In marine fish, liver concentrations vary between 0.002-0.23 mg/kg in herring and 0.003-0.03 in perch, suggesting that limnic and marine fish concentrations are approximately in the same range.

²⁹⁵ Ni concentrations in blue mussels vary between 0.04 and 1.5 mg/kg ww, which means that all values are above the Swedish trigger value of 0.02 mg/kg ww, but six out of 243 evaluated values are also above QSsec pois according to SDS (0.7 mg/kg). There is therefore a risk for non compliance also in locally exposed areas. In perch muscle it was only detected twice (0.13 and 0.05 mg/kg) out of 39 samples. Fish liver data are difficult to evaluate regarding non compliance.

22 Critical protection objectives for current priority substances

The most critical protection objectives that were identified in the current substance data sheets.

Priority substance	Most critical	Comment
Alachlor	protection objective/s Pelagic organisms	Drinking water standard is lower than overall standard; QShh food much less sensitive than pelagic; trigger to derive QSbenthic not met but benthic toxicity probably less than pelagic
Anthracene	Pelagic organisms	Benthic toxicity is unknown but can be estimated by EqP; trigger to derive QShh food not met
Atrazine	Pelagic organisms	Drinking water standard is lower than overall standard; trigger to derive QShh food not met; trigger to derive benthic toxicity not met and benthic toxicity unknown but can be estimated by EqP
benzene	Freshwater: human health, saltwater: pelagic organisms	The freshwater EQS is based on expert judgement and refers to drinking water; QShh not possible to derive, trigger to derive QSbenthic not met
PBDE	Freshwater: human health from seafood consumption; Marine: secondary poisoning	The freshwater QS for sec pois is 4 times less critical than human consumption. The different assessment for marine and limnic env is due to the use of a required assessment factor ²⁹⁹
Cadmium	Pelagic organisms (?)	Benthic toxicity data are available for freshwater sediment and would actually generate a lower water QS but there were uncertainties in the EQP estimation method - therefore pelagic QS has major influence on overall EQS; The highest MPA corresponds to QS sec pois (incl background); QShh food not as sensitive as pelagic
C10-13 chloroalkanes	secondary poisoning	Benthic toxicity unknown but can be estimated by EqP; pelagic almost as sensitive as secondary poisoning, but human health

²⁹⁶ Concentrations vary e.g. between 0.0003 and 0.0048 mg/kg ww at Brofjorden 2 (heavily exposed area), all values being above the Swedish trigger value (0.0002 mg/kg ww), suggesting that non compliance is to expect in locally exposed areas. From effect based tools, moderate to very high imposex frequencies are reported (varying between 36 and 100%) although in general lower frequencies were observed in last years.
²⁹⁷ variable trends are seen depending on species and area

²⁹⁸ The trends for Ni, Cu and Zn are not decreasing but conclusions are difficult to make due to analytical problems. Also the Cr trend is unclear. There was a change in metal analysis (except for Hg) in 2004, so values between 2003 and 2007 should be interpreted with care (Bignert et al 2010).

²⁹⁹ Please note that draft revised EQS will possibly be expressed for biota and the most critical objective is QShh and be significantly lower than the previously calculated QSsec pois and PNEC (1 and 0.3 mg/kg respectively). Furthermore, according to the draft dossier on PBDE, QShh would be considered much more sensitive than QS sec pois, in both marine and fresh water environments.

Priority substance	Most critical protection objective/s	Comment
		through consumption much less sensitive
chlorfenvinphos	pelagic organisms	sec poisoning almost as sensitive but human health consumption not; drinking water standard on same level. Trigger to develop QSbenthic not met.
Chlorpyrifos	pelagic organisms	sec poisoning almost as sensitive but human health consumption not; drinking water standard on same level as MAC; benthic toxicity known and covered by pelagic QS but unclear if/how much less sensitive
1,2-dichloroethane	human health	The overall EQS is based on drinking water criterion although the drinking water standard is lower than overall standard; data not available to derive QShh food but probably less sensitive than from drinking water. Trigger to develop QSbenthic not met.
Dichloromethane	human health	related to drinking water standard (same level); human health through food consumtion is about 4-5 times less sensitive, Trigger to develop QSbenthic not met.
DEHP	secondary poisoning	Difficult to rate benthic toxicity vs pelagic. pelagic toxicity not determined because chronic studies performed at conc above solubility level, benthic toxicity known but water conc calculated by EqP is above solubility limit. Human health through food consumption almost as sensitive (esp for mussels) as secondary poisoning.
diuron	pelagic organisms	human health through food consumption much less sensitive but drinking water standard somewhat lower value than overall EQS; Trigger to develop QSbenthic not met.
endosulfan	pelagic organisms	benthic toxicity unclear (expressed in unit ug/l and provided by rapporteur but no background document available); sec pois and human health related to food consumption less sensitive than pelagic, drinking water standard also less sensitive
fluoranthene	benthic organisms	Other organisms probably less sensitive; trigger to derive QShh not met but also insufficient data available
НСВ	water EQS: pelagic organisms; biota EQS: human health. If monitored in water a stricter overall EQS should be used	Benthic toxicity data in long term test unavailable and effects in acute assays are unbounded (no effect observed in highest tested conc), but can be estimated by EqP from pelagic data; pelagic much less sensitive than human health related to food intake as well as secondary poisoning (slightly less sensitive than human) but large variability in conversion factors are the reason for two separate EQS values.
НСН	pelagic organisms	secondary poisoning more or less equal sensitivity for gamma HCH (lindane) and only slightly less sensitive for other HCHs compared to pelagic organisms; also human health risks related to food consumtion of lindane only slightly less sensitive than pelagic; benthic toxicity unknown but EqP can

Priority substance	Most critical protection objective/s	Comment
		be used to estimate such toxicity
	water EQS: pelagic organisms; biota EQS: human health. If monitored in water a stricter overall EQS	secondary poisoning almost as critical as human health through food consumption; benthic toxicity unknown but can be estimated from EqP. pelagic much less sensitive than human health related to food intake as well as secondary poisoning (slightly less sensitive than human) but large variability in BCF values is the reason for two separate EQS
Hexachlorobutadiene	should be used	Values.
Isoproturon	pelagic organisms	Drinking water standard is lower than overall standard; human health related to food consumption much less critical than risks for pelagic organisms; Trigger to develop QSbenthic not met.
Lead	secondary poisoning (?) - unclear because only draft is published	Benthic toxicity values available (MPA value calculated) and benthic organisms seem to be somewhat more sensitive than pelagic, and human health risk nearly as high as secondary poisoning, the greatest risks identified occur from the consumption of insects (sec poisoning) and crustaceans (human consumption) - in these cases probably more sensitive than pelagic community
Mercury	water EQS: pelagic organisms; biota EQS: secondary poisoning, If monitored in water a stricter overall EQS should be used	secondary poisoning more critical than human health through food consumption; limited data available on benthic toxicity but EqP can be used to derive estimate from pelagic data; the one chronic toxicity test available suggests pelagic organisms to be more sensitive than benthic though; pelagic much less sensitive than secondary poisoning, large variability in estimating bioaccumulation potential and transformation of inorganic to organic forms are the reasons for two separate EQS values.
Naphthalene	pelagic organisms	Benthic toxicity unknown and trigger value was not met, but PNEC was calculated by EqP; secondary poisoning less sensitive and risks to human health from food consumption even less sensitive than pelagic communities. Drinking water standard is lower than overall EQS
Nickel	all objectives more or less as critical (?) - unclear because only draft is published	Overall EQS corresponds to drinking water criteria but pelagic organisms seem to be more sensitive (MPA is one order of magnitude lower), limited data available on benthic toxicity but EqP can be used to derive estimate from pelagic data; the few toxicity data available suggests pelagic organisms to be as sensitive as benthic; secondary poisoning and human health from food consumption seem to be only slightly less sensitive as pelagic
Nonylphenol	pelagic organisms	Benthic toxicity unknown but can be estimated by EqP although trigger value actually not met (KpSPM-water is only 536, i.e. trigger value of 1000 not met; but calculated anyhow because

Priority substance	Most critical protection objective/s	Comment
		EqP value was developed for octylphenol); secondary poisoning and human health through consumption equally critical but less critical than pelagic
		Benthic toxicity largely unknown but can be estimated by EqP (snails may be particularly sensitive and EqP may underestimate toxicity); sec poisoning slightly less sensitive than human health through food consumption
Octylphenol	pelagic organisms	but both less sensitive than pelagic organisms Drinking water standard is actually lower for BaP; benthic toxicity data not available but derived by EqP; not possible to derive values for secondary poisoning; although QShh food was calculated for BaP, the corresponding water concentration was not calculated - thus
РАН	pelagic organisms	sensitivitiy of other protection objectives unclear
Pentachlorobenzene	secondary poisoning	Pelagic sensitivity much lower than secondary poisoning, benthic toxicity unknown but can be estimated by EqP, QS hh food very uncertain and therefore difficult to estimate relative sensitivity
		Drinking water standard is acually lower than overall EQS; secondary poisoning and human health related to food consumption less sensitive than pelagic; benthic toxicity unknown but can be estimated from EqP
PCP	pelagic organisms	(although Koc varies with pH).
Simazine	pelagic organisms	Drinking water standard is lower than overall EQS. QShh food much less sensitive than pelagic sensitivity, trigger for calculating sensitivity of secondary poisoning not met; although trigger value for deriving Qsbenthic is also not met, due to 30% will be present in sediment after 28d an EqP derived value is available
	polagio organionio	The trigger for deriving QS sec pois is not met, and not possible to derive QShh food but suspected carcinogen; pelagic community less sensitive than benthic; however trigger
Trichloromethane	benthic organisms	for deriving benthic QS is actually not met Qshhfood is less sensitive than pelagic, sec pois is even more less sensitive. Benthic toxicity is unknown, but an EqP value has been derived, although Koc values exhibit large variability.
Trichlorobenzenes	pelagic organisms unclear because based on already existing community legislation and overall value deviates from all QS values, but from QS values it seems that marine pelagic community is most sensitive; for freshwater human	trigger value to derive sediment QS is not met but EqP value anyhow calculated; QShh food and QS sec pois on approx same level of sensitivity and somewhat more sensitive than freshwater pelagic community but less sensitive than marine pelagic community

Priority substance	Most critical	Comment
	protection objective/s	
	consumption and	
	secondary poisoning	
	are more sensitive	
		Benthic toxicity data available but less
		sensitive than all other objectives; food
		consumption more sensitive than sec pois but
Trifluralin	pelagic organisms	less than pelagic

23 Assessment of accumulation potential in different compartments of the current priority substances

Assessment of accumulation potential in different compartments of the priority substances based on inherent properties as well as detection frequency³⁰⁰ of substance in different compartments according to available Swedish monitoring data.

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
Alachlor	Limited bioaccumulation (both according to Kow and BCF), not readily biodegradable. Water solubility ca 240 mg/l.	Preferred: water Optional: Sediment/SPM Not recommended: Biota	Not detected in any off shore sediment samples in 2008 Pesticide database: never detected in the large rivers investigated or in water and sediment samples in any of the type areas. Screeningdatab ase: Very low detection in water and sediment No	Substance properties and available data suggest that water monitoring is most relevant

³⁰⁰ Detection frequencies are considered very low if below 2%, low if 2-10%, moderate if 10-30%, high if 30-50% and very high if above 50%.

³⁰¹ Triggered by low water solubility, high particle affinity, logKow>3 (but not too high MW), BCF>100, low level of degradation/metabolisation/hydrolysis. Conclusion based primarily on information according to SDS in CIRCA and/or CIS no 19 (water solubility). Other references may also have been used.

 ³⁰² According to Mackay fate modelling tool in Episuite. Not calculated for metals/organometals.
 ³⁰³ In the text it is also stated that: Preferred=Monitoring should be performed in this matrix (in some cases both sediment and biota are included – the choice should be made on the basis of local contamination and on the EQS derived); Optional= can be performed in this matrix but also in others; the choice will be made on the basis of the degree of contamination of a particular matrix; Not recommended= unless there is evidence of the possibility of accumulation in this matrix.

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
			biota data available.	
Anthracene	Accumulates in biota (both according to Kow and BCF) but probably does not biomagnify. Photolysis in water but not readily biodegradable (particularly not in sediment). Water solubility ca 0.04 mg/I. Fate modelling indicates about equal distribution between water and sediment. Accumulates	Optional: Water, Sediment/SPM, biota	Biota database: detected in all blue mussel samples Detected in all off shore sediment samples (2003) Screeningdatab ase: Moderate detection in water, Very high detection in biota. Moderate detection in	Substance properties and available data suggest that biota and sediment may be the most relevant compartments to monitor but water is also applicable.
Atrazine	according to art 3.3.	Preferred: water	sediment. Pesticide	Substance
		Not recommended: Sediment/SPM, Biota	database: Low to moderate detection in water in the two large rivers investigated, never detected in sediment samples in any of the type areas.	properties and available data suggest that water is the most relevant compartment to monitor
	Limited bioaccumulation (both according to Kow and BCF), but also low water solubility and some accumulation in sediment may occur. Information on biodegradability variable, but inherent degradability suggested. Water solubility ca 33 mg/l Limited	Preferred: water	Not detected in any off shore sediment samples in 2008. Screeningdatab ase: Very low detection in water and sediment. No biota data available. Detected in all	Substance

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
	bioaccumulation (both according to Kow and BCF). Expected to degrade in surface water, Water solubility ca 1800 mg/l	Not recommended: sediment/SPM, biota	off shore sediments in 2008 in varying concentrations (no data registered for 2003). Screeningdatab ase: Low detection in water, moderate detection in biota (but less than 20 records available and only detected in Fucus vesiculosus). Very low detection in sediment (but analysed in less than 20 samples).	properties suggest that water is the most relevant compartment to monitor, but available data indicate that substance can also appear in sediment and biota (Fucus).
PBDE	Accumulates (both according to Kow and BCF), probably biomagnifies ³⁰⁴ Not readily biodegradable. Water solubility ca 13 ug/l. Fate modelling indicates strong risk for accumulation in sediment. Accumulates according to art 3.3.	Preferred: biota, sediment/SPM Not recommended: water	Sediment database: High detection frequency of most congeners in off shore sediments Biota database: High detection frequency in both fish muscle, liver and blue mussels Screeningdatab ase:	Substance properties and available data suggest that both sediment and biota would be relevant to monitor but available data indicate that substance can also appear in water.

³⁰⁴ Burreau S, Zebühr Y, Broman D, Ishaq R. 2004. Biomagnification of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) studied in pike (Esox lucius), perch (Perca fluviatilis) and roach (Rutilus rutilus) from the Baltic Sea. Chemosphere 55: 1043-1052

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
			Moderate detection in water. Very high detection in biota. Moderate detection in sediment	
Cadmium	Accumulates (according to BCF and partition coefficient for water- SPM) but does not seem to biomagnify. Water solubility depends on compound. Accumulates according to art 3.3.	Not included in the assessment	Biota database: Very high detection frequency in blue mussels and fish liver but not detected in fish muscle from reference sites. 100% detection frequency in off shore sediments SLU water databases Very high detection frequency in rivers that end at sea. Screeningdatab ase: Very high detection in water, biota and sediment.	Substance properties and available data suggest that all compartments could be relevant to monitor. In biota, fish liver and not muscle should be monitored.
C10-13 chloroalkane s	Accumulates (both according to Kow and BCF), biomagnification shown, no information on degradability. Water solubility ca 0.1 - 0.5 mg/l. Fate modelling indicates strong risk for accumulation in sediment.	Preferred: Sediment/SPM and biota Not recommended: water	Data not yet available for off shore sediment analysed in 2008 and were not analysed in 2003. Screeningdatab ase: Very low detection in	Substance properties suggest that both sediment and biota could be relevant to monitor. Only limited data availability.

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
	Accumulates according to art 3.3.		water and biota (but less than 20 records available and only analysed in fish muscle) and low detection in sediment.	
chlorfenvinph os		Optional: Sediment/SPM, biota, water	Pesticide database: never detected in the large rivers investigated or in water and sediment samples in any of the type areas. Not detected in any off shore sediment samples in 2008	Substance properties and available data suggest that water and biota would be the most relevant compartments to monitor.
	Does not accumulate in sediment according to partition coefficient but bioaccumulation is indicated by BCF, not ready biodegradable. Water solubility ca 150 mg/l		Screeningdatab ase: Low detection in water. No biota data available. Very low detection in sediment but analysed in less than 20 samples.	
Chlorpyrifos	Accumulates (according to both BCF and Kow), not ready biodegradable. Water solubility ca 0.8 mg/l. Fate modelling indicates strong risk for accumulation in sediment.	Optional: Sediment/SPM, biota, water	Pesticide database: never detected in the large rivers investigated or in water and sediment samples in any of the type areas.	Substance properties suggest that biota and sediment could be the most relevant compartments to monitor but there is no monitoring data available for

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
			Not detected in any off shore sediment samples in 2008	biota and the substance was never detected in sediment (or water).
			Screeningdatab ase: Very low detection in water. No biota data available. Very low detection in sediment but analysed in less than 20 samples.	
1,2- dichloroethan e	Limited bioaccumulation (both according to Kow and BCF), not ready biodegradable. Water solubility ca 9 g/l	Preferred: Water Not recommended: sediment, biota	Not detected in any off shore sediment samples in 2008 Screeningdatab ase: Low detection in water. No biota data available. Very low detection in sediment but analysed in less than 20 samples.	Substance properties and available data suggest that water is the most relevant compartment to monitor
Dichlorometh ane	Limited bioaccumulation (both according to Kow and BCF),	Preferred: Water Not recommended: sediment, biota	Not detected in any off shore sediment samples in 2008 Screeningdatab ase: Very low detection in	Substance properties and available data suggest that water is the most relevant compartment to monitor
	highly volatile, not ready biodegradable. Water solubility ca 20 g/l		water, no values above AA-EQS in freshwater. Low detection	

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
			in biota but less than 20 records available and only analysed in Fucus vesiculosus and fish muscle. Very low detection in sediment but only analysed in 3 samples.	
DEHP	Accumulates (according to both Kow and BCF) but biomagnification not indicated by monitoring data and BCF higher for mussels and amphipods than fish, may become metabolised. Readily biodegradable. Water solubility ca 3 ug/l. Fate modelling indicates strong risk for accumulation in sediment. Accumulates according to art 3.3.	Optional: Sediment, biota Not recommended: Water	Detected in almost 50% of off shore sediment samples from 2008. Screeningdatab ase: Moderate detection in water. Moderate detection in biota (detected in Fucus vesiculosus, blue mussels and muscle from perch, herring and eelpout but fish liver does not seem to have been analysed ³⁰⁵). Very high detection in sediment.	Substance properties and available data suggest that sediment and biota would be the most relevant compartments to monitor, but taking into account an expected aerobic microbial degradation and metabolization.
diuron	Limited bioaccumulation (both according to Kow and BCF), not ready biodegradable. Water solubility ca	Preferred: water Not recommended: sediment/SPM, biota	Pesticide database: moderate detection in the large rivers investigated, high detection	Substance properties and available data suggest that both water and sediment in locally exposed

³⁰⁵ Tissue and species not always specified

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
	40 mg/l		frequency in sediment from one of the type areas ³⁰⁶ Not detected in any off shore samples in 2008. Screeningdatab ase: Low detection in water. No biota data available. Very high detection	areas could be the most relevant compartments to monitor.
endosulfan	Accumulates according to Kow and BCF Not ready biodegradable. Water solubility ca 0.3 mg/l	Optional: sediment/SPM, biota, water	in sediment Pesticide database: high detection of endosulfan sulphate in both water and sediment samples from one of the type areas, otherwise low detection in water and moderate in sediment Not detected in any off shore sediments in 2008, but LOQ probably does not fulfil QAQC requirements. Screeningdatab ase: Very low detection in water, sediment and	Substance properties and available data suggest that both water and sediment could be relevant to monitor in exposed areas. Inherent properties also suggest biota to be relevant but there is limited data available.

³⁰⁶ But data are only available from 7 samples

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
			biota (but less than 20 records available).	
fluoranthene	Accumulates according to Kow and BCF (but for fish the value was calculated, probably metabolised to high degree); no information on biodegradability. Water solubility ca 0.3 mg/l. Fate modelling indicates strong risk for accumulation in sediment. Accumulates according to art 3.3.	Preferred: Sediment/SPM, biota Not recommended: water	Biotadatabase: Very high detection in blue mussels. Sediment database: very high detection frequency (2003) Screeningdatab ase: Moderate detection in water. Very high detection in biota and sediment.	Substance properties and available data suggest that sediment and biota would be the most relevant compartments to monitor.
НСВ	Accumulates according to Kow and BCF. Biomagnification. No info on biodegradability. Water solubility ca 6 ug/I. Fate modelling indicates strong risk for accumulation in sediment. Accumulates according to art 3.3.	Preferred: sediment/SPM, biota Not recommended: water	Biota database: Very high detection in herring, pike and marine perch muscle and cod liver, and high in blue mussels Detected in all off shore sediments in 2008. Screeningdatab ase: Moderate detection in water and sediment. Very high detection in biota.	Substance properties and available data suggest that sediment and biota are the most relevant compartments to monitor
	Accumulates according to Kow	Preferred: biota	Biota database: Very high	Substance properties and
НСН	and BCF; degradability unclear	Optional: sediment/SPM,	detection in blue mussels of	available data suggest that

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
	but half life in sediment mesocosm was 48 d. Water solubility ca 5-10 mg/l. Fate modelling indicates about equal distribution between water and sediment. Accumulates according to art 3.3.	water	all three congeners. Moderate detection in marine perch muscle of all three congeners in the same sample. In limnic perch ³⁰⁷ muscle very low detection of the beta-HCH and alpha-HCH congeners, but very high detection of gammaHCH. Very high detection frequency of all three congeners in cod liver. Pesticide database: Never detected in sediment samples but low detection in water samples from the type areas. Detected in most off shore sediments in 2008 (in particular beta and gamma). Screeningdatab ase: Moderate detection in	both sediment and biota could be the most relevant to monitor but water could also be relevant to monitor.

³⁰⁷ For pike, data are only available from 1995-2000 but again there was very low detection frequency of beta-HCH, whereas alphaHCH

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
			water. Very high detection in biota. Very low detection in sediment.	
Hexachlorob utadiene	Accumulates according to Kow and BCF. Biomagnification not observed, half life in water 4-50 weeks. Water solubility ca 2.5 mg/l. Fate modelling indicates strong risk for accumulation in sediment. Accumulates according to art 3.3.	Preferred: Biota Optional: Water, sediment/SPM	Screeningdatab ase: Low detection in water. Very low detection in biota but only 4 records available and all on Mytilus edulis. Very low detection in sediment but analysed in less than 20 samples.	Substance properties suggest that biota is the most relevant compartment to monitor but also water and sediment could be relevant. There is very limited data available.
		Preferred: water Not recommended: sediment/SPM, biota	Pesticide database: Low sediment detection but LOQ probably does not fulfil QA QC requirements, High detection frequency in water. Not detected in any off shore sediment samples from 2008. Screeningdatab	Substance properties and available data suggest that water is the most relevant compartment to monitor
Isoproturon Lead	Limited bioaccumulation (both according to Kow and BCF), half life in water 40 d, 150 d in water/sediment. Water solubility ca 70 mg/l Accumulates	Not included in the	ase: Very low detection in water, Very low detection in sediment but analysed in less than 20 samples. Biota database:	Substance

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
	(according to BCF and partition coefficient for water- SPM) but does not seem to biomagnify. Water solubility depends on compound. Accumulates according to art 3.3.	assessment	Very high detection in fish liver ³⁰⁸ and blue mussels but very low detection frequency in char muscle (n=7) and moderate detection in perch muscle (n=39). Detected in all off shore sediment Very high detection in water (rivers that end at sea) ³⁰⁹ Screeningdatab ase: Very high detection in water, biota and sediment.	properties and available data suggest that all compartments could be relevant to monitor, but in fish preferably liver.
Mercury	Accumulates according to BCF and partition coefficient for water- SPM, biomagnification also occurs. Water solubility depends on compound. Accumulates according to art 3.3.	Preferred: biota Optional: sediment/SPM Not recommended: water 310	Biotadatabase: very high detection frequency (in fish muscle and liver as well as in blue mussels) Very high detection in water (rivers	Substance properties and available data suggest that all compartments could be relevant to monitor, but for organic mercury preferably biota.

³⁰⁸ From all limnic perch liver data (n=1850), Pb was e.g. detected in all but 315 samples if including all years registered. It was detected in all herring liver data. ³⁰⁹ Detected in all but 17 samples including all years and stations registered (n=8170) but background not

taken into account ³¹⁰ This assessment is based only on organic mercury

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
			that end at sea) ³¹¹ Detected in all off shore sediment Screeningdatab ase: Moderate detection in water. Very	
		Optional: water, biota, sediment/SPM	high detection in biota and sediment. Biotadatabase: Detected in all blue mussels Detected in all off shore sediment samples	Substance properties and available data suggest that all compartments could be relevant to monitor.
Naphthalene	Accumulates (according to Kow and BCF for some species) but probably does not biomagnify. Half life 2-4 weeks. Water solubility ca 30 mg/l		Screeningdatab ase: Moderate detection in water and sediment. Very high detection in biota.	
Nickel	Accumulates according to partition coefficient for water- SPM. Probably moderate bioaccumulation but varies, between species and concentration levels, generally higher in bivalves and at low concentrations, probably not subject	Not included in the assessment	Biota database: Very high detection in blue mussels and in fish liver ³¹² but very low detection frequency in fish muscle. Very high detection in rivers that end	Substance properties and available data suggest that all compartments could be relevant to monitor, but in fish preferably liver, not muscle

³¹¹ Detected in all but one sample, n=4964 data included, based on all years and stations registered. Background concentrations not taken into account
 ³¹² Nickel was e.g. detected in all but 315 samples from perch liver (n=1184 registered data).
 ³¹³ Nickel was detected in 12 out of 16 off shore sediment samples in 2003.

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
	to biomagnification. Water solubility depends on compound		at sea Very high detection in off shore sediment ³¹³ Screeningdatab ase: Very high detection in water, sediment and biota.	
Nonylphenol	Accumulates according to Kow and BCF, somewhat higher in mussel than fish. Inherently biodegradable but half life 150 d and influenced by several factors. Water solubility ca 6 mg/l. Fate modelling indicates strong risk for accumulation in sediment.	Preferred: Water, sediment/SPM Optional: biota	Not detected in any off shore sediment samples in 2008 (only 4-n- nonylphenol was analysed), but in 2003, also 4-iso- nonylphenol was analysed and frequently detected Screeningdatab ase: Moderate detection in water. Very high detection in biota (perch) but tissue not specified. Moderate detection in sediment	Substance properties and available data suggest that all compartments could be relevant to monitor
Octylphenol	Accumulates according to Kow and BCF, but fish studies show low to moderate accumulation. Biomagnification studies scarce but seem to be low risk. Inherently	Preferred: Water, sediment/SPM Optional: biota	Not detected in any off shore sediment samples in 2008 but more than half of samples in 2003. Screeningdatab	Substance properties and available data suggest that all compartments could be relevant to monitor

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
	biodegradable, but fails 10d window criterion and adaptation seems to be necessary. Degradation in anaerobic sediment unclear. Water solubility ca 5 mg/l. Fate modelling indicates strong risk for accumulation in sediment.		ase: High detection in water. Very high detection in biota but for many fish samples tissue is not specified. Very high detection in sediment.	
РАН	Accumulates according to Kow and BCF (although not available for all individual PAHs that are included in the EQS), no info on biodegradability in SDS. Water solubility ca 1 ug/l (BaP, Benzo(b/k)fluoranthe ne); 0.2 ug/l (benzo(ghi)perylene, indeno(123,cd)peryl ene. Fate modelling indicates strong risk for accumulation in sediment. Accumulates according to art 3.3.	Preferred: Sediment/SPM, biota ³¹⁴ Not recommended: water	Biota database: high detection blue mussels Screeningdatab ase: Moderate detection in water. Moderate detection in biota. High detection of BaP, benzo(k)fluoran thene, benzo(ghi)peryl ene, and very high of Benzo(b)fluora nthene and indeno(1,2,3- c,d)perylene in sediment.	Substance properties and available data suggest that sediment and biota are the most relevant compartments to monitor. In biota, preferably organisms with low level of metabolisation (e.g. blue mussel)
Pentachlorob enzene	Accumulates according to Kow and BCF, no information on biomagnification, not ready biodegradable. Water solubility ca 0.8 mg/l. Fate modelling indicates strong risk for	Preferred: Sediment/SPM Optional: biota Not recommended: water	Not detected in any off shore sediment samples in 2008. Screeningdatab ase: Moderate detection in water, biota	Substance properties and available data suggest that sediment and biota would be the most relevant compartments to monitor

³¹⁴ Taking metabolisation in higher trophic levels into account.

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
	accumulation in sediment. Accumulates according to art 3.3.		(but fish liver not analysed) and sediment.	
РСР	Accumulates according to Kow and BCF. Aerobic aquatic half life 5d, anaerobic 34d. Water solubility ca 14 mg/l. Fate modelling indicates strong risk for accumulation in sediment.	Optional: water, sediment/SPM, biota	Not detected in any off shore sediment samples in 2008. Screeningdatab ase: Moderate detection in water, Very high detection in biota (not detected in Fucus vesiculosus but in all fish muscle samples; tissue not specified for all), but less than 20 recorded data available. High detection in sediment	Substance properties and available data suggest that all compartments could be relevant to monitor
Simazine	Limited bioaccumulation (both according to Kow and BCF), not ready biodegradable. Water solubility ca 6 mg/l	Preferred: water Not recommended: sediment/SPM, biota	sediment. Pesticide database: Low water detection but not detected in sediment. Detected in two samples from off shore sediment in 2008. Screeningdatab ase: Very low detection in water, No biota data available. Very low	Substance properties and available data suggest that water is the most relevant compartment to monitor

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
			detection in sediment.	
Trichlorometh	Limited bioaccumulation (both according to Kow and BCF), anaerobic degradation (half life expected to be 15 days in sediment). Water solubility ca 9	Preferred: water Not recommended: sediment/SPM, biota	Detected in all off shore sediments in 2008 (not analysed in 2003). Screeningdatab ase: Low detection in water. High detection in biota but less than 20 records available (only analysed in fish muscle and Fucus vesiculosus). Very low detection in sediment but analysed in less than 20	Substance properties suggest that water is the most relevant compartment to monitor but substance was also detected in off shore sediment and biota.
(chloroform)	mg/l	Preferred: Biota	samples. Biota database:	Substance
	Accumulates according to Kow	Optional: sediment, water	Very high detection frequency in gastropods Very high detection in off shore sediment	properties and available data suggest that biota and sediment are the most relevant compartments
ТВТ	and BCF. Biomagnification not observed. Not ready biodegradable but degrades in 1-3 months in aerobic conditions. Water solubility ca 2 mg/l. Accumulates according to art 3.3.		Screeningdatab ase: Moderate detection in water. Very high detection in biota and sediment.	to monitor but water could also be relevant to monitor
Trichlorobenz enes	Accumulates according to Kow and BCF. Regarded as inherently	Optional: sediment/SPM, water, biota	1,2,4- trichlorbenzene detected in 50% of off	Substance properties and available data suggest that

Substance	Accumulation potential according to SDS ³⁰¹ , estimated distribution between sediment and water ³⁰² and "accumulating" according to art 3.3. in 2008/105/EC	Preferred, optional and not recommended compartment/s to monitor ³⁰³ according to table 1 in CIS 25; based on inherent properties	Detection frequency etc according to available national monitoring data, incl screening database	Conclusion regarding accumulation in sediment and/or biota
	degradable. Water solubility ca 6-19 mg/l. Fate modelling indicates about equal distribution between water and sediment.		shore sediment samples in 2008 (not analysed in 2003). Screeningdatab ase: Low detection in water, Very low detection in biota (analysed in fish muscle and bivalves but not detected in any samples). Moderate detection in sediment.	the most relevant compartments to monitor are sediment and water
Trifluralin	Accumulates according to Kow and BCF but bioaccumulation was questioned due to rapid dissipation and depuration, probably no biomagnification, not ready biodegradable. Water solubility ca 0.3 mg/l. Fate modelling indicates strong risk for accumulation in sediment.	Preferred: Sediment/SPM Optional: biota Not recommended: water	Pesticide database: never detected in the large rivers investigated or in water and sediment samples in any of the type areas. Not detected in any off shore sediment samples in 2008. Screeningdatab ase: Moderate detection in water, No biota data available. Very low detection in sediment but analysed in less than 20 samples.	Substance properties suggest that sediment is the most relevant compartment to monitor but this was not confirmed by monitoring data (not detected). No biota data are available.

24 CA concept indices

HI, TUS, PODI

The HI, TUS, PODI and RPF are based on the CA concept and described in more detail in the report by Kortenkamp et al (2009). The TUS (Toxic Unit Summation) is directly based on the CA formula. Therefore

$TUS = {}_{i=1}\Sigma^n (c_i / ECx_i)$

Where c_i is the concentration of the individual substance I in a mixture. The ratio c_i/ECx_i is called the TU, Toxic Unit and x is typically 50% (i.e. the TU is based on EC50 values). If TUS=1, the total expected effect is x, but if TUS>1 larger effects are expected. The Σ PAH model developed by Swartz et al (1995) is based on TU calculations related to LC50 values of PAHs tested in sediment.

The HI, Hazard Index is based on EL, the exposure level, and AL, the acceptable exposure level:

$HI=_{i=1}\Sigma^{n}(EL_{i}/AL_{i})$

Where and n is the number of chemicals in the mixture. If HI exceeds 1, the mixture components exceed the level considered to be acceptable.

The Point of Departure Index (PODI) is based on EL, the exposure level, and Point of Departure POD_i

$PODI=_{i=1}\Sigma^{n} (EL_{i}/POD_{i})$

Where POD_i can be e.g. NOAEL or BML. The major difference between the HI index and the PODI index is that in the latter case, the uncertainty factors are removed from the calculation.

Table TEF values for nonylphenol ethoxilates.

Nonylphenol (NP)	1
NP1EO	0,5
NP2EO	0,5
NPnEO (3≥n≤8)	0,5
NPnEO (9≥n)	0,005
NP1EC	0,005
NP2EC	0,005

Table TEF values for planar PCBs, dibenzodioxins and dibenzofurans according to WHO. TEFfish is used for the evaluation of sediment data, and TEFmammal for the evaluation of fish data.

Kongener	TEFfish	TEFmammal
2,3,7,8-TCDD	1	1
1,2,3,7,8-PCDD	1	1
1,2,3,4,7,8-HCDD	0,5	0,1
1,2,3,6,7,8-HCDD	0,01	0,1

1,2,3,7,8,9-HCDD	0,01	0,1
1,2,3,4,6,7,8-HCDD	0,001	0,01
OCDD	<0,0001	0,0001
2,3,7,8-TCDF	0,05	0,1
1,2,3,7,8-PCDF	0,05	0,05
2,3,4,7,8-PCDF	0,5	0,5
1,2,3,4,7,8-HCDF	0,1	0,1
1,2,3,6,7,8-HCDF	0,1	0,1
1,2,3,7,8,9-HCDF	0,1	0,1
2,3,4,6,7,8-HCDF	0,1	0,1
1,2,3,4,6,7,8-HCDF	0,01	0,01
1,2,3,4,7,8,9-HCDF	0,01	0,01
OCDF	<0,0001	0,0001
PCB77	0,0001	0,0001
PCB81	0,0005	0,0001
PCB126	0,005	0,1
PCB169	0,00005	0,01
PCB105	<0,00005	0,0001
PCB114	<0,00005	0,0005
PCB118	<0,00005	0,0001
PCB123	<0,00005	0,0001
PCB156	<0,00005	0,0005
PCB157	<0,00005	0,0005
PCB167	<0,00005	0,00001
PCB189	<0,00005	0,0001

25 Workshop on effect based tools (Göteborg January 2011)

In total there were about 70 participants attending the workshop. The first day was divided into two major parts: the first included 15 flash presentations³¹⁵ of different type of effect based tools. A brief presentation of the outcome of a French similar worshop³¹⁶ on biomarkers arranged by Ineris was also presented. In the afternoon, participants were divided into four discussion groups to discuss matters related to the four categories of effect based tools: in vitro bioassays, biomarkers, in vivo bioassays and "higher organisational level tools". Each group was provided with a questionnaire and the list of nominated tests and asked to select the most relevant tools and evaluate these. Invited speakers had also received the questionnaires before the workshop and several had filled in responses to the questions related to the tools that they presented. The time to fill in the questionnaire was very limited but participants had the chance to submit information to the organizers also after the workshop.

The nominated tools are listed below.

- acetylcholinesterase in vitro
- ALAD
- Ames

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- umuC
- Micronucleus
- Comet
- Benthic diatoms
- benthic flux measurements
 Chronic subletbal taxiaity tasts on addit
- Chronic sublethal toxicity tests on sediment dwelling organisms
- Daphnia magna
- diatom shell malformations
- dioxin-like effects,
- EROD
- FET-test (fish embryo toxicity)
- fish embryos, fish reproduction, fish behaviour, fish physiology
- Fish gill biomarker
- Fish Sexual Development Test
- Genetic adaptation and pollution tolerant ecotypes
- genotoxicity,
- health indices in mussels
- Heart rate as a sublethal indicator of stress in mussels
- Integrated (fish) monitoring
- LDH
- Lipid peroxidation Lysosome stability

³¹⁵ Link to workshop agenda and presentations: <u>http://www.vattenmyndigheterna.se/Sv/vasterhavet/deltagande-och-dialog/seminarier-och-konferenser/Pages/default.aspx</u>

³¹⁶ Programme, presentations, participant list and report can be fount at <u>http://www.ineris.fr/fr/dossiers-</u> thematiques-ineris/756

- metallothionein •
- microarrays, physiological aging and reproduction (fecundity, egg quality) •
- microcosms •
- Microtox,
- molecular tools, metagenomics
- Mouth deformation chironomids
- MTT
- Neutral red
- Oxidative damage •
- physiological aging
- PICT
- reproduction (fecundity, egg quality) •
- Reproductive success in fish •
- SPEAR
- SWIFT Periphyton test •
- The use of sticklebacks as a tool in biomonitoring
- TTR-binding assay and TH-responsive reporter gene assays
- V fisherii screening test

Those tools that were evaluated in day 1 or questionnaires were filled in at later stages and sent to the author were (contact persons in paranthesis):

- Biomarkers (contact person for fish biomarkers: Lars Förlin, Göteborg University and Anders Sjölin Toxicon):
 - Oxidative damage: protein carbonylation _
 - Oxidative damage: lipid peroxidation
 - Oxidative damage: DNA oxidation
 - Oxidative damage: molecular antioxidants (i.e. GSH)
 - Lysosomal stability (Åke Granmo, Marine Monitoring)
 - Scope for growth (Åke Granmo, Marine Monitoring)
 - Reproductive success in fish
 - EROD
 - Metallothionein
 - induction of vitellogenin in male fish
 - Diatoms shell malformations (Maria Kahlert, University of Agricultural
 - Sciences)
 - Imposex (Marina Magnusson, Marine Monitoring)
 - Higher organisation levels:
 - PICT (Hans Blanck, Göteborg University)
 - Swift (Hans Blanck and Sara Brosché, Göteborg University)
 - SPEAR (Willem Goedkoop, University of Agricultural Sciences;
 - Mattias Liess, Peter van der Ohe from Leipzig University in Germany)
 - In vitro bioassays (contact persons Timo Hamers, IVM Netherlands; Magnus
 - Engwall Örebro University, Peter Behnisch BDS Netherlands, Karl Lilja IVL):
 - AR CALUX
 - DR CALUX
 - **ER CALUX**
 - ERa CALUX
 - **GR CALUX**
 - PAH CALUX
 - PR CALUX
 - TRb CALUX

- Acetylcholinesterase inhibition assay
- Carboxylesterase inhibition assay
- Ames fluctuation assay
- umuC

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– TTR-binding

In vivo bioassays (contact persons Britta Eklund, Stockholm University; Göran Dave Göteborg University, Anders Sjölin Toxicon, Kerstin Magnusson N Research, Karl Lilja IVL):

- Ceramium tenuicorue
- Fish embryo toxicity
- Daphnia magna
- Bacterial luminescence Vibrio fischeri
- Fish sexual development test
- Nitocra spinipes

The second day included presentations on related topics, such as the TRIAD approach, mixture effects assessments based on chemical data, and BLM modelling. The focus was switched towards the regulatory needs and the possibilities to use alternative monitoring tools (including effect based tools) was disussed within the WFD context. Also the upcoming work within the CMEP³¹⁷ activity 3.2.C was presented.

During a summing up session it could be concluded that there are tools available for both marine and limnic Swedish environments and for the study of several trophic levels (fish-invertebrates-plants) and levels of organisation. However, from the questionnaires a lack of assessment criteria, necessary to increase transparency in the evaluation of data, could be identified. Several of the tools, especially on higher organisational levels also still need further development and validation studies. Although not specifically required, there are many potential uses of the effect based monitoring tools presented during the workshop also within the WFD. However, there was some disagreement about e.g. whether certain tools actually would fulfil the requirement to truly measure effects on "ecological level" and thus be used as biological quality elements within the WFD context. It was however agreed that several of the tools discussed could be used to predict negative effects on the ecology, and therefore used in a similar way as the current use of chemical data (within WFD context, this would refer to river basin specific pollutants) to predict ecological status.

The active participation of the two coordinating parties of this subgroup in the workshop could be considered as a kick off of the activities in the CMEP drafting group on effect based tools with the mandate to write a technical state of the art report. The outline of this workshop was shortly also presented at the WGE meeting in Brussels in March (2011, 15-16th) as well as the CMEP Prague meeting (June 2011).

³¹⁷ CMEP=Chemical Monitoring and Emerging Pollutants, a subgroup to the WFD Common Implementation Strategy (CIS) Working Group E (Chemical Aspects, formerly called Priority Substances), operational since 2007 when it superseded the Expert Advisory Forum. Activity 3.2.C is related to effect based tools and a technical state of the art report will be finalised by December 2012..

26 Benthic community index, BQI

Sensitive species tend to dominate in undisturbed environments, while tolerant species in disturbed areas. The BQI, Benthic Quality Index, is a tool to measure this and was developed for soft bottom coastal benthic communities. It is based on three factors:

- species composition the proportion between tolerant and sensitive species
- number of species
- abundance number of individuals

The higher the index value, the larger is the proportion of sensitive species. The index value varies from 0 (no benthic organisms) to approximately 22 (high status) and is calculated using the following equation:

 $BQI_m = [\sum_{i=1}^{Sklassade} (N_i/N_{totklassade} * Känslighetsvärde_i)]*log(S+1)*[N_{tot}/(N_{tot}+5)]$

Where S=total number of species, $S_{klassade}$ =number of sensitivity classified species; N_{tot} =total number of individuals per 0,1 m²; $N_{totklassade}$ =total number of sensitivity classified individuals; N_i =number of individuals of the species i.

The proportion between sensitive and tolerant species varies between approximately one to fifteen. The sensitivity values³¹⁸ of the observed species thus have a heavy impact on the value of the index.

The sensitivity values for the same species vary between geographical areas. On the west coast, sensitivity is ranked according to its presence in different types of environments: species that are common in areas with low number of species obtained a low sensitivity value. This approach was not applicable to the Baltic Sea because of a natural low number of benthic organisms. Instead it is primarily based on expert judgement. On the West Coast, the sensitivity value of blue mussel (Mytilus edulis) is e.g. 7.05 whereas it is 5 on the East Coast.

Different species may be sensitive to one type of stress and not another type of stress. In general, the main reason for finding certain species at particular coastal sites is determined by parameters related to organic load (nutrients, oxygen levels etc) and not toxic substances. The BQI is also generally higher below the halocline on the West Coast because of a more stable salinity environment, whereas larger depths in the Baltic have been exluded from the evaluation due to the risks of oxygen depletion.

³¹⁸ The sensitivity values are listed in annex B to "Naturvårdsverkets handbok 2007:4. Status, potential och kvalitetskrav för sjöar, vattendrag, kustvatten och vatten i övergångszon": Bedömningsgrunder för kustvatten och vatten i övergångszon.

The BQI can also be applied to benthic communities of lakes and is then based on the sensitivity of different chironomid species to low oxygen leveles

$BQI=\sum_{i=0}^{5} (k_i * n_i)/N$

Where k_i varies between 0 and 5 for different species and n_i =number of individuals within indicator group i and N=total number of individuals in all indicator groups. Also BQI values for lakes therefore are primarily measuring effects of eutrophication.

27 Some common biomarkers with regular use

In order to facilitate a suitable design of monitoring approach for a certain objective, some of the more commonly used biomarkers are described in more detail below to illustrate advantages and disadvantages and practical aspects (including costs and restrictions in sampling season etc). Major part of the information is based on OSPAR (2007)³¹⁹, JAMP guidelines, method descriptions for the national fish monitoring programs³²⁰, workshop questionnaire and personal communication with experts. Nevertheless, before including a certain biomarker the most updated guidelines should be consulted.

27.1.1 Metallothionein (MT)

Short description: Metallothionein (MT) is a protein that is present in most vertebrate and some invertebrate cells. One third of the MT protein consists of cysteine, an amino acid that contains sulphydryl groups that can bind to metals. MT therefore acts in metal detoxification, but also in regulation of the intracellular bioavailability of essential elements (Zn, Cu). MT is induced (concentrations elevated) in cells exposed to metals but also free radicals. Fish livers usually have high levels of metals

Endpoint (unit): Concentration of hepatic MT (ug/mg cytosolic protein). Analysing the amount of MT protein is preferred instead of MT mRNA³²¹. An intercalibration study showed similar results were obtained if using metal saturation and polarographic analyses whereas ELISA and colorimetric assay results deviate from overall median.

Standard/scientific reference to test protocols: The protocol used within the Swedish national monitoring programme is based on Hylland (1999)³²² and MT content in liver is measured by ELISA. JAMP recommends any of three methods (ELISA, differential pulse polarography or spectrometric assay).

Species that can be investigated: Can be used for both marine, brackish and limnic fish species. In the national (marine) monitoring programme, perch and eelpout are used.

Season to be avoided/recommended: Preferrably in early autumn, but must be collected within a period of one month outside the spawning season. In the Swedish national monitoring programme, perch is sampled in September and eelpout in April/November.

Tissue/cells examined: Liver cells

Use within regular monitoring: Routinely used within the Swedish marine integrated fish monitoring programme.

³¹⁹ OSPAR Commission 2007. Background Document on Biological Effects Monitoring Techniques. Assessment and Monitoring Series.

http://www.ospar.org/documents/dbase/publications/p00333_Background%20Document%20of%20biologioca 1%20effects.pdf

³²⁰ Hälsotillstånd hos kustfisk – biologiska effekter på subcellulär och cellulär nivå <u>http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/hav/fiskhals</u> <u>o.pdf</u>

³²¹ Because physiological/toxicological response depends on MT concentration and not mRNA. Also the half life of MT is longer.

³²² Hylland, K. 1999. Biological effects of contaminants: Quantification of metallothionein (MT) in fish liver tissue. ICES Tech.Mar.Environ.Sci. 26

Assessment criteria: Because MT is an exposure biomarker rather than an effect indicator, it is recommended to compare the results to suitable reference sites. Absolute criteria may be used if there is background knowledge on the species, sampling is performed during same and appropriate time period and same sex and size are selected. In the OSPAR review (2007), the upper 90 percentile for baseline values was identified as the range 13-16 ug MT/mg cytosolic protein for cod, flounder, dab and plaice, based on data from JAMP (Joint Assessment and Monitoring Program). The following threshold values therefore indicate an external stressor affecting hepatic MT: cod 16 ug/mg cytosolic protein (baseline value 6,5-16), flounder 15 ug/mg cytosolic protein (baseline value 8,4-15), dab 13 ug/mg cytosolic protein (baseline value 7,2-13), plaice 14 ug/mg cytosolic protein (baseline value 6,5-14). The assessment should be based on analysing at least 25 individuals. MT is monitored in eelpout on the Swedish west coast annually but there is an increasing trend observed (from about 10 ug MT/mg in 2005³²³).

Specificity: Generally responds to metals (Cu, Zn, Cd, Hg³²⁴, Ag³²⁵) but also responds to oxidative stress (free radicals) and organics.

Other influencing factors: Sex, season, temperature, age (OSPAR 2007), local conditions, analytical procedures (Hylland 1999), fish size, condition and GSI (JAMP guideline)

Sensitivity/variability/response time: Relatively sensitive, variability between laboratories observed (OSPAR), probably significant response within a few days after exposure (Hogstrand et al 1991³²⁶) **Ecological relevance:** Exposure biomarker. Measures exposure to metals (Cu, Zn, Cd) and disturbance of copper and zink metabolism.

Swedish contact person: Lars Förlin, Göteborg University (Dept Zoophysiology)³²⁷

Complexity/learning period: Easy to learn

Costs: The major costs related to studying this biomarker are related to fish catch (field studies) or costs for fish/cages (cage studies). The actual costs for MT analysis are low

Comments: Can act as early warning biomarker to indicate risks of physiological response due to metal (Cu, Zn, Cd) exposure. Results do not confirm the presence of effects but could be valuable as part of a weight of evidence approach. Swedish marine baseline data for eelpout are available for comparison and regional studies could potentially be coordinated with this national program, but for studies on other species, season etc and in limnic areas reference sites would be needed for comparison. Because major costs are related to sampling rather than analysis, adding MT would not substantially increase the costs and parallel measurements of Cd, Zn and Cu are strongly recommended in order to evaluate the results. Because livers are used for both analyses, care should be taken to make sure sufficient material is available.

³²³ Corresponding increase in confidence limits from 8-12 to 11-15 ug/mg. Parallell increase in cadmium concentration has also been observed.

³²⁴ However, the response to methylated Hg is unclear and MT is therefore currently not recommended as a biomarker for Hg contamination.

³²⁵ MT was shown to be induced by AgS2O3, AgCl and AgNO3, but the latter compound is suspected to cause osmoregultory disturbance preceding MT induction (Hogstrand et al 1991).

 ³²⁶ Hogstrand et al 1991. Significant response after 2d of injection of 2 mg of Cd, maximum response after 6d.
 ³²⁷ In national program, MT analysis is performed by NIVA.

27.1.2 Cytochrome P450 1A activity (EROD)

Short description: The excretion of organic hydrophobic substances in vertebrates is facilitated by a stepwise transformation into more water soluble compounds. The first step (phase I) in this process is usually catalysed by the enzyme system called Cytochrome P450 oxygenases³²⁸. There are many forms of this enzyme family, also divided into subfamilies. One such subfamily is the CYPA1, considered to be particularly important and involved in transforming planar molecules. Besides being a substrate, planar molecules can also bind to the cytosolic Ah receptor, inducing the P450 1A system. This induction can be measured by adding the substrate 7-ethoxyresorufin, which will become de-ethylated into resorufin (product) by EROD (7-ethoxyresorufin-O-deethylase).

Endpoint (unit): Resorufin production (pmol/min/mg protein)

Standard/scientific reference to test protocols: The protocol used within the Swedish national monitoring programme is based on Burke & Mayer 1974³²⁹. JAMP refers to analytical procedures described by Stagg and Macintosh (1997).

Species that can be investigated: Can be used for both marine, brackish and limnic fish species. In the national (marine) monitoring programme, perch and eelpout are used. Works less well on invertebrates.

Season to be avoided/recommended: preferably same two week period and sex should be sampled each year. In the national monitoring programme, perch is sampled in September and eelpout in April/November. Sampling should take place at least one month after spawning.

Tissue/cells examined: Most commonly measured in liver extracts but also in gill preparations. **Use within regular monitoring:** Probably the most frequently analysed biomarker. Routinely used within the Swedish integrated fish monitoring programme, with annual measurements in female perch and female eelpout on both west and east coast. Used also in DK, maybe UK, NL, NO, DE, FR. It has also been analysed within regional monitoring programmes (Bohuskustens vattenvårdsförbund analysed EROD in eelpout at three locations (Stenungsund, Göta Älv estuary, Brofjorden; and Blekingekustens Vattenvårdsförbund and Vattenvårdsförbundet för västra Hanöbukten included EROD analyses in the monitoring program outside paper mills³³⁰) The assay was also used in investigative monitoring in contaminated areas in both limnic³³¹ and marine environments³³². Other references to monitoring studies performed in Sweden include.

Assessment criteria: It is always recommended to compare the results to suitable reference sites. The OSPAR review (2007) suggests that a two-fold induction compared to the upper limit of baseline data indicates influence by planar organic contaminants in marine organisms. Some freshwater species have been found to have less variable baseline activities between studies whereas baseline autumn values

³³⁰ <u>http://www.hanobukten.org/resultat1999/rapport99.pdf</u> and http://www.enlevandefjord.se/information/Tanglakerapport.pdf

och nedströms Borås, hösten 2002

³²⁸ Previously called MFO, Mixed Function Oxidase

³²⁹ The method used is described in Allmänna råd för vattenrecipientkontroll vid skogsindustrier. 1994. Allmänna råd 94:2. Naturvårdsverket Solna but originally evolved from Burke, M.D., and Mayer, R.T. 1974. Ethoxyresorufin: Direct fluorometric assay ofmicrosomal dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metab. Disp. 2:583-588. See also: Andersson, T., Förlin, L., Härdig, J., and Larsson, Å. 1988. Physiological disturbances in fish living in coastal water polluted with bleached kraft mill effluents. Can. J. Fish. Aquat. Sci. 45:1525-1536. Förlin, L., Goksøyr, A., Husøy, A.M. 1994. Cytochrome P450 monooxygenase as indicator of PCB/dioxin like compounds in fish. In: Kramer K.J.M., editor. Biomonitoring of coastal waters and estuaries. CRC Press, Boca Raton, Florida. pp 135-150.

³³¹ See e.g. Viskan studies, Förlin et al 2002. Rapport om Fiskfysiologiska undersökningar i Viskan, uppströms

http://www.lansstyrelsen.se/vastragotaland/SiteCollectionDocuments/sv/miljo-och-klimat/verksamheter-medmiljopaverkan/fororenade-omraden/bidrag/viskan/FiskfysiologiskastudierViskan20022003.pdf ³³² Andersson et al., 1988; Fish exposed to craftmill effluents were impacted up to 4.5 km from discharge;

³³² Andersson et al., 1988; Fish exposed to craftmill effluents were impacted up to 4.5 km from discharge; Sturve et al., 2005. Disturbances during dredging were found to be more pronounced (due to remobilized pollutants) than under normal conditions.

along the Norwegian coast are varying between approximately 10-100 pmol/min/mg protein for Atlantic cod, 10-40 pmol/min/mg protein for flounder, 120-530 pmol/min/mg protein for dab, 30-150 pmol/min/mg protein for plaice. In the Baltic Kvädöfjärden station, EROD has been observed to increase significantly in female perch (from about 0.05 to 0.2 nmol/mg protein min between the late 80s and 2008³³³). **Specificity:** Responds to Ah-receptor agonists. Strong EROD inducers³³⁴ are dioxins, planar PCBs and PAHs (such as Benzo(a)pyrene).

Other influencing factors: developmental stage, sex, age, reproductive status of the organism, as well as temperature, season and dietary factors (lack of food can cause lack of EROD respons3333e). Possibly also low oxygen. EROD induction can be inhibited by too high concentrations of the Ahreceptor agonists as well as other chemicals like metals and xenooestrogens³³⁵.

Sensitivity/variability/response time: Relatively sensitive, can act as an early warning signal for planar aromatic hydrocarbons. Induction was detected 40 km from source (pulp mill) Intercalibration studies show large variability in results, thus emphasizing the application of internal quality assurance procedures, such as the use of references for all batches. The reponse gradually decreases with time in caged studies. Gills more sensitive to water borne substances than liver.

Ecological relevance: Exposure biomarker.

Swedish contact persons: Lars Förlin, Göteborg University (Dept Zoophysiology), Anders Sjölin, Toxicon.

Complexity/learning period: Easy to learn

Costs: The major costs related to studying this biomarker are related to fish catch (field studies) or costs for fish/cages (cage studies). The actual costs for analysis are low³³⁶.

Comments: Can act as early warning biomarker to indicate risks of physiological response related to an exposure to AH receptor agonists such as dioxins, planar PCBs and certain PAHs (including Benzo(a)pyrene). If used in gradient studies in areas heavily and chronically exposed one needs to be aware of the possibility to observe lower EROD induction (tolerance development). Results do not confirm the presence of ecologically relevant effects but could be valuable as part of a weight of evidence approach. Swedish marine baseline data are available for comparison but a reference site is considered to be essential in all studies. Regional monitoring could be coordinated with national programs if performed within the same period. Because major costs are related to sampling rather than analysis, adding EROD would not substantially increase costs. Because liver may be necessary for the chemical analysis care should be taken to make sure sufficient material is available. However, OSPAR considers 1g sufficient for EROD and DNA adducts, histopathology and chemical analysis.

³³³ See annual reports from the monitoring program, but also Hansson T, Lindesjöö E, Förlin L, Balk L, Bignert A, Larsson Å. 2006. Long-term monitoring of the health status of female perch (Perca fluviatilis) in the Baltic Sea shows decreased gonad weight and increased hepatic EROD activity. Aquatic Toxicology 79: 341-355. Although not statistically significant, an increasing trend is also suggested in data from the Baltic Holmön station: EROD varies between 0.07 and 0.18 nmol/mg protein min but are generally higher in data from last 10 years.

³³⁴ "Strong inducer"=substance causing >100 fold induction compared to control.

³³⁵ Brüsch Weiler BJ, Würgler FE, Fent K. 1996. Inhibitory effects of heavy metals on cytochrome P4501A induction in permanent fish hepatoma cells. High chronic exposure was also suspected to be the cause for low EROD induction at exposed sites in the Stockholm area; Hansson T, Schiedek D, Lehtonen KK, Vuorinen P, Liewenborg B, Noaksson E, Tjärnlund U, Hanson M, Balk L. 2006. Biochemical biomarkers in adult female perch (Perca fluviatilis) in a chronically polluted gradient in the Stockholm recipient (Sweden). Marine Pollution Bulletin 53: 451-468.

³³⁶ Less than 100 Euro per sample

27.1.3 DNA adducts

Short description: DNA adducts are covalent structures that indicate exposure to genotoxic compounds, integrating several factors, including uptake, metabolism and DNA repair. Target tissues are e.g. liver. The most sensitive method is 32P postlabelling³³⁷, being able to detect a wide range of carcinogens (prior characterisation is not necessary).

Endpoint (unit): number of adducted nucleotides per number of undamaged nucleotides. Also analysed as diagonal radioactive zones, DRZs (composite of multiple overlapping DNA adducts if exposed to complex mixture of genotoxic compounds)

Standard/scientific reference to test protocols: JAMP guidance OSPAR recommends methodology described in Stein et al (1993, 1994). Interlaboratory QA programmes have been conducted (BEQUALM&IARC) and there is a standardised protocol available (Times technical document, IARC publications).

Environments that can be investigated: Can be used for both marine, brackish and limnic fish species and the 32P post labelling method is not species specific. In the national (marine) monitoring programme, perch and eelpout are used.

Season to be avoided/recommended: No indiations that season would influence results. **Use within regular monitoring:** Routinely used³³⁸ within the Swedish integrated fish monitoring programme. It has also been used within regional monitoring programmes (Bohuskustens vattenvårdsförbund;

Assessment criteria: Standards not available but background data usually show no detectable adducts or very faint DRZs. Baseline levels in the national programs have not been analysed but in perch, detected values varied between 0.2 and 1.8 nmol/mol in 2005 but DNA adducts were not detected in 2008 at Kvädöfjärden. DNA adducts were also detected in regional monitoring of the Göteborg and Brofjorden areas in 1999 and presence of adducts were reconfirmed in 2006 in the Göteborg area (approximately 4 nmol/mol). DNA adducts were also detected in Stockholm gradient study at up to about 5 nmol/mol concentrations (Hansson et al 2006³³⁹)

Specificity: DNA adducts can be caused by several genotoxic compounds but PAHs are known to cause genotoxicity by the formation of adducts.

Other influencing factors: The levels are not significantly affected by age, sex, season or dietary status. However, because detoxicification systems (e.g. CYP1A) are influenced by changes in environmental variables, these factors should always be considered (including salinity and temperature). **Sensitivity/variability/response time:** DNA adducts are persistent (several months) and the response is thus cumulative, although they can be removed by repair processes and cell death. In chronic exposures, they often reach steady state concentrations. Variability between labs reasonable but important to use external standard.

Ecological relevance: Effects biomarker, possible predictor of pathology (correlations have been observed between DNA damage and certain lesions, such as neoplastic liver disease, foci of cellular alteration and neoplasia).

Swedish contact person: Lennart Balk, ITM.

Complexity/learning period: P32 labelling is most specific and sensitive but time consuming method. **Costs:** Depends on method, P32 labelling considered expensive³⁴⁰.

Comments: If DNA adducts are detected, it should be considered an early warning response and higher concentrations indicate effects (correlation found with certain lesions). Does not alone suggest effects can be expected on population level but could be valuable as part of a weight of evidence approach as it suggests decreased fitness could occur. Some Swedish data are available for comparison but reference sites should be included and threshold levels would need to be established. Because liver may be necessary for the chemical analysis care should be taken to make sure sufficient

³³⁷ A semi quantitative method; not all DNA adducts are labelled with the same efficiency and enrichment/chromatographic steps will select certain adducts over others.

³³⁸ However, the use is more limited in later years.

³³⁹ Hansson T, Schiedek D, Lehtonen KK, Vuorinen P, Liewenborg B, Noaksson E, Tjärnlund U, Hanson M, Balk L. 2006. Biochemical biomarkers in adult female perch (Perca fluviatilis) in a chronically polluted gradient in the Stockholm recipient (Sweden). Marine Pollution Bulletin 53: 451-468.

³⁴⁰ Sternbeck et al 2008 refers also to less sensitive but less complex immunological method by Oost et al 2004.

material is available. However, OSPAR considers 1g sufficient for EROD and DNA adducts, histopathology and chemical analysis.

27.1.4 Reproductive success in eelpout

- **Short description:** The eelpout ("viviparous blenny") is a viviparous species, i. e. the females give birth to living larvae. Each female carries between 20- 300 embryos/larvae. Reproductive success and malformations can therefore easily be studied.
- Endpoint (unit): The most common endpoints are malformed³⁴¹ larvae (% of broods with >5% malformed larvae, or mean frequency of malformed larvae), late dead larvae³⁴² (% of broods with >5% late dead larvae, or mean frequency of late dead larvae) and growth retarded larvae³⁴³ (mean frequency of growth retarded larvae). The latter endpoint is considered less useful. In previous studies, "frequencies of females with at least one abnormal larvae present in the brood" was also used but this measure is not included in the proposal for assessment criteria of reproductive success. In addition, it is possible also to analyse the sex ratio, that under normal conditions is 50:50.
- **Standard/scientific reference to test protocols**: The protocol used within the Swedish national monitoring programme is based on Fiskeriverket, 2004³⁴⁴. JAMP guidance refers to Jacobsson et al 1986; Neuman et al 1999 and Strand & Dahllöf 2005.
- **Environments that can be investigated:** The eelpout is distributed along the whole Swedish coast, covering both marine and brackish environments. However the species is not abundant in all areas.
- **Season to be avoided/recommended:** In the national monitoring programme, eelpout is sampled in November.
- **Use:** Routinely used within the Swedish integrated fish monitoring programme, with annual measurements in females. Used also in DK, Germany, Portugal (to be confirmed). It has also been used within regional monitoring programmes (Bohuskustens vattenvårdsförbund analysed reproductive success in eelpout at three locations (Stenungsund, Göta Älv estuary, Brofjorden). The assay was also used in investigative monitoring in an area contaminated by paper mill effluents (Larsson&Förlin 2002)³⁴⁵. Other references to monitoring studies performed in Sweden include Veetema et al. 1997, Ådjers et al. 2001, Strand et al. 2004, Gercken et al. 2006; finding high variability in malformed larvae at both Swedish and German sites.
- Assessment criteria: The following preliminary criteria are suggested in the OSPAR 2007 review, divided into three classes, I representing the background response (upper limit representing 90 percentile of response at reference sites); II representing sites where effects cannot be excluded and III representing sites with significant effect levels compared to background response. The assessment should be based on at least 40 pregnant females, and to assess the malformation frequency it is necessary to have at least 40 larvae in each brood.
 - o
 % of broods with >5% malformed larvae: I: 0-5%; II: >5-20%; III: >20%

 o
 mean frequency of malformed larvae: I: 0-1%; II: >1-2%; III: >2%

 o
 % of broods with >5% late dead larvae: I: 0-5%; II: >5-20%; III: >20%

 o
 mean frequency of late dead larvae: I: 0-2%; II: >5-20%; III: >20%

 o
 mean frequency of late dead larvae: I: 0-2%; II: >2-3%; III: >3%
 - mean frequency of growth retarded larvae: I: 0-4%; II: >4-6%; III: >6%

³⁴¹ Malformations include yolk sac or intestinal defects, bent spine, spiral shapes of spinal axis, eye defects including rudimentary or missing eye, crano-facial effects, Siamese twins, clumps of larvae. ³⁴² Late dead larvae are defined as larvae without malformations and having lengths >15 mm or >10 mm in Denmark.

³⁴³ Growth retarded larve are defined as normal developed larvae that are smaller than the three highest length classes in the broods.

³⁴⁴ Fiskeriverket, 2004. Handboksblad för Tånglakeprovtagning 2004-03-10.

³⁴⁵ Larsson DGJ & Förlin L. 2002. Male-biased sex ratios of fish embryos near a pulp mill:

Temporary recovery after a short-term shutdown. Environmental Health Perspective 110(8): 739-742

- **Specificity:** General biomarker, can respond to several different types of xenobiotics, including organochlorines, pesticides, PAH, metals.
- **Other influencing factors:** Increased temperature and oxygen depletion events. Different factors can influence different endpoints
 - Sensitivity/variability/response time: Year to year variability can occur.
- **Ecological relevance:** Effect biomarker of high ecological importance. Reproductive success is directly related to expected negative effects on population level, although other factors (such as eelpouts being caught in fvke nets) can also be significantly influencing the population.
- Swedish contact persons: Lars Förlin, Göteborg University; Anders Sjölin Toxicon,
- **Complexity/learning period:** Not very complex
- **Costs:** Depend on how many individuals that will be examined³⁴⁶
- **Comments:** High responses suggest population effects are likely to occur although data alone do not confirm that hazardous substances are involved³⁴⁷. Would therefore be of significant importance in a weight of evidence approach. To identify causes, investigative studies are needed (based on chemical analyses etc). Swedish baseline data are available for comparison and there are also draft absolute evaluation criteria. Costs are high but regional studies could potentially be coordinated with the national program to utilize data from these reference sites. Availability may vary between sites.

³⁴⁶ about 100 individuals can be examined by three persons in one working day; price per fish about 20-30 Euro (commercial basis); if analysing about 40 individuals (minimum recommended) total costs for the analysis (excluding sampling) would become about 1000 Euro

³⁴⁷ The development of a library would probably also be possible, to facilitate the possibilities to link certain types of malformations to particular groups of substances. Förlin L, Göteborg university, pers comm..

27.1.5 Vitellogenin

- Short description: Vitellogenin (VTG) is normally produced in females in the liver and transported by blood stream to the ovary. As a result the concentrations in blood plasma rise up to 6 orders of magnitude as compared to immature females or male fish. The protein is stored in the egg and used as nutrient by the embryo. Production is regulated in several steps, initially triggered by environmental factors such as photoperiod, temperature and food availability. The hypothalamus secretes hormones that in turn stimulate the pituitary gland to secrete other hormones that cause the ovary to release a third hormone, 17β -estradiol (E2) into the blood stream. In the liver cells, E2 binds to the oestrogen receptor (ER). The ER dimerises and binds to a promoter region of E2 inducible genes, including VTG and ER genes. Thus the E2 binding triggers both VTG and ER production. The hepatically produced VTG then is transported in blood to the ovary, causing an increase in the size of the ovaries. Normally, males do not produce VTG but because they posess the VTG genes, upon exposure to xenooestrogens it can be produced in the liver and accumulate in blood plasma. Upon artificial stimulation from E2, the VTG levels can rise up to a million fold in magnitude, thus exhibiting a very high range of response and VTG therefore being an extremely sensitive biomarker. Xenoestrogens can trigger the VTG synthesis by binding to the ER receptor and some pharmaceuticals, such as EE-2, are even more potent than E2. Other xenooestrogens, such as alkylphenols, some phthalates, parabens and phytosterols posess weak activity, whereas others are actually ER agonists (including metabolites from certain PCBs, o,p-DDT and methoxychlor). It is possible to measure either the protein itself or the gene expression. OSPAR recommends using ELISA method that can detect concentrations below 10 ng/ml.
- Endpoint (unit): VTG concentration in blood plasma (ng/ml). There are different types of VTG and some species possess several types. Supporting parameters are e.g. GSI and intersex occurrence of gonads (assessed by histological examination).
- Standard/scientific reference to test protocols: The protocol used within the Swedish national monitoring programme is based on Larsson et al 1999 and Parkkonen et al 1999 ³⁴⁸ (OSPAR recommends using an ELISA³⁴⁹ method that can detect concentrations below 10 ng/ml, and for cod lipovitellin (Iv) should be used. It is important that the same source of Vtg antibody and antigen is used. Hepatic VTG mRNA is measured by either RT³⁵⁰-PCR or Q-PCR³⁵¹ but there is currently no external QA scheme available and it is therefore not yet recommended by OSPAR. Because the VTG is unstable, careful handling is important, including centrifugation of blood samples within 30 minutes.
- **Species that can be investigated:** Can be used for both marine, brackish and limnic fish species. In the national (marine) monitoring programme, perch and eelpout are used. OSPAR recommends cod or dab (off shore, although the ELISA method for VTG in dab needs to be developed because lower levels of VTG in this species) as well as flounder (estuaries). Investigations in Swedish contaminated limnic areas (Viskan) used caged juvenile rainbow trout.
- Season to be avoided/recommended: In the national monitoring programme, perch is sampled in September and eelpout in April/November. Sampling should be performed outside the breeding season and always at the same time of the year. Flounder should be sampled in January/February before offshore migration.
- **Tissue/cells examined:** Measured in blood plasma of male fish.

³⁴⁸ Larsson D.G.J., Adolfsson-Erici M., Parkkonen J., Petterson M., Berg A.H., Olsson P.-E. and Förlin L. 1999 Ethynyloestradiol - an undesired fish contraceptive? Aquat. Toxicol. 45, 91-97.

Parkkonen J., Larsson D.G.J., Adolfsson-Erici M., Petterson M., Berg A.H., Olsson P.-E. and Förlin L. 1999. Contraceptive pill residues in sewage effluent are estrogenic to fish. In Proceedings of 6th International symposium on the reproductive physiology of fish. Eds Norberg, Kjesbu, Taranger, Andersson and Stefansson. pp 362-364.

³⁴⁹ Enzyme Linked ImmunoSorbent Assay; an immunoassay based on the fact that VTG is highly antigenic; the assays are very sensitive and have high specificity

³⁵⁰ Reverse Transcription Polymerase Chain Reaction; semi quantitative analysis

³⁵¹ Realtime PCR; quantitative analysis

- Use: Frequently included in regular monitoring programs (incl DK, UK, NL, NO, DE, USA, FR) Routinely used within the Swedish integrated fish monitoring programme, with annual measurements in perch and eelpout (both west and east coast). It has also been used within regional monitoring programmes (Bohuskustens vattenvårdsförbund analysed VTG in eelpout at three locations (Stenungsund, Göta Älv estuary, Brofjorden) The assay was also used in investigative monitoring in contaminated areas. Other references to monitoring studies performed in Sweden and abroad include Sturve et al., 2005³⁵²; Houtman et al., 2007³⁵³; Vethaak et al., 2005; Sanchez and Porcher, 2009.
- **Assessment criteria:** A reference site is recommended although for some species it would probably be possible to evaluate the VTG levels in absolute terms. Basal levels of VTG in male blood plasma are low (<10 ng/ml in flounder based on UK data); in cod a more tentative value of 0.2 ug/ml is proposed (based on 90 percentile data from caged males from reference sites in the North Sea), whereas very high concentrations were observed in exposed fish (>50 mg/ml). Based on 90 percentile of UK flounder data, a provisional background concentration of 0,13 ug/ml is suggested. At least twelve fish of a constant size range are needed and they should be sampled outside the breeding season and at the same time every year. One ring test of VTG in cod has been performed with comparable results among participants.
- **Specificity:** Highly specific biomarker for oestrogenic exposure.
- Other influencing factors: low oxygen can modulate vitellogenin synthesis in some species. Season is an important factor for male plasma VTG in flounder (being lower in June/July and highest in February/March according to UK data). The VTG concentrations can also correlate with fish size if caught away from point sources, so picking fish from a narrow size range is recommended. For cod e.g. smaller individuals (30-45 cm) are recommended to assess recent xenoestrogen exposure in the JAMP guidelines. It has also been suggested that coexposure to certain compounds, such as planar compounds that interact with the AH-receptor, can inhibit VTG synthesis³⁵⁴
- Sensitivity/variability/response time: Very sensitive. No response probably means no xenoestrogens at least in effective concentrations. Variability can be large at contaminated sites, possibly being related to varying genotypes, size, migration and prey selection, making statistical evaluation difficult and emphasizing the implementation of careful field designs. The response time is slow (several days) for VTG in blood plasma. Also the half life after exposure has ceased is daysweeks. Therefore, low VTG levels can either indicate low recent exposure or previous larger exposure. The response time of hepatic mRNA is much shorter (transcription can be measured within a few hours and decay halflife is 3-4 days).
- Ecological relevance: Measures reproductive impairment. However, it is probably not possible to estimate negative effects on population level by measuring VTG alone although elevated levels should be considered an early warning signal. Supportive parameters that should be recorded include weight and gonad weight, enabling the calculation of GSI³⁵⁵ (indicating sexual maturity of the individual). Gonads should also be preserved, to determine intersex (presence of oocytes in testes) in case of high VTG levels and to confirm sex. Otolith samples can be used for age determination and EDA/TIE fractionation can be performed on oestrogenic metabolites in bile (or sediment extracts from the affected area).
 - Swedish contact person: Lars Förlin, Göteborg University
- **Complexity/learning period:** Easy to learn (if measuring protein levels); kits are available for several fish species such as salmon and cod (although not using Lv as recommended for this species).
- **Costs:** The major costs related to studying this biomarker are related to fish catch (field studies) or costs for fish/cages (cage studies). These are considered moderate-high. The actual costs for analysis are low-moderate.

Comments: Can act as early warning biomarker to indicate risks of physiological response due to endocrine disruptors. Significant effects do not confirm the presence of effects but could be valuable as part of a weight of evidence approach and should be interpreted together with histological

³⁵² Suggesting that dredging activitites can have larger impact than previously anticipated.

³⁵³ Correlation found with gatrointestinal tract estrogenic activities.

³⁵⁴ due to increased CYP1A mediated metabolism of estrogens alternatively, due to an inhibiting AHR-ER crosstalk. In contrast, other coexposure of estrogens with other compounds such as antifungal azoles that inhibits CYP1A and CYP3A enzymes can enhance VTG synthesis due to decreased CYP metabolism (Gräns et al 2010).

³⁵⁵ Gonadosomatic index; gonad weight as percentage of body weight

examination of intersex of gonads, GSI and sex ratio of the population in order to make conclusions about effects. Because highly sensitive, lack of significant response probably indicates lack of effects from xenoestrogens. Swedish marine baseline data are available for comparison but a reference site is considered to be essential in all studies. Regional monitoring could be coordinated with national programs but analysis need to be performed shortly after sampling. Because major costs are related to sampling rather than analysis, adding VTG would not substantially increase costs. Because blood samples are used for the VTG analysis, there is probably no conflict with chemical analyses to obtain sufficient material.

27.1.6 Lysosomal stability

Short description: The lysosome is a sub-cellular organelle with similar processes in all organisms. It has a stable membrane, and contains acids and enzymes that can degrade a vast range of molecules, including waste materials. Recent studies also suggest that the lysosome can act as an antioxidant (Cuervo, 2004; Moore et al., 2006). The lysosomal stability can be affected by several stressors, including metals and organic contaminants, and indicates early toxic effects and health status. The lysosomal membrane stability test is thus an integrative parameter that would respond to the combined impact to most contaminants.

Endpoint (unit): Minutes (either measured by NRR, Neutral Red Retention, or cytochemically) **Standard/scientific reference to test protocols**: JAMP guideline available.

Environments that can be investigated: Primarily used so far in marine mussels but also fish **Season to be avoided/recommended:** No significant seasonal variability for NRR has been observed (Castro et al., 2004). In fish, none or only very small effects on lysosomal membrane stability was observed (Köhler, A. 1991). However, periods of spawning in mussels should be avoided (Moore et al., 2004).

Use: Lysosomal stability was so far not included in any of the regular national monitoring programs. However, it is used on international level (including as 1st tier screening parameter in the Mediterranean Sea), on occasion in the Baltic and will be included in a west coast coastal SRK program

Assessment criteria: Suggested (same values for both mussels and fish), low values corresponding to less disturbed conditions. Upper and lower limits are ≤ 20 and ≥ 10 for the cytochemical method; and for NRR ≤ 120 and ≥ 50

Specificity: General biomarker

Other influencing factors: Can also be influenced by physical stressors (hypoxia e.g.) and nutritional status. Should be taken into account during sampling.

Ecological relevance: Damaged membranes induce strong alterations in cellular function and is thus an indicator of early toxicity (fitness indicator). Has been used to predict fish liver damage and hepatopancreas damage in molluscs, larval viability, scope for growth and macrobenthic community diversity.

Performers available in Sweden: Åke Granmo Marine Monitoring

Complexity/learning period: Relatively non-complicated.

Costs: Low cost.

Comments: Because blue mussels are regularly monitored within the national program there are good possibilities to add this biomarker at low additional costs to obtain a general measure of stress levels. Because only minor amounts of sample (blood) are needed for the NRR analysis, there would be no conflict with chemical analysis. In fact, sampled individuals would be possible to return to the same site (the method is non destructive) for future sampling from the same individual. However, blood sampling and analysis should take place as soon as possible after retrieval of the individuals (within 24h).

28 Assessment criteria for effect based tools

28.1 Contaminated sites

Assessment criteria suggested in Naturvårdsverket 1999³⁵⁶, for the evaluation of some in vitro assays at contaminated sites.

Test	Criteria	Comment
Microtox, EC50 15 min vol %	Ground water: <50: very high impact from point source 70-50: high impact 95-70: probable impact >95: no or low impact Sediment (pore water): <50: very high impact from point source 70-50: high impact 90-70: probable impact >90: no or low impact	Based on 24 samples
Microtox, EC20 15 min vol %	Ground water: <15: very high impact from point source 50-15: high impact 90-50: probable impact >90: no or low impact Surface water: <50: very high impact from point source 70-50: high impact 80-70: probable impact >80: no or low impact	Based 31 surface water samples, and 25 ground water samples.
	Sediment (pore water): <15: very high impact from point source 25-15: high impact 50-25: probable impact 90-50: no or low impact	
Microtox whole sample 30 min vol% EC50	Sediment: <1: very high impact from point source 3-1 high impact 10-3 probable impact >10 no or low impact	

³⁵⁶ Naturvårdsverket 1999. Metodik för inventering av förorenade områden. Bedömningsgrunder för miljökvalitet, vägledning för insamling av underlagsdata. Report number 4918. <u>http://www.naturvardsverket.se/Documents/publikationer/620-4918-6.pdf</u>

Test	Criteria	Comment
Microtox whole sample 30 min vol% EC20	Sediment: <0,3: very high impact from point source 1-0,3 high impact 3-1 probable impact >3 no or low impact	
UMU Ctest -S9, dilution f 1	Ground water: No or very little impact from point source: 0,9-1,55	Based on 10 samples
UMU Ctest -S9, dilution f 3	Ground water: No or very little impact from point source: 0,9-1,36	Based on 10 samples
UMU Ctest +S9, dilution f 1	Ground water: No or very little impact from point source: 0,79-1,36	Based on 10 samples
UMU Ctest xS9, dilution f 3	Ground water: No or very little impact from point source: 0,85-1,23	Based on 10 samples
EROD In vitro bioassay	Soil: >125 ng/TEQ/g TS: very high impact from point source 25-125 ng/TEQ/g TS: High impact 5-25 ng/TEQ/g TS: Probable impact <5 ng/TEQ/g TS: No or very little impact from point source Sediment: >50 ng/TEQ/g TS: very high impact from point source 10-50 ng/TEQ/g TS: High impact 2-10 ng/TEQ/g TS: Probable impact <2 ng/TEQ/g TS: No or very little impact from point source	Based on 10 soil and 9 sediment samples

Assessment criteria suggested in Naturvårdsverket 1999³⁵⁷, for the evaluation of in vivo bioassay results from analysing contaminated sediment.

Test	Criteria	Comment
Algal growth 72h EC50 (vol %)	<25: very high impact from point source 70-25: large impact 95-70: probable impact >95: low or no impact	Based on 18 samples
Algal growth 72h EC10 (vol %)	<10: very high impact from point source 50-10: large impact 95-50: probable impact >95: low or no impact	Based on 18 samples
Mussel test, % mortality	 >10: large impact from point source 3-10 probable impact <3 low or no impact 	
Mussel test, development factor	 >45: large impact from point source 40-45 probable impact <40 low or no impact 	

28.2 **Proposed marine monitoring criteria**

Proposed assessment criteria for in vivo bioassays in the marine environment (from ICES, 2008).

Assay	Endpoint (unit)	Background ³⁵⁸	Elevated	High and cause
		response range	response	for concern
			range	
Sea urchin	% abnormality	≤10	>10-≤50	>50
embryo				
(water)	% growth	≤20	>20-≤50	>50
Bivalve	% abnormality	≤20	>20-≤50	>50
embryo				
(water)				
Copepod	% mortality	≤10	>10-≤50	>50
(water)	5			

³⁵⁷ Naturvårdsverket 1999. Metodik för inventering av förorenade områden. Bedömningsgrunder för miljökvalitet, vägledning för insamling av underlagsdata. Report number 4918. <u>http://www.naturvardsverket.se/Documents/publikationer/620-4918-6.pdf</u>

³⁵⁸ Upper limit normally based on either 90 or 95 percentiles of reference sites

Corophium (sediment)	% mortality	≤30	>30-≤60	>60
Arenicola (sediment)	% mortality	≤10	>10-≤50	>50

28.3 Proposed preliminary assessment criteria for biomarkers in the marine environment (from ICES 2008).

Biomarker,	Unit	Background ³⁵⁹	Elevated	High and cause
species	Umt			for concern
	.ug/1	$\begin{array}{ c c } \hline \textbf{response range} \\ \leq 2 \end{array}$	response range	
VTG, cod,	ug/l	≥ 2		
flounder		- 1	> 1.0	
Reproduction,	% Malformed	≤1	>1-2	>2
eelpout	larvae			
	% Late dead	≤2	>2-3	>3
		≥ 2	~2-3	~3
	larvae			
	% Growth	≤4	>4-6	>6
	retarded larvae		- + 0	2.0
EROD	pmol/mg	$\leq 80 \pmod{100}$		
	protein			
	1	\leq 40 (dab)		
		. ,		
		≤ 10 (flounder)		
Bile	ug/ml	\leq 220 (dab)		
metabolite: 1-	_			
OH pyrene		\leq 0,95 (cod)		
DNA adducts	Number	\leq 7.86 (dab)		
	adducts/mol			
	DNA	≤ 6.84		
		(haddock)		
		\leq 7.90 (saithe)		
		. ,		
Lysosomal	minutes	>20	<i>≤</i> 20 - <i>≥</i> 10	<10
stability		(Cytochemical)		
-				
		>120 (Neutral	≤120 - ≥50	<50
		red retention)		
FDI, Fish		< 2.5% quantile	2.5-97.5 %	>97.5 %
Disease Index			quantile	quantile

³⁵⁹ Upper limit normally based on either 90 or 95 percentiles of reference sites

29 Proposed assessment scheme for biomarkers to be used in a weight of evidence approach

The following proposed (not yet published) assessment scheme was presented in 2010for the biomarkers monitored in the national Swedish monitoring program (Larsson et al 2010)³⁶⁰. The table is an extract to illustrate the suggested weight (ecological significance) of individual biomarkers (the higher the number in the second column, the more strongly the biomarker is considered to measure response of the particular functional group) and the main functions (reproduction disturbances are e.g. considered more relevant than ion regulation impacts). Additional weighting procedures are suggested and the authors should be consulted for the full presentation.

Physiological function	"Weight" of individual biomarker (the higher the number the heavier weight in the particular function)	
Reproduction (total weight: 3)		
	Reduced gonads: 1	
	Delayed maturity: 1	
	Dead or malformed embryos: 2	
	VTG induction in males: 2	
	VTG reduction in females: 1	
Condition and metabolism (total weight: 2)		
	Reduced CF: 2	
	Increased CF: 1	
	Change in liver size: 1	
	Change in glucose: 1	
	Change in lactate: 1	
Liver function (total weight: 1)		
	Tissue alterations (cell death e.g.): 3	
	Change in liver size: 2	
	Structural changes (vakuols, parasites): 1	
	DNA-addukter: 2	
	EROD: 1	
	Glutathionrecuctase:1	
	MT: 1	
Immune response (total weight: 1)		
	White blood cells (change in numbers): 2	
	Macrofague centers (increased number): 2	
	Lymphocytes (change in numbers): 1	
	Trombocytes (change in number): 1	
	Granulocytes (change in number); 1	
Red blood cells (total weight: 1)		
_	Hematokrit: 2	
	Hb: 2	
	Red blood cells (change in numbers): 2	
	Immature red blood cells (change in number): 1	
Ion regulation (total weight: 1)		
	K change: 2	
	Ca changes: 2	
	CI AND Na changes: 3	
	CI OR Na changes: 1	

³⁶⁰ Havsmiljöseminariet i Borgholm i april 2010. Proposal developed by Åke Larsson, Niklas Hansson, Lars Förlin and Jari Parkkonen (Göteborg University).

30 Some novel tools

30.1 SPEAR – invertebrate community index that is sensitive to hazardous substances

Whereas the BQI can rather be used to estimate effects related to eutrophication, German researchers recently developed an index based on the relative sensitivity of different invertebrate species to hazardous subtances (Liess & von der Ohe 2005). The index is called SPEAR index (SPEcies At Risk) and measures the proportion between sensitive (SPEAR) and less sensitive (SPEnotAR, "SPEcies not At Risk") species, and is expressed as a percentage.

SPEAR index (%) =[{number of SPEAR}/{number of SPEnotAR}]*100

Thus, the higher the SPEAR index value, the less impacted the area is anticipated to be. The sensitivity aspects includes an assessment of both physiological sensitivity and the recovery potential, see table below.

Species that are considered potentially sensitive to pesticide exposure based on an assessment of recovery potential need to fulfil criteria for all three traits in order to be further assessed.

Potentially sensitive to pesticide exposure (further assessment is done)	Classified as SPEcies NOT At Risk
Generation time exceeds 0,5 year	Generation time less than 0,5 year
Poor migration potential	Good migration potential
Aquatic larval stages during high	Adult stages emerged before May
exposures	(during high exposures): no aquatic
	exposure

In the study by Liess & von der Ohe (2005), species fulfilling the above criteria to be classified as potentially sensitive to pesticide exposure, were further assessed regarding their relative physiological sensitivity. This assessment was based on the relative sensitivity observed when comparing EC50 values of the particular species to a certain substance, to the corresponding EC50 for Daphnia magna for the same substance. The relative sensitivity, S, is calculated by the following equation (von der Ohe & Liess 2004)³⁶¹:

S= log (LC50 _{Daphnia magna} / LC50_i)

The obtained median relative sensitivity observed (-0.36) was used as cut off to finally identify species that should be considered SPEcies At Risk and thus included in the final calculation of the index. Thus the species are grouped

³⁶¹ The sensitivity of the species to toxic stress is in this study ranked relative the sensitivity of Daphnia magna for the same compound (metals and organics respectively).

according to their sensitivity to toxicants (based on the relative species sensitivity distribution rank) and their life cycle traits.

In the study by Liess and van der Ohe (2005), twenty central European streams were investigated and showed that a measured pesticide concentration of 0,1*EC50 led to a short and long term reduction of abundance and number of SPEAR and a corresponding increase in species not at risk (SPEnotAR). Even concentrations of 0,01*EC50 correlated with long term change in community composition. The SPEAR increased when there were undisturbed stream sections available upstreams, thus highlighting the need to also take conditions upstreams and recolonisation aspects into account.

After the publication in 2005, another SPEAR index; the SPEARpesticides has been introduced (Beketov et al 2009). There are therefore at present two SPEAR indices available. The SPEARpesticides index responds to insecticides and SPEARorganic responds to petrochemicals and synthetic surfactants (Beketov & Liess 2008).

Using the SPEAR approach on invertebrate data from Swedish streams monitored within a national program, the SPEAR index varied between 60-80%³⁶². Chemical data are not available and the obtained data can therefore only be compared to other parameters such as percentage farmland cover. After recalculation uwing the new version of the index, the correlation between obtained SPEAR values and the percentage farmland cover is improved. This correlation was also observed if using PCA (Principal Component Analysis). The Swedish dataset used is from the year 2000 and the type of crops used at that time are now being investigated in order to predict retroactively what type of pesticides that were present. From a Swedish perspective, the SPEAR metric is therefore considered promising but needs to be adapted to northerly conditions regarding landscape and climate and compared to other metrics that quantify ecological change.

The SPEAR concept is applicable to assess the effects on invertebrate communities in rivers but not lakes or coastal areas and also not to temporary streams. Sampling should be performed in fall to take insect emergence into account. It is necessary to have access to effect data for the species that are considered "potentially sensitive" to pesticide exposure in order to be able to evaluate the physiological sensitivity. There is only a very limited effect dataset available for other invertebrate species than the ones that are commonly used for chemicals testing for regulatory purposes, such as Daphnia magna. This also limits the use of the index. However, it would also be possible to base a preliminary assessment on "read across" or QSAR data. Validation studies were so far performed in Finland (Schäfer et al 2007), Australia (Schäfer et al 2011) and France. In Sweden there is a need for further validation before the SPEAR indices can be used on a regular basis and as part of the WFD classification.

The sensitivity rank of different species is relative and specific for a certain dataset. Therefore, a species that is considered to be "SPEAR" in one particular dataset, can be considered "SPEnotAR" in another dataset depending on the relative

³⁶² Willem Goedkoop pers comm

frequency distribution. This approach limits the possibility to establish absolute assessment criteria for the SPEAR index. However, a SPEAR index below 40% has been suggested as a sufficiently significant response to state that the site is disturbed³⁶³.

There is now a website with a "SPEAR calculator", developed by Helmholtz Centre for Environmental Research (UFZ) available³⁶⁴.

30.1.1 Literature cited

Beketov MA, Liess M 2008. An indicator for effects of organic toxicants on lotic invertebrate communities: Independence of confounding environmental factors over an extensive river continuum. Environmental Pollution 156 : 980–987

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Liess M, von der Ohe PC 2005. Analyzing effects of pesticides on invertebrate communities in streams. Environmental Toxicology and Chemistry. 24, (4): 954-965.

Schäfer R, Caquet T, Siimes K, Mueller, R, Lagadic L, Liess M. 2007. Effects of pesticides on community structure and ecosystem functions in agricultural headwater streams of three biogeographical regions in Europe. Science of the Total Environment. 382, 2-3, 272-285

Schäfer, RB., Pettigrove, V, Rose, G, Allinson, G, Wightwick, A, Von Der Ohe, PC, Shimeta, J, Kühne, R, Kefford, BJ. 2011. Effects of pesticides monitored with three sampling methods in 24 sites on macroinvertebrates and microorganisms. Environmental Science & Technology

Von der Ohe, P & Liess, M. 2004. Relative sensitivity distribution of aquatic invertebrates to organic and metal compounds. Environmental Toxicology and Chemistry, Vol. 23, No. 1, pp. 150–156

30.2 PICT

The general belief is that community function is less sensitive to toxicants than community structure. However, PICT (Pollution Induced Community Tolerance) has been suggested as a sensitive tool to track changes in the community function that can be attributed to toxic substances. The PICT approach was developed by Blanck & Wängberg and Blanck et al (1988). It aims at measuring an increase in average tolerance of a community to the substance/s that initially restructured the community.

Because tolerance is measured, it is important that the effects observed only reflect the changes developed during the selection phase. The quantification of "average tolerance" is made by using short term tests, such as measuring effects on photosynthesis, nutrient cycling, degradation of organic matter, energy conversion, survival etc, on a "community sample" that is challenged with known toxicants in the laboratory. Common endpoints are therefore e.g. thymidine incorporation into nucleic acids of bacteria, but also nematode lethality (Millward & Grant 1995,

³⁶³ Workshop enquiry

³⁶⁴ <u>http://www.systemecology.eu/SPEAR/index.php</u>

2000), and abundance in benthic invertebrate assemblages (Courtney & Clements, 2000)

Thus, the approach works for any community that can be sampled, and PICT was so far used to assess the tolerance developed by invertebrates, periphyton, phytoplankton, bacteria and nematode communities in both marine and limnic environments (Blanck 2002). PICT was primarily used for risk assessment purposes to assess the risks of indivual contaminants on community levels (as opposed to the traditional approach to measure effects on single species) by exposing sampled communities collected from clean sites in the laboratory (Blanck 2002). PICT was also used in retroactive risk assessment studies on marine peryphyton communities, by sampling communities from contamination gradients and exposing the samples to single chemicals known to be contained in these gradients, such as TBT and irgarol (Blanck & Dahl 1996; Grönvall et al 2001). Recovery was also studied before and after TBT ban (Blanck & Dahl 1998). In recent years, PICT combined with the transplantation of periphyton communities has been suggested as a promising tool to identify impaired sites by detecting an induced tolerance after transplantation Transplantation techniques of periphyton communities are facilitated by utilizing the rapid colonization occurring on deployed glass discs. In situ PICT assays using transplanted communities has been suggested as a promising tool that can link ecological and chemical status in the WFD context (Pesce et al 2010a, b; Tlili et al 2010, 2011).

An important limitation was that the toxicant/s need to give rise to a response in a short term test in order to be able to quantify the induced tolerance. The optimal endpoint should therefore also be sensitive and sensitivity of a short term chronic test increases with a short generation time of the species. Therefore measuring bacteria thymidine was so far considered the most useful endpoint. Bacterial ecology parameters were so far not considered in the WFD context although it is clear that impacts on bacterial functions would indeed have the potential to cause impact on ecosystem level.

Also the identity of the toxicants needed so far to be known/suspected in order to know what substance or mixture that should be used in the "challenge test". In an environment that is influenced by complex coctails from many sources, including substances that are rapidly degraded or transformed it may be difficult to decide which single substances that are the most relevant to use in the challenge test. However, by exposing communities sampled from "clean" environments to water or sediment samples from contaminated (downstreams) environments, either in the laboratory or in the field, and measure effects on relevant functions, it would be possible to actually avoid the step of identifying the suspected contaminants before being able to measure the effects (Rotter et al 2011). The lack of knowledge on causing agents from the start, would necessesitate the use of endpoints that can provide integrated response from several potential mode of actions. So far, tymidine incorporation in bacteria, would be the only such identified endpoint used within PICT studies (Blanck 2002). However, Montuelle et al (2010) concluded that OMICS methodologies such as genetic fingerprints³⁶⁵ can be used in the PICT concept to assess chemical effects and ecosystem resilience.

³⁶⁵ OMICS refers to effect based tools that study effects on genetic levels. Analogous to classical biomarkers, OMICS methods can be used to identify the presence of induced mode of action/s on a genetical or protein

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30.3 Metagenomics to study ecosystem function³⁶⁶

Novel tool to monitor microbial communities

Microbial communities are important for ecosystem functions at many levels, as they serve as the most important primary producer in aquatic environments (Price 2001) and therefore provide the fundament of the ecosystem services we rely on. Changes in community function at the microbial level could easily propagate and perturb the ecosystem as a whole. Hence, it is important to monitor changes in community structure and function, for the understanding of ecosystem health.

level. One branch of this family of method is the proteomics and microarray tools. These assays are now being performed on routine and commercial basis. Sequencing has advanced even further but there is a research need identified in how to analyse the data. There is a need to develop standardised bioinformatics tools to study resistance genes. Software needs to be developed as the challenge is in the interpretation. ³⁶⁶ The description was largely provided by Johan Bengtsson and Joakim Larsson, Göteborg University and only minor changes has been made.

Traditionally, monitoring microbial communities have been troublesome, even though approaches such as PICT (Blanck et al. 1988) has successfully been used as a tool to inspect functional changes in response to toxicant selection pressure. However, in recent years a tremendous increase in DNA sequencing capacity, combined with an unprecedented drop in price per obtained nucleotide sequence, have made it possible to study the functional elements of an ecosystem at the levels of the actual genes responsible for these functions. Such sequencing studies of the total DNA content of an environment is generally referred to as metagenomics (Riesenfeld et al. 2004).

Total microorganism community function now possible to analyse

Metagenomic studies can be targeted towards specific genes of interest, such as taxonomic markers (16S rRNA) or genes involved in detoxification or antibiotic resistance development. However, it is often of interest to study the total functional content of a community of microorganisms to get a broader picture of the ecosystem function (Tringe et al. 2005). To assess such broad questions about the total composition of organisms, genes and functions represented within a community in a single experiment would have been impossible without the leap forward in sequencing technology seen in the last decade (Metzker 2010). Metagenomics provides a means to analyse complete communities of microorganisms, regardless of whether they can be cultured in the laboratory or not. This is a huge benefit as it has been estimated that only one or a few per cent of the microorganisms in nature can be readily grown in the laboratory (Amann et al. 1995).

Standardisation potential and reproducibility

One of the main benefits of using DNA sequencing and metagenomics for community analysis is that there are easily implemented and standardized protocols for DNA extraction and amplification, and that the methods produce reliable and reproducible results. There are several laboratories and companies that include most of the DNA preparation as part of the sequencing service, making the process from extracted DNA sample to resulting sequences highly standardized and more or less transparent to the end-user.

Data interpretation and computational capacity to decide functional role of each sequence

Because of the large amount of sequences generated in a single sequencing run, most often on the order of hundreds of thousand to hundreds of million sequences, the major challenge of metagenomics is the post-sequencing analysis in which the functional role of each sequence is determined. This means that there is a great need for bioinformaticians to make sense of the DNA information. Currently, there is no easy-to-use software solution that can harness the power of metagenomics without the need for some degree of bioinformatics expertise. Such solutions are, however, undoubtedly in development, and progress has been made with packages such as MEGAN (Huson et al. 2011). Nevertheless, either in-house bioinformaticians or consultants are today a requirement to be able to draw conclusions from the vast amount of sequence data generated using the modern sequencing techniques. In addition, the large amount of DNA sequenced in these studies requires substantial storage and computing capacities.

Possible to measure species composition in a very precise way

An important benefit of using metagenomics for community studies is that the amount of sequences generated enables very precise measurements of e.g. the species composition in a community. Based on a genetic marker approach³⁶⁷ it is possible to study the species composition in the community, in terms of which species or groups of species that are present and how different toxicant selection pressures affect the community structure. Previous knowledge of the exact sequence of the genetic marker in a given species is not needed to be able to identify it and give an estimate of its taxonomical association.

Ecological relevance and possibilities to identify previous exposure to toxicants

The study of species composition changes in a community is not a sub-lethal endpoint but rather an analysis of effects on the community and ecosystem levels. The ability to investigate highly and lowly represented genetic functions in a community translates into an understanding of which functions and organisms that are required to maintain ecosystem stability, and in the end the sustainability of the ecosystem services³⁶⁸ provided by the community.

The ability to study functions present or absent in a community, as well as the abundance of the genes responsible for these functions, also makes it possible to assert something about previous exposure to various toxicants. This assessment is either based on sensitive versus non-sensitive indicator species found, or by looking at the abundance of genes encoding specific detoxification functions³⁶⁹, cf SPEAR and PICT. It would also be possible to e.g. combine studies of community tolerance, such as PICT, with metagenomics to investigate the underlying changes in genetic composition responsible for inducing a response to the toxicant at the phenotypic level.

A major benefit of using metagenomics to study the composition of microbial communities is the possibility to not only focus on specific genes and functions, but to look broader into *all* available genes and functions. In practice, this means that it is not necessary to propose a hypothesis before conducting the actual experiments. Instead, the content of organisms and functions detected in the

³⁶⁷ Such approaches use well-studied genetic markers such as the 16S rRNA gene, and the vast amount of data analyzed makes the method very robust compared to e.g. gel-based techniques. One way of retrieving 16S DNA from a community sample is to use PCR to amplify the 16S gene specifically, although data on the 16S and 18S genetic markers can also be obtained from metagenomic samples of total community DNA using bioinformatics utilities (Bengtsson et al. 2011).

³⁶⁸ This does not only have ecological implications, but can also serve as a basis when assessing which aquatic areas that are more suitable for e.g. food production.

³⁶⁹ To find the genetic elements that encode the functions of interest in an ecosystem, the obtained sequences must be mapped to a database of functionally known genes using bioinformatics tools. Such databases could be more general, such as the protein family databases Pfam (Finn et al. 2010) and COG (Tatusov et al. 2003), or more specific, e.g. the antibiotic resistance gene database ARDB (Liu and Pop 2009). It is also possible to map genes in e.g. the manually annotated protein database SwissProt (UniProt Consortium 2010) to Gene Ontology (GO) terms (Ashburner et al. 2000). The GO terms represent a gradually more specific terminology that can be used to accurately describe the functions of a gene to the extent that these functions are known.

sample can be used to trace effects of toxicants, regardless of whether the presence of any specific toxicant was known on beforehand³⁷⁰.

Of particular concern: antibiotic resistance genes in the environment

Some antibiotics were previously on the WFD candidate list of priority substances. However, a major concern related to antibiotics in the environment (especially if released from sewage treatment plants where there are biological treatment steps with recirculation) is probably rather related to a potential release of antibiotic resistant genes, that at least with time could end up also in pathogenic bacteria and pose a health hazard, rather than toxic effects of the original compounds per see. A possible use of metagenomics is to monitor the presence and abundance of antibiotic resistance genes in the environment. While this has been possible for a long time using culturing techniques, metagenomics provides a means to look into the resistance profile of *all* bacteria in a community³⁷¹. This is a benefit because of the limited number of cultivable microorganisms in nature, as pointed out earlier.

Costs

For the last decade, sequencing costs has dropped dramatically while the capacity of the sequencing machines has grown (Schuster 2008). Today, it is possible to obtain more than a hundred million sequences in a single run on a single machine. The cost of one such run is below 10 000 Euro and it is also possible to buy smaller parts of a sequencing run for around 1000 Euro per part.

Multiple samples can be analysed in one run by a process called "multiplexing". Multiplexing adds a small DNA sequence to every sequence in the sample, which functions as a tag describing to which sample the sequence belongs. In this way, the sequences derived from one experiment can easily be separated from those in another experiment after sequencing has finished. The numbers of samples that can be multiplexed in one run varies with sequencing technology, but usually at

³⁷⁰ However, a prerequisite for retrieving statistical significance for changes related to environmental stress is that the sequencing has been "deep" enough, i.e. that the sequencing effort has produced a sufficiently large number of sequences. This can be a problem in more complex communities, inhabited by many different species, especially if these organisms have large genomes. Hence, it is always of interest to keep the number of sequences obtained at the maximum within the available budget. It is desirable to produce on the order of at least tens of millions of sequences from a single sample to get sufficiently accurate results in these broader studies, which is easily achieved through e.g. Illumina sequencing. Using a more narrow scope, on the other hand, limits the number of investigated genes and functions, but allows for smaller sequencing efforts. Here, Roche 454 sequencing is a useful alternative as it provides longer DNA reads, more suitable for analysis of sequence variants. The narrow approach still allows for monitoring presence of specific pathogens, resistance genes, detoxification systems, and species composition with high precision, but relies on a priori knowledge of the components of the system to a much larger extent than the broader approach to metagenomics.

³⁷¹ For this purpose, both the narrow and the broad approach are useful. For example, specific resistance genes such as Sul1 and Sul2, both responsible for sulphonamide resistance, can be amplified and their abundance quantified specifically. It would also be possible to study the diversity of these specific genes to find which variants that are present. However, it would be simple to extend the study into a broad one, investigating all known types of resistance genes, by using a database such as ARDB (Liu and Pop 2009) and thereby screen for more than 350 types of resistance and thousands of variants of resistance genes in one experiment. This allows for a much more detailed look of the spread of antibiotic resistance in the environment. Such studies have, for example, been carried out in areas polluted with pharmaceuticals in India, finding high abundances of many different classes of resistance genes (Kristiansson et al. 2011). However, this kind of broad resistance surveys requires sufficient depth to be able to draw conclusions on any differences between different communities and environments.

least 8-10 samples can be run simultaneously. For example, it is today possible to sequence 75 DNA samples from sediments, to a depth of around four million sequences per sample, for about 20 000 Euro. It is expected that sequencing prices will continue to drop over the next years, making metagenomics analysis even cheaper, and facilitating its use for a broader range of inquiries.

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