Document title	HELCOM indicators on diclofenac and estrogen
Code	3-2
Category	CMNT
Agenda Item	3 - Organization of work and Group's working methods
Submission date	19.6.2017
Submitted by	Secretariat
Reference	

Background

Diclofenac and estrogen were suggested to be used as HELCOM indicators of the ecosystem health of the Baltic Sea. The work on these indicators was launched in the frame of HELCOM COREST II project. Draft descriptions of both indicators in accordance with an agreed structure of HELCOM core indicators were elaborated in 2015. Current status of the indicators can be identified as "pre- core" indicators. This means that the indicators were identified as necessary by the HELCOM Contracting Parties for BSAP and MSFD purposes. The indicators were not adopted as core indicators, due to the fact that some aspects of the indicators were under development.

When the work on the Regional status report on pharmaceuticals in the Baltic Sea environment was launched CORSET II project group decided to put both indicators on hold until observation data on these substances in the marine environment has been collected.

HELCOM 38-2017 agreed on the ToR for the CG Pharma group and suggested to include the task on further elaboration of indicators on diclofenac and estrogen into the CG Pharma ToR.

This document contains draft descriptions of indicators on diclofenac (Annex 1) and estrogen (Annex 2).

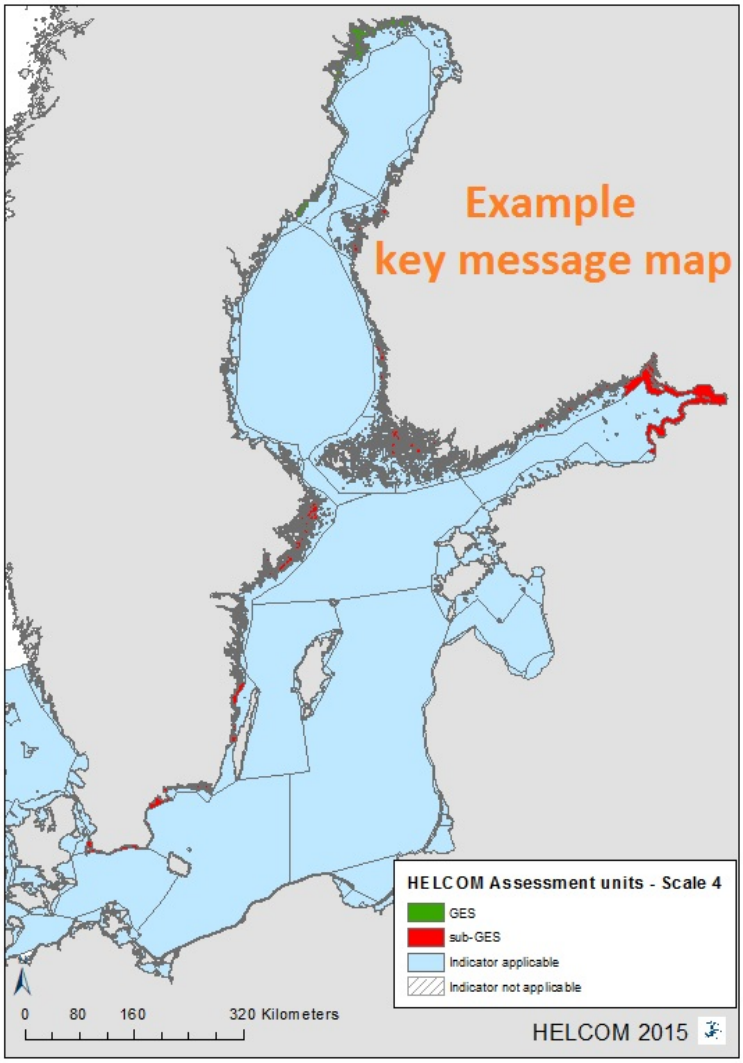
Action requested

The Meeting is invited to take note of the current state of HELCOM indicators on diclofenac and estrogen and agree on the next steps towards elaboration of HELCOM core indicators.

Annex 1.

Diclofenac concentration

Key message



The occurrence and concentration of diclofenac in the Baltic Sea marine environment is currently very poorly known with information only from screening studies available, and accurate evaluations of the status of the environment cannot be made at this time. Detecting any trends in the status will only be possible after monitoring has been carried out for a few years to come.

Initial screening studies have indicated that the diclofenac concentration can be high close to waste water treatment plants and that high concentrations of diclofenac may also be detected in rivers draining into the Baltic Sea. Additional studies on how far the effects of the increased concentration affects the environment from the source is needed to guide status evaluations in the future.

Relevance of the core indicator

Diclofenac is a widely used pharmaceutical in the Baltic Sea region. Studies have shown toxic effects in marine organisms to high concentrations of diclofenac.

Policy relevance of the core indicator

	Primary importance	Secondary importance
BSAP Segment and Objective	• Healthy wildlife	
MSFD Descriptors and Criteria	8.1. Concentration of contaminants	
Other relevant legislation: EU EQSD		

Authors

Ekaterina Chernova, Lena Avellan, Kari Lehtonen, Jakob Strand

Cite this indicator

[Author's name(s)], [2015]. [Indicator name]**.** HELCOM core indicator report. Online. [Date Viewed], [Web link].

Indicator concept

Good Environmental Status

The EU Environmental Quality Standards for diclofenac is used as the GES-boundary. The concentration in water and biota that form the GES-boundary are also in line with the scientific PNEC or NOEC values implying that no harmful effects can be found. However, as the issue is of an emerging nature, it is proposed that the GES-boundary would initially be evaluated as a trend so that no increase in the concentration would be allowed.

As a general rule, the GES-boundaries in HELCOM core indicators are to rely primarily on sampling in biota. The primary GES-boundary of the indicator is a concentration of diclofenac in biota being below 1 µg kg^{-1} ww as derived in the QS biota. If evaluation using biota sampling is not feasible, the status evaluation can be done against the secondary GES-boundary 0.01 µg L^{-1} in average in water as derived for EQS water (AA).

The EU directive on environmental quality standards (2008/105/EC), Article 3, states that also long-term temporal trends should be assessed for substances that accumulate in sediment and/or biota which is currently not possible due to poor data availability.

Anthropogenic pressures linked to the indicator

	Strong connection	Secondary connection
General	Introduction of pharmaceuticals	
MSFD Annex III, Table 2	- Introduction of synthetic compounds	-

Assessment protocol

Due to the fact that no commonly agreed monitoring strategy has been developed for pharmaceuticals, only data from screening studies and scientific literature can at this time be considered.

The indicator is tentatively evaluated on the HELCOM assessment unit level 4, as the status of the marine environment is presumed to be affected mainly from point sources constituted by the waste water treatment plants. In screening studies, concentrations exceeding the GES-boundary have generally been found to drop over a distance of a few kilometres. As knowledge on the spatial extent of the effects from increases, it may be possible to appropriately evaluate a larger coastal area and then applying the HELCOM assessment unit level 3. The indicator is in theory applicable in all assessment units throughout the Baltic Sea, as diclofenac is used in all HELCOM CPs.

Relevance of the indicator

Policy Relevance

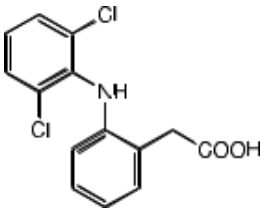
The Moscow Ministerial Declaration 2010 gives HELCOM a clear obligation to 'further assess the environmentally negative impacts of pharmaceuticals and other substances that are not monitored regularly, with the aim as a first step to assess in a coordinated manner their occurrence in the Baltic Sea and evaluate their impacts on the Baltic biota'. The commitment was followed up by the 2013 HELCOM Ministerial Declaration in which the Contracting Parties agreed to collect information on pharmaceuticals and assess the status of contamination of pharmaceuticals and their degradation products in the marine environment. The aim of the core indicators on pharmaceuticals is to support the agreed assessments.

There are no restrictions or bans on the use of diclofenac in the Baltic Sea region. India is phasing out the use of diclofenac due to the documented detrimental effects on vultures.

Diclofenac is included on the first watch list of the EU Priority Substances (2013/39/EU) with the aim to gather monitoring data for the purpose of facilitating the determination of appropriate measures to address the risk posed by those substances. Inclusion on the first watchlist is done when there is concern that the substance will have negative impacts on the environment based on results from the prioritization process of the hazardous substances under the WFD, research results and similar. For those HELCOM CPs that are also EU countries, the inclusion on the first watch list obligates the countries to commence monitoring at selected representative monitoring stations for a 12-month period, starting in 2015.

The effects of diclofenac in the ecosystem

Diclofenac is an active pharmaceutical ingredient belonging to a group called nonsteroidal anti-inflammatory drugs (NSAIDs) that works by reducing hormones that cause inflammation and pain in the body. The pharmaceutical is widely used in the Baltic Sea region as different pharmaceutical preparations, both in the format of tablets to be ingested as well as creams for topical application.



Introduction of diclofenac to the Baltic Sea is believed to take place mainly through the sewage systems with high concentrations of diclofenac having been detected in effluent water from waste water treatment plants (WWTP). In the Baltic Sea, diclofenac has also been detected in water samples far from sources (HELCOM 2014). Also globally diclofenac is frequently detected in the aquatic environment (Zang et al. 2008). Diclofenac is sufficiently persistent to pass through the processes in the sewage water treatment plants to reach surface waters (Tixier et al. *2003*). However, recreational use of surface water bodies can temporarily result in a higher pharmaceutical input compared to that of WWTP especially in lakes and rivers (Atlasi et al 2012). Thus, it could be of relevance to take into consideration all possible sources of pharmaceutical loading. Sources such as hospitals, pharmaceutical manufacturers, sewer overflow or leakage, septic tanks, agriculture and storm water run-off are also relevant to consider as potential pathways for introduction of pharmaceuticals to the Baltic Sea.

Current processes in the waste water treatment plants do not reduce the concentration of diclofenac, thus the concentration of diclofenac in the incoming waters generally equals that of the effluent waters. Concentration of diclofenac in the effluent has often even been higher than in the incoming unpurified sewage water (HELCOM 2014). This phenomenon might be explained by the liberation of diclofenac from conjugated metabolites during bacterial treatment (HELCOM 2014). In other words, diclofenad is partially present in the sewage in a conjugated form due to the metabolic processes in the organism that first ingested the substance as well as the 'cocktail effect' of transformation in the sewage, cojugates are not formed during the treatment process (Peres and Bacrelo 2008).

Diclofenac has been shown to bioaccumulate in fish exposed to treated sewage waters (Brown et al. 2007). There is evidence showing detrimental effects of diclofenac on aquatic organisms. Diclofenac shows toxic effects on kidneys in fish, the concentration at which no effects are detected (NOEC) is 1 micro gram /liter water (e.g. Schwaiger et al. 2004). There is also evidence of impairing osmoregulation ability of crabs due to diclofenac (Eades & Waring 2010). At the concentrations of diclofenac in the marine environment that have been reported, acute toxic effects are not expected to occur however careful consideration of potential chronic effects are needed since little is currently known about the pathways of diclofenac in the environment.

The strongest evidence of the detrimental effects of diclofenac stems from the terrestrial environment. Residues of diclofenac causing kidney failure is considered to be the main cause for a decline of >95 % in the population of oriental white-backed vulture, one of the (previously) most common raptors in India and Pakistan (Oaks et al, 2004, Shultz et al, 2004; Reddy et al, 2006; Swan et al, 2006; Cuthbert et al, 2006, 2007).

Results and confidence

The HELCOM BASE pilot project on pharmaceuticals in the St Petersburg area showed that the average concentration of diclofenac in the effluent waters from WWTP varied from 355 ng/L in the summer of 2013 to 510-550 ng/L in the winter of 2014 (HELCOM 2014). The upper limit for daily release of diclofenac from the city of St Petersburg was estimated at 1.1 kg, suggesting an annual load of approximately 400 kg of diclofenac to the Baltic Sea from the city (HELCOM 2014). Averaging the load to the annual water volume of 78.9 km^3 (2,500 km^3/s)from the river Neva, gives an average expected surface water concentration of diclofenad in the water flowing into the Gulf of Finland of 4-5 ng/L (HELCOM 2014). The expected concentration is below the GES-boundary of 10 ng/L.

At the central waste water treatment plant in St. Petersburg the concentrations in the influent were 150-740 ng/L with an average of 408 ng/L and concentrations in the effluent were 150-490 ng/L with an average of 355 ng/L (Table 1). The influent water is a mix of domestic wastewater and rainwater, which results in the meterorological conditions having an impact on the processed water volume.

Table 1. Primary analytical results and estimations of daily discharge of diclofenac in the central St. Petersburg WWTP (reproduced from HELCOM 2014).

Date	primary analytical concentration of Diclofenac [ng/L] (values in brackets – on LTQ OrbiTrap)		rain	water volume	estimated diclofenac discharge [g/day]	
	influent	effluent	[mm]	[1000 m^3]	influent	effluent
Phase 1						
Sun 21.07.2013	**396.1**	**374.7**	8	1076	426	407
Mon 22.07.2013	**253.2** **256.4**	**493.8 (326)** **304.6**	18	1216	310	485
Tue 23.07.2013	**377.7**	**320.2 (239)**	6	1030	389	330
Wed 24.07.2013	**220.0**	**513.9 (286)**	12	881	194	453
Thu 25.07.2013	**332.2**	**436.5**	0	911	303	399
Fri 26.07.2013	-	-	0	942		
Sat 27.07.2013	-	-	0	877		
Sun 28.07.2013	**373.3**	**491.1**	0	869	304	400
Mon 29.07.2013	**413.3 (494)** **441.1**	**445.7 (452)** **321.9**	0	814	371	334
Tue 30.07.2013	**685.5**	**369.9**	0	718	492	266
Wed 31.07.2013	**250.0 (259)** **251.1**	**203.2** **200.3**	0	892	224	180
Thu 01.08.2013	**154.2**	**247.1**	6	894	138	221
Fri 02.08.2013	-	-	8	1117		
Sat 03.08.2013	-	-	0	916		
Sun 04.08.2013	**481.5 (452)** **491.8**	**153.8** **299.2 (269)**	0	850	414	193
Mon 05.08.2013	**741.0**	**344.4**	0	830	615	286
Tue 06.08.2013	**428.8 (378)** **487.7**	**310.2** **318.6**	0	877	402	275
Wed 07.08.2013	**400.0**	**385.4**	0	841	336	324
Thu 08.08.2013	**555.5**	**318.3**	0	905	503	288
AVERAGE	**408**	**355**			**362**	**323**
Phase 2						
Tue 19.02.2014	**590**	**470**		1264		
Sun 23.02.2014	**270**			1244		
Mon 24.02.3014	**160**	**470**		1044		
Tue 25.02.2014		**630**		991		
Wed 26.02.2014				1054		
Sun 16.03.2014	**320**			1000		
Mon 17.03.2014	**1700**	**440**		894		

Tue 18.03.2014		**530**	835	
Sun 23.03.2014	**450**		908	
Mon 24.03.2014	**310**	**520**	737	
Tue 25.03.2014		**500**	675	
Wed 26.03.2014			648	
Sun 30.03.2014	**400**		790	
Mon 31.03.2014	**350**	**580**	793	
Tue 01.04.2014		**600**	781	
Wed 02.04.2014			733	
Sun 06.04.2014	**390**		861	
Mon 07.04.2014	**300**	**430**	827	
Tue 08.04.2014		**500**	804	
Wed 09.04.2014			798	
AVERAGE	**350**	**530**		

The average concentration of diclofenac in the sewage water was found to be higher in Phase 2 than in Phase 1 of the sampling of the pilot project (Table 1). One reason for this could be a higher daily discharge observed in the cold season (Phase 2) than in the warm season (Phase 1) - 323 g/day and 477 g/day, respectively, due to increased use of diclofenac during the cold period and a significant part of metabolites being hydrolysed back to diclofenac in the warm period.

Information on sales of diclofenac is considered to be a relevant proxy to be used in the evaluation of environmental status. In the HELCOM BASE pilot project sales statistics were collected for Russia where 87 pharmaceutical preparations that include diclofenac have been registered for use in 2009-2010, and calculations were made to estimate the total usage of diclofenad in the St.Petersburg region.

Sales of creams for topical (external) application Non-Steroid Anti-Inflammatory drugs with during one year (May 2012-April 2013) in Russia totalled 33.9 million packages (RUB 3.9 billion). Voltaren and Diclofenac are the two leaders with 4.7 million packages each. One 20 g unit of 1% Voltaren Gel (RUB 190) contains 0.2 g diclofenac, with larger packages of 50g, 75g and 100 g also available on the market. Diklak gel is available as similar packages with a 1% concentration and in addition as a 5% preparation in 50 g tubes. Yearly sales of Diklak (tot RUB 145 million, unit price RUB 170) corresponds to 2.13 tonnes of diclofenac. However, the proportion of Diklak sold with an active ingredient of either 5% or 1% is not known. Therefore, the total consumption of diclofenac for external application is estimated from these data as 14 million units per year or 2.8 tonnes. Based on calculations, the external application use of diclofenac in St. Petersburg is estimated at 170 kg. If all the diclofenac were to enter the sewage system, the concentration could reach 200 ng/L. (HELCOM 2014)

Sales of pills, injections and suppositories by three leading brands were considered based on indicative numbers provided by an independent source (Table 2) (HELCOM 2014). The total annual consumption of diclofenac through pharmaceutical preparations other than creams for topical application is estimated at 20 tonnes, with an estimated sale of 27 million diclofenac units sold in 2011 (HELCOM 2014). In 2010 hospitals purchased 900,000 packages for injection and <100,000 in the form of pills, totalling an annual consumption of 20 tonnes (HELCOM 2014). Calculating the share consumption of these pharmaceutical preparations for the St. Petersburg area provides an estimate of 700 kg per annum. If all the diclofenac were to enter the sewage system, the concentration could reach 850 ng/L (HELCOM 2014).

Table 2. Indicative annual consumption of three brands of pharmaceutical preparations containing diclofenac in Russia (reproduced from HELCOM 2014).

	Injections	Pills	Suppositories
Diclofenac	6,731,799	5,958,925	900,616
Ortophen	144,030	6,155,850	26
Voltaren	1,168,177	232,503	489,748
Diclofenac per package	0.5 g	1 g	0.5 g
Diclofenac per form	**4 tonnes**	**12 tonnes**	**0.75 tonnes**

Summarizing the findings of the HELCOM BASE pilot study of sales and concentrations of diclofenac in empirical sampling in WWTP, shows that the actual concentration of diclofenac in the waste water treatment plant is only two times lower than the calculated potential concentrations if all sold diclofenac were to enter the sewage system. In the pilot study it was noted that as most of the consumed diclofenac is metabolised before excretion into the waste water, the main source for diclofenac in waste water is likely to stem from the creams for topical application. However, if only the sales statistics of diclofenac from the topical application products were to be considered, it would not explain the empirically detected concentrations. Thus the pilot study concluded that (a) the accuracy currently reached in the use of the sales statistics might not be high enough to make strong conclusions, (b) the presumed rate of metabolisation of consumed diclofenac is inaccurate, or (c) diclofenac is transformed on a massive scale in the sewage to free diclofenac from its metabolised state of different conjugates.

Existing screening data and scientific studies in rivers and estuaries point to the direction that diclofenac concentrations exceed the threshold level in several areas of the Baltic Sea (Table 3). Influent and effluent waters in waste water treatment plants had very high diclofenac concentrations and only an 11–28 % retention has been noticed in two large Swedish screening studies (Andersson et al. 2006, Fick et al. 2011).

Table 3. Diclofenac concentrations from screening studies and scientific literature. The GES boundary for surface water is 10 ng L^{-1} and for biota 1µg kg^{-1} ww. Exceeding concentrations are shown by red colour. The limit of quantification (LOQ) in the Swedish screenings studies was 10 ng L^{-1}

	Biota (µg/kg ww)	WWTP influent (ng/L)	WWTP effluent (ng/L)	Surface water (ng/L)	Sediment (µg/kg dw)	Reference
River Vantaa, Finland				16 – 65		Vieno et al. 2007
River Aura, Finland				at WWTP 26, 1km 9, 5km 6, 8km 5, 4km 4, 23km 2, 32km 2		Vieno 2007
River Kokemä-enjoki, Finland				at WWTP 11, 10km 1		Vieno 2007
River Kyrö, Finland				at WWTP 39 2km 23, 90km ND		Vieno 2007
Finland's WWTPs		Mean 0.42 (range 0.23–0.64)	Mean 0.35 (range: 0.14–0.62)			Vieno 2007
Germany			Mean: 810, max: 2100	Median: 150 (max 1200)		Ternes 1998
Germany			1300 ±100			Ternes et al. 2003
Germany		2330	1360			Quintana & Reemtsma 2004

Sweden			Ellinge 140, Öhns 81, Rya 100, Henriksdal 270			Lilja et al. 2010
Sweden			14–710			Andersson et al. 2006
Sweden			220, 230			Remberger et al. 2008
Sweden	Uppsala: <LOQ Vallentuna: <LOQ	Stockholm: 900, 1400, 1800 Umeå: 970, 1700, 2800 Uppsala: 1200, 1500, 7000	Stockholm: 420, 450, 590 Umeå: 430, 1000, 1300 Uppsala: 1500, 2100, 3900	Uppsala: 28, 90, 290, 880 Vallentuna: <LOQ		Fick et al. 2011
Sweden		Karlshamn: 570 Karlskrona: 500, 670 Ronneby: 200 Sölvesborg: 200 Luleå: 100 Piteå: 300	Karlshamn: 400 Karlskrona: 310, 700 Ronneby: 260 Sölvesborg: 300 Bollnäs: 600 Hudiksvall: 200 Sandviken: 200 Luelå: 200 Piteå: 200 Ängelholm: 200 Helsinborg: 300 Landskrona: 300 Malmö: 300 Trelleborg: 200 Trosa 200, 400 Skellefteå: 100			Andersson et al. 2006
River Piteå, Sweden	N.D.	0.19, 0.54	0.23, 0.22	N.D.	3.5, 0.19, 0.85, 3.1	Remberger et al. 2009
Sweden		160	120	120 (max)		Bendz et al. 2005

Confidence of the indicator status evaluation

Due to the fact that monitoring of pharmaceuticals is only commencing in many of the HELCOM CPs, no actual status evaluation has yet been made for the indicator. It is recommended that monitoring would be carried out for a few years before a status evaluation is done. Currently the data is spatially scattered with only single screening studies carried out.

When a status evaluation has been carried out, the confidence of the status evaluation is believed to be low to moderate throughout the HELCOM region at first. The spatial and temporal coverage of the available data is poor currently poor, as is the knowledge of how well a sample represents a larger area. Historical data on diclofenac concentrations in the Baltic Sea is not available and thus trend based evaluations are not possible.

Monitoring Requirements

Monitoring methodology

Diclofenac can readily be analysed in water and in fish plasma. Pharmaceuticals are generally present at very low concentrations in the environment. Thus it is very important that selected analysis methods take detection limits into consideration, also the analytical procedure should be easy to carry out on the specified level.

The HELCOM BASE pilot project carried out sampling in waste water treatment plants according to the following methodology:

- WWTP personnel carried out sampling
- In influent water by sampling over a 24-hour period on which the average was calculated. Effluent samples were grab samples taken on the same mornings.
- On several days, two samples were taken and analysed independently.
- Samples were collected by SRCES personnel immediately afterwards and extracted within 24 hours.
- $^{13}C_6$-Diclofenac was used as a surrogate standard and was introduced into the raw sample.
- Samples were analysed for Diclofenac on a secondary HPLC-HRMS, IT-TOF.
- Several of the samples were also analysed for Diclofenac on LTQ OrbiTrap; however, these results cannot be considered quantitative - they were used to confirm the range of concentrations and collect the test data for future analysis on the LTQ OrbiTrap.

Description of optimal monitoring

Due to the lack of coherent data from the marine environment and especially from biota, it is not possible to give recommendations on optimal monitoring at this time. Generally it can be stated that monitoring should be commenced in coastal areas close to cities with large wastewater treatment plants (WWTPS) and that intercalibration between all involved laboratories would be very beneficial

Screening studies of surface water show concentrations generally dropping to below the GES-boundary at a distance of 10 km from WWTPs. It might be appropriate to monitor concentrations at the WWTP and at a specified distance from the plant in sea water and biota, however specifying the distance will require further work. The change of diclofenac concentration over a distance from source is highly dependent on the hydrological, hydro-chemical and hydro-biological regimes in the assessment unit. It might not be possible to provide guidelines as the appropriate design of the sampling strategy that would be generally applicable in all areas. However, it could be relevant to consider more closely the patterns in changing concentrations versus distance from source from several locations, to consider whether min-max and median values at a certain location or from several locations could be used when evaluating the status of the environment. Examining concentration trends over time more closely is also considered to be relevant.

The pilot study in the St Petersburg area carried out in the HELCOM BASE project (HELCOM 2014) clearly demonstrated the benefit from coupling environmental monitoring data with proxy data from pharmaceutical sales. However the pilot study underlines the importance of accessing high quality sales data for making relevant proxy evaluations. Improving the availability of this proxy data from pharmaceutical sales, would be a cost effective method to improve the knowledge base of the environmental status assessment of the Baltic Sea.

Current monitoring

Diclofenac is currently not included in the regular environmental monitoring of any HELCOM CP. The substance is considered an emerging issue, and as such it has been included in national screening programmes. Swedish EPA has carried out screening studies.

As diclofenac has been included on the first watch list under the EU list on priority substances (2013/39/EU), HELCOM CPs that are also EU Member States are to commence monitoring at selected representative monitoring stations in 2015. Monitoring shall be carried out at least at one monitoring station with more stations to be added according to guidance in the directive.

Description of data and up-dating

Metadata

The data is very variable. Most of the measurements are from WWTPs, while a few have been taken from rivers or estuaries or biota.

Arrangements for up-dating the indicator

The proposal is to settle on the up-dating procedures of the indicator once the monitoring efforts have become more established. The same applies to the operationalization of data-flow. Currently there is no HELCOM expert group dealing with pharmaceuticals, so the up-dating would likely need to be assigned to a specific CP or institute.

Publications and archive

(Archive)

- pdf:s of the currently published- and older versions of this indicator

Publications used in the indicator

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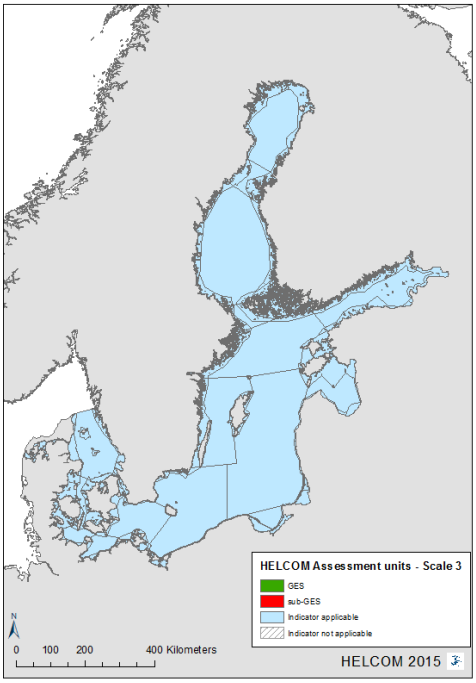
Additional relevant publications

Annex 2.

Exposure to and effects of estrogenic chemicals

Key message



No evaluation has been made yet as the concept is still under development. Monitoring data is available for some of the included estrogenic substances and effects have been measured in some areas of the Baltic Sea. Chemicals with estrogenic properties are widely used in society and, thus, spread to the environment. However, it is unclear if, or to what extent, they affect the ecosystem. A few chemicals with estrogenic properties are routinely monitored, but there are numerous chemicals with estrogenic properties that are not monitored. In many cases the estrogenic properties of a chemical may not be known. To deal with this, an indicator is suggested that includes environmental concentrations of known estrogens as well as biological responses of exposure to estrogens.

Relevance of the core indicator

The core indicator includes concentrations of known estrogen like chemicals as well as biological responses to estrogenic exposure. The indicator will, thus, take account of the most well-known estrogenic chemicals, as well as all other chemicals that cause estrogenic effects in the environment. The indicator is useful for determining if the environment is exposed to estrogen like chemicals.

Policy relevance of the core indicator

Endocrine disrupting chemicals have been on the political agenda for about 15 years. In 1999, the European Commission adopted the 'Community strategy for endocrine disruptors' (EC 1999). The strategy focuses on research and testing methods to allow better risk assessment of chemicals with endocrine disrupting properties. This has resulted in a priority list of known or suspected endocrine disruptors. However, endocrine disrupting properties are still not routinely evaluated in risk assessments, with the exception of plant protection products and biocides. This means that it is likely that endocrine disruptors are and will be released into the environment. Environmental monitoring that is focused on endocrine disruptors is necessary to avoid unpleasant surprises in the future.

The Moscow Ministerial Declaration 2010 gives HELCOM a clear obligation to further assess the environmentally negative impacts of pharmaceuticals and other substances that are not monitored regularly, with the aim as a first step to assess their occurrence and effects in the Baltic Sea.

	Primary importance	**Secondary importance**
BSAP Segment and Objective	Hazardous substances	Biodiversity
MSFD Descriptors and Criteria	Concentrations of contaminants give no effects	Biodiversity is maintained
WFD	Ecological status	Chemical status

Authors

Niklas Hanson

Department of Biological and Environmental Sciences

University of Gothenburg

Sweden

Cite this indicator

HELCOM [2015]. Estrogen indicator**.** HELCOM core indicator report. Online. [Date Viewed], [Web link].

Indicator concept

The core indicator includes concentrations of some of the most well-known environmental estrogens as well as biological indicators of estrogenic exposure. The inclusion of biological indicators is important to also cover other chemicals with estrogenic properties, known or unknown. For example, the European Commission has developed a priority list of 564 chemicals with known or suspected endocrine disrupting properties[1]. Only a handful of these chemicals are regularly monitored in the Baltic Sea.

The chemicals that are included in the indicator are ethinyl estradiol (EE2), 4-nonylphenol and its ethoxylates, 4-octylphenol, bisphenol A, and Bis(2-ethylhexyl) phthalate (DEHP). The biological responses that are included in the indicator are vitellogenin in blood plasma, sex ratio of eelpout fry, and occurrence of intersex. All indicators are measured in eelpout (*Zoarces viviparous*), with the exception of vitellogenin which can also be measured in perch (*Perca fluviatilis*).

Vitellogenin is one of the most well studied biological indicators for exposure to estrogenic chemicals. Vitellogenin is an egg yolk precursor protein expressed in the females of nearly all oviparous species. Normally, the concentration is very low in male blood plasma, but is increased in the presence of estrogenic chemicals. In environmental monitoring, vitellogenin in male fish has therefore been used to detect effects of estrogenic chemicals (e.g., Larsson et al 1999, Jobling et al 1998). Experiments have shown that vitellogenin in blood plasma responds before effects are seen on the population level (Palace et al 2009). Vitellogenin in male blood plasma can therefore be used as an early warning signal for effects of estrogens.

Exposure to endocrine disruptors can also affect sexual differentiation in fish. Female-biased sex ratios have been observed in natural populations close to sewage treatment effluents (Vadja et al 2008). This can be linked to the release of estrogenic chemicals from the sewage treatment plant, particularly synthetic estrogens (Larsson et al 1999). Eelpout is a suitable species for studying effects on sexual differentiation because it is viviparous, which means that the eggs hatch and the fry develop inside the female's body. Thereby, it is possible to determine the primary sex ratio by capturing eelpout during the period when the fry develop inside the female eelpout. This method has been used successfully to show male-biased broods in recipients for pulp-mill effluents (Larsson and Förlin 2002).

Occurrence of intersex is another biological indicator that has been shown to react to estrogens (e.g., Jobling et al 2006, Kidd et al 2007). Intersex describes a state where male fish develop female reproductive tissue. Experiments have shown that also intersex can act as an early warning signal that responds before effects are seen on the population level (Palace et al 2009).

Good Environmental Status

The concentrations of estrogenic chemicals in the marine environment along with estrogenic effects in marine organisms are used in this core indicator to evaluate whether an area reflects good environmental status (GES) or not. Both chemical and biological indicators are needed to form a comprehensive view on estrogenic exposure. The concept for defining good environmental status is that no estrogenic effects or concentrations of estrogenic substances that can cause such effects are detected. This means that the GES-boundaries for both the chemical and the biological measurements must be met.

For an area to be evaluated as having achieved GES, the summed concentration of estrogenic substances in Table 1 must be below the specified GES-boundary. Furthermore, at least one of the biological indicators needs to be analyzed and found to be below the specified GES-boundary. If two or three of the biological indicators are analyzed, they all have to be below the GES-boundary.

[1] http://ec.europa.eu/environment/chemicals/endocrine/strategy/substances_en.htm

GES-boundary for concentration of estrogenic-like compounds

The GES-boundary for the concentration of estrogenic-like compounds is achieved when the exposure to estrogenic chemicals is below 0.18 ng/l ethinyl estradiol equivalents (EEQ). The total exposure expressed as EEQ is calculated according to Equation 1, where C is the concentration of each compound (in ng/l) and P is the potency of each compound according to Table 1.

$$\boldsymbol{EEQ} = \sum \boldsymbol{C} \times \boldsymbol{P} \qquad \text{Equation 1.}$$

All the included estrogenic chemicals have, or can be assumed to have, similar modes of action (i.e. binding to the estrogen receptor). The potency of the compound differs, and in order for the total exposure estimate to be accurate, the differences in estrogenic potency are to be taken into account in accordance to Equation 1 and Table 1.

Table 1. Estrogenic potency of relevant environmental estrogens.

Estrogenic chemical	Potency	Reference
Ethinyl estradiol (EE2)	1	Gutendorf et al 2001
4-Nonylphenol	0,00001	Gutendorf et al 2001
4-Nonylphenol ethoxylates	0,000001	Metcalfe et al 2001
4-octylphenol	0,000066	Gutendorf et al 2001
Bisphenol A	0,00002	Gutendorf et al 2001
DEHP	0,0000008	Jin et al 2008

The acceptable exposure to estrogenic chemicals of EEQ <18ng/l is based on a species sensitivity distribution (SSD) for ethinyl estradiol (Caldwell et al 2008). From the SSD it was estimated that 95% of species are unaffected at a concentration of 0.35 ng/l. To account for uncertainties in the estimate, an assessment factor of two was added. This gives an acceptable environmental concentration of ethinyl estradiol of 0.18 ng/l. To account for the estrogenic activity of the other chemicals in Table 1, they are to be added according to Equation 1.

GES-boundary for biological effects

Vitellogenin and sex ratio have been monitored at Swedish reference sites since 2001 and 1997, respectively. This means that there is good data available to determine natural variation. The normal range can then be defined as the range that contains 95% of the results from reference sites. Because of the natural variability, there is always a risk that a value erroneously falls within (false negative) or outside (false positive) this normal range. GES-values for vitellogenin and sex ratio were set so that the risk for false positives and false negatives were equal when 25 fish are sampled.

For the concentration of vitellogenin in blood plasma, GES target values were set to 700 ng/ml for both species. Vitellogenin levels below these limits indicate that there is no estrogenic effect. The limit was based on the natural variation that has been observed at the reference sites Holmön, Kvädöfjärden and Torhamn during 14 years of Swedish environmental monitoring. All three sites are located far from large population centers and are unlikely to be exposed to estrogenic chemicals. The target value for GES was set so that the probability for false positives and false negatives were equal (5% and 7% for perch and eelpout, respectively).

For the sex ratio of eelpout fry, the GES-target value was set to 52,6% females. When the proportion of females is below this level, there is no reason to assume estrogenic effects. This target value was based on 18 years of environmental monitoring at the site Kvädöfjärden, which is located far from point sources. The target values for GES were set so that the probability for false positives and false negatives were equal (14%).

For intersex in male eelpout, data from reference sites is not available to determine GES in the same way as for vitellogenin. However, previous studies have shown no occurence of intersex at reference sites. At exposed sites, however, intersex prevalence of 20% or more has been observed (Gercken and Sordyl 2002,

Lyons et al 2004). The GES-limit should be set so that it does not point out unexposed sites as affected, and so that truly exposed sites are not missed. Based on the available data, GES was set to 5%. It is unlikely that truly unaffected sites will exceed this level. Furthermore, it is not likely that sites with less than 5% intersex are significantly exposed to estrogens.

Figure 1 shows a schematic decision framework for the estrogen indicator.

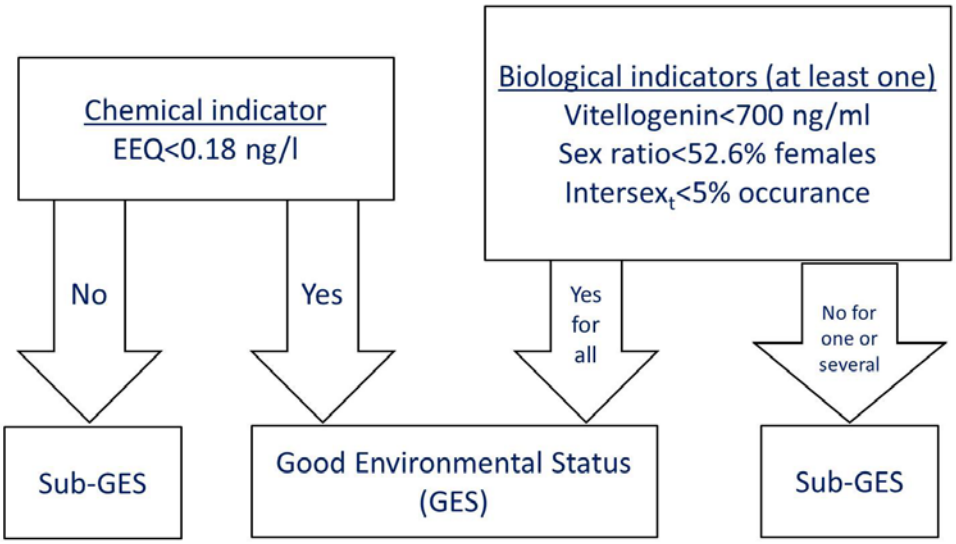


Figure 1. Decision framework to determine if good environmental status (GES) is achieved. All chemicals and at least one biological indicator needs to be included in the assessment.

Anthropogenic pressures linked to the indicator

Chemicals with estrogenic potential are found throughout society, e.g. in plastics and textiles. However, it is the synthetic estrogen ethinyl estradiol that has caused the greatest concern. This is because the estrogenic potency is comparable to that of natural estrogen, while the estrogenic chemicals in other consumer products have much lower potency. In the environment, the estrogenic impact reflects the sum of all estrogens. Many of the chemicals that are used in society end up at waste water treatment plants (WWTP). It is therefore not surprising that the most prominent estrogenic effects have been seen in recipients for WWTPs (e.g., Jobling et al 2006, Larsson et al 1999).

Assessment protocol

Due to the potential exposure to estrogenic chemicals throughout the Baltic Sea region, the indicator is considered relevant and applicable in the whole Baltic Sea. Because of the natural distribution of the fish species, all biological indicators are not possible to use everywhere. However, at least one of the two species is present at most coastal locations in the Baltic Sea. This means that at least one of the biological indicators is possible to use in each of the assessment units (HELCOM assessment unit Level 3 is considered the most appropriate assessment scale).

Chemicals must be measured in accordance with the directive on technical specifications for chemical analysis and monitoring of water status (2009/90/EC). Measurements should be performed in water and have sufficient limit of quantification (LOQ) to detect relevant levels of estrogens. The most crucial analysis is EE2, which due to its high potency requires a low LOQ. Required LOQ to detect a concentration that contributes approximately one sixth to the accepted estrogenicity (0.18 EEQ) are shown in Table 2. If these LOQs are used (or better), results below LOQ can with confidence be interpreted as no impact.

Table 2. Aproximate limit of quantification (LOQ) required for detecting a concentration that contributes to one sixth of the accepted estrogenicity. Note that LOQ for EE2 is presented as ng/l.

Estrogenic chemical	LOQ
Ethinyl estradiol (EE2)	0.03 ng/l
4-Nonylphenol	3 µg/l
4-Nonylphenol ethoxylates	30 µg/l
4-octylphenol	0.3 µg/l
Bisphenol A	1.5 µg/l
DEHP	30 µg/l

To accurately use the GES-values for the biological indicators, mean values from ten individuals should be used.

For the northern and the southern part of the Baltic Sea (divided by the Åland Sea), vitellogenin in perch is to be measured during the first and the last week of September (± one week), respectively. For eelpout, vitellogenin should be measured during the second week of November (± one week). After capture, the fish should be allowed to recover from stress caused by capture and handling for 3-5 days before sampling. Blood samples are centrifuged at site during sampling to separate blood plasma from the blood cells. The blood plasma is then frozen until analysis at the laboratory. The samples are analyzed using an enzyme-linked immunosorbent assay (ELISA) as described by Sturve et al (2005) and Parkkonen et al (1999).

The GES-values for sex ratio are based on monitoring results from eelpout during the second week of November ((± one week). Since sex dependent survival of fry cannot be excluded, it is advisable to determine sex ratio at this time of the year to allow use of the GES-value. The fry should be sex determined using to the method described by Larsson et al (2000).

Samples for determination of intersex should be performed in the spring/summer in accordance with Gercken and Sordyl (2002).

Relevance of the indicator

Policy relevance

The effect of estrogens in the ecosystem

An indicator for estrogenic exposure is highly relevant considering the current lack of knowledge regarding exposure to and effects of estrogenic chemicals in the ecosystem. Endocrine disrupting chemicals pose a potential risk to the environment and to human health. However, without proper indicators it is not possible to determine the level of risk. Just as it is important to identify high risks and guide management action, it is also important to show when the risk is low and no management action is needed. The core indicator can serve both purposes.

There is extensive evidence that shows a causal link between exposure to ethinyl estradiol and feminization of fish at environmentally relevant concentrations (up to a few ng/l in recipients to waste water treatment plants). For example ethinyl estradiol at sub ng/L levels has been shown to cause both vitellogenin induction (Purdom et al 1994, Thorpe et al 2003, Jobling et al 2003) and intersex/sex-change in fish (Örn et al, 2003; Parrot and Blunt, 2005). Long-term exposure of 5-6 ng/l has been shown to cause population collapse in fish (Kidd et al 2007). Furthermore, amphibians have been shown to be approximately as sensitive as fish to ethinyl estradiol. An exposure of 1.7 ng/l resulted in skewed sex ratios of adult frogs and malformations of their gonadal duct system (Pettersson and Berg 2007). Other estrogens included in the indicator are significantly less potent. However, the concentrations in high exposed areas may still be high enough to contribute to the total exposure of estrogens.

Results and confidence

No evaluation results for the indicator as a whole are currently available. Some descriptive results are described to support further development of the indicator concept.

Table 2. *Concentrations of ethinyl estradiol found in the Baltic region.*

Site	Ethinyl estradiol (ng/L)	Reference
Sweden, Vallentuna	<LOQ	Fick et al. 2011
Sweden, Uppsala	<LOQ	Fick et al. 2011
Germany, Inner Wismar Bay	20 (2004) 3 (2003)	Beck et al. 2006
Germany, Eggers Wiek	9 (2004) 2.5 (2003)	Beck et al. 2006
Germany, Outer Wismar Bay	2 (2004) <LOQ (2003)	Beck et al. 2006
Germany, Salzhaff	3 (2004) 1.5 (2003)	Beck et al. 2006
Germany, Darss Peninsula	1.5 (2004) 2.5 (2003)	Beck et al. 2006

Table 2. *Biological indicators for estrogen exposure in the Baltic region.* Data to be added.

Indicator	Site	Effect	Reference
Intersex in eelpout	Germany, Wismar	25%	Gercken and Sordyl 2002
Intersex in eelpout	Germany, Salzhaff	28%	Gercken and Sordyl 2002
Intersex in eelpout	Germany, Rostock	24%	Gercken and Sordyl 2002
Intersex in eelpout	Germany, Darss	0%	Gercken and Sordyl 2002
Sex-ratio in eelpout	Sweden, Kvädöfjärden	50.34%	Swedish program for integrated fish monitoring, 2013
Vitellogenin in eelpout	Sweden, Kvädöfjärden	267 ng/ml	Swedish program for integrated fish monitoring, 2014
Vitellogenin in perch	Sweden, Kvädöfjärden	93 ng/ml	Swedish program for integrated fish monitoring, 2014
Vitellogenin in perch	Sweden, Holmön	97 ng/ml	Swedish program for integrated fish monitoring, 2014
Vitellogenin in perch	Sweden, Torhamn	192 ng/ml	Swedish program for integrated fish monitoring, 2014

Confidence of indicator status evaluation

As no evaluation has yet been made it is not yet possible to evaluate the confidence. However, it should be noted that the availability of monitoring data for all the relevant parameters varies between regions in the Baltic Sea. Thus it is anticipated that the confidence of future evaluations may differ regionally.

Monitoring Requirements

Description of optimal monitoring

Because effects mainly can be expected in hot spots, especially close to waste water treatment plants, it is important to include such sites.

Current monitoring

The indicator is currently not used. However, the chemical analyzes are performed for other monitoring or screening purposes and two of the biological indicators (vitellogenin and sex ratio) are monitored at reference sites in Sweden.

Publications and archive

Publications used in the indicator

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Additional relevant publications