Manual for Marine Monitoring in the

COMBINE

Programme of HELCOM

Part D

Programme for monitoring of contaminants and their effects

Last updated: 31.03.2006

PART D. PROGRAMME FOR MONITORING OF CONTAMINANTS AND THEIR EFFECTS

D.1. OBJECTIVES AND GOALS FOR CONTAMINANTS MONITORING

More specifically the aims of COMBINE mean:

For contaminants:

To compare the level of contaminants in selected species of biota (including different parts of their tissues) from different geographical regions of the Baltic Sea in order to detect possible contamination patterns, including areas of special concern (or ´hot spots´).

To 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.

To 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.

Into the aims of this monitoring programme the assessment of quality of seafood with regard to the human consumption is not included. That is the responsibility of appropriate national authorities.

Measurements of contaminants in Sea Water are an important tool to detect trends in space and time in a nonbiotic matrix. The data provide a basis to understand the bioaccumulation pattern of contaminants and to establish mass balances or contaminants.

The contaminant monitoring programme can never achieve the full extent of the geographical resolution so the all parts of the Baltic environment is covered. This mainly because the cost of the analytical programme but also because of zoogeographical reasons. Still there is a need to cover the main subregions with comparable results but because of above mentioned reasons the sampling network has to be sparsely distributed. The core variables within the contaminant programme are thus variables that are studied over the entire area and provide the best available comparable information on time trends as well as spatial distribution.

D.2. SELECTED COMPARTMENTS

D.2.1. BIOLOGICAL COMPARTMENTS

The following criteria are *taken into account for* the selection of monitoring species:

* the species should *be of reasonable size to* allow analyses of individuals
* the species *collected for core studies* should have broad distribution in the Baltic Sea
* the species should represent the sampling site *or defined areas*
* biological effects studies should be possible
* good knowledge of the ecology, physiology etc. should be available
* the species should have good accessibility.

Selected species: Open Sea

Herring (*Clupea harengus)*
Cod (*Gadus morhua)*
Guillemot (*Uria algae)*

Selected species: Coastal zone, to be decided

Blue mussel (*Mytilus edulis)*
Bladder wrack (*Fucus vesiculosus)*
Macoma balthica
Saduria entomon
Flounder (*Platichtys flesus)*
Perch (*Perca fluviatilis)*
Viviparous blenny (*Zoarces viviparus)*
Common tern (*Sterna hirundo)*
Grey Seal (*Halichoerus grypus)*
Ringed seal (*Pusa hispida)*
Common seal (*Phoca vitulina)*
White tailed sea eagle (*Haliaetus albicilla)*

Table D.1 shows an overview of sampling requirements for contaminants.

Selected tissues

All details concerning tissue selection with regard to the various analytes are presented in Table D.2. For the analysis of lipid-soluble compounds the concentration shall be reported both on lipid weight basis as well as fresh tissue basis. For metals concentrations shall be reported both on fresh weight basis and dry weight basis.

- Pooled samples of the growth of the year of algae are analysed for contaminants.
- In invertebrates pooled homogenised samples of soft tissues are analysed.
- In fish chemical analysis on individuals are carried out on muscle and liver tissues.
- Bird eggs are analysed on an individual basis as homogenised egg content.

Table D.1. Overview of sampling requirements for contaminants

Species (n)	Location	Depth (m)	Time	Age, year (N or S)	Size cm	Sex
Herring (12-15)	open sea	n.s.	Aug - Sept	N/2+,3+	n.s.	female
Cod (12-15)	open sea	n.s.	Aug - Sept	S/1+,2+	24-35	female
Macoma baltica 80 g	open sea	n.s.	Sept	n.s.	>0.5	n.s.
Saduria ent. 80	open	n.s.	Oct -	n.s.	4-6	n.s.

Species (n)	Location	Depth (m)	Time	Age, year (N or S)	Size cm	Sex
g	sea		Nov			(***)
Uria aalge (10) eggs	islands		1-15 May	n.s.	n.s.	n.s.
Flounder (10-15)	coast (**)	< 20 m	Aug - Sept	2+	n.s.	female
Viviparous blenny (10-15)	coast (**)	< 20 m	Nov - Dec	n.s.	17-30	males (*)
Perch (10-15)	coast	n.s.	Aug - Sept	n.s.	15-20	females
Sterna hir. (10) eggs	coast		June - July		n.s.	n.s.
Mytilus 80 g	coast	N/2-5 S/<15	Oct - Nov	n.s.	3-4	n.s.

* Males collected for chemical analysis before spawning (Aug), females collected in October for studies on reproductive outcome and fry development
** Samples shall be collected away from river mouth
*** Egg-carrying females avoided

S Southern Baltic
N Northern Baltic
n.s. not specified

D.2.2. SEA WATER

Contaminants occur in the dissolved phase as well as associated with suspended particulate matter. Since both fractions are taken up by biota in a specific manner, it is recommended to analyse dissolved and particulate contaminants separately. Surface sea water should be analysed since it is directly affected by input of contaminants via rivers and atmospheric deposition.

D.2.3. SAMPLING SITES

Sampling sites for fish, biota, bird eggs and water for contaminant analysis are shown in Figures D.1, D.2 and D.3 and Tables D.2, D.3 and D.4. In Figures D.1 and D.2 selected species at the various sampling sites are shown and in Table D.2 responsible countries are indicated. For the coastal areas a selection of sites covering hot spots as well as reference areas needs to be elaborated. The reference area system should be internationalized as far as possible, and be available for comparison for all Contracting Parties. The establishment of Baltic Sea Protected Areas (BSPA) implies a need for monitoring in these areas and they should be considered in the Coastal Monitoring Programme. Thus for the coastal areas the programmes are still under development. However, in some countries there are already programmes running.

TABLE D.2.Information on variables and matrices committed to be measured in the contaminants programme by the Contracting Parties (Danish monitoring programme included in the coastal programme)

[OPEN SEA]

Species	Matrix	Variable	DK	EE	FI	DE	LV	LT	PL	RU	SE
Core programme											
Herring	liver	Cu, Cd, Pb, Zn		+	+	+		+	+		+
	muscle	Hg;		+	+	+		+	+		+
		DDTs;		+	+	+		+	+		+
		CBs (IUPAC Nos.28, 52, 101,118,138, 153 and 180);		+	+	+		+	+		+
		HCB; alpha + gamma HCH		+	+	+		+	+		+
Main programme											
Cod	liver	Cu, Cd, Pb, Zn;		+	+	+		+	+		+
		DDTs;		+	+	+		+	+		+
		CBs (IUPAC Nos.28, 52, 101,118,138, 153 and 180);		+	+	+		+	+		+
		HCB; alpha + gamma HCH				+		+			
	muscle	Hg		+	+	+		+	+		+
Macoma baltica	homogenized soft tissue	Hg, Pb, Cd, Cu;		+				+			
		DDTs;		+				+			
		CBs(IUPAC Nos. 28, 52, 101, 118, 138, 153 and 180);		+			+(metals)	+			
		alpha + gamma HCH		+				+			

Species	Matrix	Variable	DK	EE	FI	DE	LV	LT	PL	RU	SE
Saduria entomon	homogenized whole organism	Hg, Pb, Cd, Cu;		+							
Guillemot	egg content	Hg, Pb, Cd, Cu;									+
		DDTs;									+
		CBs(IUPAC Nos. 28, 52, 101, 118, 138, 153 and 180);									+
		HCB; alpha + gamma HCH									+
Sea water	dissolved phase	Cu, Cd, Pb, Zn				+		+			
	particulate matter	Cu, Cd, Pb, Zn				+					
	total water	Hg, DDTs;				+		(+)			
		CBs(IUPAC Nos.28, 52, 101, 118, 138, 153 and 180); HCB; alpha-,beta-, gamma-HCH, PAHs				+		(+)			
Supporting programme											
Herring	different age classes	Hg, Pb, Cd, Cu;			+						
		DDTs;			+						
		CBs (IUPAC Nos. 28, 52, 101, 118, 138, 153 and 180);			+						
		HCB; alpha + gamma HCH;			+						
Blue mussel	homogenized soft tissue	DDT, DDE, DDD, CBs, HCH		+							
Sea water		tot. oil hydrocarbons (fluorom.)		+	+	+	+	+			
Herring;		brominated flame									+

Species	Matrix	Variable	DK	EE	FI	DE	LV	LT	PL	RU	SE
guillemot eggs		retardants									

[COASTAL ZONE] [will be updated according to the information from the CMP]

Species	Matrix	Variable	DK	EE	FI	DE	LV	LT	PL	RU	SE
Core progrramme											
Mytilus	homogenized soft tissue	Hg, Zn, Cu, Cd, Pb;	+		+	+		+	+		+
	homogenized soft tissue	DDTs;	+	(+)	+	+		+	+		+
		CBs(IUPAC Nos.28, 52, 101, 118, 138, 153 and 180)	+	(+)	+	+		+	+		+
		HCB; alpha +gamma HCH;	+	(+)	+	+		+	+		+
Viviparous blenny	liver	Cu, Cd, Pb, Zn	+			+	+		+		+
	muscle	Hg;	+			+	+		+		+
		DDTs;	+			+			+		+
		CBs(IUPAC Nos.28, 52, 101, 118, 138, 153 and 180);	+			+			+		+
		HCB; alpha + gamma HCH;	+			+			+		+
Perch	liver	Cu, Cd, Pb, Zn				+	+		+		+
	muscle	Hg;			+	+	+		+		+
		DDTs;			+	+			+		+
		CBs(IUPAC Nos.28, 52,			+	+			+		+

Species	Matrix	Variable	DK	EE	FI	DE	LV	LT	PL	RU	SE
		101,118, 138, 153 and 180);									
		HCB; alpha + gamma HCH;			+	+			+		+
Main programme											
Flounder	muscle	Hg	+					+			
	liver	Zn, Pb, Cd, Cu	+					+			
Supporting programme											
Seals	blubber	Hg, Pb, Cd, Cu;			+						+
		DDTs;			+						+
		CBs(IUPAC Nos. 28, 52, 101, 118, 138, 153 and 180);			+						+
		HCB; alpha + gamma HCH;			+						+
		biomarker studies on			+						+
		population			+						+
Mytilus	homogenized soft tissue	TBT	+								
	homogenized soft tissue	PAH	+	+							
Fucus	growth of the year	Cu, Cd, Hg, Pb, Zn		+							
Macoma	homogenized soft tissue	total oil hydrocarbons		+							
Mytilus		(fluorom.)		+							

[COASTAL ZONE] [will be updated according to the information from the CMP]

Biological effect monitoring for supporting programme

Species	Variable	DK	EE	FI	DE	LV	LT	PL	RU	SE
Perch	physiology, population parameters, reproduction, biomarkers			+	+			(+)		+
Viviparous blenny	physiology, biomarkers							+		+
	reproduction, population parameters							+		+
Fish community	population parameters			+				+		+
Seals	population dynamics, reproduction			+				+		+
White-tailed eagle	population dynamics, reproduction			+						+
Whelk Buccinum	imposex	+								

TABLE D.3. Areas for collecting biota for contaminant analysis

Species	Stat. Rect.
Herring	60/H2
	57/H2
	51/H0
	50/G8
	49/H6
	48/H6
	48/H4
	48/H3
	45/H3
	46/G7
	39/G8
	38/G6

Species	Stat. Rect.
	40/G5
	38/G4
	43/G1
Cod	57/H2
	51/H0
	49/H6
	48/H6
	48/H4
	48/H3
	45/H3
	42/G8
	39/G8
	38/G6
	38/G4
	43/G1
Viviparous blenny	57/H1
	43/H3
	44/G7
	38/G8
	38/G2
	40/G2
Perch	57/H1
	43/H3
	44/G7
Flounder	38/G2
	40/G2
	39/G0

Species	Stat. Rect.
Mytilus edulis	48/H4
	45/H4
	43/G1
Macoma baltica	43/H3
	48/H4
	45/H4
Saduria entomon	48/H4
Guillemot eggs	43/G7

TABLE D.4. Stations for collecting sea water for contaminant analysis

(At the stations with BMP designation, samples are taken for depth profiles, at the stations without BMP designation, only two depths above the halocline are sampled.)

Heavy metals			
BMP designation	**National designation**	**Latitude**	**Longitude**
	225005	54E42,90'	10E08,00'
N3	225006	54E36,00'	10E27,00'
	225059	54E27,55'	10E14,70'
	225057	54E06,10'	11E10,50'
M2	225058 / O12	54E18,90'	11E33,00'
M1	46	54E28,00'	12E13,00'
K8	30	54E43,40'	12E47,00'
K7	69	55E00,00'	13E18,00'
K5	113	54E55,50'	13E30,00'
K4	109	55E00,00'	14E05,00'

K2	213	55E15,00'	15E59,00'
	222	55E13,00'	17E04,00'
	256	55E19,60'	18E15,10'
K1	259	55E33,00'	18E24,00'
	253	55E50,40'	18E52,00'
	250	56 05,00'	19E10,00'
	263	56E20,80'	19E22,70'
	260	56E38,00'	19E35,00'
	272	57E04,30'	19E49,80'
J1	271	57E19,20'	20E03,00'
	WB3	53E57,00'	11E24,50'
	UW4	54E10,00'	12E06,00'
	KHM	53E49,50'	14E06,00'
	OB4	54E00,40'	14E14,00'

Organic contaminants			
BMP designation	**National designation**	**Latitude**	**Longitude**
	701	54E50,00'	09E30,00'
	704	54E50,00'	09E54,00'
N3	706	54E36,10'	10E27,00'
	708	54E33,00'	10E12,00'
	710	54E25,10'	10E13,30'
	715	54E03,00'	10E50,90'
	FB	54E36,00'	11E09,00'
N1	717	54E30,50'	11E25,00'
M2	718 / O12	54E18,90'	11E33,00'
M1	719 / O46	54E29,10'	12E17,00'
K8	30	54E43,40'	12E47,00'

K7	69	55E00,00'	13E18,00'
K5	113	54E55,50'	13E30,00'
K4	109	55E00,00'	14E05,00'
K2	213	55E15,00'	15E59,00'
	222	55E13,00'	17E04,00
	256	55E19,60'	18E15,10'
K1	259	55E33,00'	18E24,00'
	253	55E50,40'	18E52,00'
	250	56E05,00'	19E10,00'
	263	56E20,80'	19E22,70'
	260	56E38,00'	19E35,00'
	272	57E04,30'	19E49,80'
J1	271	57E19,20'	20E03,00'

D.3. SELECTED CONTAMINANTS

D.3.1. OPEN SEA

Core variables, herring:

- mercury, copper, cadmium, lead, *zinc*
- DDT and metabolites,
- CBs (Nos. 28, 52, 101, 118, 138, 153, and 180),
- hexachlorobenzene (HCB), and
- alpha- and gamma-hexachlorocyclohaxane (HCH)

The core programme for contaminants is given in Table D.2.

Main variables, cod, guillemot eggs:

- mercury, copper, cadmium, lead, zinc
- DDT and metabolites,

- CBs (Nos. 28, 52, 101, 118, 138, 153, and 180),
- hexachlorobenzene (HCB), and
- alpha- and gamma-hexachlorocyclohaxane (HCH)

Main variables, sea water:

Concentration in suspended particulate matter

- copper, cadmium, lead, zinc

Figures not avaiable yet.

FIGURE D.1. Sampling sites for fish

FIGURE D.2. Sampling sites for biota

FIGURE D.3. Sampling sites for water

Concentration in the dissolved phase

- copper, cadmium, lead, zinc

Total concentration

- mercury
- DDT and metabolites,
- CBs (Nos. 28, 52, 101, 118, 138, 153, and 180),
- hexachlorobenzene (HCB), and
- PAH
- alpha-, beta-, and gamma-hexachlorocyclohaxane (HCH)

D.3.2. COASTAL ZONE (EC MON 1/96, ANNEX 7)

This programme is still under development and the recommendations here are only tentative.

Contaminants in Mytilus, Macoma, perch viviparous blenny, eggs of common tern, guillemot and seal tissue are:

- mercury, copper, cadmium, lead, zinc,
- DDT and its metabolites,
- CBs (Nos. 28, 52, 101, 118, 138, 153, and 180),

- hexachlorobenzene (HCB),
- alpha- and gamma-hexachlorocyclohexane (HCH)

Contaminants in flounder, Danish coastal programme

- mercury, copper, cadmium, lead, zinc,
- DDT and its metabolites,
- CBs (Nos. 28, 52, 101, 118, 138, 153, and 180),
- hexachlorobenzene (HCB),
- alpha- and gamma-hexachlorocyclohexane (HCH)

The compilation of the contaminants to be analysed in the BMP and in the CMP is contained in Table D.2.

D.4. SUPPORTING STUDIES:

- determination of contaminants in herring of different age classes to follow the accumulation
- brominated flame retardants in selected species
- studies aiming to give information on effects of contaminants on Baltic top predators
- toxaphene in selected species of fish and invertebrates, guillemot eggs, seal tissues. Toxaphene is transported via the atmosphere and has a well-documented toxicity
- dioxins and furans are very toxic and among the most serious risk substances in the Baltic. Monitoring is, however, very expensive and there are few laboratories carrying out these determinations on a routine basis. The national time series already started need to be continued, as this is the only information available on these substances
- planar CBs are recommended by ICES not to be included in the monitoring programmes, but should be the subject of research programmes
- baseline study of TBT in biota, sediment and water
- determination of petroleum hydrocarbons (UVF) in sea water as well as biota.

D.5. SELECTION AND NUMBER OF SPECIMENS

Detailed information on sample size, age and size of collected specimens, sex and sampling time is given in Table D.1. At the analysis of fish samples females shall be used. Exception to this is viviparous blenny where males are used for chemical analysis and females for determination of biomarker parameters.

Experience from the BMP shows that individual data in long-term monitoring increase the possibilities of detecting both temporal and spatial variations. This explains the criteria that have been taken into account for the selection of species. In addition analysis of individual specimens will allow studies of the

relationships between different contaminants. This will also provide the possibility of calculating ratios between contaminants and allow correlations to be made with biological variables such as length, body condition, etc.

HERRING

Core variables for contaminant monitoring in biota of the Baltic Sea is herring. A narrow definition of sampling population is required. Young herring would more likely represent the sampling area compared to old ones showing a migration behaviour. Since individual analysis shall be performed, herring large enough to allow analysis of organic contaminants instead of individual fish should be selected. In practise, 1+-3+ year (north-south) old herring should be selected, depending on the geographical location of the sampling site. Sampling of spring spawning herring should be carried out in autumn to avoid changes of the physiology during spawning. The maturity of the gonads should be recorded and the same degree of maturity should ideally be sampled every year. The number of specimens could be reduced from the present recommendation (20) to at least 12 specimen. However, when new sampling localities are established where the within-year variation should be investigated 20 specimens are recommended. Depending on the rather low within-year variation, this would in general not cause any significant loss in the reliability of the average values. In order to reduce the influence of small-scale spatial and temporal variations on the inter-annual variation, it might be beneficial to collect samples several times during the sampling .

FLOUNDER

At studies of flounder, age is a selection parameter. For flounder analysed specimens shall be 2+ years. 10-15 specimens shall be collected at each sampling site.

COD

Cod can only be found on an regular basis southern and central Baltic Proper. Thus sampling and analysis for monitoring purposes shall only be carried out in these areas. For cod length-stratified sampling may be maintained where it has been successfully applied in the past, and then 25+/- 10% specimens shall be analysed. For new time series, however , it may be more appropriate to sample and analyse individually at least 12 fish of a limited size range (24-35 cm) from each sampling site in order to minimize natural variability within the sample.

PERCH, VIVIPAROUS BLENNY

To obtain samples from homogenous populations, a short length interval shall be used at the collection of these two species. For perch the length interval is 15-20 cm and for blenny 17-30 cm. 10-15 specimens shall be collected at each sampling site.

INVERTEBRATES

Because of the variation in salinity within the Baltic no general advice can be given as to size of the invertebrates. However, in Kattegat 20 specimens of Mytilus at a size of 3-6 centimetres shall be used. This

imply a possibility to compare obtained data with data recorded within OSPARCOM (OSPARCOM, 1996). For the rest of the study area it is important to keep to the same size at the specific sampling site between years and most probably the number of specimens in the pools has to be increased in certain areas to obtain a sample big enough to allow chemical analysis. For all invertebrates a pooled sample of 80 g is needed to allow the chemical analysis.

BIRD EGGS

Guillemot eggs shall be collected in the early part of the reproduction period to avoid analysis of replacement eggs. This will minimize the within year variation in contaminant concentrations of the collected samples. Ten eggs shall be collected per sampling site.

Below are listed the biological measurements that shall be taken on the various matrices.

- Age: fish
- Total weight: fish, mytilus, bird eggs
- Total length: fish, Mytilus. Macoma, bird eggs
- Total width: bird eggs
- Liver weight: fish
- Gonad maturity: fish
- Sex: fish

D.6. FIELD SAMPLING AND STORAGE (FOR MUSSELS ALSO SAMPLE PREPARATION FOR ANALYSIS)

Fish samples can be dissected for chemical analysis immediately after they have been caught. However, of practical reasons this can often be difficult and the laboratory conditions at the locality of collection might imply a risk for contamination during the preparation or available personal are untrained or lack sufficient practical experience. It is then more convenient to deep freeze the fish specimens before transport to a laboratory with adequate conditions. If the samples are deep frozen before transport, the outstretched specimens shall be put individually in polyethylene plastic bags and the bags labelled individually. The samples shall be kept frozen during the transport. If the specimens are prepared for chemical analysis in field recommendations given in Part D.7 have to be followed.

For bivalves, it is best to carry out the initial postsampling procedures on board the vessel to avoid a two-step procedure of freezing and re-freezing (which causes variable water losses). Thus, it is recommended that a person skilled in these procedures collect the bivalves and carry out the initial procedures as soon as possible thereafter.

When the organisms have been collected, they should be rinsed externally in clean water from the area of collection to wash away sediments and other foreign matter. They should then be allowed to remain in

clean sea water from the area of collection for 12-24 hours to allow them to remove sediments and other foreign matter as pseudofaeces. The specimens should be kept alive at a temperature similar to that observed at the sampling site (preferably in a refrigerator). The storage tank should preferably be of glass.

When this time is over, the total length of each organism should be measured and the information recorded.

After draining off the shell liquor, the whole soft body of the organism including the adductor muscle should be carefully removed from the shell and combined with the others to be included in the sample. Care should be taken to avoid excessive tissue damage and thus cause water loss during this procedure.

Eggs of birds shall be transported to the laboratory for preparation immediately for further preparation. Egg measurements (weight, length and width) shall be done before opening of the egg with drilling a hole at the equatore. The egg content shall be blown out with a glass pipette (the egg shall not contain an embryo) and brought to the freezer, where it shall be kept until final preparation for chemical analysis. The empty eggshell shall be stored and dried in room temperature for future measurements of eggshell parameters. Homogenized egg content shall be analysed.

Storage of specimens or individual tissue samples as well as pooled samples

Material of single specimens shall be stored in polyethylene plastic bags.

Material from single tissue samples or pooled tissue samples shall be stored in the following way:

- Samples for trace element analyses can be stored in precleaned polyethylene, polypropylene, polystyrene or glass containers.
- Samples for analysis of organic contaminants should be stored in precleaned glass containers.

Tissues can deteriorate in a rather short time span at room temperature. Consequently, samples should be frozen as soon as possible after packaging. They can be frozen rapidly by immersion in liquid nitrogen or blast freezing, but both these techniques need care. Whatever system is used, freezing a large bulk of closely packed material must be avoided. The samples in the centre will take longer to cool and will therefore deteriorate more than those in the outer layer.

Once frozen, samples can be stored in a deep freezer at temperatures of -20 ^{o}C or below. The laboratories should validate their storage procedures. Each sample should be carefully labelled. The label should contain at least the sample's identification number, the type of tissue, and the date and location of sampling.

Mussel samples shall always be prepared for chemical analysis without freezing. After that the soft tissue samples can be frozen.

D.7. SAMPLE PREPARATION FOR CHEMICAL ANALYSIS

GENERAL REMARKS

Tissue samples have to dissected while they are in good condition. Uncontrollable losses of determinands or cross-contamination from other deteriorating tissues and organs may occur, because biological tissue deteriorates. To avoid this, individual fish specimens must be dissected at sea if adequate conditions prevail on board, or be frozen immediately after the collection and transported frozen to the laboratory where they are dissected later. The dissection room should be kept clean and the air should be free from particles. If clean benches are not available on board, the dissection of fish should be carried out in the land-based laboratory under clean room conditions. If the option chosen is dissection on board the ship, two criteria must be met:

1. the work must be carried out by personnel capable of identifying and removing the desired organs according to the requirements of the investigations, and
2. there must be no risk of contamination from working surfaces or other equipment.

EQUIPMENT AT PREPARATION

Crushed pieces of glass or quartz knives, and scalpels made of stainless steel or titanium are suitable dissection instruments.

Colourless polyethylene tweezers are recommended as tools for holding tissues during the dissection of biological tissue for trace metal analysis. Stainless steel tweezers are recommended if biological tissue is dissected for analysis of organic contaminants.

After each sample has been prepared, including the samples of different organs form the same individual, the tools should be changed and cleaned.

The following procedure is recommended:

a) for analysis of inorganic contaminants:
1) Wash in acetone or alcohol and high purity water.
2) Wash in HNO_3 (p.a.) diluted (1+1) with high purity water. Tweezers and haemostats in diluted (1+6) acid.
3) Rinse with high purity water.
b) for analysis of organic contaminants:
1) Wash in acetone or alcohol and high purity water.

The glass plate used during dissection should be cleaned in the same manner. The tools must be kept dust-free between working hours.

TREATMENT OF THE SPECIMENS

Before any tissue preparation start, the individual specimens shall be weighted and the total length shall be determined (outstretched specimens)

MUSCLE TISSUE PREPARATION

For analysis of fish muscle, the epidermis and subcutaneous tissue should be carefully removed from the fish. Samples should be taken under the red muscle layer. In order to ensure uniformity of samples, the right side dorso-lateral muscle should be taken as the sample. If possible, the entire right dorsal lateral filet should be used as a uniform sample, from which subsamples can be taken after homogenizing for replicate dry weight and contaminant determinations. If the fish is small both entire filets shall be used. If, however, the amount of material so obtained would be too large a sample, a specific portion of the dorsal musculature should be chosen for the sample. It is recommended that the portion of the muscle lying directly under the first dorsal fin be utilized in this case. As both fat and water content vary significantly in the muscle tissue from the anterior to the caudal muscle of the fish, it is important to obtain the same portion of the muscle tissue for each sample. This is necessary in order to ensure comparability (Oehlenschläger, 1994).

Muscle tissue samples collected from frozen fish specimens shall always be prepared from samples that are half frozen. Preparation of tawned tissue will imply a risk of body liquid losses and shall be avoided as much as possible. Determination of sex and preparation of liver tissue shall also, as much as possible, be done when the fish specimen is only half tawned.

LIVER TISSUE PREPARATION

To sample liver tissue, the liver must be identified in the presence of other organs such as the digestive system or gonads. The appearance of the gonads will vary according to the sex and the season and the status shall be recorded (sex, maturity). After opening the body cavity with a scalpel, the connective tissue around the liver should be cut away and as much as possible of the liver is cut out in a single piece together with the gall bladder. The bile duct is then carefully clamped and the gall bladder dissected away from the liver.

Either the fish have been frozen before preparation (and the preparation is done on half frozen tissue) or sampling preparation of liver is done on unfrozen fish samples, the entire liver shall be weighted and after that brought to the freezer before further preparation for analysis. This is particularly important at preparation of the fatty cod liver. Tawned cod liver tissue will loose fat at the preparation because of squeezing. Any loss of body liquid or fat at the preparation before analysis will make the determination of fat and dry weight incorrect.

BIVALVES AND BIRD EGG

Preparation procedure presented under the Chapter "Field sampling and storage (for mussels also sample preparation for analysis)".

REFERENCE

Oehlenschläger, J. 1994. Quality assurance during sampling onboard. *In* ICES/HELCOM Workshop on Quality Assurance of Chemical Analytical Procedures for the Baltic Monitoring Programme. Ed. By G. Topping and U. Harms. Balt. Sea Environ. Proc. No. 58: 82-84.

D.8. STATISTICAL COMMENTS

Based on an assessment carried out by the Statistics and Data Treatment group of EC BETA the detection of a 5% annual change over a time period of 15-20 years is required in temporal trend monitoring programmes. This stress the need of long term studies when changes are not expected to be dramatic. The existing BMP monitoring has a rather long history, and the time period already covered by this series provides an excellent platform for future trend studies.

The Statistics and Data Treatment group of EC BETA recommend that the annual samples at individual sites can be reduced from the present 20 - 25 specimens to 10-15. The OSPARCOM ad hoc Working Group on Monitoring (MON) recommend at least 12 specimens per site and year. This is why 12 specimens at each sampling site and year have been recommended for the open sea programme. The advice by ICES (1995) is that based on the fact that calculations have been conducted on only a very limited data set and that there is a need for better estimates of the variance components associated with time series data, no definitive advice on optimal sample size for temporal trend monitoring programmes can be provided at the present time.

A power analysis was conducted by German scientists on basis of the German time series for Cd and Cu in surface sea water of the Baltic proper. It could be shown that 10-20 samples taken at a fixed season are sufficient to detect a 10% trend within 10 years with a power of >0.9.

D.9. SPATIAL DISTRIBUTION STUDIES

New studies are under way to develop statistical aspects of geographical distributions of contaminants. Information on statistical aspects of spatial distribution will be included at a later stage when available. The present programme of the core variable herring will provide us a possibility to make a spatial distribution study of the contaminants investigated in the Baltic area. In a similar way the main variables cod and blue mussel will give us a possibility to compare the contamination burden as well as a comparison of the ecological risk for the species of concern in the North Sea area and the Baltic.

D.10. ESTABLISHING OF NATIONAL SPECIMEN BANKING PROGRAMMES

There is often need for material from periods in the past to assist retrospective studies. A recognition of "new" contaminants and the ability to carry out retrospective studies to rapidly obtain information on trends has to be stressed. Also, there is often a need of re-analyse earlier collected samples in long time series to confirm the reliability of previously performed analyses. The establishment of specimen banking programmes provides the possibility of meeting these two needs.

D.11. BIOLOGICAL EFFECTS MONITORING

More specifically the aims of COMBINE mean:

FOR THE EFFECTS OF CONTAMINANTS:

"To carry out biological effects measurements at selected locations in the Baltic Sea, particularly at sites of special concern, in order to assess whether the levels of contaminants in sea water and/or suspended particulate matter and/or sediments and/or in the organisms themselves are causing detrimental effects on biota (e.g., changes in community structure)."

The objectives of the biological effect monitoring programme is to study the relationships between concentrations and effects.

In the Baltic Sea area, studies related to the effects of contaminants in biota have often been performed as monitoring of changes at population or community level. However, to reach an understanding of the actual causes of these changes, knowledge on health parameters is essential. Although in an operative use in various sea areas, the effect studies have been sporadic in the Baltic Area.

Biological effects monitoring should integrate measurements from the level of effects of contaminant concentrations at the tissue level up to effects at the population level. It shall also cover different levels in the food web as well as different time scales in manifestations of the effects of exposure (acute and chronic responses). All studies should include simultaneous measurements of the levels of relevant contaminants in the study organism and relevant environmental matrix. The species chosen so far for the chemical analysis programme have, to a large extent, been selected on the basis of experiences from pilot studies.

RECOMMENDED STUDIES

With regard to biological effects monitoring and being aware of the existing projects among the Contracting Parties to the Helsinki Convention, ICES was invited to advise on methods for determining effects primarily on reproduction, immunology and metabolism of marine organisms. In addition, the recommendations of OSPARCOM on the parameters used should be taken into considerations to harmonize the programmes and to make use of the expertise relevant for Baltic species and the Baltic environment. It is important that monitoring data, whether they are contaminant or effect data, are produced with high enough quality. QA is an important requirement to ensure a consistently high data quality.

METHODS

Since the applicability of several of the contamination-related biomarkers in current use (e.g. EROD induction, histopathology) has not been adequately investigated in most Baltic Sea organisms that are potentially useful as monitoring species, studies providing information on this are of high value.

AchE (acetylcholinesterase) inhibitors such as organophosphate and carbamate pesticides may be substances of concern in some highly contaminated areas of marine environment (as pointed out by ICES). At present, however, the importance of these pesticides as marine pollutants is not known. Studies focusing of the applicability of AchE activity inhibition in different group of organisms should be encouraged.

In addition, the development of chronic sediment and water bioassays are considered useful for studies in heavily contaminated areas.

ORGANISMS

Bivalves (*Macoma balthica*, *Mytilus edulis*) are regarded as the most useful indicators of the degree of regional contamination and in this view recommendable for the studies of biological effects.

Coastal fish (e.g. *Perca fluviatilis*, *Zoarces viviparus*) are good candidates for biological effects monitoring. Physiological monitoring has been carried out since 1989 on national level.

Top predators (e.g. seabirds, seals) are very sensitive indicators of contaminant influence. Continuation of national studies on pathology and population size of the three Baltic seal species is considered important.

D.12. GENERAL STRATEGY FOR THE STUDY OF NEW CONTAMINANTS

A general strategy for new contaminants should include the conduct of a baseline study in which biota or other media should be sampled from both reference sites and expected hot spots, providing a first indication of the maximum range of spatial variation within the Baltic Sea area.

D.13. SAMPLING PROGRAMME AS COMMITTED BY THE CONTRACTING PARTIES

The sampling programme as committed by the Contracting Parties is summarized in Table D.2.

DENMARK

The Danish monitoring programme is under revision. The sampling strategy of the new suggested preliminary programme (not yet approved by the appropriate authorities) is based on five different groups of harmful substances taking into account the known sources. These five groups of substances are:

1) Persistent, toxic, bioaccumulating substances (half life of 2-10 years)

- hexachlorobenzene (HCB), DDT, hexachlorocyclohexane (HCH) (i.a. chlordane, dieldrin), PCBs,
- heavy metals Hg, Cd, Pb, Cu and Zn,
- PAH and organotin

2) Substances via waste water

- p-nonylphenols (+ethoxylates), phthalates (DEHP), Linear Alkyl Sulphonates (LAS) (detergents), PAH, certain metals (Be, Li, Ag, Sb, Tl) and organotin compounds

3) Substances from different diffuse sources, mainly agriculture

- pesticides (atrazine, simazine), organotin compounds

4) Anti-fouling agents

- Tributyltin, Cu-compounds, Irgarol

5) New problem substances (other sources)

- brominated flame retardants, tris(4-chlorophenyl)methanol and tris(4-chloro-phenyl)methane, planar CBs, toxaphene

Group 1 substances will be analysed in biota from 5-7 sampling sites once per year. Only coastal areas will be monitored. Biological effects monitoring (imposex in whelk, Buccinum) from about 5-7 stations once per year. For substances in group 2-5, preliminary surveys of existing concentrations will be made before any new monitoring programme is started covering these substances.

ESTONIA

The Estonian programme includes:

- monitoring of fish (herring and if available cod): (Cu, Cd, Pb, Hg, Zn) and organochlorines (DDTs, PCB,"-,$- and (-HCH) once per year at 3 locations
- monitoring of invertebrate and algae (*Macoma*, *Saduria*, *Fucus*): Cu, Cd, Pb, Hg, Zn once per year at 3 locations
- monitoring of total oil hydrocarbons in sea water (fluorometric analysis) twice a year

FINLAND

The Finnish programme includes:

- monitoring of total oil hydrocarbons in sea water (fluorometric analysis) annually from the representative stations (6 stations)
- monitoring of biota (herring and if available, cod): heavy metals (Cu, Cd, Pb, Hg and Zn) and organochlorines (DDTs, CBs, HCB, "-,$- and (-HCH) at 4 locations
- in addition contaminants for different age classes of herring will be analysed annually from one sampling site
- the selected organisms at the coastal area are Macoma, Mytilus, Saduria, perch, pike and herring; and they are collected from altogether seven areas at every or every third year. Analyses: Cd, Cu, Hg, Pb, Zn, DDT compounds, CBs, HCHs and HCB

GERMANY

The German programme includes:

A. for open sea monitoring:

- monitoring of biota (herring and cod): heavy metals (Cu, Cd, Pb, Hg and Zn) and organic contaminants, DDTs, CBs, "-,$- and (-HCH, HCB from one sampling site once per year (area west of Bornholm),

- monitoring of sea water:

Basic programme:

- heavy metals (Cu, Cd, Pb, Zn and Hg) and organic contaminants (9 CB congeners, DDTs,"-,$- and (-HCH, HCB, PAHs (15 compounds)) once a year at 10 stations along the line J1, K1 and K2, and at M2, M1, K8, K5, K7 and K4
- Additionally: heavy metals (basic programme) at N3 and M2, twice a year;
- organic contaminants (basic programme + 30 petroleum hydrocarbons) at stations N3, N1, M2, M1 and FB, once a year.

B. for coastal monitoring:

monitoring of biota:

- perch: heavy metals Cd, Hg, Pb, Cu, Zn and organic contaminants HCB, HCHs and PCBs at three sampling sites twice a year
- Mytilus: heavy metals Cd, Hg, Pb, Cu, Zn, Cr, Ni, As and organic contaminants HCB, HCHs, DDTs and PCBs at four stations once a year and at two stations twice a year. Additionally at one station twice a year heavy metals, pesticides, PCBs and PAHs using different methods
- Viviparous blenny: heavy metals, pesticides, PCBs and PAHs using different methods at one station once a year
- Herring Gull eggs: heavy metals, pesticides and PCBs using different methods at one station once every second year

monitoring of seawater:

- heavy metals: Cu, Cd, Pb, Zn, Ni and Hg at four stations 8 to 12 times a year AND Cu, Cd, Pb, Zn and Hg at two stations once a year and at one station more than 15 times a year
- organic contaminants: 9 CB congeners, DDTs, "-,$- and (-HCH, HCB, PAHs (15 compounds) and 30 petroleum hydrocarbons at 5 stations once a year

C. coastal zone biological effect monitoring

perch: population parameters from three sampling sites twice a year

LATVIA

The Latvian programme includes:

The Gulf of Riga

sea water:

- total oil hydrocarbons (fluorometric, determination); 7 stations sampled 4 times per year (February, May, August, November)

fish:

- Viviparous blenny, liver, metals - Zn, Cu, Cd, Pb (muscle Hg); 2 stations, once in August
- Perch, liver, metals - Zn, Cu, Cd, Pb (muscle Hg); 2 stations, once in August

molluscs:

- Macoma baltica, metals - Zn, Cu, Cd, Pb (muscle Hg); 2 stations, once in August

sediments:

- metals - Zn, Cu, Cd, Pb, (Hg); 9 stations, once in August

LITHUANIA

The Lithuanian programme includes:

In water:

- Hg - 3 stations;
- Cu, Cd, Pb, Zn - 3 stations;
- Organochlorines - 3 stations;

In biota:

- Hg - 1 station once per year;
- Cu, Cd, Zn, Pb - 1 station, once per year;
- Organochlorines - 5 stations, 1 time per year.

POLAND

The Polish programme includes:

In biota:

- heavy metals (Hg, Cd, Pb, Cu, Zn) in herring muscle and cod muscle and liver, perch (muscle and liver), viviparous blenny (muscle and liver) and Mytilus edulis; from 6 sites
- DDTs, CBs; -HCH, HCB in herring muscle, cod muscle and liver, perch (muscle and liver), viviparous blenny (muscle and liver) and Mytilus edulis; once per year from 6 sampling sites

In sediment:

- metals (Cu, Zn, Cd, Hg, Pb) and organic toxicants once in five years from 9 sites

RUSSIA

No information available.

SWEDEN

The Swedish programme for contaminant monitoring includes:

- Selected organisms in coastal areas: perch, viviparous blenny, blue mussels, all from two locations.
- Selected organisms in open sea: herring (5 sites), cod (2 sites) and guillemot egg (one site).
- Contaminants studied: Cd, Cu, Hg, Pb, Zn, DDT compounds, CBs, HCHs, HCB in perch, viviparous blenny, blue mussel, herring, cod and guillemot egg. Dioxins and planar CBs in herring (3 sites) and brominated compounds in guillemot (1 site).

Study area, Bothnian Bay, Bothnian Sea, the Baltic Proper, the Kattegatt. Samples collected in autumn but two time series started in the beginning of 1970s are collected in spring as well as the guillemot egg. All sampling sites are located in areas locally unaffected from local pollution.

The contaminant monitoring programme is integrated with the ecological and physiological fish monitoring programmes.

The Swedish programme for studies on biological effects of eutrophication and toxic substances monitoring includes:

- ecological coastal fish monitoring twice a year (July-August and October) in the Gulf of Bothnia and the Baltic Proper (minimum number of stations: 6 for gill nets and maximally 18 for fyke nets). Variables: stock analysis (species composition, catch per unit effort, age composition) and individual analysis (growth, gonad weight, fecundity, condition factor, external indication of diseases
- physiological coastal fish monitoring once a year (summer) in the Gulf of Bothnia and the Baltic Proper. Samples of stationary fish collected in one coastal area. Variables: gonadosomatic index, liver somatic index, hematocrit value, leucocyte count, plasma ions, cytochrome P-450, EROD activity, blood lactate and tissue glycogen.

Monitoring of population status of top predators (white tailed sea eagle, ringed, common and grey seals) are incorporated in the Swedish Marine Monitoring Programme. Reproduction as well as population size is followed by annual countings.

REFERENCES

ICES, 1995. Report of the ICES Advisory Committee on the Marine Environment, 1995. ICES Cooperative Research Report No. 212.

OSPARCOM, 1996. Summary record of the ad hoc Working Group on Monitoring (MON), MON 96/9/1, Annex 5.