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Interim Report of the Working Group on the Biological Effects of Contaminants (WGBEC)

7-11 March 2016

Lisbon, Portugal

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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Executive summary

The Working Group on the Biological Effects of Contaminants (WGBEC), chaired by Bjørn Einar Grøsvik (NO) and Ketil Hylland (NO), met at IPMA, Lisbon, Portugal, 7–11 March 2016. There were 10 attendees through the week, representing 6 countries.

WGBEC aims to address effects of chemical stressors on the marine environment and subsequent consequences for human health and resource use, taking into account other stressors and environmental factors. The group aims to develop, evaluate and quality assure methods and frameworks for environmental quality.

WGBEC raison d´être and communication with other expert groups. The group aims to expand the scope of its activities to include other ecosystem components, i.e. seabirds and mammals, as well as linking up to human health issues. The revised strategy includes a focus on environmental interactions and multistressor assessment. The group will continue to develop methods and strategy in support of EU legislation, e.g. MSFD. Members of WGBEC participated in a joint workshop with WGEEL (WKBECEEL) with an aim to discuss contaminant-related health effects in the European eel, reported separately. Work will be continued on proxy species. WGBEC has continuous contact with MCWG and will be in touch with relevant groups concerning new ToRs.

ToR a - review effecs of chronic oil exposure on marine organisms. WGBEC has extensive competence on oil effects and has recently produced a guideline to monitor effects of acute spills. A paper is planned to review chronic effects of oil in marine ecosystems.

ToR b - review available studies on marine seabird ecotoxicology. WGBEC recognises the extensive research already in progress on seabird ecotoxicology. Focus will be on including seabirds in a wider framework, investigate links with human health and specific mechanisms of effects, e.g. genotoxicity.

ToR c - review available studies on marine mammal ecotoxicology. WGBEC recognises the extensive research already in progress on marine mammal ecotoxicology. Recent observations for toothed whales are very disturbing. Focus will be on including marine mammals in a wider framework, investigate links with human health and specific mechanisms of effects, e.g. genotoxicity.

ToR d - review effects of contaminants on community composition. There has been a discussion over the past couple of decades on how contaminant-specific community studies may be. Work will be continued in collaboration with appropriate ICES groups and directed towards linking biomarkers and community responses.

ToR e - develop methods to evaluate effects of acute spills on marine organisms. There exist a plethora of national guidelines on how to monitor acute spills, but there is a scarcity of effect methods. WGBEC aims to produce a synthesis guideline paper on acute spill monitoring, which also integrates effect methods.

ToR f - develop methods to evaluate effects of ocean acidification on marine organisms. WGBEC acknowledges the activity of a range of organisations in this research area. The group have highlighted the current status on effects and identified possible monitoring targets, but will continue work in this area as one factor in interactions/combined effects (ToR i).

ToR g - review interactions between essential nutrients or vitamins and contaminants in marine organisms. WGBEC has an on-going interest in how and whether contaminants interact with vitamin and trace mineral homeostasis, until now predominantly thiamine (B1) and vitamin A (including metabolites). No new information was presented at the meeting, but the group has agreed to hold a workshop on thiamine measurements back-to-back with the 2017 meeting, due to the dramatic consequences deficiencies in this vitamin has been shown to have in natural populations. The link, if any, with contaminants is still tenuous.

ToR h - review progress with marine plastic ecotoxicity to marine organisms. This research area was discussed at length during the WGBEC 2014 meeting (with MCWG and WGMS). Updates were provided by Paula Sobral (PT), Dick Vethaak (NL) and Bjørn Einar Grøsvik (NO). The group notes that there are both national and international projects underway with strong participation from WGBEC.

ToR i - review and update knowledge of environmental interactions and combined stressors in marine ecosystems. The group reviewed studies presented by Ketil Hylland (NO), using mesocosms to elucidate interactions, and Juan Bellas (ES) on how natural processes affect how mussels respond to contaminants. WGBEC views this as one of the most central work items of the next few years.

ToR j - review effects of emerging contaminants on marine organisms. Over the last decade, WGBEC has had fruitful discussions on the mechanism of action of emerging contaminants (see text for description of "emerging"). The group agreed to ask MCWG to keep it updated on relevant emerging substances, taking on discussion on a case-by-case basis.

ToR k - review the use of passive samplers and dosing in marine ecotoxicity studies. This topic was discussed comprehensively with MCWG and WGMS during WGBEC 2014. Dick Vethaak (NL) and Thierry Burgeot (FR) presented new data on novel samplers and applications.

Review the status of publications and consider requirements for new publications. One new TIMES manuscript on "Supporting variables for biological effects measurements in fish and blue mussel" had been submitted and was reviewed by the group. The manuscripts in process for publication in Marine Environmental Research from the ICON project were briefly presented. The volume is expected to be published early autumn 2016. Six manuscripts are currently available on the net.

AQC activities for biological effect methods. WGBEC will initiate a round of intercalibrations in 2016 on EROD (liver), AChE (muscle), PAH-metabolites (bile) and micronucleus frequency (blood smears). The process will be run under the BEQUALM umbrella, and will primarily be undertaken at the University of Oslo.

Communication with the ICES Data Centre. WGBEC notes that it is possible to submit biological effects data to the ICES database using a simplified procedure. This will facilitate the inclusion of effect data in the database.

1 Administrative details

Working Group name

Working Group on Biological Effects of Contaminants (WGBEC)

Year of Appointment

2016

Reporting year within current cycle (1, 2 or 3)

1

Chair(s)

Bjørn Einar Grøsvik, Norway

Ketil Hylland, Norway

Meeting venue

Lisbon, Portugal

Meeting dates

7–11 March 2016

2 Terms of Reference a) – z)

ToR a - review effecs of chronic oil exposure on marine organisms

ToR b - review available studies on marine seabird ecotoxicology

ToR c - review available studies on marine mammal ecotoxicology

ToR d - review effects of contaminants on community composition

ToR e - develop methods to evaluate effects of acute spills on marine organisms

ToR f - develop methods to evaluate effects of ocean acidification on marine organisms

ToR g - review interactions between essential nutrients or vitamins and contaminants in marine organisms

ToR h - review progress with marine plastic ecotoxicity to marine organisms

ToR i - review and update knowledge of environmental interactions and combined stressors in marine ecosystems

ToR j - review effects of emerging contaminants on marine organisms

ToR k - review the use of passive samplers and dosing in marine ecotoxicity studies

3 Summary of Work plan

Year 1

The development of methods to assess effects of acidification is an ongoing issue, to be reported each year. Effects of emerging contaminants will be finalised (there has been activity on this issue over the last 3-year period). The group will finalise recently initialised work on interaction between contaminants and vitamins. Work will also focus on items to be reported in year 2 with status updates this year (a, b, c).

Year 2

This is an important reporting year during this 3-year cycle with a final reporting, i.e. review papers, on items a, b (chronic oil exposure, seabird toxicity), in addition to status updates for items f and h (effects of acidification and plastics).

Year 3

Final reporting (i.e. review papers) on items c, d and i (marine mammal ecotoxicology, effects on communities and interactions/combined effects) as well as status reports for ocean acidification.

4 List of Outcomes and Achievements of the WG in this delivery period

The report of the "Trial application of the OSPAR JAMP integrated guidelines for the integrated monitoring and assessment of contaminants 2012–2015" was presented during the last MIME meeting in 2015 by France. Thierry Burgeot (FR) introduced a discussion concerning the challenge for the biological effects application in OSPAR CEMP.

The Working Group on Eel (WGEEL) and the Working Group on Biological Effects of Contaminants (WGBEC) met in Os, Norway on 25–27 January 2016 during its first workshop WKBECEEL under the subject "Are contaminants in eels contributing to their decline?", chaired by Caroline Durif (Norway) and Bjørn Einar Grøsvik (Norway). There were 19 participants (18 attendees and one remote) representing 10 countries. The outcome of the discussions and the literature review according to the ToRs will be presented in a report May 2016.

Publications from the ICON workshop

The ICON project was initiated from WGBEC. The main objective of the project was to provide a run-through of the framework for integrated contaminant monitoring developed for OSPAR (Vethaak *et al.*, 2016), introduced in Hylland *et al.* (2016a) and including chemical analyses (Robinson *et al.*, in press), PAH-metabolite analyses (Kammann *et al.*, 2016), methylmercury analyses (Lang *et al.*, in press), as well as biological effects (Almroth *et al.*, in press; Hylland *et al.*, 2016b, Martinez-Gomez *et al.*, 2016; Vethaak *et al.*, in press). Case studies were included from the UK (Lyons *et al.*, 2016), Seine estuary (Burgeot *et al.*, in press) and the Mediterranean (Martinez-Gomez *et al.*, in press) and a final assessment performed (Hylland *et al.*, in press).

5 Progress report on ToRs and workplan

ToR a: Review effects of chronic oil exposure on marine organisms (ToR a)

Oil pollution is one of the major chemical and physical challenges for coastal (and to some extent offshore) marine ecosystems worldwide. WGBEC has a unique competence in this field, having contributed to developing guidelines for OSPAR and individual countries concerning monitoring, as well as performing research (see reference list for the group. Although it has been shown that early life stages of fish are particularly susceptible to oil pollution, there is limited understanding of the extent to which such pollution impacts natural fish populations and virtually no knowledge of effects on invertebrate early life stages.

Over the past 15 years, WGBEC has addressed issues relevant to oil effects and the group recently produced a guideline for acute oil spill monitoring (Martinez-Gomez *et al.*, 2011). The group wishes to share this competence in the form of a review paper, the draft of which is planned to be available by WGBEC 2017. Bjørn Einar Grøsvik will be first author and co-ordinate the paper. WGBEC members are invited to write up drafts for one or more of the following (and hence be co-authors): (i) description of chronic exposure (BE); (ii) tissue and environmental concentrations following chronic exposure (SB), (iii) comparison acute – chronic, (iv) effect methods – fish (BE, KH, SB), (v) effect methods – invertebrates/communities (JB, SB), (vi) effect methods – seabirds (KH), (vii) effect methods – marine mammals (KH), (viii) comparison lab – field; thresholds/assessment values (BE), (ix) the relevance of phototoxicity, (x) prognostic vs diagnostic – prediction/models, (xi) degradation and environmental modification.

ToR b: Review available studies on marine seabird ecotoxicology (ToR b)

Ketil Hylland (NO) introduced the subject, referring to earlier studies showing ecologically relevant impacts from contaminants on seabirds, e.g. Bustnes *et al.*, 2012, 2015. He further referred to ongoing studies at the University of Oslo on genotoxicity in seabirds, measured using the comet assay (DNA strand breaks). Preliminary results indicate that there are clear species differences in baseline DNA strand breaks in different species, i.e. eider duck, two skua species, glaucous gull, kittiwake, but there was no clear relationship between concentrations of contaminants in blood with DNA strand breaks in lymphocytes. A similar study with herring gull will be performed in 2016 with sites along the Norwegian coast. There are strong research groups working on contaminant effects in seabirds, see e.g. Fisk *et al.*, 2005, Dietz *et al.*, 2013. WGBEC will clarify by 2017 whether there are issues that can be addressed with the competence available to the group, e.g. concerning inclusion of seabirds in an ecosystem assessment framework, interactions and/or monitoring.

Dick Vethaak (NL) reminded WGBEC that some seabirds are among the species most at risk from environmental plastic pollution and suggested that WGBEC consider future activity in this area, but also highlighted that many groups are already active in this field internationally.

The WGBEC activity on thiamine deficiency is highly relevant to seabirds and will be followed up in 2017 (see section 11 below), necessitating deferring the final reporting on the issue to 2018 (year 3 in the cycle). WGBEC Chairs will contact JWGBIRD and other experts to elicit feedback prior to WGBEC 2017 with a view of clarifying whether the group can fill a niche in evaluating aspects of contaminant effects and/or integrated monitoring.

ToR c: Review available studies on marine mammal ecotoxicology

Ketil Hylland (NO) described a study on genotoxicity in polar bear, in which 47 individuals were sampled in the autumn 2013 in collaboration with the Norwegian Polar Institute. Lymphocytes were isolated and comet analyses (DNA strand breaks) performed in the field. The blood of a volunteer was used as a reference for the analyses. Whole blood was used for contaminant analyses. Contrary to expectations, preliminary results suggest that individuals with high concentrations of contaminants had less DNA damage than individuals with lower concentrations of contaminants. Age, gender and condition did not appear to affect DNA damage in polar bear. There are already strong research groups who have been addressing effects of contaminants on marine mammals, see e.g. Dietz *et al.*, 2013, Jepson *et al.*, 2015. Particularly the results in Jepson *et al.* (2015) are very disturbing, suggesting that orcas south of the UK do not reproduce, possibly due to contaminant stress. WGBEC Chairs will contact WGMME and other experts for possible future collaboration.

ToR d: Review effects of contaminants on community composition

Over the past decades there has been a discussion as to whether community composition is sufficiently contaminant-specific to merit inclusion on a list of recommended methods for contaminant-oriented assessment. There is a clear need for further discussion on this topic, which will need to also involve other ICES working groups (i.e. benthic ecology). Biological effects measurements that can potentially be used to establish the link between exposure to toxic substances and effects on organisms must meet three basic conditions, to be sensitive, to have ecological relevance, and to be simple, standardisable and of limited cost. The responses measured at the lower levels of organisation (molecular or cellular levels) are sensitive and specific responses, but the significance of these responses as to the structure and function of the populations or biological communities is not easily quantified.

Ultimately, the impact of pollution can be manifested at the highest level of biological organisation, i.e. natural benthic or pelagic communities. Benthic communities are well suited to study the effects of marine pollution at medium-to-long term. Because of its relative stability and due to the relatively long life cycle of macrobenthic species, biological communities are less sensitive to short variations of the physico-chemical water characteristics, compared for instance to the high sensitivity of planktonic communities (Reish 1986). Moreover, the composition of benthic communities reflects in a comprehensive manner environmental conditions, not only at a given moment, but may integrate the conditions during a certain period of time (Gray 1992, Pearson and Rosenberg 1978). The most frequent responses of a biological community to pollution are changes in structure, i.e. the relative abundance of some species increases while for other species decreases, and for others remains stable. Impacted benthic communities are usually dominated by organisms adapted to environmental changes, which are considered to be opportunistic species, such as spionid and capitellid polychaetes (Reish 1955, Beiras *et al.* 2012). It may be appropriate to include the study of benthic communities in integrated monitoring programs of marine pollution, especially taking into account the requirements of the current European legislation (WFD, MSFD) aiming to assess the current state of the marine environment and to identify areas that potentially cannot achieve good environmental status. Although changes in populations or communities are relevant in terms of ecological significance, however, they cannot easily be related to pollution. That is, as we ascend in the organisation level the ecological relevance increases, but specificity, rapidity of response and easiness of standardisation as a routine technique for environmental monitoring decrease, and vice versa. Therefore, more effort is needed for the validation and application of these techniques in biomonitoring (efficiency, reliability, and cost-effectiveness). The way forward for their use may arise in part from their combined use with biomarkers, which would help to establish the link between cellular and molecular specific changes and the effect at the individual/population level. WGBEC Chairs will contact BEWG to discuss collaboration.

ToR e: Develop methods to evaluate effects of acute spills on marine organisms

This ToR is connected to MSFD 8.2.2: Occurrence, origin, extent of significant acute pollution events *(e.g.* slicks from oil and oil products) and their impact on biota physically affected by this pollution. Members of WGBEC have addressed issues relevant to acute oil spills effects and the group recently produced a guideline for acute oil spill monitoring (Martinez-Gomez *et al.*, 2011). All countries with a coastline have some guideline as to how to monitor acute spills, but there is a scarcity of effect methods. Upon request, Jim Readman (UK) contributed input to this item, summarising methods on water analyses and monitoring strategies.

Acute spills have conceivably different patterns of effects than diffuse, chronic inputs. This is a fundamental question in environmental science, and very important for environmental assessment. Global frequencies of acute accidents related to exploration, production and transport of oil has been presented by Eckle *et al.*, 2012. They show that accidental tanker spills have significantly decreased over the past four decades both in terms of numbers and volumes of spills. Key factors are improvements in navigation technology, the automatic identification system (AIS), double hull as requirements after the Exxon Valdez accident and decrease in average age of the world tanker fleet. Less data are available for acute spills of other types of contaminants.

Some accidental oil spills have shown strong effects on early stages of fish as an important effect after acute oil spills, for examples as shown after the Exxon Valdez spill in 1989. Similar effects have also been shown in smaller spills with bunker oil, as reported after the container ship Cosco Busan discharged 200000 l of bunker fuel oil in the San Francisco bay in 2007. This discharge gave high mortality in Pacific herring embryos in the area, and points to the importance of enhanced phototoxicity due to uv-irradiation after uptake of components in bunker oil (Incardona *et al.*, 2012). The study also indicates the importance of including effect studies on early developmental stages of marine animals when assessing acute accidents.

The need for an integrated contaminant-oriented guideline paper on acute spill assessment and monitoring will be clarified by WGBEC 2017. This will depend on the quality of existing guidelines from international organisations and national authorities. WGBEC members should send national guidelines on monitoring following acute spills to Bjørn Einar Grøsvik.

ToR f: Develop methods to evaluate effects of ocean acidification on marine organisms

Recent progress on ocean acidification research has been reported in a special issue of the ICES Journal of Marine Science (Volume 73 issue 3). Over the past few years, another ICES group has addressed issues relating to acidification, SGOA (Study Group on ocean acidification). No clear indicators for acidification have emerged from the work by that group (SGOA, 2015). Possibly that is due to several reasons: the pH decrease so far has been ~ 0.1 unit and the expectation is 0.3 unit until the end of the century. In coastal areas, the pH ranges much more on a diurnal basis, by other influences such as deposition, changes in the catchments and changes in eutrophication status. The observed effects on organisms have not been very clear in a "noisy" environment. However WGBEC agrees that the development of physiological or chemical related indicators for OA is the way forward.

The last IPCC report (2014) gives a good overview of the sensitivity and tolerances of different organisms for two IPCC scenarios. Available data shows that the most sensitive taxa are coral, echinoderms (especially larvae), bivalves and gastropods. The effects are reduced calcification and survival.

Dick Vethaak referred to an evaluation that suggested a shortlist of pteropods, coccolithophores and foraminifera as the most sensitive. It indicated that the Arctic pteropod *Limacina helicina* would be most appropriate as an indicator species. The advice from the SGOA was to archive pteropods (in ethanol). Mussels (*Mytilus edulis*) were not included in the shortlist because they do not appear to be sensitive to the pH levels that we can expect by 2100 and they also inhabit waters with higher pH ranges. Brittlestar *Ophiothrix fragilis* larvae appear to be sensitive to acidification, with reduced survival and growth (see Dupont *et al.*, 2008, 2010). So far, most laboratory exposure studies are short-term experiments. They do not allow the investigation of adaptation to reduced pH, which is poorly understood. In areas with naturally low pH or pH gradients, such as around underwater volcanoes, long-term effects can be observed. In general, there is evidence of a decrease of the number of calcifiers and an increase of non-calcifiers near the source (volcano) (Hall-Spencer *et al.*, 2008).

Pelagic habitats experience natural diurnal variation in pH through natural cycles of photosynthesis and respiration of primary producers. It would be interesting to clarify why such variation does not cause damage to e.g. brittlestar larvae.

As referred to above, there is already substantial research on possible effects of acidification on marine organisms. The main focus of WGBEC will be how pH changes interact with other environmental factors or stressors in affecting marine organisms or processes (see section 13).

ToR g: Review interactions between essential nutrients or vitamins and contaminants in marine organisms

This ToR was extensively discussed during the 2015 WGBEC meeting in Bergen, with particular reference to thiamine (Lennar Balk, SE) and vitamin A (Jakob Strand, DK). Except for recent studies indicating the relevance of retinoid pathways for invertebrates (Andre *et al.*, 2015), no new information had been forthcoming for this meeting. The apparent seriousness of the issue of thiamine deficiency has prompted WGBEC to initiate large-scale analyses of relevant parameters. A WGBEC member, Lennart Balk, is a leading expert in the field and will hold a workshop for WGBEC members in 2017. It is anticipated that we will accrue data on thiamine and any contaminant interactions in 2017/2018, so WGBEC wish to extend the ToR to the third year. WGBEC will arrange a workshop on thiamine analyses in co-ordination with the 2017 meeting.

ToR h: Review progress with marine plastic ecotoxicity to marine organisms

An invited presentation concerning studies with plastics over the past decade was given by Paula Sobral (PT). In this brief presentation marine litter was put in context regarding the impacts of its degradation in the oceans which leads to fragmentation into smaller and smaller pieces, the so called microplastics (particles smaller than 5 mm) which also include particles that are produced as such, i.e. resin pellets from the industry and microbeads present in personal care and household products. A summary of the research by her team (MARE-NOVA) within the scope of the POIZON project (2010–2014) and subsequent work was provided. Microplastics were detected in beaches, coastal waters and coastal sediments, and found in the gut contents of commercial fish on the Portuguese coast, posing great concern about possible transfer along the food web, which will ultimately reach humans through fish and shellfish consumption. Contamination by adsorbed pollutants (PAH, PCB and DDT) was detected in all samples of beached resin pellets, with high values found near industrial facilities and port facilities. As there are no reference values for contaminated plastic (such as threshold or probable effects levels, TEL and PEL) risk assessment is not possible at the moment.

Dick Vethaak presented new data on microplastic pollution from the Dutch coast and inland waters. Initial studies from VU-IVM Amsterdam and Deltares (NL) confirm the presence of microplastics in the Dutch coastal waters and delta metropolis (wastewater, sewage sludge, canal water and sediment) and riverine surface waters of the continent (Leslie *et al.*, 2013; IVM, unpublished data). Relatively high microplastic levels in riverine suspended particulate matter (1400–4900 kg^{-1} dry weight (dw)) and in urban canal sediments (<68 to 10,500 particles kg^{-1} dw) and from 100 up to 3600 particles kg^{-1} dry sediment along the Dutch North Sea coast. Amsterdam canal water sampled at different urban locations contained microplastic concentrations (48–187 L-1), similar to those observed in wastewater that is emitted from sewage treatment facilities in the area. The results show that rivers are major transport pathways for microplastisc to be discharged into the sea, The high microplastic enrichment in marine sediments compared to most literature data for seawater at the surface supports the hypothesis of a seabed sink for these materials, but at least partial settling of the materials occurs in freshwater as well. Marine biota are heavily exposed to plastic particles. Body residues between 10 and 100 particles g^{-1} dw were measured in benthic macro invertebrate species inhabiting the Dutch North Sea coast, including filter-feeding mussels and oysters, species for human consumption as well as other consumers in the marine food chain. Further it was concluded that urban delta cities are significant emitters of microplastics in the aquatic environment. Moreover, evidence was presented that plastic particles can be a carrier for the dispersal of hormone disrupting and other toxic chemicals, and pathogens (including potential human agents) and may pose new environmental and human health threats in coastal and urban delta areas.

Bjørn Einar Grøsvik (NO) presented data of registration of litter from the Norwegian Sea bed mapping program MAREANO and registration of litter from the yearly ecosystem surveys in the Barents Sea. The MAREANO program records and quantifies observations of seabed litter from video inspections from the continental shelf along the Norwegian coast from the area off Møre and northwards. Some parts of the Barents Sea have also been mapped in this program. Litter is observed at 25 % of the 1626 video transects mapped in MAREANO. More than half of the litter observations are of objects related to fishing activities including lost fishing gears. Highest frequency of litter observations were registered in marine valleys and ravines. The registrations indicate an average amount of litter of 200 kg per km^2 in the investigated area.

Since 2004 the Institute of Marine Research (IMR), Norway and the Polar Research Institute of Marine Fisheries and Oceanography (PINRO), Russia have collaborated on ecosystem based surveys in the Barents Sea. From 2010 registration of marine litter as surface observations and by-catch in bottom- and pelagic trawls has been a part of this collaboration. Highest occurrence of litter was observed in areas of intensive fishery and navigation. Plastic prevailed among observed litter and litter was observed more frequently in bottom trawls than in pelagic trawls. Other types of litter (metal, paper, rubber, textile, glass) were sporadically observed. Such repeated measurements are interesting to develop as indicators for litter in this region.

ToR i: Review and update knowledge of environmental interactions and combined stressors in marine ecosystems

All environmental scientists are aware that there is a multitude of both natural and anthropogenic factors in natural ecosystems that affect organisms, of which contaminants is one. To manage contaminant inputs into marine ecosystems, it is crucial to understand how and whether other factors increase or decrease the toxicity of contaminants, whether contaminants affect other processes, e.g. modulating immune processes and hence causing increased disease, as well as the relative importance of contaminants in relation to other stressors, e.g. fisheries and habitat modification. To make matters even more complex, there is never only two factors or stressors involved and there is always more than one contaminant.

Members of WGBEC have addressed the above issues over the past two decades and are in a position to provide significant contributions to the scientific community and environmental managers. WGBEC sees this ToR as one of our major work items during the present three-year period.

Marine pollution monitoring programmes aim to establish the link between chemical pollution and harmful effects on marine organisms. This requires the development of background levels and assessment criteria for both pollutants and biological responses, which provide the scientific basis to design measures for improving environmental quality. However, biological effects data obtained in monitoring studies are sometimes difficult to interpret due to the large number of natural variables affecting the biological processes considered as indicators of pollution, such as temperature, food availability, reproductive cycle, shore location or salinity. It is therefore indispensable to understand the importance of sources of variability other than pollution affecting biological responses, and integrate this information with that of chemical analyses and biological responses to better understand the variance of the data, particularly in large-scale monitoring programs covering a wide range of environmental scenarios. This calls for a more holistic approach in which pollutants are not considered as the only source of variability on the biological responses of organisms, but as an additional variable.

Ketil Hylland (NO) presented a pelagic mesocosm study in which interactions between oil, a pharmaceutical (emamectin) and Si (to facilitate diatom growth) were investigated using community processes (Vestheim *et al.*, in prep). Although emamectin treatment was effective in incapacitating zooplankton grazers, this only had a transient effect on primary production in the system. Interestingly, the presence of oil appeared to favour bluegreen bacteria. An earlier study investigated interactions include organic material/Si, oil and a pharmaceutial in pelagic mesocosms (Vestheim *et al.*, 2010), organic material, hypoxia and contaminants in sediments (Hylland *et al.*, 1996), parasite and organochlorine interaction in seabirds (Bustnes *et al.*, 2006), plastic accumulation and contaminant exposure (Hertzke *et al.*, 2015), interactions between contaminants and UV, i.e. phototoxicity (Choi & Oris, 2000), the use of booster pesticides in antifouling agents or pesticides and finally particles from mining activities and chemicals. This is by no means an exhaustive list, but should serve to show the wide range of possible interactions and combined effects that exist. A further complicating factor is the density effect: should a population be decimated by e.g. contaminant exposure, the remaining individuals in that population will have improved conditions with regard to habitat and food availability, potentially reducing or even abolishing the effect of the contamination.

In addition to interaction as described above, properties of an individual, generally reflecting one or more of the above factors, e.g. food availability, will modulate effect responses due to contaminant exposure. Such confounding factors have been described earlier (e.g. Hylland *et al.*, 1996) and are discussed in a TIMES-document by Hanson *et al.* (in prep).

Marine pollution monitoring programs aim to establish the link between chemical pollution and harmful effects on marine organisms. This requires the development of background levels and assessment criteria for both pollutants and biological responses, which provide the scientific basis to design measures for improving environmental quality. However, biological effects data obtained in monitoring studies are sometimes difficult to interpret due to the large number of natural variables affecting the biological processes considered as indicators of pollution, such as temperature, food availability, reproductive cycle, shore location or salinity (González-Fernández *et al.* 2015a). It is therefore indispensable to understand the importance of sources of variability other than pollution affecting biological responses, and integrate this information with that of chemical analyses and biological responses to better understand the variance of the data, particularly in large-scale monitoring programs covering a wide range of environmental scenarios. This calls for a more holistic approach in which pollutants are not considered as the only source of variability on the biological responses of organisms, but as an additional variable.

Recent laboratory studies conducted by the Spanish Oceanographic Institute (Instituto Español de Oceanografía, IEO), confirmed that mussel condition (nutritive condition and reproductive status), present a strong effect on molecular and physiological biomarkers, masking the responses to pollution. In order to identify the effect of food availability and consequently, of mussel nutritive condition, on biomarker responses, mussels (*Mytilus galloprovincialis*) from an unpolluted site from Galicia (NW Spain) were conditioned to three different food rations for 2 months, in order to obtain three groups of mussels with different nutritive conditions: high, intermediate and low (nutritive stress) conditions. The experiment was carried out in early summer, time of year far from gametogenesis (autumn) or spawning (spring), in order to isolate the experimental variable 'nutritive condition' from gonadal development (reproductive state). Then, each mussel group was exposed to the three-ring PAH fluoranthene (FLU) for three weeks. The results obtained, evidenced that most of the studied biomarkers (clearance rate, respiration rate, Scope for Growth, SOD, CAT, GPx and PO) were more affected by mussel nutritive state than by toxicant exposure, showing higher values the group with lower nutritive condition (nutritive stress), whereas the effect of toxicant was not always evident, masked by the nutritive condition effect (González-Fernández *et al.* 2015b). The effect of the reproductive state on pollution responses was studied in mussels from the same population, sampled at two different periods of the reproductive cycle (reproductive and resting stages), and were conditioned to the same laboratory conditions to avoid the influence of external factors. Mussels were at the same nutritive status (equal mussel quantitative condition) but differed on the developmental stage of their gonads. After conditioning, mussels were exposed to FLU for three weeks. The reproductive status was assessed by the measurement of gonadal development and reserves composition. The obtained results indicate that the effect of the reproductive status was greater than the effect of the toxicant in most of the studied biomarkers (respiration rate, SOD, CAT and GPx), with values higher during the reproductive stage than during the resting stage. On the other hand, the effect of the toxicant was observed for SFG, CAT and GPx, but this effect was only apparent during the resting period. Moreover, there was a deterioration of mussel gonadal tissue following FLU exposure during the reproductive stage. FLU accumulation in mussel tissues was also found to be dependent on reproductive status, with higher bioaccumulation of FLU in mussel tissues during the resting period (González-Fernández *et al.*, manuscript under review).

A manuscript will be prepared in time to be evaluated at WGBEC 2017. WGBEC members should get in touch with Ketil Hylland to contribute to this manuscript, preferably with discussion of particular interactions. Identified additional contributors: Juan Bellas, Concepcion Martinez-Gomez, Dick Vethaak. Ketil Hylland will initiate a first review manuscript 2016-2017 to be further progressed during WGBEC 2017 with a view to submit spring 2017; the Chairs will follow-up on progress by 1.10.16.

ToR j: Review effects of emerging contaminants on marine organisms

The group discussed the various definitions of the term "emerging", which is used to give the impression of substances which has recently been detected in the environment, but which is widely used for substances that have been around for decades. "Emerging" may sometimes reflect detection in new contexts of matrices or new effects, and the groups of substances are generally referred to by their use pattern or source, e.g. flame retardants, pharmaceuticals, antifouling agents, pesticides, household chemicals, cosmetics, munitions and industrial chemicals. As will be apparent, most of them are used at low concentrations and concentrations in the environment are generally low. WGBEC has been addressing this issue for a period with a particular focus on effects of "emerging" substances. While this has often led to fruitful discussions of mechanisms of toxicity for such substances, this is no longer a prioritised area for the group.

ToR k: Review the use of passive samplers and dosing in marine ecotoxicity studies

Passive samplers have some obvious areas of use, i.e. monitor concentrations of substances of interest in environment, monitor concentrations in experiments (see below) and finally as a sampler for complex mixes of substances in water or sediment for subsequent bioassay testing (see below).

Ecotoxicological applications were developed in France with the POCIS deployment in order to evaluate the biological effects of herbicides along the Atlantic coasts. POCIS (Polar Organic Chemical Integrative Sampler) integrative samplers were combined with classical techniques of analytical chemistry (HPLC UPLC-MS) in water sample. The POCIS brings an alternative to the study of the herbicides in the field and in experimental studies. The herbicides are hydrophilic molecules which appear in a strong concentration in water after storm or rainy events and some herbicides can also diffuse regularly. The integrative properties of POCIS allow to quantify herbicides in water during a continuous period and allows to solve some analytical limits of detection. POCIS extraction can also be done for bioassays application.

Two different type of POCIS application in ecotoxicology study were discussed:

1) The POCIS were used in exposed and assay tanks (Barranger *et al.*, 2013) to confirm the waterborne concentration of diuron reached in water during the experiment with oyster from 0.2 to 0.3 µg/L. This application of POCIS emphasizes the relevance of the result obtained and the risk associated to chemical contamination for oyster recruitment.
2) The POCIS were also applied in production areas of oysters in Marennes Oléron (Gulf of Biscay-France). A range of concentrations from 0.2 to 17 ng/L were quantified among the herbicides (atrazine, atrazine 2 hydroxyde, nicosulfuron, DMST, acethochlore, acethochlore ESA, methalochore, metalochore ESA, metalochore EA) the most frequently detected in water.

Passive samplers have been deployed in an autonomous benthic station for assessing the impact of weather events on chemical contamination of water. The FRAME benthic station is an autonomous measuring and sampling system that can be deployed and recovered using a coastal craft. This system is designed to acquire in situ data for a period of about two months, detect "events" (storms, flooding, etc.) and evaluate chemical contamination (trace metals and organic contaminants) in the water column (before, during and after the event).

In a novel approach, silicon-based samplers were used to mimic the bioavailable fraction of organic contaminants in sediments during the recent ICON project, reported in Vethaak *et al.* (in press). Contaminants were extracted and subsequently tested for toxicity using a range of *in vitro* methods.

WGBEC raison d´être and communication with other expert groups

Dick Vethaak (NL) initiated a discussion on the direction and future of the WGBEC. In his view, there are good reasons to broaden the scope and ambitions of WGBEC to comply with the needs and objectives of ICES's integrated ecosystem approach. In addition, it is important to put the work of the group in a perspective of recent developments on human health implications related to the marine environment. Future work could include a wider ecological perspective of understanding to quantify the risks of chemicals, chemical mixtures and metabolites and their contribution to impacts on population and ecosystem levels. WGBEC has already aimed for a wider ecosystem approach through our new ToRs (b), (c) and (d), concerning seabirds, marine mammals and communities, respectively. In addition to addressing different organism groups or biological systems, this strategy requires a multi-stressor approach to include non-chemical stressors in order to quantify the contribution of chemical effects to marine ecosystem status, ecosystem health, biodiversity and other ecosystem services and eventually implications for human health. This fits well with the current ToRs (g) and (i) on interactions of contaminants with essential nutrients/vitamins and combined effects/interactions with other environmental stressors/factors.

Substances that we use every day are turning up in our estuaries and seas, where they can impact marine life and eventually ourselves. Current risk assessments are based on a single chemical and will underestimate impacts on marine wildlife since chemicals interact with other chemicals as well as with non-chemical stressors in the environment. Priority and emerging chemicals affect marine environments and may come back to haunt us in unanticipated ways. The above will require more emphasis on the impact of chemical stress on lower trophic levels (primary and secondary producers) and microbial communities and nutrient cycles. One of our long-term challenges is the translation of contamination-related effects from species to population and communities, which at the moment is best addressed using mesocosm experiments and modelling approaches. This question can also be addressed through ToR (d). The impact of chemical contaminants on energy allocation and the flows of energy and nutrients though the food web and their impact on productivity is another challenge, partly addressed as part of the ToRs on nutrients/vitamins and interactions/combined effects.

The concept of marine sentinel organisms provides one approach to evaluating marine ecosystem health. A sentinel marine species can provide early warning of existing or emerging health hazards from the marine environment due to their ability to concentrate contaminants or integrate effects from contaminant exposure within a food web or ecosystem. Sentinel species will need to include marine organisms with physiology and/or diet similar enough to humans such that they may provide early indication of potential adverse health effects and provide insight into toxic mechanisms of any given hazardous agent. Such sentinels and indicators are thermometers for current or future impacts on individuals or populations, both with regard to animal and human health. A well-known example is endocrine disrupting chemicals. These compounds are known to act on endocrine systems of echinoderms, molluscs, fish, marine mammals and humans in analogous ways. Wildlife-human connections between marine sentinels and humans permits better characterisation and management of impacts that ultimately may affect marine animal and human health associated with the marine environment.

There is a range of sensitive biological effect methods (biomarkers, bioassays) for chemical contaminants such as those developed by ICES for OSPAR monitoring purposes. Such impacts may however also include the biological effects of natural toxins. The impact of algal toxins on marine organisms may be perhaps greater than those of priority and emerging chemical contaminants in some areas and periods, but our knowledge concerning this is still very limited. There is some scope to include this under ToR (i) on interactions, but impacts of natural toxins, alone or in combination with other stressors should be considered for future WGBEC work programmes.

As some marine mammal species share the coastal environment with humans and consume the same food, they also may serve as effective sentinels for public health problems. A better understanding of the impacts of emerging contaminants will lead to improved management decisions for the environment, especially coastal ecosystems that are already battling such a multitude of stresses. Recent indications that southern orca populations may be so contaminated that they have stopped reproducing (Jepson *et al.*, 2015) have made this issue even more relevant. This will be tackled under ToR (c) for WGBEC over the next couple of years.

In addition to the above, long-term consequences of climate change and potential environmental degradation are likely to include aspects of general health indicators and disease emergence in marine plants and animals. In turn, these emerging diseases may have epizootic potential, zoonotic implications, and a complex pathogenesis involving other cofactors such as anthropogenic contaminant burden, genetics, and immunological dysfunction. A draft (scoping) paper on the potential impacts of chemical stressors on marine ecosystems and associated human health implications will be prepared by WGBEC 2017, led by Dick Vethaak and Ketil Hylland.

Joint workshop of the Working Group on eel and the working group on Biological Effects of Contaminants (WKBECEEL)

Members of WGBEC met with WGEEL members at Solstrand, Os, Norway, in January 2016 to discuss the relative importance of contaminants for the observed decline in European eel stocks. WGEEL has through many years developed a knowledge base for eel ecology and contaminant concentrations and wished for WGBEC to contribute towards discussions on toxicokinetics and -dynamics involved in the redistribution and internal exposure of hydrophobic contaminants stored in muscle. The challenge in working with European eel is the lack of direct observation on mature and spawning individuals as well as early larval stages. Silver eel (the migrating stage) has been made to mature following hormone treatment. Spawning and culturing eggs and yolk-sac larvae have been achieved in laboratory, but the challenge to grow the larvae through start feeding has not been solved. All European eel have similar genetics and there thus appears to be a homogenisation during spawning. There is however a geographical separation in that there is a dominance of female eels in northern Europe (where they have to swim or be transported the furthest as larvae) and male eels in southern Europe. The concept for WKBECEEL was good, but there was not sufficient time for an in-depth discussion of all relevant topics during the meeting. This should however be pursued at a later time, possibly with direct allocation of specific tasks to participants prior to the meeting.

WGBEC discussed proxy species for eel with similar life histories, although not as extreme. All marine fish invest in gonadal development and may conceivably be models. Alternative species suggested by the group included salmon, lamprey and flounder, all known to migrate in relation to spawning. To prepare for a follow-up on the issue, WGBEC will request data from the ICES data centre to evaluate temporal changes in relevant parameters in monitored species (dab, flounder, cod).

WGBEC Chairs will contact the Chairs of JWGBIRD, WGMME and BEWG to clarify possibly collaboration on ToRs b, c and d for the 2017 meeting.

Intercalibration of biological effects methods

Any biological effect method to be used for national or international monitoring programmes should be subject to appropriate internal and external AQC, particularly as this is a requirement for submitting data to the ICES database. It is likely that the role of AQC will take on an even greater importance with the use of biological effect methods for monitoring GES (Descriptor 8) in the EU MSFD.

Depending on uptake by European laboratories, WGBEC decided to perform intercalibrations for EROD (cod liver), AChE (cod muscle), PAH-metabolites (cod bile) and micronucleus analyses (mussel haemocytes). Registration for the activity will be done in April under the BEQUALM umbrella, organised by Steven Brooks (NO).

Organisms will be collected by Ketil Hylland in May, kept at the University of Oslo and treated appropriately to generate three groups for each method (phenanthrene, pyrene and benzo(a)pyrene for EROD and metabolites). Liver and muscle tissue will be homogenised under liquid nitrogen, bile mixed, diluted and aliquotted, and blood smears prepared from mixed haemolymphs. Samples will be distributed to participating laboratories at their own cost. Analyses will need to be finalised by the end of the year and results communicated to WGBEC Chairs, facilitating evaluation at WGBEC 2017 and reporting back to participants immediately following that meeting. Ketil Hylland will initiate an intercalibrations for selected methods in 2016-2017.

Communication with the ICES Data Centre

The data centre has provided WGBEC with an excel spreadsheet format to facilitate reporting of biological effects data. This has been made possible through communication between WGBEC (Thierry Burgeot) and the ICES data centre.

Status of publications and consider requirements for new publications

ICES TIMES

WGBEC reviewed status with ICES TIMES manuscripts and revised deadlines as appropriate. One new manuscript was produced in draft for the meeting to review (2012/1/SSGHIE09, Supporting variables for biological effects measurements in fish and blue mussel; Hansson, Thain, Martinez-Gomez, Hylland, Gubbins, Balk. The group found the quality of the submitted manuscript to be satisfactory. Some comments were forwarded to the authors, who will then submit to WGBEC Chair Bjørn Einar Grøsvik.

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6 Revisions to the work plan and justification

ToR b: activity on contaminant effects on seabirds to be extended to year 3 of the cycle (2018).

ToR e: Work with acute spills to be extended to year 3 of the cycle (2018), including finalisation of a guideline manuscript.

ToR g: Activity on vitamin and nutrient interactions to be extended to year 3 of the cycle (2018).

ToR i: This is going to be one of the most important and extensive issues for WGBEC over the next few years and we wish to extend the final reporting to year 3 of the cycle (2018).

7 Next meetings

The Working Group on Biological Effects of Contaminants (WGBEC), chaired by Bjørn Einar Grøsvik, Norway, and Ketil Hylland, Norway, will meet in Reykjavik, Iceland, 13–17 March 2017.

Annex 1: List of participants

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Annex 2: Recommendations

Recommendation	Adressed to
1. WGBEC requests data for selected marine fish species (dab, flounder, cod) for as many years and regions as possible: body wt, liver wt, gonad wt, gender, length, age, liver lipid, muscle lipid.	ICES Data Centre
2. MCWG is requested to keep WGBEC informed about emerging substances of toxicological interest.	MCWG

Annex 3: MIME integration and national programmes

As part of each annual meeting, WGBEC reviews contaminant monitoring programmes implemented in European countries and integration with or compliance with OSPAR (MIME) and EU (MSFD). Comprehensive overviews were presented by many countries, e.g. Sweden, Spain, France, Norway and Germany, during the last reporting period. For data or overviews reported earlier the reader is referred to WGBEC (2015) or earlier reports.

The report of the "Trial application of the OSPAR JAMP integrated guidelines for the integrated monitoring and assessment of contaminants 2012–2015" was presented during the last MIME meeting in 2015 by France. Thierry Burgeot (FR) introduced a discussion concerning the challenge for the biological effects application in OSPAR CEMP.

MIME 2014 proposed the following steps to arrive at the final product:

(a) analyse short- and long-term datasets from local/national studies and apply BACs and EACs,

(b) conduct a simple assessment of the SGIMC approach to integrate biological effect and chemical data

The revised report was written with the contribution of Thierry Burgeot (FR) and Brandon McHugh (IR). It includes extra data countries submit during 2014/2015 and provides details of activities completed during 2015 to further progress approaches suitable for the application of biological effects tools, in line with the OSPAR recommended approach to assess pollution effects and to provide a better overview of ecosystem health.

While the need to integrate biological effects and contaminant concentrations has been identified by OSPAR in previous Quality Status Reports, there is a particular need to include biological effects in all assessment of contaminant impacts, as has been reinforced by the EU MSFD, Descriptor 8.2.2. This requires data on biological effects in order to evaluate whether harm is occurring when assessing environmental status of contaminants.

A core set of biological effect techniques has been recommended by SGIMC (2011) following a comprehensive process. These recommendations were taken forward by the HELCOM CORESET programme.

Although not all biological effect data can currently be uploaded to the ICES database due to issues with the reporting formats, a wide range (spatial plus inshore versus offshore) of data exist and can be included in an assessment using the integrated approach.

While much data exists, not all core contaminants, biomarkers and bioassays suggested by SGIMC are included in the monitoring programmes by different Contracting Parties. The SGIMC integrated approach can provide an overall ecological assessment based on a selection of contaminants, biomarkers and bioassays.

Appropriate guidelines, AQC and assessment criteria are available for some contaminants and all core biological effects techniques. A higher number of EAC and BAC are established for biological effects compared with chemicals levels.

Continued focus on harmonisation of the selection of a minimum and appropriate contaminant and biomarkers/bioassay analysis programme, the on-going ecological validation of the chemical and biological EAC and BAC applied in all areas of monitoring and the continued development of new EAC and BAC for contaminants and biomarkers/bioassays are also key to the further enhancement/application of integrated approaches within CEMP and in support of D8 of the MSFD.

Moving biological effects techniques from pre-CEMP to a mandatory CEMP basis would be expected to further facilitate uptake of these methodologies and would likely allow for an integrated assessment of contaminants and biological effects on (sub)regional or specific scale. The example of imposex demonstrates clearly that when techniques are made mandatory harmonisation quickly follows. The development of a metric to quantifying contaminant-related effects in marine ecosystems is a key deliverable of this approach.

A weight-of-evidence approach is appropriate in the most common field scenario with chronic and diffuse contamination. In this case, the dynamic responses of the core biomarkers illustrates a stress which can integrate the combined effects of a mixture of contaminants.

The efforts undertaken by OSPAR Contracting Parties has allowed the development of a comprehensive suite of assessment criteria (BAC/EAC) which is unique in Europe and OSPAR has the scientific legitimacy to propose an integrated approach with a standardised interpretation based on assessment criteria in biology and chemistry.

This report only reflects an assessment of "Integrated" data acquired through the standardised spreadsheet and is reflective of data that Contracting Parties consider as having been collected in an integrated manner.

The paper does not include all data existing in the database as this needs to be further screened to evaluate if there are contaminants data associated with these sites and, if so, whether these series can then be considered as being suitable for integrated assessment.

At the end of the Trial application 2012-2015, HASEC granted MIME experts a one-year extension of the 'pilot activity' that tested the Integrated Guidelines and the practical application of biological effects monitoring techniques, including the issue of enhanced access to biological effects measurement data. The best challenge during this period of pilot activity is to integrate further chemical and biological data with short and long term studies. The ICES developed a simplified excel sheet which was presented to the WGBEC. This simplified ICES excel sheet will be populated from March to the 1st September 2016 in order to collect more data. All the contracting parties are requested to sending data. Portugal and Iceland will be contacted by WGBEC Chairs in order to send their data and the ICON data will be added before September 2016. A roll-over will be done during the MIME 2016 in November.