Summary of the Belspo BRAIN Project NewSTHEPS:

The NewSTHEPS project focused on the use of alternative sampling techniques (passive samplers, sediment traps), combined with state-of-the-art analytical methodologies to quantify a wide variety of chemical compounds, including legacy pollutants, contaminants of emerging concern (CECs), metals as well as their potential marine ecotoxicity and risks. This was combined with stable isotope analysis of suspended particulate matter (SPM) and modelling techniques in harbours and along the Belgian coast. The main goal was to offer policy support tools that allow to monitor a multitude of chemicals remaining previously undetected to enable risk assessment, combined with distance to target and Margin of Safety (MoS) assessments of mixtures of substances in the field.

A wide suite of novel methods and innovative approaches were developed to evaluate the Descriptor 8 Good Environmental Status related to the Marine Strategy Framework Directive. These methods were applied in the Belgian Part of the North Sea (BPNS) through monitoring and modelling, yielding new insights into exposure, effects and ecological risks of CECs, legacy organic compounds and metals.

There are multiple key achievements, related to both developed methods and findings on the current state of the BPNS. The novel passive sampling technique of Diffusive Gradients in Thin-Films (DGT) was for the first time applied in the BPNS and therefore the first database about labile metal concentrations was obtained in this coastal area. The organic DGT (o-DGT) samplers, followed by *in vitro* testing of the extract with ERA-CALUX, was for the first time used to measure estrogenic activity in marine waters.

Speedisk® extractions followed by validated UHPLC-Q-Orbitrap analytical methods allowed (ultra)-trace measurement of a broad range of CECs in the marine environment, including pharmaceuticals, personal care products (PCPs), pesticides, steroidal Endocrine Disrupting Compounds (EDCs), plasticizers and plastics additives. Lower method detection limits (< 1 ng L^{-1}) were obtained in comparison with commonly applied methods using Oasis HLB (Hydrophilic lipophilic bond) based extractions. Hydrophilic divinylbenzene (h-DVB) sorbent, a novel and highly promising sorbent for passive sampling of CECs with a broad range of physicochemical properties (Log K_{ow} from 0.6 to 8.5) was physico-chemically characterized. A novel "Simple Teabag Equilibrium Passive Sampler" (STEPS) for polar CECs was developed, and data obtained in a static exposure batch set-up showed fast uptake rates (R_s from 0.3 to 12 L d^{-1}, with a median value of 1.5 L d^{-1}) and high sampler-water equilibrium partitioning coefficients (Log K_{sw} between 4.1 and 6.5). The simple and cost-efficient design of STEPS and its versatility (ease of incorporating various sorbent materials) to capture a wide range of compounds offer great potential to holistic monitoring of CECs, although more in-depth in situ calibration is recommended to fully understand the underlying uptake mechanisms under field conditions.

A multi-ratio method to estimate bioavailability of non-polar (legacy) compounds works well for chlorinated biphenyls and pesticides. Most legacy pollutants were demonstrated to be close to equilibrium partitioning between different environmental compartments (water, SPM, sediment). This basically means that any environmental compartment selected for monitoring offers information on all others.

An effect-based monitoring approach for environmental risk assessment, linking passive sampling with Speedisk® and marine sample enrichment with ecotoxicity testing was developed. A strong correspondence of the relative CEC composition between original field samples and enriched test media was demonstrated. Enrichment Factors (EF) of up to 44 were obtained in a microplate ecotoxicity test with the diatom *Phaeodactylum tricornutum*, enabling full concentration-response testing of environmentally realistic contaminant mixtures (ERCMs). We further propose that, if toxic effects are detected below an EF of 10, this translates to the occurrence of ecological risk, considering the currently applied minimum safety factor of 10 to the most sensitive ecotoxicity data according to marine risk assessment guidance.

An initial high-resolution 3D chemical fate and exposure model for the BPNS was developed and technically verified, by adding degradation of chemicals and their adsorption to suspended matter and sediment to the "COupled Hydrodynamic Ecological model for REgioNal Shelf seas (COHERENS)". The model was successful under several testing conditions and was checked for mass conservation, but its application requires further refinement in terms of more precise riverine chemical inputs, degradation and adsorption components and validation against chemical monitoring datasets.

We developed an automated algorithm and R code to compute marine-specific predicted no effect concentrations (PNECs) of chemicals. This algorithm uses extracted ecotoxicity data from the publicly available and regularly updated US-EPA online ecotoxicity database as input, and then applies EU guidance on deriving a marine PNEC based on the type and amount of data available for each substance. This algorithm allows fast screening-level ecological risk assessment for any chemical of interest and allows straightforward and rapid updates of PNECs with every update of the US-EPA database.

Several key results were obtained in conjunction with existing methods and laboratory experiments. All new methods and approaches mentioned above were applied, across 5 monitoring campaigns at 4 locations in the BPNS, i.e. two coastal sites and two harbour sites.

Maximum dissolved trace metal concentrations were 0.40 μg L^{-1} for Co,10.1 μg L^{-1} for Cu, 2.83 μg L^{-1} for Ni, 0.19 μg L^{-1} for Cd, and 0.54 μg L^{-1} for Pb. Measured dissolved concentrations of Ni, Pb, and Cd were all below EQS (Environmental Quality Standards). Labile metal concentrations were lower and ranged up to 0.22 μg L^{-1} for Co, 0.60 μg L^{-1} for Cu, 0.64 μg L^{-1} for Ni, 0.049 μg L^{-1} for Cd, and 0.23 μg L^{-1} for Pb. Trace metal species showed both seasonal and spatial variation in the BPNS. The labile trace metal concentration, measured with DGT, are time averaged and different from the total, dissolved or particulate metal concentrations measured by spot sampling technique. Labile trace metal concentrations are therefore likely more appropriate for marine risk assessment and recommended as complementary data to total, dissolved and particulate metals, and as a basis for improved EQS.

Particulate Organic Carbon in the open sea ranged from 1.5 - 3.6 mg L^{-1}. The $\delta^{13}C$ signatures were used to trace the origin of suspended organic matter and indicated both terrestrial and marine sources to suspended organic matter in the BPNS, albeit mostly from marine origin. This is important to consider to further improve the marine fate and exposure model.

Occurrence data for 63 pharmaceuticals, personal care products and pesticides (up to 680 ng L^{-1}), 97 steroidal EDCs (up to 104 ng L^{-1}) and plastics additives (up to 6.5 μg L^{-1}) are reported, many of which have been detected for the first time in the marine environment. Ecotoxicity testing of CECs for which no marine toxicity data were available in scientific literature or databases revealed low toxicity for most substances to the diatom *P. tricornutum* ($n = 20$, 72h-EC50 $\geq$ 0.6 mg L^{-1}) and the copepod *N. spinipes* ($n = 23$, 48h-LC50 $\geq$ 4.8 mg L^{-1}). As an exception, 4 neonicotinoids showed high toxicity to copepods, with no observed effect concentrations (NOECs) from 0.31 - 1.1 μg L^{-1} when considering mobility, survival or reproduction of *N. spinipes*. Mixtures risk assessment revealed ecological risk of neonicotinoids, mainly in harbour sites.

No evidence of toxicity to the copepod *N. spinipes* at concentrations approximating those actually occurring in the BPNS was found. The effects-based monitoring approach with the diatom *P. tricornutum* revealed significant growth inhibition at Relative Enrichment Factors (REF) between 3.2 and 33 and a Margin of Safety between only 1.1 and 11 across 8 marine samples. Using conventional risk assessment approaches, these data suggest ecological risks due to realistic mixtures of CECs in the BPNS, both in harbours and open sea. However, linking the toxicity with the targeted CECs was not possible. Comparison of analytical data of CECs from passive and grab samples with existing EQS or predicted no effect concentrations (PNECs), revealed no EQS exceedance for any of the 7 WFD priority substances, exceedance of the PNEC for 2 out of 18 EU watchlist substances (both steroidal EDCs, i.e. EE2 and E2), and no exceedance of 15 PNECs for antibiotics established to avoid antibiotic resistance. However, some substances showed risk quotients (RQ) > 0.1, pointing to their potential importance when considering risk associated with mixtures.

Application of our automated algorithm and code allowed to calculate screening-level PNECs for 97 substances. Comparison against analytical data of CECs revealed no obvious difference of RQ distributions between sampling locations or sampling method. There were 21 substances presenting an ecological risk to the BPNS, i.e. with median RQ > 1 : 10 steroids, 5 pesticides (2 neonicotinoid insecticides and 3 herbicides), 2 pharmaceuticals, 2 phthalates, 1 PCP and Bisphenol A. Fifteen of those, currently not being part of the EU watchlist, are at risk to escape regulatory action, and thus require urgent specific attention. For substances with the highest RQs (including for many steroids), fish are often the most sensitive taxonomic group. Our screening-level risk assessment results can help delineating priorities for refined assessment or risk reduction in the future.

In a case study, the marine fate and exposure model was applied to clothianidin and showed that the degradation rate has a strong impact on predicted concentrations in the BPNS, as well as on the relative contribution of each riverine input to it. Both the Scheldt, Rhine and Meuse rivers

were shown to significantly influence exposure in the BPNS. This initial model validation is encouraging, but more extensive validation is needed in future work, together with the refinement of the degradation and adsorption components in this model, in order to evaluate the effectiveness of policy measures proposed to reduce CEC exposure and risks in the BPNS.

Keywords:
passive sampling – high-resolution mass spectrometry – ecotoxicity – environmental risk assessment – marine chemical fate and exposure model