



Recommended Operating Procedure (ROP) Aim of ROP (tick box) Munition detection or identification In situ exposure studies ☐ Sampling □ Chemical analysis □ Bioassays □ Bioindicators/biomarkers 18. Data analysis and assessment version 2.0 Kari K. Lehtonen Finnish Environment Institute (SYKE) Latokartanonkaari 11, 00790 Helsinki, Finland email: kari.lehtonen@ymparisto.fi Scope To obtain a comprehensive view of the contamination status of areas possibly affected by dumped munitions the data collected from field measurements needs to be assessed not only per single parameter but also in an integrated manner. However, currently there are no specific integrative or statistical methods specially designed for environmental assessments of dumped munitions, which can mostly be regarded similar to any contaminant point source present in the seabed and leaking out toxic substances. Thus, some recently developed and applied data integration methodologies are briefly presented in this document with key references to literature. In addition, one of the methodologies, the Integrated Biomarker Response (IBR), is described in more detail since it has recently been successfully applied in studies at munition dumping areas in the Baltic Sea. Summary of the method/ROP The data integration methods presented here to be used for the assessment of contamination status of dumping areas consist of measured chemical (concentrations) and biological (effects) parameters. The choice of the method applied depends on the properties of the data set at hand, and, e.g., the scope and representativeness of the parameters and availability of specific threshold values, and therefore no

Safety aspects

Not relevant.

Documentation

The data for used for any of the integrative assessments should ideally be completely independent from each other. The data should also be quality checked and available for users as original data per single parameter; this is important for transparency (avoiding misinterpretations and misuse of the integrated indices), possible recalculations and comparisons using different methodologies available, and also the possibility to examine only the single parameters and their interactions.

general recommendations on the preferred method can be presented.





Methods

Examples of data integration approaches

OSPAR Integrated Monitoring and Assessment of Contaminants approach

Developed by the Oslo and Paris Convention (OSPAR Commission) and Expert Groups of the International Council for the Exploration of the Sea (ICES), the methodology^{1,2} is based on a multistep "traffic light" data aggregation from different matrices using equivalent background (BAC) or environmental (EAC) assessment criteria both for contaminants and biological effects, allowing a simple graphical presentation. The initial comparisons determine whether the parameter and site combinations are < BAC (blue), between the BAC and EAC (green), or > EAC (red). This summarized status indicator defined for each parameter can then be integrated with others in different ways.

Chemical Status Assessment Tool (CHASE)

Developed and applied by the Helsinki Commission (HELCOM), CHASE³ integrates data on hazardous substances in water, sediments and biota as well as bioeffect indicators and is based on a substance or bioeffect specific calculation of a "contamination ratio", which is the ratio between an observed concentration/bioeffect and a threshold value.

Weight-of-Evidence (WOE) approaches

Different WOE approaches have been developed during the years. One of the recent ones⁴ has been very successfully applied in marine areas and consists of logical flow-charts elaborating results from single "lines of evidence" based on several chemical and biological parameters, normative guidelines or scientific evidence. The data are then summarized into specific synthetic indices prior to their integration into an overall evaluation.

Integrated Biomarker Index (IBR)

The IBR was originally developed as a graphical tool based on the calculation of Star Plot areas and applied to biochemical biomarkers⁵ but its use has since been extended to cover all kinds of biological effects⁶. In studies of effects of dumped munitions in the Baltic Sea it has been applied to laboratory-exposed⁷ and field-caged mussels⁸ and wild caught fish^{9, 10}. The same methodology based on parameter standardisation has also been applied in the description of total tissue load of contaminants with comparisons with parallel biological effects data¹¹.

Calculation procedure

For each biomarker:

- (1) Calculation of mean and SD for each sampling site (or in case of an experiment, treatment);
- (2) Standardisation of data for each site: $x_i' = (x_i \text{mean } x) / s$, where x = standardised value of the biomarker, $x_i = \text{mean value of a biomarker from each site}$, mean x = mean of the biomarker calculated for all the sites, and s = standard deviation calculated for the site-specific values of each biomarker. Result: variance = 1, mean = 0.
- (3) Using standardised data, addition of the value obtained for each site to the absolute (=non-negative) value of the minimum value in the data set: $B = x_i' + lx_{min}l$. Result: adjusts the lowest value in the set to zero.

For all the biomarkers treated this way:

- (4) Calculation of Star Plot areas by multiplication of the obtained value of each biomarker (B_i) with the value of the next biomarker, arranged as a set, dividing each calculation by 2.
- (5) Summing-up of all values: $\{[(B_1 \times B_2)/2] + [(B_2 \times B_3)/2] + ... [(B_{n-1} \times B_n)/2]\}$. Result: IBR (average of different arrangements of biomarkers in the set).





Since the value of IBR is obtained by summing up the parameters derived from the actual biomarker values, i.e., after the calculation steps 1–4, it is directly dependent on the number of biomarkers in the set. Thus, in the results section the values of IBR are given divided by the number of biomarkers used in each case and termed as IBR/n. It should also be noted that because the calculation method of IBR is based on relative differences between the biomarker responses in each given data set it is necessary to re-calculate all the index values each time when making new comparisons, e.g., adding new sites or comparing seasonal values.

Conclusions (if applicable)

The selection of the integration method should be made objectively and according to the suitability of the available data. All the methodologies have their pros and cons and the user should be aware of the basis of the calculations, and, if possible, even try out different methodologies for the same data sets. Importantly, expert advice in selecting the most suitable method is always required. Careful documentation of the methodology used is of utmost necessity.

References

¹Davies, I.M., Vethaak, A.D., ICES Cooperative Research Report No. 315, **2012,** 277 pp.

²Vethaak, A.D., Davies, I.M., Thain, J.E., Gubbins, M.J., Martínez-Gómez, C., Robinson, C.D., Moffat, C.F., Burgeot, T., Maes, T., Wosniok, W., Giltrap, M., Lang, T., Hylland, K., **2017**, *Marine Environmental Research* 124, 11-20.

³Andersen, J.H., Murray, C., Larsen, M.M., Green, N., Høgåsen, T., Dahlgren, E., Garnaga-Budrė, G., Gustavson, K., Haarich, M., Kallenbach, E.M.F., Mannio, J., Strand, J., Korpinen, S., **2016**, *Environmental Monitoring and Assessment* 188, 115.

⁴Piva, F., Ciaprini, F., Onorati, F., Benedetti, M., Fattorini, D., Ausili, A., Regoli, F., **2011**, *Chemosphere* 83 (4), 475-485.

⁵Beliaeff, B., Burgeot, T., **2002**, Environmental Toxicology and Chemistry 21, 1316-1322.

⁶Broeg, K., Lehtonen, K.K., **2006**, *Marine Pollution Bulletin* 53, 508-522.

⁷Schuster, R., Strehse, J.S., Ahvo, A., Turja, R., Maser, E., Bickmeyer, U., Lehtonen, K.K., Brenner, M., **2020**, *Marine Environmental Research* (in press/Sept 2020).

⁸Lastumäki, A., Turja, R., Brenner, M., Vanninen, P., Niemikoski, H., Butrimavičienė, L., Stankevičiūtė, M., Lehtonen, K.K., **2020**, *Marine Environmental Research* (in press/ Sept 2020).

⁹Ahvo, A., Lehtonen, K.K., Lastumäki, A., Straumer, K., Kraugerud, M., Feist, S., Lang, T., Tørnes, J.A., **2020**, *Marine Environmental Research* (in press/Sept 2020)

¹⁰Niemikoski, H., Straumer, K., Ahvo, A., Turja, R., Brenner, M., Rautanen, T., Lang, T., Lehtonen, K.K., Vanninen, P., **2020**, *Marine Environmental Research* (in press/ Sept 2020).

¹¹Lehtonen K.K., Leiniö S., Schneider R., Leivuori M., **2006**, *Marine Ecology Progress Series* 322, 155-168.

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