***DAIMON Toolbox Fact Sheets:***

*Methods to Study the Impact of Dumped Munitions on Marine Biota*

**Assessment category 3: Biological effects**

**Toolbox component: Genotoxicity**

## Fact Sheet 3.27: Gene transcription

*Authors: Raisa Turja and Kari Lehtonen, Finnish Environment Institute (SYKE)*

### What is it?

Exposure to xenobiotics induces changes in gene transcription. Genes involved in stress responses such as the antioxidant defence system (ADS) and DNA damage regulation are among the important genes to be analyzed to assess the potential effects of xenobiotic exposure.

### What does it tell you?

Transcriptional changes correspond to up- and downregulation of the target genes in response to xenobiotic exposure. Regulated gene expression may have effects at the protein level, e.g., in the concentrations of enzymes and chaperone molecules. If target genes specifically respond to exposure to munitions-related hazardous substances, gene transcription may be used as a CWA- or TNT-specific indicator.

**Type of Indicator** (tick box)

☐ non-specific stress indicator
☐ specific for groups of contaminants incl. CWA or explosives
☒ CWA-specific indicator
☒ specific for substances related to explosives (e.g. TNT)

### How to measure it?

***Species*:** Transcriptional responses can be measured in a large variety of organisms, including fish and mussels. To design primers, existing sequence data on the target gene is required, preferably on the study species or close relatives. Published primers on target genes of the study species are an advantage.

***Matrix*:** Fish liver and mussel digestive gland tissue homogenates.

***Equipment*:** Commercial kits for RNA extraction (TRIZOL reagent, Invitrogen); Bioanalyzer (Agilent RNA 6000 Nano kit); cDNA synthesis (e.g., SuperScript III First-Strand Synthesis System, Invitrogen), nucleic acid concentration measurements (QUBIT RNA and ssDNA assay kits, Invitrogen) and qPCR (e.g. Platinum SYBR Green qPCR SuperMix-UDG, Invitrogen); Primers (Forward and Reverse) for the target genes ordered from, e.g., Integrated DNA technologies; homogenisator (FASTPREP,

MPBIO), Bioanalyzer (Agilent); PCR machine; qPCR machine; fluorometer (e.g., Qubit); super freezer. All plastic ware must be sterile (RNase/DNase free) and suitable for utilized machines. Pipets must be sterilized under UV-light. Additional reagents used in reactions or dilutions must be suitable for molecular work (e.g., ultrapure water).

***Measurements and units***: Measurements at different stages are performed according to manufacturers' instructions. Briefly, for sample quality control, extracted RNA is analyzed with Bioanalyzer and after that 3 µg of RNA is utilized in the cDNA synthesis reaction. For qPCR standard curve, a small subset of each cDNA sample (1-2µL) is pooled together and the pooled sample is diluted in ultrapure water: 1:1, 1:2, 1:5 and 1:10. For each analyzed gene, except 18S, 1µL of undiluted cDNA sample with 9 µL of reaction mix are pipetted to the qPCR plate in three replicates. Reaction mix contained forward and reverse primers (both 10µM). For 18S standards and samples were diluted 1:100. qPCR program was adjusted according to the utilized primers. Data is given as Ct-values: the fractional PCR cycle number at which the fluorescence is greater than the threshold (Non template control). cDNA concentration of each sample was measured.

***Calculations***: Efficiency is calculated from slope of the standard curve where Ct values were plotted against cDNA concentration. Efficiency € = $(10^{(-1/ |slope|)}) - 1$. Ct values of all samples were normalized with the control treatment values. ΔCt = Ct (sample) - mean Ct (control). ΔCt values are corrected with efficiency. Eff(sample)=$(E+1)^{\Delta Ct(sample)}$. Relative quantification value (RQ) (sample)=Eff/[cDNA].

***Sample size***: Measurements were made from 6 or 12 individual specimens from each study site or experimental treatment.

**How to analyze and assess the data?**

Relative quantification (RQ) method is used to assess the fold change of the target gene mRNA in tissue of the exposed vs. control mussels. This can be analyzed using efficiency calculated from the standard curve and normalized with control treatment values.

**References**

Giuliani, M.E., Benedetti, M., Arukwe, A., Regoli, F. 2013. Transcriptional and catalytic responses of antioxidant and biotransformation pathways in mussels, *Mytilus galloprovincialis*, exposed to chemical mixtures. *Aquatic Toxicology* 134-135:120-7.

Lacroix, C., Coquillé, V., Guyomarch, J., Auffret, M., Moraga, D. 2014. A selection of reference genes and early- warning mRNA biomarkers for environmental monitoring using *Mytilus* spp. as sentinel species. *Marine Pollution Bulletin* 86: 304–313.