A. Volpi Ghirardini, A. Arizzi Novelli, C. Losso, M. Picone, G. Libralato, D. Marchetto, G. Frangipane, P.F. Ghetti

Environmental Science Department, University of Venice, Campo della Celestia, 2737/b - 30122 Venice, Italy. voghi@unive.it

EARLY LIFE STAGES OF SEA URCHINS AND BIVALVES IN MARINE ECOTOXICOLOGY: VALIDATION OF METHODS USING A QUALITY ASSURANCE/QUALITY CONTROL PROCEDURE

The use of toxicity bioassays is fundamental in monitoring programs aiming to evaluate the quality of transitional and marine environments subjected to chemical contamination (European Water Directive 2000/60/EC). The choice of species must be based mainly on a profound understanding of biology, on availability, handling and sensitivity. The search for the most representative species for specific areas of concern should aim at indigenous species instead of surrogate species in order to increase the ecological relevance of a toxicity bioassay. Sea urchins and bivalves are considered the best candidates among marine organisms (ICES, 2003) since early life stages of the life-cycle are easily obtainable for many months of the year. Methods based on these stages are effective because they combine rapidity of response with an high sensitivity, comparable to that of chronic exposures. Only toxicity bioassays that are accurately evaluated and validated using QA/QC procedures should be used for biomonitoring purposes. A good QA/QC program requires the control of various phases, including both biological materials (adult quality used for obtaining gametes, positive and negative controls necessary to accept or reject experimental data, sensitivity and discriminatory capacity towards pure substances) and abiotic matrices for testing (sampling methods that ensure representative samples of environmental media, sample storage, standardization of procedures to obtain test matrices, evaluation of confounding factors). Moreover, before being applied for any purpose, a toxicity bioassay must be subjected to an iterative methodological evaluation phase, in order to gather information on its performance and to define its field of application. Therefore, a step-by-step procedure was designed to evaluate methods: a) precision, intra-laboratory reproducibility and comparability with standard methods; b) sensitivity, discriminatory ability and comparability with other toxicological methods using pure compounds; c) applicability to specific matrices (e.g. superficial water, elutriate, pore water) from different environments.

This procedure was applied to validate methods based on the sea urchin *Paracentrotus lividus* (sperm cell and embrio toxicity) and the bivalve *Mytilus galloprovincialis* (embryo toxicity). Intralaboratory reproducibility was calculated and recorded in control charts using a reference toxicant (copper), taking into account all variables (operators, sampling sites and periods, batches of adult animals). All methods demonstrated a good reproducibility, in spite of the use of field populations: the sea urchin sperm cell test showed a mean EC50 of 0.056±0.008 mg/L (CV=15%, n=37), the sea urchin embryo toxicity test a mean EC50±SD of 0.065±0.007 mg/L (CV=12%, n=20); the bivalve embryo toxicity test a mean EC50 of 0.019±0.002 mg/L (CV=12%, n=10). Comparative sensitivity and discriminatory ability towards pure toxicants (data expressed as EC50 and NOEC) was accurately investigated, providing information about the ability of bioassays (comparing environmental exposure concentrations and NOEC) to detect contaminants causing pollution in transitional and marine environ-

nents. Sensitivity and discriminatory ability in assessing toxicity of sediment elutriates and pore water from the Venice lagoon were then verified applying methods to sites covering different types and levels of contamination. Results revealed the efficacy, particularly of both embryotoxicity bioassays, in discriminating between differing pollution/bioavailability conditions among stations and periods. Finally, definitive validation of these methods for monitoring purposes has required accurate studies on the contribution to toxicity due to ammonia and sulphides as possible confounding factors, by comparing exposure concentrations in environmental samples and sensitivity limits of methods measured experimentally (for sea urchin methods) or reported in standard methods (for bivalve methods) for each toxicant.