



UNITED NATIONS ENVIRONMENT PROGRAMME

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*Estimation of the toxicity of
pollutants to marine phytoplanktonic
and zooplanktonic organisms*

*Reference Methods For Marine Pollution Studies
No. 44 (draft)*

Prepared in co-operation with



FAO



IAEA

NOTE: This document has been prepared in co-operation between the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA) and the United Nations Environment Programme (UNEP) under project FP/5101-84-01.

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PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources and has requested the development of regional action plans. The Regional Seas Programme at present includes ten regions and has over 120 coastal States participating in it. (1), (2)

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment and of its resources, and of the sources and trends of the pollution, and the impact of pollution on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies are being developed and are recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialized bodies of the United Nations system as well as other organizations and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines the style used by the International Organization for Standardization (ISO) is followed as closely as possible.

The methods and guidelines, as published in UNEP's series of Reference Methods for Marine Pollution Studies, are not considered as final. They are planned to be periodically revised taking into account the development of our understanding of the problems, of analytical instrumentation and the actual need of the users. In order to facilitate these revisions the users are invited to convey their comments and suggestions to:

International Laboratory of Marine Radioactivity
International Atomic Energy Agency
c/o Musee Oceanographique
MC9R000 MONACO

which is responsible for the technical co-ordination of the development, testing and intercalibration of Reference Methods.

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- (1) UNEP: Achievements and planned development of the UNEP's Regional Seas Programme and comparable programmes sponsored by other bodies. UNEP Regional Seas Reports and Studies No. 1 UNEP, 1982.
- (2) P. HUIJ: A Strategy for the Seas. The Regional Seas Programme: Past and Future UNEP, 1983.

This issue of the Reference Method for Marine Pollution Studies No. 44 was prepared in co-operation with the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA). It includes comments received from a number of scientists who reviewed the method. The assistance of all those who contributed to the preparation of this reference method is gratefully acknowledged.

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1. SCOPE AND FIELD OF APPLICATION

This reference method describes procedures for estimating the toxicity of poisons to marine phytoplankton and zooplankton. Procedures are given for estimating the median effective concentrations (EC50) of poisons to phytoplankton, and the minimum algistatic concentration (MAC-5). For zooplankton, procedures are given for determining median lethal concentrations.

2. REFERENCES

- REISH, D.J. and OSHIDA, P.S. (1986) Manual of Methods in Aquatic Environment Research. Part 10. Short-term static bioassays. FAO Fish. Tech. Pap. (No.247) 62pp.
- UNEP/FAO/IAEA (1986) Estimation of the acute lethal toxicity of pollutants to marine fish and invertebrates: Determination of the survival time-concentration relationship (toxicity curve) and the estimation of median lethal concentrations. Reference Methods for Marine Pollution Studies No.43, UNEP.
- WARD, G.S. & PARRISH, P.R. (1982) Manual of Methods in Aquatic Environment Research. Part 6. Toxicity Tests. FAO Fish. Tech. Pap. (No.185) 23pp., Rome, FAO.

3. PRINCIPLES

Organisms are exposed to each of a range of concentrations of the poison under test. For phytoplankton, the median effective concentration (EC50) is estimated in terms of the number of individuals surviving, the biomass of individuals surviving, or the chlorophyll content of the individuals surviving. For zooplankton, the median lethal concentration (LC50) is estimated by conventional log-probit analysis of the mortality data.

4. REAGENTS

4.1 For phytoplankton tests

Filtered sea water

Distilled water

KNO₃

KH_2PO_4

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

$\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$

$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$

Sodium EDTA

Crystalline vitamin B12

Biotin

Thiamine hydrochloride

Toxicant stock solution

4.2 For zooplankton tests

Filtered sea water

Toxicant stock solution

NOTE: Maintenance of zooplankton in the laboratory frequently requires the maintenance of phytoplankton cultures as a food source.

5. APPARATUS

5.1 For phytoplankton tests

Autoclave

Erlenmeyer flasks (various sizes)

Microscope

Constant temperature room or enclosure

Fluorescent lighting system

Haemocytometer

Automatic particle counter (optional)

Orbital shaker

Fluorometer (optional)

Homogeniser (optional)

Logarithmic probability graph paper

Salinometer or salinity probe of adequate accuracy

5.2 For zooplankton tests

Culture vessels of 10-50 litre capacity

Microscope

Constant temperature room or enclosure

Pasteur pipettes

Glass test vessels of appropriate size

Logarithmic-probability graph paper

NOTE: Maintenance of zooplankton in the laboratory frequently requires the maintenance of phytoplankton cultures as a food source.

NOTE: A sample sheet of logarithmic-probability graph paper is included at the end of this manual, which may be photocopied in case of difficulty in obtaining supplies from commercial sources.

6. MAINTENANCE OF STOCK PHYTOPLANKTON CULTURES

6.1 Laboratories undertaking toxicity tests with phytoplankton should establish and maintain stock cultures of the required species. It is generally easier and more reliable to obtain the initial cultures from a reputable biological supplier than to attempt to isolate pure cultures from field collections.

6.2 Solutions required

6.2.1 Solution A - sea water: Use sea water from an uncontaminated source, filtered through a 0.45 μm filter to remove algae, bacteria and particulate matter. Artificial sea water may be used if necessary. Synthetic sea water may be made up from the following formula:

<u>Chemical</u>	<u>Quantity (g)</u>
NaCl	23.926
Na_2SO_4	4.008
KCl	0.677

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