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TOXICOLOGY RESEARCH CENTRE

DP/ROK/82/028

REPUBLIC OF KOREA

Technical report: Aquatic Toxicology*

Prepared for the Government of the Republic of Korea
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of R. R. Stephenson,
expert in aquatic toxicology

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301

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EXPLANATORY NOTES

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Abbreviations

KRICT	**-**	**Korea Research Institute of Chemical Technology**
TRC	**-**	**Toxicology Research Centre**
GLP	**-**	**Good Laboratory Practice**
SOP	**-**	**Standard Operating Procedure**
SRC	**-**	**Sittingbourne Research Centre (- base laboratory of advising expert).**
OECD	-	Organization for Economic Co-operation and Development

ABSTRACT

As part of the expert assistance programme defined for the Toxicology Research Centre (DP/ROK/82/028) a mission in Aquatic Toxicology is reported; undertaken in two phases, 24th March 1986 - 28th March 1986 and 1st June 1986 - 17th June 1986.

The broad objective of the assistance was to aid the upgrading of experimental techniques in aquatic toxicology to a level internationally acceptable for the registration of pesticides and speciality chemicals.

Assistance was given through detailed discussion of international guidelines for testing (OECD) and SOPs. Audits of methods currently being used at TRC to assess toxic effects on fish, daphnids and algae were made and the findings discussed in relation to international standards.

It is concluded that the laboratory has the basic requirements for successful aquatic toxicology testing. It is therefore recommended that the necessary documentation and approach for compliance with GLP be introduced and certain equipment and facilities be upgraded. Continued contact with facilities operating to internationally accepted standards in the field of aquatic toxicology is recommended.

CONTENTS

INTRODUCTION

This report covers a mission of one month, undertaken in two phases, 23rd - 28th March 1986 and of 1st June 1986 - 17th June 1986, to the Toxicology Research Centre of the Korean Research Institute for Chemical Technology. The TRC, established with UNDP aid and guidance, is planned to develop as the main facility for contract toxicology in the Republic of Korea. Aquatic toxicology has been identified as one area in which expertise will be required. This mission was established in order to provide assistance in acute and chronic aquatic toxicity testing and, in model ecosystem analysis.

The original specific objectives were:-

1. Review methodology for assessment of ecotoxicity of newly synthesised chemicals and introduce the latest methods in this field.
2. Provide advice on optimum research procedures and data analysis in short and long term toxicity tests with fish, daphnia and algae.
3. Advise on laboratory scale rearing of test fish and daphnia.
4. Introduce the concepts of ecosystem analysis which tests the likely behaviour of chemicals in the environment and advise on making an aquatic model ecosystem, and on test procedures and data analysis.

These objectives were redefined after discussions with Dr Roh (Director of the Centre) and members of his staff during the initial visit. The revised objectives were:-

1. To upgrade the experimental techniques in aquatic toxicology to a level which would allow data produced to be internationally acceptable. Particular attention being paid to the requirements of GLP.

2. To plan a technical development programme for the aquatic toxicology laboratory over the next 2-10 years focussing on requirements for the registration of pesticides and speciality chemicals.
3. To contribute to the development of personnel and facilities necessary to achieve objective 2.
4. To initiate a collaborative research project in the area of aquatic toxicology.

The objectives of the mission were fulfilled by individual and group discussion, observation and auditing of experimental procedures, practical demonstrations and seminars.

RECOMMENDATIONS

1. Steps to be taken to bring the aquatic toxicology testing into compliance with GLP. A phased approach is recommended.

2. The design of new laboratories should take note of the detailed recommendations made regarding the nature and scope of facilities required for providing a full range of aquatic toxicology testing (Annex I).

3. Construction or purchase of certain items of equipment are recommended, not all of which are major capital items.

4. Provision should be made to ensure that the chemical support which will be required by aquatic toxicology studies is available.

5. Provision should be made for continued contact between the TRC and other laboratories involved in aquatic toxicology for regulatory purposes. Contact could be through training visits by TRC staff members, by the establishing of collaborative research projects and by further UNDP/UNIDO supported missions.

I. ACTIVITIES

A. First visit

The state of development of the aquatic toxicology laboratory was reviewed (Annex I) and an action plan for the second visit produced. In addition a number of actions were agreed to be carried out between the visits.

State of development of the aquatic toxicology laboratory

The scientific staff in the laboratory is well qualified and able to perform the tasks necessary to bring the standards of the laboratory upto an internationally acceptable level. The documentation of international guidelines and standard methods in aquatic toxicology is good and the staff have a sound knowledge of them, though only limited experience in their practical application. The equipment and facilities available are somewhat limited but this will be improved when the group moves to new accommodation in some 12 - 18 months time.

Action plan for second visit

It was agreed that the bulk of the second visit would be given over to discussion of SOPs and OECD guidelines concerned with the culture of and, testing of toxicity to, fish, Daphnia and algae. Methods at present used in TRC would be reviewed and audited in the light of GLP requirements.

Additional actions

In order to maximise the value of two visits it was agreed that certain experimental work would be carried out at the TRC before the second visit. Experiments related to the testing and culture of algae, fish and daphnia were discussed and agreed. In addition I agreed to take a sample of the TRC daphnid culture to the UK where I would carry out a series of experiments to ascertain optimum culture conditions.

All of these experimental programmes were carried out to the extent that prior commitments allowed. Work with the algal tests at the TRC was particularly successful in defining optimal cell densities for initial inocular in algal tests and the work in the UK provided details of a successful culture regime for the TRC daphnid culture.

B. Second visit

The objectives agreed during the first visit were reconfirmed. A culture of Daphnia magna, a species not available in Korea, was provided along with an SOP for its maintenance.

The majority of the second visit was spent in giving detailed advice and instruction in testing methods with special reference to, and emphasis on the requirements of GLP.

SOPs and OECD guidelines

The following SOP and/or OECD guidelines were discussed in detail with relevant staff:-

- acute toxicity test with algae
- maintenance of daphnid cultures
- acute and chronic toxicity tests with daphnids
- acute toxicity tests with fish
- early life stage tests with fish
- maintenance and culture of fish.

These discussions provided an opportunity to pass on practical advice and also to indicate the data which had to be collected to fulfil GLP requirements.

Procedural audits

Staff of the TRC carried out acute toxicity tests with algae, daphnia and fish. The studies were audited by me with advice given on errors and omissions. Detailed discussions were held following the audits and a report made to the responsible scientist. This proved an effective method of introducing the concept of procedural audits as required by GLP, at the same time providing direct comment on methods at present employed in the laboratory.

Guidance on GLP

Throughout the visit the importance and relevance of GLP was stressed. Detailed guidance was given on the implementation of GLP in the maintenance of stock cultures and in testing. It was emphasised that staff should begin immediately to phase in some aspects of GLP. Aspects to be implemented as soon as possible were, the use of protocols, the maintenance of complete files of raw data, the generation and use of SOPs.

Field trials

Visits were made to a forestry field trial (proposals for the trial had been discussed during the first visit) and comments made on the approach taken. A demonstration of a field trial to examine effects of pesticides on stream invertebrates was organised and carried out.

The future need for field testing facilities for aquatic toxicology was discussed and the use and role of field experiments in assessing the hazard of pesticides described.

Seminar

A seminar - "The sequential approach to assessing the hazard of pesticides in the aquatic environment" was given.

Facilities and equipment

The facilities and equipping of the new laboratory, at present under construction, were discussed and recommendations made (Annex I).

II. FUTURE DEVELOPMENT IN AQUATIC TOXICOLOGY

Over the next two years development should focus on the establishing of GLP, gaining experience in the conduct of acute toxicity tests with fish, daphnia and algae and chronic toxicity tests with daphnia. The move into the new laboratory will also take place during this period.

Subsequent development in the new facilities should focus on the introduction of longer term testing with fish. Two tests are of major importance, the early life stage test with fish eggs and larvae and the bioaccumulation test. Some experience with a species suitable for early life stage tests, the killifish (medaka), already exists at the TRC but there is at present no experience of bioaccumulation testing.

Alongside the development of laboratory testing, outlined above, there is a need for continued development of skills and experience at assessing the effects of chemicals using field experiments.

It is highly desirable that the new laboratory should have some temperature controlled rooms and an increased facility for continuous-flow testing with fish.

The need for adequate analytical chemistry support for aquatic toxicology studies, laboratory and field, was stressed and this should be incorporated into the development of the biological testing programme.

III. CONCLUSIONS

The aquatic toxicology laboratory has the basic resources necessary for it to develop into an international accepted laboratory. Future development should focus on three areas, the implementation of GLP, increased practical experience in the conduct of basic aquatic toxicology tests with fish, Daphnia and algae and the development of chemical monitoring of exposure concentrations during laboratory toxicity tests.

IV. ANNEX I

Detailed Assessment and Recommendations

Assessment	Recommendation
GLP	
Few of the basic necessities of GLP are in operation. Much could be done to move towards compliance in advance of the establishment of a QA unit.	i) Protocols (Study plans) should be issued for all future studies in aquatic toxicology. ii) SOPs should be prepared for all items of GLP significant equipment and for all routine testing procedures. iii) Data collection (and storage) should be of a standard compatible with GLP.
Facilities	
Laboratory	
A move to new laboratory accommodation is planned in 1-2 yrs.	i) The new facility should have capability of holding more stock fish in flowing water conditions and be capable of supporting more continuous flow testing with fish - particularly important when larger species than those used at present are needed e.g. for bioaccumulation studies.

ii) There is a need for temperature controlled rooms operating in the range 15-30°C on a year round basis.

iii) Provision should be made for the separation of culture and testing laboratories, if possible.

iv) A small chemical laboratory for making stock solutions etc. should be available separated from but adjacent to the testing laboratories.

Field

There are no experimental facilities for field testing.

i) The provision of a set of small ponds for field testing of chemicals should be considered for 2-5 yrs.

ii) In the meantime experience of field testing should continue to be developed.

Equipment

Present level of equipment will not be adequate if the laboratory is to provide a full range of aquatic toxicity tests. There will be a greater requirement for continuous-flow testing with fish and equipment for invertebrate and algal testing requires upgrading if the throughput of testing is to increase.

i) equipment necessary for establishing the following continuous flow tests with fish should be provided
   - 96 h acute
   - bioaccumulation
   - early life stage.

ii) a more adequate facility for producing algal food for daphnid cultures should be established.

iii) for algal testing an orbital incubator or system of illuminated temperature controlled shakers with a greater capacity than that available at present is required.

Knowledge and Skills	
The theoretical knowledge of staff is good and well supported by documentation of international guidelines and regulatory requirements. There is however, as would be expected in a new laboratory, a lack of experience of putting this knowledge into practice in a routine and reproducible way.	i) Provision be made for members of staff to gain practical experience of aquatic toxicology in well established laboratories operating to GLP standards. ii) The possibility of collaborative research projects be pursued.
Chemical support to aquatic toxicology studies At present time only very limited chemical support is given to laboratory aquatic toxicology studies.	i) It is essential that plans be made to ensure that the need for chemical analysis in support of aquatic toxicology studies be met. (NB in many regulatory studies in aquatic toxicology in established laboratories the cost of chemical support equals or exceeds that of the biological component).
Test Organisms An adequate selection of test organisms is now available.	