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ECOTOXICOLOGY AND MARINE ECOLOGY STUDIES IN POST-WAR KUWAIT

159p
tables
graphs
diagrams
illus.

DP/KUW/92/003

KUWAIT

Technical report: Findings and recommendations*

Prepared for the Government of the State of Kuwait
by the United Nations Industrial Development Organization
acting as executing agency for the United Nations Development Programme

Based on the work of F.S.H. Abram, Expert on Ecotoxicology

Backstopping Officer: Yong-Hwa Kim
Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

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* This document has not been edited

V.96-85398

We regret that some of the pages in this report may not be up to the proper legibility standards, even though the best possible copy was used for scanning

CONTENTS

Final Technical Report: Development of Bioassay Facility

Abstract

The Kuwait Institute for Scientific Research is developing a Laboratory to perform routine testing and research in aquatic toxicology, and the United Nations (UNDP and UNIDO) has provided technical support towards this. The laboratory premises became available in late May 1995, and were officially opened the following November. This report describes progress.

KISR scientists have received training in aquatic bioassay methods, and preliminary tests have been made, but problems have been caused by a six-month delay in completion of the premises, and lack of financial provision for scientific apparatus. Nevertheless, funding has now become available for a research project on the toxicology of oils, and this study is well in hand.

Data for sighting the study from the preliminary tests are described, with drawings of apparatus constructed in the KISR workshops and by contract in the United Kingdom. Further equipment for the partial combustion of oils under laboratory conditions is designed and under development. An extension of the project to include studies on the growth and condition of fish is reviewed.

A programme for an overseas tour of advanced training for KISR personal is described, but this has not yet been implemented for budgetary reasons.

Proposals for financing a programme of routine bioassays as suggested by the United Nations are indicated, together with suggestions for integrating this with improved fiscal procedures for environmental control.

Final Technical Report: Development of Bioassay Facility

Notes for UNDP (Kuwait) and UNIDO (Vienna).

Introduction

This report follows sequentially from an Interim Report submitted in June 1995. It records further progress in commissioning a Bioassay Laboratory for the Kuwait Institute for Scientific Research, under a UNDP/UNIDO consultancy assignment from November 1994 to December 1995.

The Laboratory became available for use at the end of May 1995 (against an original completion date of October 1994), and the official opening was performed on 20th November by Dr. Ahmed A. Al-Rubai, Minister of Education, Higher Education, and Chairman of the Board of Trustees KISR. The United Nations was represented by Mrs. Mayada Homad, UNDP.

The principal objective of the United Nations is to further the protection of the aquatic environment around the Gulf states, following commissioning of the laboratory, and to be achieved by providing an operational focus for aquatic toxicology throughout the Levant. Additional factors include the training of KISR personnel, and the development of Good Laboratory Pratice Procedures. Details are given in the job description and project outputs, reiterated for convenience in Annex I.

1. Development of the Laboratory

1.1. Toxicity Tests.

The Laboratory became functional in June 1995, and to date two toxicity evaluations have been performed in addition to much work towards setting up research. The toxicity tests were 'routine' bioassays using Environmental Protection Agency protocols, and may be regarded both as a training exercise for acute tests (Annex I, job description 1.2) or as sighting procedures for research on the toxicology of oils (job description 1.3).

The selected test species were the encysted ova and nauplii of the brine shrimp, *Artemia salina*, and a species of sheim *Acanthopagrus SPP.* Both tests were made using procedures approaching Good Laboratory Practice, as indicated in the United Kingdom Compliance Scheme. Those using brine shrimp nauplii were made in parallel with an evaluation by Dr. P. Harrison (UNDP/UNIDO Expert in Marine Ecology) on the response of planula larvae from the coral *Acropora arabensis.*

Data from these tests are given comparitively in Tables 1 and 2, and for tests using sheim in Table 3. In Table 1, data are expressed in per cent by volume of the Water Accommodated Fraction of Kuwait Crude Oil; the undiluted WAF contained 5.0 mg of oil hydrocarbon per litre using analysis by the R.O.P.M.E. method.

Table 1. Toxicity of Kuwait Crude Oil to the ova and larvae of the brine shrimp

Test	Concentrations in per cent v/v WAF, with 95 per cent confidence limits in parentheses	
	24 hours	48 hours
Toxicity to Nauplii	LC50 91.8 (70.2-[120])	-
Per Cent Inhibition of Hatching	EC50* 69.8 (16.9-[289]) EC50+ 24.0 (4.2-[128])	37.5%** inhibition in 100 per cent WAF

* Observations by M. Metwally
** Observations by H. Al-Shemmari
\+ Observation by F.S.H. Abram

Table 2. Percentage settlement of *A.arabensis* larvae in different oil concentrations and in controls.

Concentration mg oil/litre	A	B	C	D	MEAN	STD.DV.
Control	26.4	52.0	54.1	42.2	43.7	12.63
0.01	34.0	52.6	66.0	31.3	46.0	16.37
0.10	0.0	10.2	24.5	11.1	11.5	10.05
0.31	10.7	4.4	0.0	3.0	4.5	4.51
1.00	0.0	0.0	0.0	0.0	0.0	0.0

Table 3. Mortality of Sheim Following Five Days Exposure to Dispersed Oil

Nominal Concentration of Oil Per Cent by Volume	Concentration of Hydrocarbon Analysis at End of Test ppm	Per cent Mortality After Five Days
2.00	0.105	50
1.00	0.079	30
0.56	0.031	10
0.32	0.029	10
0.18	0.052	20
Control	-	0

A median period of survival of 6000 minutes (95% confidence limits, 4532 to 8942 minutes) was found for the nominal concentration of 2.00 per cent by volume, and a 4-day LC value of 2.55 per cent V/V (95% confidence limits 0.509 to 12.8 per cent) was obtained.

KISR reports on these tests are given in Annex II, and the G.L.P. documentation in Annex III.

1.2. New Apparatus.

Specialised apparatus for research on the toxicology of oils has either been set up, or is under active development for a KISR research project, Ref. VR004P on the toxicology of crude and partly combusted oils. For convenience, a copy of this project is included as Annex IV. In brief, this research is to do with the effects of oil spills into the Arabian Gulf occasioned by the Gulf War, and correlates with item 1.3. of the job description, and 2.5. in the project outputs (Annex I).

Drawings of the apparatus for exposing fish or macro-invertebrates to a continuous flow of oil-saturated water are shown in Figs 1 and 2. Conditioned sea water is recirculated at a controlled rate through an oil contact flask and a constant head tank. When the water has become saturated with oil (to produce a reasonably uniform Water Accommodated Fraction), this is diluted from a further supply of seawater to produce a range of concentrations of WAF. These are delivered into test aquaria containing batches of fish.

The volumetric efficiency of the apparatus was assessed by dosing a standardised solution of methylene blue through the system, measuring the optical absorbance of the resulting dilutions of the dye, and comparing these absorbances with the values anticipated from the setting of the apparatus. A report on the experiment is given in Annex II.

1.3. Growth Rate Assessment.

The results of the preliminary toxicity tests on sheim (Table 3 and Annex II) indicates that the acute lethal toxicity of the Water Accommodated Fraction of Kuwait crude oil is unlikely to be great. Therefore increased emphasis is suggested on the sub-lethal (chronic) effects of oils, and an extension to project VR004P has been prepared covering effects on the condition and growth rate of fish under prolonged exposure. These experiments are based on a combination of the Weatherley Index, and the Carlender Condition Factor.

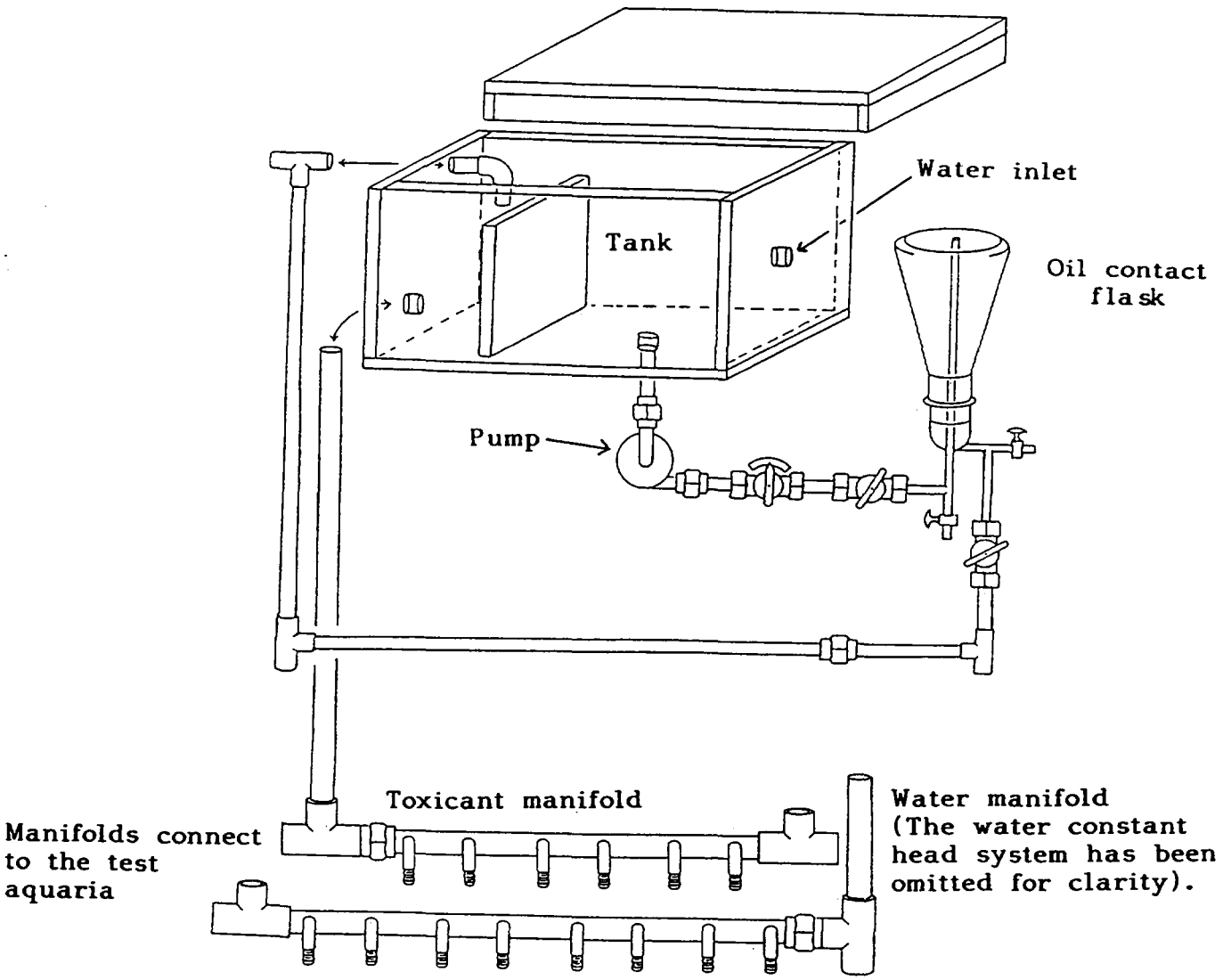
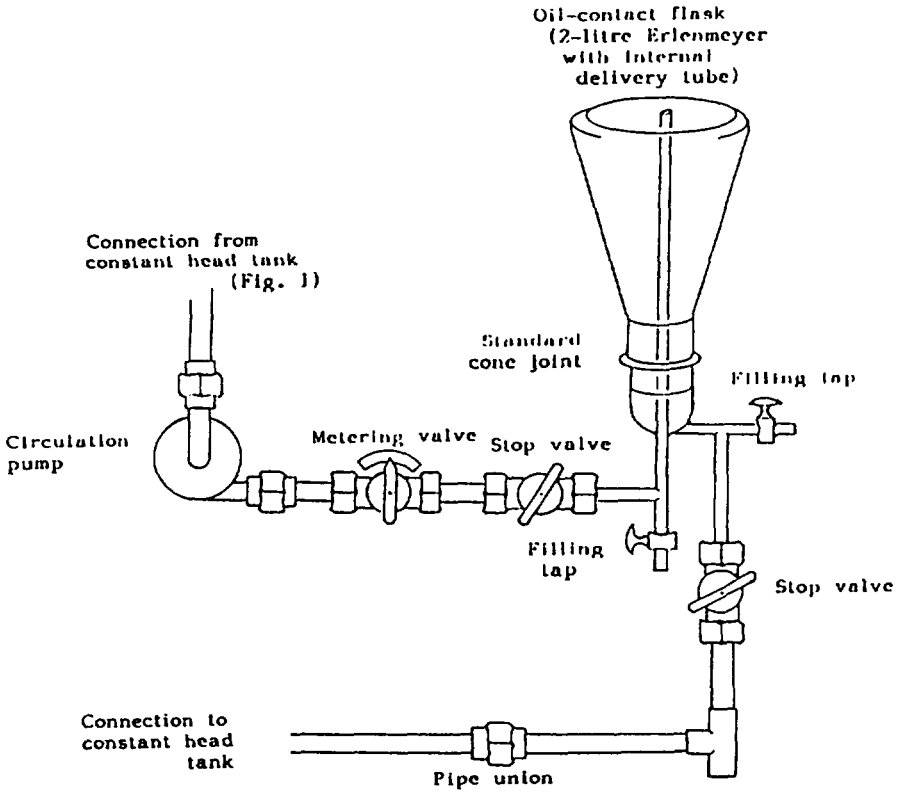
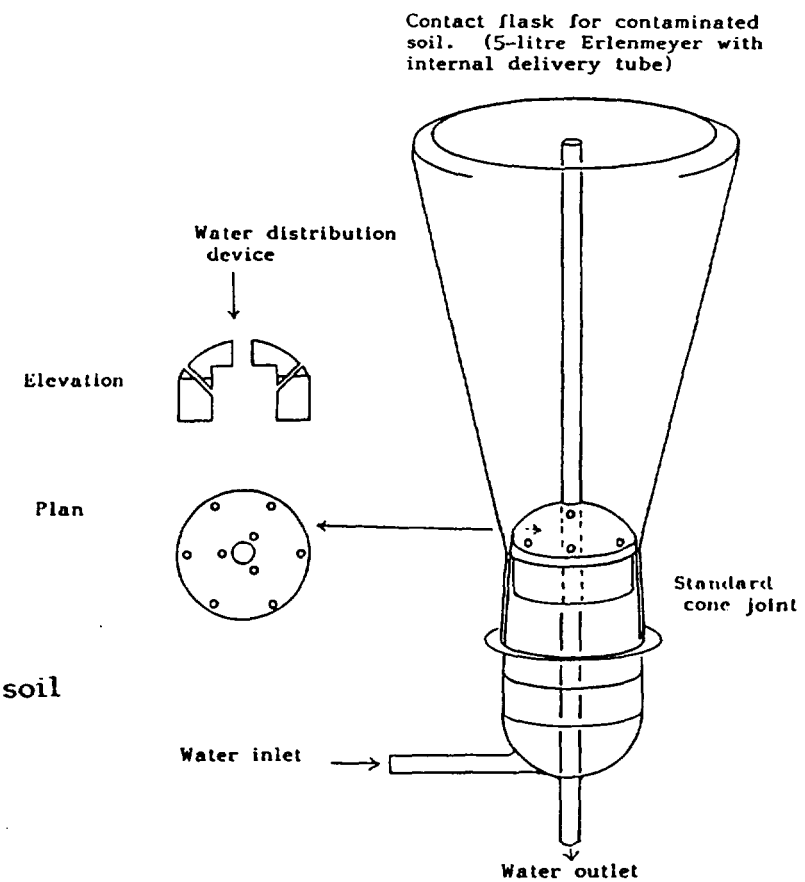


Fig. 1. Toxicity test apparatus, General diagram

This apparatus is designed to study the aquatic toxicology of oils or contaminated solid wastes. The principal is that water enters a constant head tank, is withdrawn from there by a centrifugal pump, and passed through an oil contact flask, before returning to the left-hand chamber of the tank. Hence a saturated oil/water solution is obtained. This is distributed to test aquaria at a range of controlled rates by a manifold. Water is similarly delivered; the rates are adjusted to give the desired concentrations.



A. Oil contact



B. Contaminated soil
contact

Fig. 2. Detail of contact systems

Provided that fish are fed consistently, and are free from disease or spawning stress, the Index linearises growth rate curves as a specific growth rate:

$$SGR = \frac{100 (\text{Log}_e F - \text{Log}_e I)}{D}$$

Where Log_e = Napierian Logarithm

F = Final weight of fish

I = Initial (starting) weight of fish

D = Period of test in days

The Condition Factor K relates the weight of fish to the fork length. If fish are thriving, the Factor is relatively high. It is given by:

$$K = \frac{100 \times \text{weight in grams}}{(\text{Fork length in cm})^3}$$

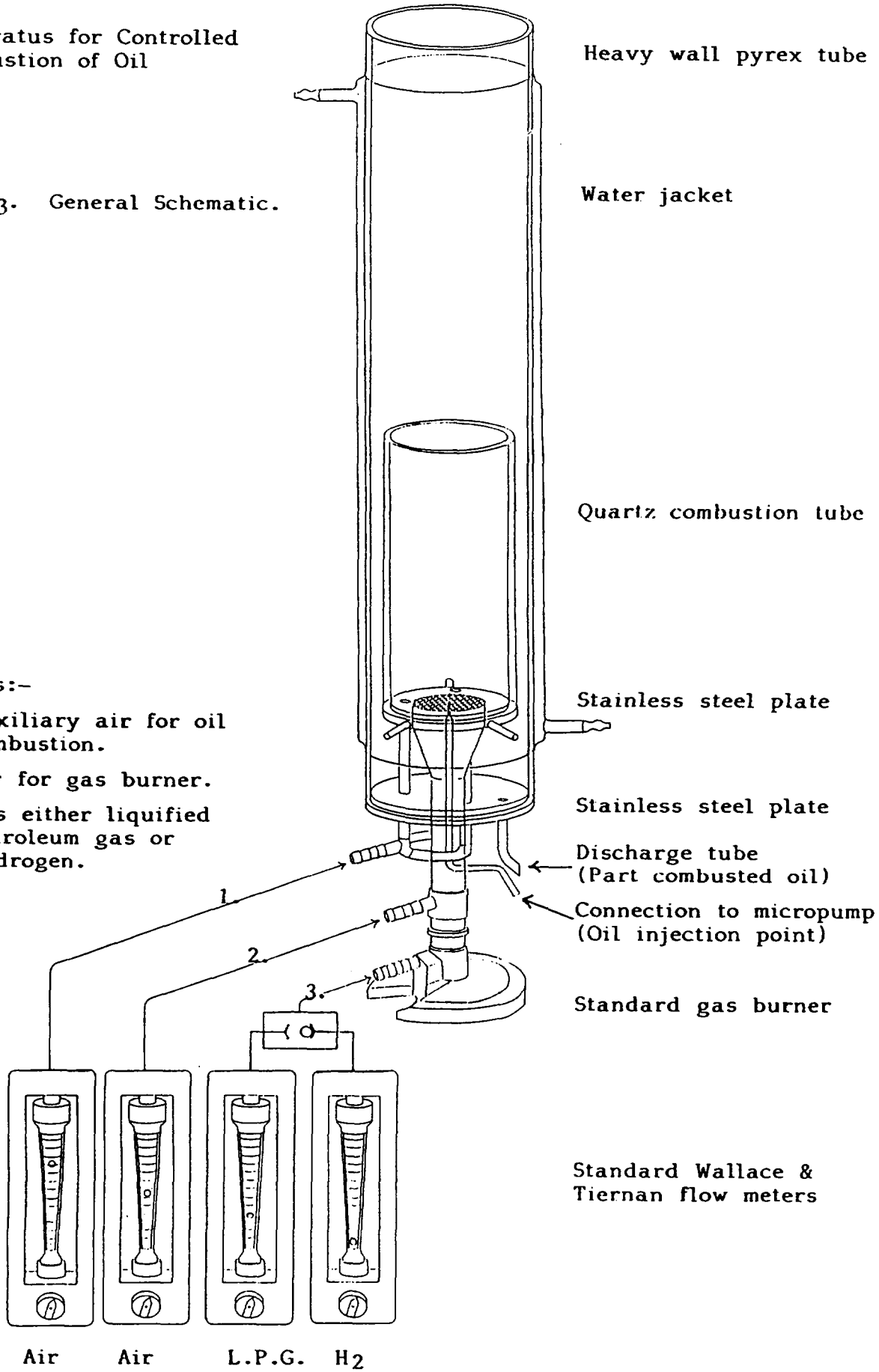
The overall effects of toxic stress on the growth of fish can be evaluated by a combination of the two formulae. Details are given in Annex II.

1.4. Development of Equipment.

During the Gulf Water, burning oil wells caused the deposition of large quantities of partially combusted oils onto the Arabian Gulf and adjacent areas, and investigation of the effects of this deposition is indicated in Project VR004P and by the United Nations, items 1.3 and 2.5 in Annex I. Samples of partly burned oils were reserved in 1991, but these are insufficient to support a research programme into the aquatic toxicology of such substances. Therefore laboratory equipment is being developed to simulate oil combustion and produce larger volumes. (These will be compared analytically with the 1991 samples).

Apparatus for Controlled Combustion of Oil

Fig. 3. General Schematic.



A sketch of the apparatus is given in Fig. 3. The principle is to induce combustion in a jet of oil by injecting it vertically through a gas flame, of which the size and temperature can be regulated by adjusting the inputs of air and a fuel gas. Secondary combustion of the oil is controlled further by regulating an additional inflow of air. A large glass condenser retains droplets and condensed vapour from the partly combusted oil.

Enquiries made to the Petroleum Division of KISR and to contacts within the United Kingdom oil industry failed to indicate a recognised standard method for the partial combustion of oils. Therefore it is considered that this apparatus could form the basis for a research paper, and possibly a protocol procedure. Further details of the design are given in Annex II.

1.5. Current Programme.

Immediate activity in the laboratory centres on an experiment to evaluate the maintenance of hydrocarbon concentrations in the fish test aquaria of the constant flow apparatus. In addition to confirming the reliability of the equipment, comparision of these data with the toxicology results from the sighting tests using sheim (Table 2 and Annex II) will enable calculation of a suitable range of hydrocarbon concentrations for the main experiments on bioconcentration and growth rates. The Study Plan (director Dr. Lulwa Ali) is included in Annex III.

2. Personnel and Operating Procedure

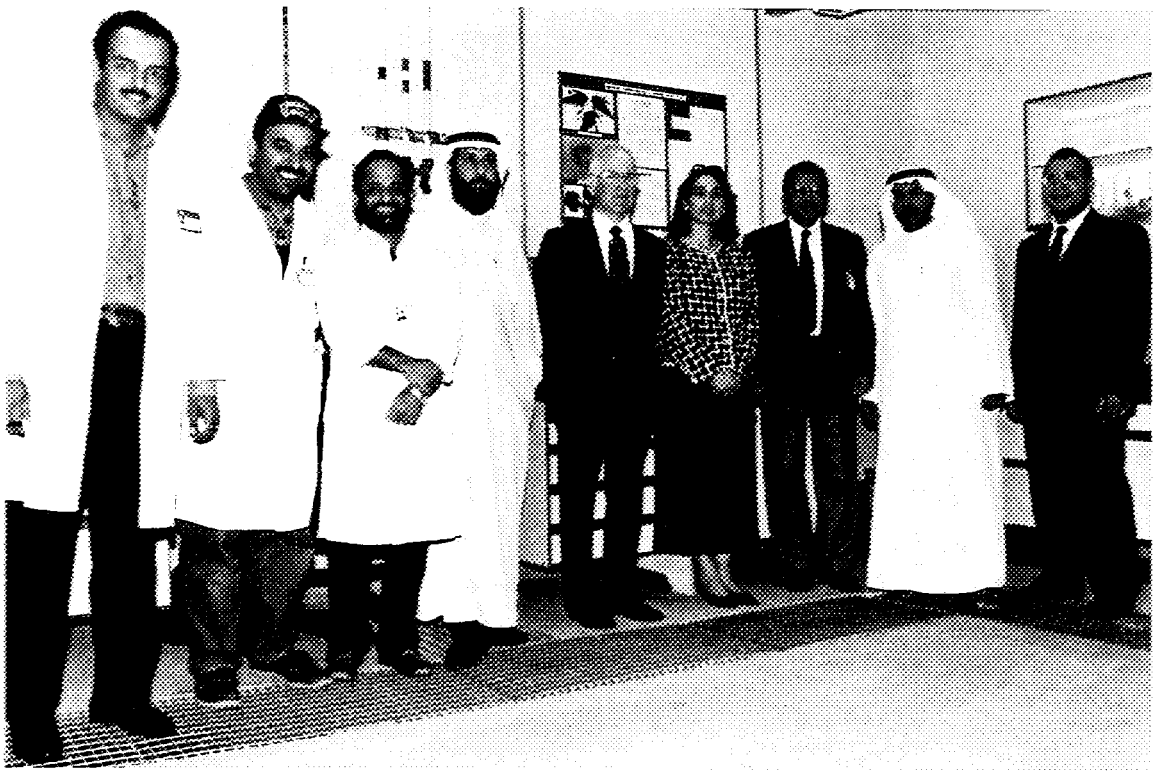
KISR staff allocated to Project VR004P are given in Table 4.

Table 4. Bioassay Laboratory Personnel

Name	Classification	
	KISR	Good Laboratory Practice
1. Dr. M. Metwally	Project Leader	Master Schedule Manager
2. Dr. L.N. Ali	Task Leader	Study Director
3. Mr. P.G. Jacob	Professional	Chemist
4. Mr. H. Al-Shemmari	Professional	Chemist
5. Mr. K. Al-Matrouk	Technician	Technician
6. Mr. W. Makarem	Technician	Technician
7. Mr. M. Bahloul	Technician	Technician
8. Mr. M.U. Beg	Researcher	Quality Assurance Officer

For convenience (e.g. for future visitors from the United Nations) a named photograph of the staff is included as Plate 1.

Lack of finance has prevented the allocation of personnel for general work on the toxicology of effluents, oil spills etc. but an operational tree has been submitted to the KISR management and is given in Fig 4.



From Left to Right

1. Mr. Waleed Makarem	Technician
2. Mr. Khalid Al-Matrouk	Technician
3. Mr. P.G. Jacob	Chemist
4. Mr. Hassan Al-Shemmari	Chemist
5. Mr. F.S.H.Abram	UNDP/UNIDO Consultant
6. Dr. Luwla Ali	Study Division
7. Dr. Mirza Beg	Quality Assurance Officer
8. Visitor	----
9. Dr. Mohammed Metwally	Master Schedule Manager

Plate 1. Bioassay Laboratory Personnel

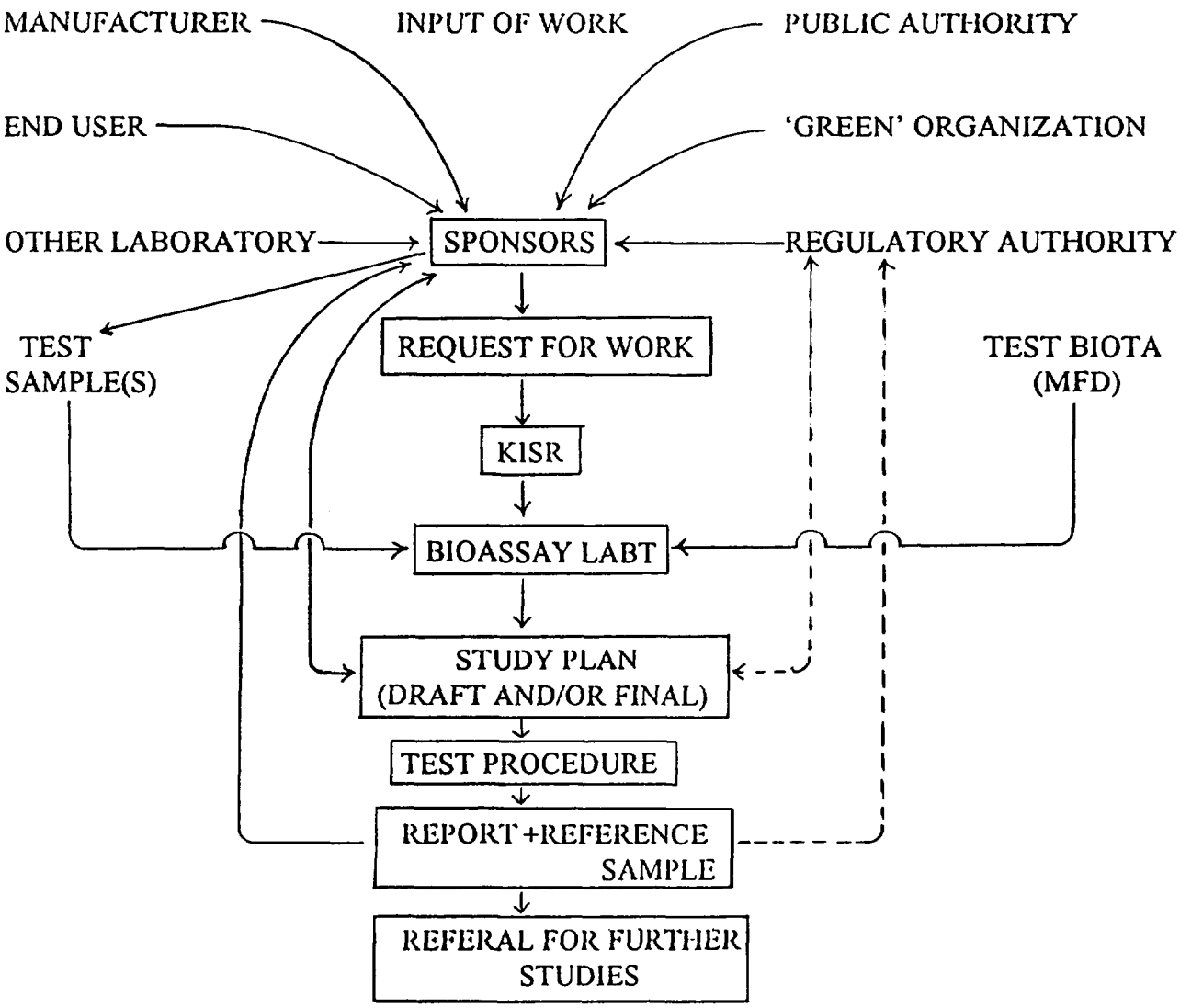


Fig. 4. Bioassay Laboratory Operational Tree

3. Inauguration of the Laboratory

As mentioned in the Introduction (1), the laboratory was formally opened on 20th November 1995, as part of the inauguration ceremony of the new Mariculture and Fisheries Department facilities on the Salmiyah site. The Bioassay Laboratory was included in the visitors tour of the premises, and great interest was expressed.

In addition to Kuwait nationals those attending included Mr. W.H. Fullerton (British Ambassador), Mrs. Mayada Homad (UNDP) and Mr. Aidan Broderick (Director, British Council).

In addition to photographs, posters were prepared illustrating the oyster embryo bioassay (under development as a modified Paris Commission Procedure) and the brine shrimp bioassay using the nauplius larva. The latter was supported by a practical demonstration. UNDP literature and posters raised considerable comment, particularly towards the protection of groundwater. This may be a important factor in the development of the Bioassay Laboratory; there is an undefined hazard from the unrestricted disposal of wastes into landfills. Mr. Abdul Wahab A. Naki (Mayor of Mansouria) expressed concern about the quality of water available for the tilapia farming in South Kuwait. A selection of photographs is included in Annex V.

4. Training of KISR Scientists

In house training has been arranged in aquatic toxicology procedures for the personnel indicated in Table 3, including detailed discussion with Dr. Mirza Beg on the development of Good Laboratory Practice Procedures. Three lectures have been given on biological statistics, as applying to bioassays and chronic toxicity tests.

Efforts have also been made in conjunction with personnel from the British Council (Kuwait and Marchester U.K.) to arrange concentrated overseas training for four Kuwaiti Scientists, namely Dr. Lulwa Ali (in charge of party) and Messrs. H. Al-Shemmari, K. Al-Matrouk and M. Bahloul. The proposed schedule includes a one-week training tour organised annually by the Chartered Institution of Water and Environmental Management, integrated with three weeks field work and practical studies in the laboratories of Hamilton Garrod Limited.

Training has not proceeded because of a lack of financial support, but it is hoped to implement the programme in 1996. Copies of correspondence are included in Annex III.

5. Good Laboratory Practice

A foundation for applying G.L.P. has been laid, but it appears improbable that full compliance will be achieved until a regular flow of effluent and industrial toxicity tests is moving through the laboratory, as indicated in Fig. 4. Nevertheless, all laboratory work has followed Study Plans written and approved to meet compliance, and using Standard Operating Procedures. Machinery has

been set up for the reception and registration of samples for testing, but is inactive because to date all work has centred upon a single input - namely Kuwait Light Crude Oil.

The principles and legal implications of GLP have been accepted by the KISR management. Copies of the paperwork are given in Annex III.

6. Future Development

Provided that financial support becomes available, it is considered that the forward implications of the bioassay facility are favourable. The principal inputs indicated by the United Nations are routine bioassays on effluents and accidental pollutants, together with applied research. Progress has been made in setting up a research programme, but lack of budgetary provision has inhibited work on arranging a programme of tests to monitor the toxicity of waste materials entering the Gulf and the groundwater aquifers.

Experience in Western Europe and the United States of America has indicated that bioassays of this type can play a central role in monitoring and controlling water pollution. Moreover, legislation is there in force which requires the biological screening of new substances before these are placed on the open market. Details of the legal position in western states are beyond the remit of this report, but essentially the company discharging the waste or seeking market clearance bears the cost of the tests.

Major industrial estates in Kuwait are located at Shuaiba, south of Kuwait City. Approximately 60 companies are active in the area, and a sketch map including effluent outfalls is given in Fig 5. Discussions with personnel from the

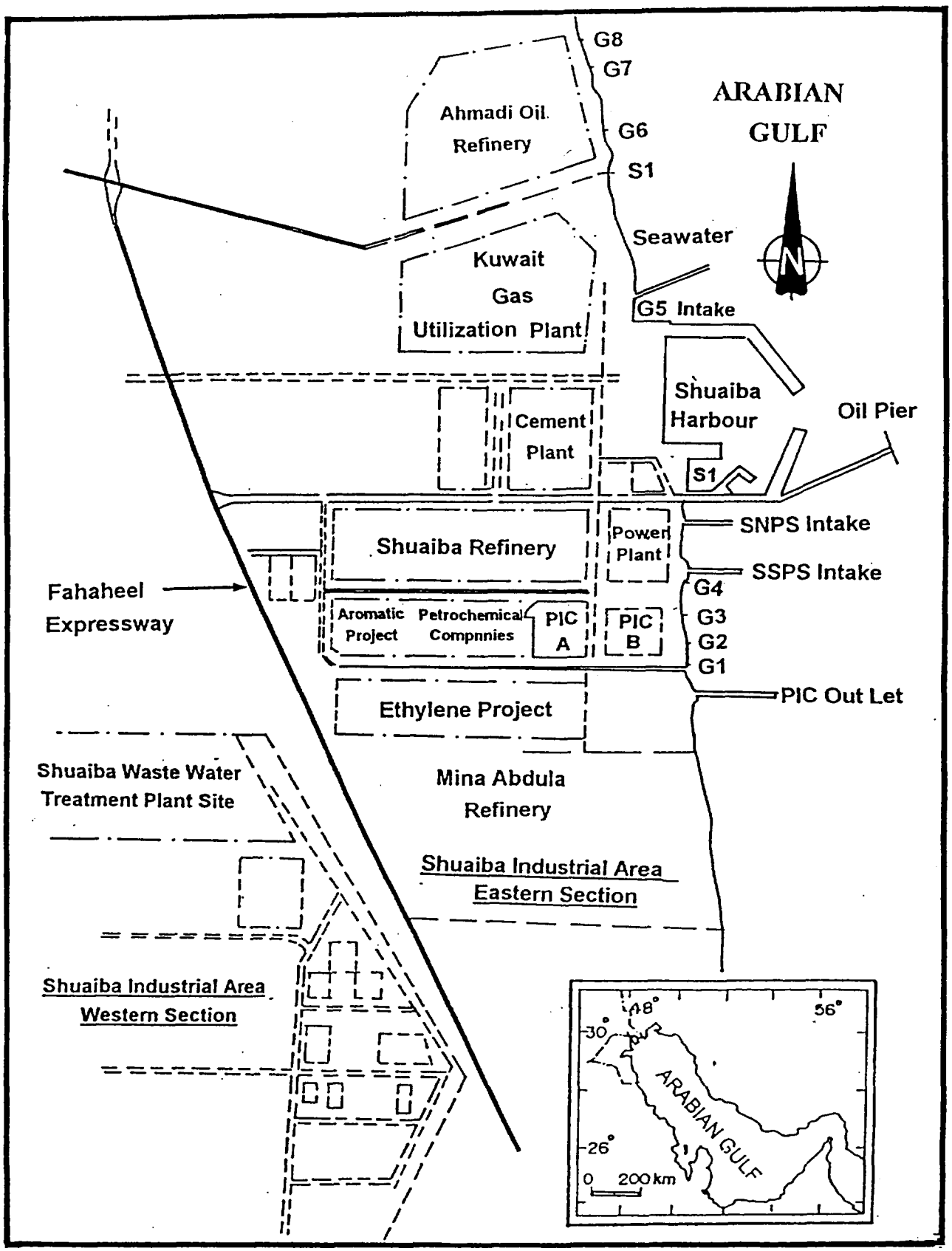


Fig.5. Shuaiba Industrial Area, showing effluent outfalls (1993).

area, and the Water Pollution Control staff of the Shuaiba Area Authority suggests that there is considerable scope for biological testing to assess water quality in the near shore zone. Also, it is recommended that emphasis should be directed to the fiscal background to environmental contamination, not only in Kuwait but within the Arabian Gulf States as a whole. Not only would this improve water quality in the Gulf, but would also provide selective finance for the operation of the Bioassay Laboratory, from providing a service facility.

Outline planning for a Workshop to support the use of the laboratory by the Gulf States has been prepared, and it is hoped to mount a suitable event in 1996.

7. Discussion

As indicated in the previous report (June 1995) development of the Bioassay Laboratory was impeded firstly by a six-month delay in the completion of the premises, and secondly by the lack by budgetary provision within KISR to purchase equipment. To some extent the position has been recovered; the laboratory is functional and a fair amount of biological work has been performed in addition to fitting out.

The tests on sheim provided information for sighting major studies using the same animal, and the brine shrimp data provide comparative results on a crustacean species which is common in the gulf, and have field significance in that they offer corelation between Dr. Harrison's work on coral larvae and a recognised protocol toxicity test.

Moreover, in the 1970-80s much work was performed using brine shrimps, mainly by Mr. P.G. Jacob (Table 4). Few recent data appear to be available, but brine shrimp tests are easy to make on a low budget, and the earlier work would provide useful comparitive data if effluent testing can be resumed - Annex I, 1.2 and 2.4.

However, financial problems remain in that the only available budget relates to a specific research project (VR004P) on the toxicology of crude and partly combusted oils - Annex IV. Thus major objectives of the United Nations in setting up routine tests for effluents and pollution incidents (Annex I, 1.2 and 2.4) have not been implemented because neither personnel nor money are available - reasons beyond United Nations control. Outside parties have expressed interest in the services potentially available in this context, recently by the M.A. Kharafi Company (Mr. Yasser Younes, Chemical Engineer) for toxicity tests on oil slick dispersants. An internal memorandum is included in Annex I.

These factors have been discussed with the middle management of KISR, and are indicated in the summary page of a UNDP Report, reproduced here as Table 5 and it is hoped that it will be possible to implement United Nations objectives within the foreseeable future by obtaining additional finance.

Table 5. Summary of Conclusions and Recommendations. Agreed with KISR.

UNITED NATIONS DEVELOPMENT PROGRAMME

PROJECT PERFORMANCE EVALUATION REPORT SUMMARY SHEET

Project Number and Title	Executing Agency	Date last report	Date this report	Planned date Tripartite Review
DP/KUW/92/003 Ecotoxicity & Marine Ecology Studies in Post War Kuwait	UNIDO	June 1995	November 1995	

	Original Budget (US$)	Latest Signed Revision (US$)
Total Budget (budget line 99)		
Government cost sharing (line 101)		
Other contributions (lines 103-8)		
UNDP contribution (line 999)		
Govt. cash contribution (from prodoc cover page)		
Govt. contribution in kind (in local currency)		

Project starting date		Project completion date	
Originally planned	Actual	Originally planned	Current estimate
November 1994	November 1994	October 1995	December 1995

SUMMARY OF CONCLUSIONS:

1. A Bioassay Laboratory, KISR, has been commissioned and is partly functional.
2. Little progress has been made in setting up a programme of routine toxicity tests.
3. A research project on the toxicology of oils is actively in hand.
4. Three technical reports have been produced from the new facility.
5. Attempts have been made to arrange overseas training for Kuwaiti scientists.
6. The project has been inconvenienced by delay in laboratory construction and lack of finance.

SUMMARY OF RECOMMENDATIONS: Referred to:-Mr. K.A. Philby (UNDP) Dr. Al-Attar (KISR)
(Whenever possible, indicate who should take the action and by when.)

1. Greater emphasis is needed on the toxicity testing of wastes; (2) above.
2. The Bioassay Laboratory should be operated additionally as a service laboratory.
3. KISR scientists should be encouraged to join appropriate learned societies.
4. Overseas training is recommended.
5. The Workshop project is commended for implementation in 1996.
6. KISR should increase the priority of this laboratory and manpower.
7. Improved provision should be made to a definite time scale.

Prepared by:		Distributed to:	Date:
Dhari Al-Ajmi Name and signature	Government Project Co-ordinator		
 Name and signature	Agency Project Co-ordinator		
F.S.H. Abram Name and signature	Other		

8. Additional Recommendation

It is suggested that KISR be advised to follow the policies of marine water quality control as developed for the North and Baltic Seas by the Paris and Oslo Commissions *(PARCOM and OSCOM). It is further recommended that the operation of the Bioassay Laboratory should be seen as part of overall environmental policy, and with attention to updating envrionmental law in Kuwait. To this end the services of a suitable expatriate lawyer (s) should be considered.

* Oslo and Paris Commissions
New Court, 48 Carey Street
London WC2A 2JQ
United Kingdom

ANNEX I

Job Description, Project Outputs, and Contact Persons
December 1995

ANNEX I

JOB DESCRIPTION

1.1. Provide advice on the equiping and start-up of a recently built Bioassay Laboratory:

1.2. Initiate and establish "routine" acute and chronic bioassays with algae, invertebrates and fish suitable for assessment of effluents and pollution incidents;

1.3. Initiate a research programme, involving laboratory, model ecosystem and ultimately field monitoring studies to determine the potential for chronic toxic effects of petroleum hydrocarbons in the Gulf region. Particular emphasis should be placed on the use of model ecosystems and field studies as a means of developing further understanding of the apparent limited impact of the recent large oil spills into the sea off Kuwait.

1.4. Train KISR stafff in the field of aquatic toxicology and aquatic toxicology and aquatic hazard assessment.

1.5. In this it is essential that international standards are maintained in following G.L.P, Q.C, Q.A and SOP in sampling, analysis, collection of data and in interpretation of the data.

ANNEX I

PROJECT OUTPUTS

2.1. Output 1

Reports on the present status within KISR of work in ecotoxicology related to the above mentioned problems, current plans experimental programmes, objectives and expertise; and a similar report for marine ecology.

2.2. Output 2

Based on the reports prepared under 3.1, detailed job descriptions and work plans for both a specific expert in marine ecology, to best address the current problems and to best integrate with other members of the proposed multi-disciplinary team.

2.3 Output 3

Kuwait Institute of Scientific Research (KISR) provided with advice to establish facilities for carrying out analytical, research work to follow the fate of toxic pollutants in the environment.

2.4. Output 4

Detailed assessment of movement of chemicals in soil, water and air made and zones mapped out according to the seriousness of problem.

2.5. Output 5

Risk assessment of toxic pollutants in the environment and their long term effects on humans, animals and aquatic life established based on known toxic data, including a specific assessment of the impact of oil pollution upon marine ecology.

2.6. Output 6

Laboratory model systems established so as to anticipate movement of chemicals and applied to field situations.

2.7. Output 7

At least 3 reports including a terminal report prepared giving various activities carried out, the results with findings and recommendations.

بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للابحاث العلمية

Date : 16th October, 1995 التاريخ :
Ref. No. : مرجع رقم

IN CONFIDENCE

Dr. Dhari Al-Ajmi
Divisional Director,
Environmental & Earth Sciences
KISR.

Dear Dr. Al-Ajmi,

Contract Toxicology

Mr. Yasser Younes, a chemical engineer employed by the Kuwait company M.A.Kharafi, has approached me regarding environmental tests on an oil slick dispersant which his company wishes to market within Kuwait. I explained that I was reasonably familiar with the European and American practices on pre-market evaluation, but was ignorant of the rules in Kuwait. Mr. Younes replied that he thought that such overseas methods would be acceptable here, provided that local species were used instead of the animals recommended in the protocols. He was grateful to receive a copy of the E.P.A. Protocol 40 CFR Part 300 II. (You will recall that this procedure has already been used in the bioassay laboratory for tests on brine shrimps and sheim). In Europe, biodegradability testing would also be required - a 28-day test to one of the O.E.C.D. protocols. Mr. Younes said that he would welcome a meeting to discuss the position.

This sort of work can be reasonably lucrative; in England my own company would charge around 2,000 Sterling - about KD 1,000. The decision is clearly one of KISR policy, and therefore I write to your good self in confidence. Nothing has been said to staff in E & ES; if you as Director are reluctant to become involved in contract testing the issue can thus be quietly abandoned without causing unproductive discussion. Perhaps you will be in touch.

With best wishes,

Yours Sincerely,

F.S.H. Abram
Consultant Biologist, UNIDO

ص.ب : ٢٤٨٨٥ الكويت - الصفاة 13109 ت: ٤٨٣٦١٠٠ - ٤٨١٦٩٨٨ - برقياً: ساينس كويت - فاكس: ٤٨٤٦٨٩١
P. O. Box 24885 - 13109 Safat - Kuwait - Telephone: 4836100 - 4816988 - Cable: SCIENCE Kuwait - Fax: 4846891

MAJOR CONTACTS, KISR

1. Dr. Mohammed H. Al-Attar	Deputy Director General KISR
2. Mr. Majed N. AlShammari	Legal Advisor, KISR

Environmental and Earth Sciences

3. Dr. Dhari N. Al-Ajmi	Divisional Director
4. Dr. Saleh Al-Muzaini	Divisional Manager, Environmental Sciences.
5. Dr. Mohammed Metwally	Associate Research Scientist
6. Dr. Lulwa N. Ali	Research Scientist
7. Dr. Mirza U. Beg	Associate Research Scientist
8. Dr. Andy Yaw Kwarteng	Associate Research Scientist
9. Dr. Sheik Talat Saad	Associate Research Scientist

Mariculture and Fisheries Department

10. Dr. Sulaiman M. Almatar	Divisional Manager
11. Mr. Shaker H. Al-Hazeem	Research Associate
12. Mr. Alan Lennox	Engineer

Additional Contacts

13. Dr. Ahmed A. Mal-Allan	Head of Water Pollution Control, Shuaiba Area Authority
14. Mrs. Kaiser Tarique	University of Kuwait
15. Mr. Yasser Younes	M.A.Kharafi Company
16. Mr. Abdul Wahab A. Naki	Mayor of Mansouria, Director of Al-Wafra Tilapia Farm
17. Mr. Aidan Broderick	Director, British Council

ANNEX II

Reports on Laboratory Studies

December 1995

Report

THE ACUTE TOXICITY OF KUWAIT CRUDE OIL TO BRINE SHRIMPS

H. Al-Shemmari
K. Al-Matrouk
M. Al-Bahloul

REPORT FOR THE ENVIRONMENTAL SCIENCES DEPARTMENT
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The Acute Toxicity of Kuwait Crude Oil to Brine Shrimps

Environmental Sciences Department, Environmental and Earth Sciences Division
Kuwait Institute for Scientific Research, Kuwait

SUMMARY

The fauna of the Arabian Gulf may be adversely affected by the discharge of petroleum hydrocarbons within the offshore zone of the Kuwaiti coast, and the Kuwait Institute for Scientific Research has been commissioned to evaluate this hazard. The investigation reported here was designed as a preparatory procedure towards a major research project - Ref. No VR004P. leader Dr. M. Metwally[1].

An assessment was made of the acute toxicity of Kuwait light crude oil (Water Accommodated Fraction (WAF)) using the brine shrimp, *Artemia salina*, as the experimental animal. Two types of tests were made, namely on the acute lethal toxicity of the WAF on the nauplius larva of the shrimps, and on the inhibition in the hatching of the shrimp eggs on exposure to the WAF.

The 24-hour LC50 value to the nauplius was found to be 91.8 per unit WAF by volume (95 per cent confidence limits 70.2 to [120] per cent). Analysis of the WAF using the ROPME[5] method, indicates equivalent hydrocarbon concentration of 105 mg oil litre.

The 24-hour EC50 value for inhibition of hatching was estimated by two workers with values of 69.8 per cent WAF, and 24.0 per cent WAF. The respective 95 per cent confidence limits were 19.9 to [289] per cent and 4.2 to [128] per cent. Therefore, the two values are not significantly different, P=0.05.

In 48 hours, 37.5 per cent inhibition was recorded in undiluted WAF.

These data are thought sufficient to indicate the acute response of Arabian Gulf crustaceae to oil contamination.

Dr. Mohammed Metwally / /1995
(Study Director)

Dr. Lulwa Ali / /1995
(Task Leader)

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1. Introduction

The main goal of this bioassay study was to train staff from the Kuwait Institute for Scientific Research, Environmental Sciences Department, in bioassay experiments. The institute has been commissioned to assess possible adverse effects of crude oil and partially combusted oils upon the fauna of the Arabian Gulf. These experiments were also made as a sighting procedure for a research project on this topic, VR004P, project leader Dr. M. Metwally[1].

Two experiments were conducted using brine shrimp (*Artemia salina*) with the water accommodated fraction (WAF) from Kuwait crude oil. The first experiment was on the toxicity of the WAF to the nauplius larva of brine shrimp as described in a protocol of the Environmental Protection Agency[2]. This is considered suitable for preparation and a baseline for project VR004P. The second experiment was a brine shrimp hatching test to assess inhibition of hatching of the shrimp eggs. This experiment is very simple and cheap to perform. The brine shrimp is the first organism ever used in toxicity tests starting from resting eggs (Soares et al., 1993)[3]. The larvae which were hatched from these cysts were cultured under controlled laboratory condition.

2. Materials and Methods

2.1. Chemical

2.1.1. Oil

The source of oil was from KISR's Petroleum Division. It was a light Kuwait crude oil with specific gravity of 1.028.

2.1.2. Preparation of Water Accommodated Fraction (WAF)

The WAF was prepared as described by Harrison [4]. Nine litres of seawater were introduced to a 10-litre borosilicate glass aspirator and 1-litre of oil was added. Thus the resultant oil to water ratio was 1:9 v/v. The aspirator was sealed and stirred magnetically on a stirring block for three hours at a rate sufficient to induce a vortex drawing in the oil downward into contact with stirring paddle.

At the time of sampling, stirring was stopped and the solution left to stand for 30 min. After discarding the first two liters, WAF was collected in 1-l glass amber bottle and stored in the refrigerator at 4°C.

2.1.3. Determination of Total Hydrocarbons of the WAF by Ultraviolet Fluorescence Spectroscopy

The concentration of total hydrocarbon in the WAF sample was determined by an ultraviolet fluorescence technique according to the ROPME Manual (ROPME, 1986)[5].

i. Construction of calibration curve

A series of standard solutions of Kuwait crude oil in a mixture of hexane and dichloromethane (7:3) were prepared in the range between 0 to 40 ppm.

UVF parameters were set as follows:-

Excitation wavelength = 310 nm

Emission wavelength = 360 nm

Width band Ex = 3.0 and Em = 10.0

Analysis was duplicated using concentration versus fluorescence reading of Kuwait crude oil standards.

Calibration is given in Table I, and shown graphically in Figure 1.

Table I. Flourescence spectroscopy of WAF calibration data:

Standard (Conc. ppm)	Fluorescence (Arbitrary Units)
0 ppm	4.9
5	11.6
10	14.5
15	22.9
20	28.2
25	34.9
30	42.3
35	46.3
40	53.3

A calibration curve was plotted which showed linearity in the range between 0 and 40 ppm (see Fig. 1). Statistical analysis of r and slope indicated a good fit (r= 0.998, p=0.05).

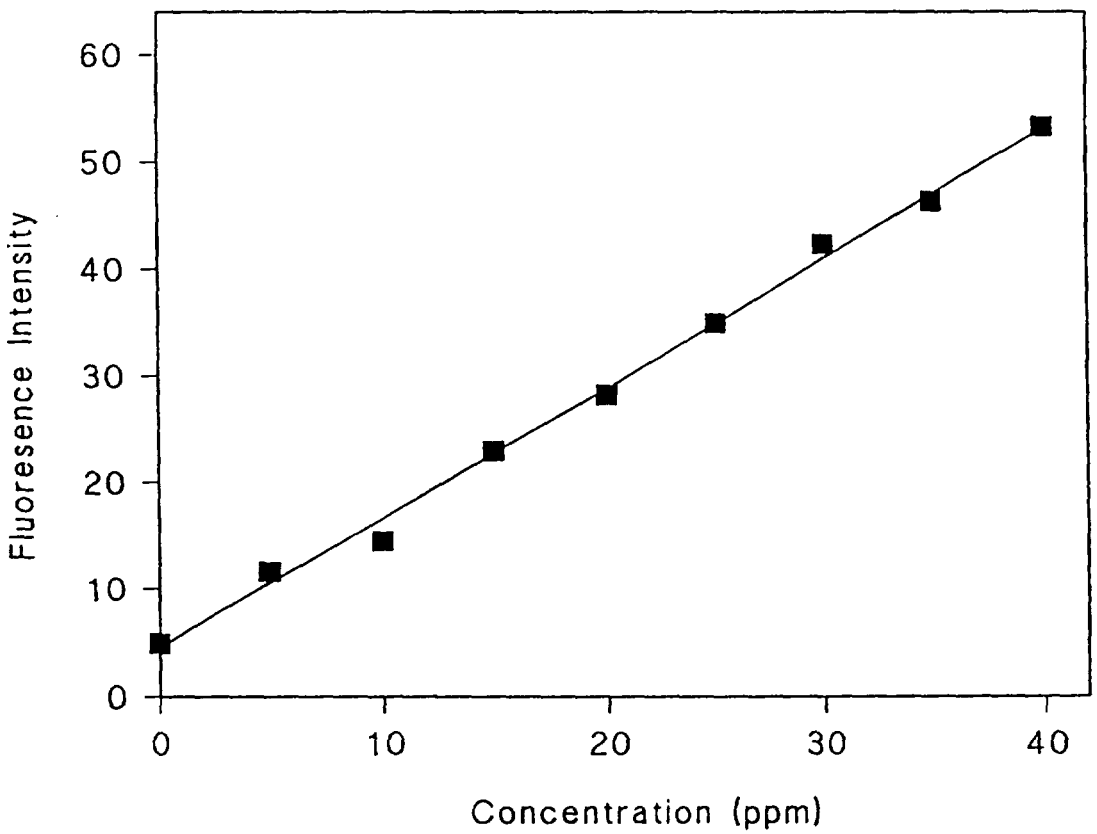

Fig. 1. UVF calibration curve of Water Accomodated Fraction .

ii. Analysis of Water Accommodated Fraction

An aliquot (100 ml) of WAF was extracted twice with 10 ml of a mixture of hexane and dichloromethane (7:3). The extract was then concentrated to 5 ml with a gentle nitrogen blow down. Analysis was made on the extract before and after certain dilutions.

2.2 Biological

2.2.1 Brine Shrimps Eggs (*Artemia salina*)

A supply of brine shrimp eggs was provided by the Mariculture and Fisheries Department (MFD) KISR, which reports that they were produced by Artemia Systems N.V. Baasrode Belgium; and were stored in a sealed container under cold conditions before use.

2.2.2 Dilution Water

The WAF was prepared and the tests performed in a natural seawater obtained from the Arabian Gulf by pumping from a near-shore well, which provides sand filtration. The salinity was found to be 24.0 grams/litre in chloride and 39.5 grams/litre in NaCl. The specific gravity was 1.028, the pH value 8.0, and the concentration of dissolved oxygen was close to the saturation value.

2.2.3 Test Methods

i) Toxicity to Nauplius Larva

As mentioned in the introduction the study using WAF to nauplius larva of brine shrimp is described in the protocol of the EPA[2]. The production of larva was in a glass beaker (about 5-litres) covered with black polythene and containing about 3-litres of natural seawater. Approximately 0.5 gram of brine shrimp eggs were added and incubated until numerous hatching was apparent (about 24 hours). Nauplii are photoactic and a light

beam was projected through a window in the polythene; to obtain nauplii, the end of a dipping pipette was located in the light beam. pH values, concentrations of dissolved oxygen and temperature in the hatching beaker were recorded. In the toxicity test (Bioassay) the nauplii were randomized into batches of 20 larva in 20 ml seawater. Five test concentrations were evaluated with five replicates for each and a control. (The method is described in the Standard Operating Procedure KISR Ref No: 0200 dated 17th June 1995). The experimental schema is given in Table 2.

Table 2. Toxicity of Kuwait Crude Oil to the Nauplius of the Brine Shrimp.

Experimental Lay Out with Concentration Expressed in Per Cent by Volume of the Water Accommodated Fraction (WAF)

No.	Concentration Per Cent of WAF	Volumes, ml		Total ml*	No. Replicates	No. of Total Nauplii
		WAF	Seawater			
1	80.0	80.0	-	100	5	100
2	56.0	56.0	24.0	100	5	100
3	32.0	32.0	48.0	100	5	100
4	17.8	17.8	62.2	100	5	100
5	10.0	10.0	70.0	100	5	100
6	Control	-	80.0	100	5	100

* Includes 20 ml containing the nauplii

pH values were recorded using a freshly calibrated ELE pH meter and concentration of dissolved oxygen using a polarographic electrode attached to the same instrument. Temperatures were recorded using a mercury in glass thermometer. Data are given in Annex Table 1.

ii) Hatching Test

This test is described in the Standard Operating Procedure KISR TOX 0100 dated 6th June 1995. Twenty mg aliquots of brine shrimp eggs were weighed into individual beakers. Each concentration was tested in duplicate over the concentration range 10 to 100% of WAF.

Five concentrations were set up at geometric intervals and incubated in the dark at a temperature of 20°C for 48 hours. pH values and concentration of dissolved oxygen were recorded. The evaluated parameter was the percent hatching by visual comparison with the controls.

2.2.4 Mathematical Methods

Individual nauplii were recorded as dead when there was no visible movement nor reaction to gentle stimulation. Mortalities were recorded at 24 hours from the first introduction of toxicant and nauplii which had disappeared from the test flasks were counted as dead, disintegration after death being assumed.

Per cent hatching of eggs was estimated by visual comparison between the test flasks and the controls following one and two days incubation.

LC50 and EC50 values with 95 per cent confidence limits were estimated using the method of Gramo and Larsstuvold[6].

3. Results

3.1 Determination of the Concentration of Oil in WAF

The WAF sample was analyzed undiluted and after dilution. The concentration as read of the calibration curve were as follows:

Undiluted sample = 105 ppm

After 5 times dilution = 19.3 ppm

After 4 times dilution = 27.4 ppm

These results showed that the prepared WAF contained 5 mg oil/L.

3.2 Toxicity of WAF to Brine Shrimps

LC50 and EC50 values with 95 per cent confidence limits are given in Table 2.

Table 2. Toxicity of Kuwait Crude Oil to Brine Shrimps.

Concentrations Expressed in Per Cent of the Water Accommodated Fraction

Test	24 Hour Values (95% Confidence Limits in Parenthesis)	48 Hour Values
Toxicity to Nauplii	LC50 91.8 (70.2-[120])	-
Per Cent Inhibition of Hatching	EC50* 69.8 (16.9-[289]) EC50+ 24.0 (4.2-[128])	37.5%** inhibition in 100 per cent WAF

* Observations by M. Metwally
** Observations by H. Al-Shemmari
\+ Observation by F.S.H. Abram

Individual records of mortality and per cent hatching are given in Annex Table III, together with details of statistical analysis for LC50 and EC50 values.

4. Discussion

The toxic effects of petroleum hydrocarbons upon the biota of the Arabian Gulf is clearly important for reasons which include possible adverse environmental effects, depletion of the fishery, and potential hazards to human health from substances taken up into fish by bioconcentration[7]. The tests described here were designed as a preliminary experiment to indicate the order of lethal toxicity associated with the WAF of Kuwait crude oil, as prepared by a standardised procedure. This procedure[4] is described under materials and methods and is considered to be reasonably comparable with that designed for use in Research Project VR004P[1], as shown in Fig. 2.

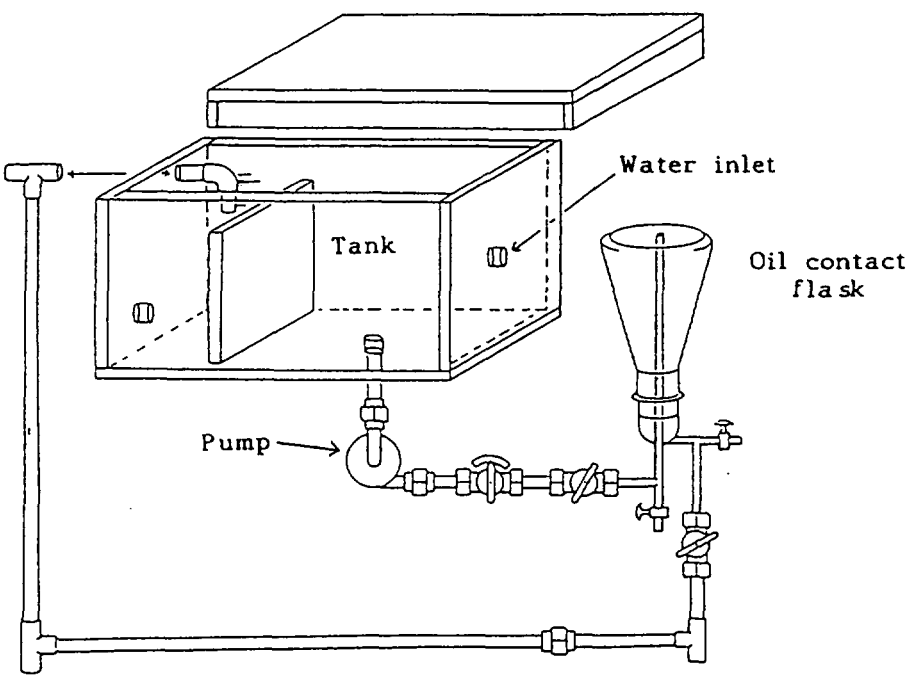

Fig. 2. Deatil of oil saturation assembly (Project VR004P).

In this apparatus an aliquot of oil is constrained in an inverted Erlenmeyer flask, and oil/water contact maintained by pumping a stream of water through a vertical tube. As indicated in materials and methods, the method used here was to stir the mixture mechanically in a closed aspirator bottle.

The results of UVF analysis showed that WAF, as prepared by high stirring rate contained oil at a concentration of 5 mgL^{-1}. However, since the fluorescence method relies essentially on the fluorescence of the more soluble and amulsified aromatic components of oil in water, this concentration therefore, represents a measure of this particular group in the accommodated fraction of the oil in seawater. Aromatics are known to be among the most toxic compounds of crude oil[7,8]. Although one cannot predict accurately the toxicity of a complex mixture such as WAF since oil hydrocarbons vary markely in their toxicity[9,10]. Nevertheless, useful information could be gained on the overall toxicity of oil fraction upon exposure to marine organisms at diffirent exposure concentrations.

The biological results indicate that concentrations of this order are acutely lethal to brine shrimp nauplii, and also have an inbitory effect on the hatching of the encysted ova of the species. It is dangerous to forecast the long-term response of animals from short-term tests, but nevertheless, these data confirm that a significant hazard exists. Therefore, more extensive work is necessary.

5. Conclusion

5.1 Tests have been made to assess the toxicity of the WAF of Kuwait crude oil, using the brine shrimp as the experimental organisms.

5.2 Procedures were used to evaluate the lethal response of the nauplius larva of the brine shrimp to WAF, and the inhibitory effects of WAF on the hatching of the ova of the brine shrimp.

5.3 The 24-hour LC50 value to nauplius larvae was found to be 91.8 per cent WAF by volume, with 95 per cent confidence limits of 70.2 to [120] per cent.

5.4 Two 24-hour EC50 values were obtained for the inhibition of hatching of brine shrimp ova, namely 69.8 per cent WAF, with confidence limits of 16.9 to [289] per cent, and 24.0 per cent, limits 4.2 to [128] per cent. (The confidence limits are wide, and therefore these values are not significantly different, P=0.05).

5.5 An inhibition in hatching of 37.5 per cent was recorded in 48 hours in the undiluted WAF.

5.6 WAF experiment was done by using the mechanical extraction method, then analyzed by spectrofluorophotometer yield using a concentration of 105 ppm.

ANNEX

Table IA. pH Values

Nauplius Test.

	Reference No. and Concentration, Per Cent WAF					
Replicate	I 80.0	II 56.0	III 32.0	IV 17.8	V 10.0	VI Control
Start						
A	7.7	7.9	7.8	8.0	8.0	8.0
B	7.8	7.9	8.0	8.0	8.0	8.0
C	7.8	7.9	8.0	8.0	8.0	8.0
D	7.7.	7.9	8.0	8.0	8.1	8.0
E	7.8	7.9	8.0	8.0	8.1	8.0
24 Hours						
A	7.9	8.0	8.1	8.0	8.1	8.0
B	8.1	8.1	8.0	8.1	8.1	8.1
C	8.0	8.0	8.0	8.1	8.0	8.1
D	7.8	8.0	8.0	8.0	8.0	8.1
E	7.8	8.0	8.1	8.0	8.1	8.1
Mean Values	7.84	7.96	8.00	8.02	8.05	8.04

Overall mean value = pH 7.79 Standard deviation = ±0.101

The pH value of the dilution water was 8.0, and the (undiluted) WAF was pH 7.6.

ANNEX

Table 1B. pH values

Hatching Test

	Reference No. and Concentration in Per Cent WAF					
Replicate	I 100	II 56.0	III 32.0	IV 17.8	V 10.0	VI Control
Start						
A	7.6	7.7	7.9	7.8	8.0	8.0
B	7.7	7.6	7.9	7.9	7.9	8.1
24 Hours						
A	7.8	7.9	7.8	8.0	8.0	8.1
B	7.8	7.8	7.9	7.9	8.0	7.9
48 Hours						
A	7.3	7.6	7.6	7.7	7.7	7.7
B	7.5	7.6	7.7	7.6	7.7	7.8
Mean values	7.62	7.70	7.80	7.82	7.88	7.93

Overall mean value = pH 7.79 Standard deviation = ±0.18

ANNEX

Table II. Temperature, Degrees Celsius

Nauplius Test

The temperature at the start was adjusted to 19.5°C throughout

	Reference No. and Concentration, Per Cent WAF					
Replicate	I 80.0	II 56.0	III 32.0	IV 17.8	V 10.0	VI Control
Start						
A	19.6	19.5	19.4	19.5	20.0	20.5
B	19.6	19.7	19.5	19.5	20.0	20.4
C	19.4	19.5	19.5	19.5	19.8	20.0
D	19.5	19.4	19.5	19.4	19.6	20.0
E	19.4	19.4	19.4	19.4	19.7	19.8
Mean values	19.5	19.5	19.5	19.5	19.8	20.1

Overall mean value* = 19.57°C standard deviation = ±0.22°C

* Includes start temperature 19.5°C

ANNEX

Table IIIA. Summary of Biological Observations

Nauplius Test. Individual Per Cent Mortalities

	Reference No. and Concentration, Per Cent WAF					
Replicate	I 80.0	II 56.0	III 32.0	IV 17.8	V 10.0	VI Control
24 Hours						
A	60	25	25	0	25	20
B	55	25	25	10	30	0
C	40	0	50	30	0	10
D	60	40	0	35	0	0
E	55	65	10	5	15	0
Mean values	54%	31%	22%	16%	14%	6.0%

ANNEX

Table IIIB. Summary of Biological Observations

Hatching Test. Estimated Per Cent Hatching

	Reference No. and Concentration in Per Cent WAF					
Replicate	I 100	II 56.0	III 32.0	IV 17.8	V 10.0	VI Control
24 Hours						
A	40	60	80	80	90	100
B	50	50	70	75	100	90
Mean Per Cent*	45	55	75	77.5	95	-
A	30	20	40	60	60	100
B	20	30	30	70	80	100
Mean Per Cent+	25	25	35	65	70	-
48 Hours						
A	60	75	75	90	90	100
B	65	75	75	85	80	100
Mean values#	62.5	75	75	87.5	85	-

* Observations by M. Metwally
+ F.S.H. Abram # H. Al-Shemmari

Toxicity of Material to Fish

LC50 Values. Test Substance = Kuwait Crude Oil , WAF in Seawater Test Species = Artemia salina nauplii

Period of Exposure = One day, 1440 minutes, 24 hours

Conc. % v/v	Per cent mortality				X^2
	O	E	C	D	
80.0	54	46.4		7.6	0.022
56.0	31	38.2		7.2	0.022
32.0	22	26.3		4.3	0.010
17.8	16	18.7		2.7	0.006
10.0	14	9.8		4.2	0.021

ΣX^2 0.081 N= 20 N' =
= Significant
LC
16 = 17.2
50 = 91.8 S = 5.337
84 = >100 Exponent = 1.307
LC50 value = 91.8% WAF
95% confidence limits
upper = [120.0]
lower = 70.2

Period of Exposure = Two days, 2880 minutes, 48 hours

Conc. % v/v	Per cent mortality				X^2
	O	E	C	D	
80.0	42				
56.0	35				
32.0	46				
17.8	51				
10.0	58				

ΣX^2 N= N' =
LC
16 =
50 = S = 5.337
84 = Exponent = 1.307
LC50 value =
95% confidence limits
upper =
lower =

Period of Exposure =

Conc. % v/v	Per cent mortality				X^2
	O	E	C	D	

ΣX^2 N = N' =
LC
16 =
50 = S =
84 = Exponent =
LC50 value =
95% confidence limits
upper =
lower =

Toxicity of Material to Fish

EC50 Values. Test Substance = Kuwait Crude Oil , WAF in Seawater Test Species = Artemia salina nauplii

Period of Exposure = One day, 1440 minutes, 24 hours

Conc. % v/v	Per cent mortality				
	O	E	C	D	X^2
100.0	45	39.2		5.8	0.014
56.0	45	57.0		8.0	0.025
31.6	75	72.0		3.0	0.004
17.8	77.5	85.0		7.5	0.044
10.0	95	92.5		2.5	0.009

ΣX^2 0.096 N= [20] N' = [100]

EC

16 = -

50 = 69.8%] S = 3.835

84 = > 18.2%] Exponent = 4.14

LC50 value = 69.8%

95% confidence limits

upper = [289]

lower = 16.9

Period of Exposure = One day, 1440 minutes, 24 hours

Conc. % v/v	Per cent mortality				
	O	E	C	D	X^2
100.0	25	22.3		1.7	0.001
56.0	25	32.2		7.2	0.024
31.6	35	43.8		8.8	0.030
17.8	65	56.6		8.4	0.030
10.0	70	68.0		2.0	0.002

ΣX^2 0.087 N= [20] N' =[100]

EC

16 = -

50 = 24.0%] S = 5.714

84 = 4.2%] Exponent = 5.33

EC50 value = 24.0%

95% confidence limits

upper = [128]

lower = 4.51

Period of Exposure =

Conc. % v/v	Per cent mortality				
	O	E	C	D	X^2
100.0	62.5	62.5		0.0	-
56.2	75.0	73.8		1.2	0.001
31.6	75.0	81.6		6.6	0.020
17.8	87.5	88.0		0.5	-
10.0	85.0	84.2		0.8	-

ΣX^2 0.027 N = [20] N' = 100

EC

16 = -

50 = >100] S =

84 = 25.5] Exponent =

EC50 value =

95% confidence limits

upper =

lower =

Toxicity of Material to Fish

EC50 Values. Test Substance = Kuwait Crude Oil , WAF in Seawater Test Species = Artemia salina ova

Period of Exposure = One day, 1440 minutes, 24 hours

Conc. % v/v	Per cent inhibition of hatching				X^2
	O	E	C	D	
100.0	45	39.2		5.8	0.014
56.0	45	57.0		8.0	0.025
31.6	75	72.0		3.0	0.004
17.8	77.5	85.0		7.5	0.044
10.0	95	92.5		2.5	0.009

ΣX^2 0.096 N= [20] N' = [100]

EC

16 = -

50 = 69.8%] S = 3.835

84 = > 18.2%] Exponent = 4.14

LC50 value = 69.8%

95% confidence limits

upper = [289]

lower = 16.9

Period of Exposure = One day, 1440 minutes, 24 hours

Conc. % v/v	Per cent inhibition of hatching				X^2
	O	E	C	D	
100.0	25	22.3		1.7	0.001
56.0	25	32.2		7.2	0.024
31.6	35	43.8		8.8	0.030
17.8	65	56.6		8.4	0.030
10.0	70	68.0		2.0	0.002

ΣX^2 0.087 N= [20] N' =[100]

EC

16 = -

50 = 24.0%] S = 5.714

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upper = [128]

lower = 4.51

Period of Exposure =

Conc. % v/v	Per cent inhibition of hatching				X^2
	O	E	C	D	
100.0	62.5	62.5		0.0	-
56.2	75.0	73.8		1.2	0.001
31.6	75.0	81.6		6.6	0.020
17.8	87.5	88.0		0.5	-
10.0	85.0	84.2		0.8	-

ΣX^2 0.027 N = [20] N' = 100

EC

16 = -

50 = >100] S =

84 = 25.5] Exponent =

EC50 value =

95% confidence limits

upper =

lower =

Report

THE ACUTE TOXICITY OF KUWAIT CRUDE OIL TO SHEIM

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The Acute Toxicity of Kuwait crude Oil to Sheim

Contents

The Acute Toxicity of Kuwait Crude Oil to Sheim

SUMMARY

This report describes the Task 2 activities of the project VR004P, "Toxicity and Uptake of Crude Oil and Partially Combusted Oil by Selected Marine Organisms in Kuwait".

The Kuwait Institute for Scientific Research is investigating the aquatic toxicity of petroleum-based hydrocarbons, in a project to assess the effects of such substances upon the biota of the Arabian Gulf. In the tests described here the vertebrate fish, sheim (*Acanthopagrus spp*) was used in sighting procedure for long-term experiments. This method was based on a protocol of the Environmental Protection Agency. Batches of ten fish were exposed to dispersions of oil over the range 0.178 to 2.00% by volume. Concentrations of hydrocarbons dispersed in the test beakers were assessed analytically.

A median period of survival of 6,000 minutes (about 3½ days) was obtained in the concentration of 2.00%. The hydrocarbon content by analysis was 0.105 ppm. A 4-day LC_{50} value of 2.55% was estimated, the corresponding concentration of hydrocarbon was estimated to be 0.134 ppm.

The data suggest that under the condition of the test the sample of oil was not acutely lethal to fish and that response of the test animals was not greatly concentration dependent.

Hassan Al-Shemmari

Bioassay Laboratory

Khalid Al-Matrouk

10th October 1995

1. INTRODUCTION

The Environmental Sciences Department of the Kuwait Institute for Scientific Research (KISR) has been commissioned to evaluate possible adverse effects of crude oil and partially combusted oils upon the fauna of the Arabian Gulf. These experiments were made as a sighting procedure for a research project on this topic, VR004P, project leader Dr. M. Metwally[1].

The tests were conducted using sheim (*Acanthopagrus spp*) on oil/water dispersions of a Kuwait crude oil[2]. Procedures were as described in a protocol of the Environmental Protection Agency[3]. In this method dispersions of oil are prepared in water by agitation on a shaking table under controlled conditions for five minutes.

2. MATERIALS AND METHODS

2.1. Chemical

2.1.1. Oil

The source of oil was from the Petroleum Division[2] of KISR. It was a light Kuwait crude oil; the specific gravity was found to be 0.897.

2.1.2. Analysis

Total hydrocarbons of the oil dispersed in the water were measured by ultraviolet fluorescence spectroscopy, according to the ROPME Manual (ROPME, 1986)[4].

i. Construction of calibration curve

A series of standard solutions of Kuwait crude oil in a mixture of hexane and dichloromethane (7:3) were prepared in the range between 0 to 3.00 ppm.

UVF parameters were set as follows:-

Excitation wavelength = 310 nm

Emission wavelength = 360 nm

Width band Ex = 3.0 and Em = 1.5

Analysis was duplicated using concentration versus fluorescence reading of Kuwait crude oil standards.

Calibration is given in Table I, and shown graphically in Fig. 1.

Table I. Fluorescence spectroscopy of Water Accommodated Fraction, Calibration Data

Standard (Conc. ppm)	Fluorescence (Arbitrary Units)
0 ppm	0.330
0.025	1.143
0.050	1.746
0.100	9.080
0.250	7.450
0.500	14.001
1.000	28.108
2.000	54.835
3.000	81.231

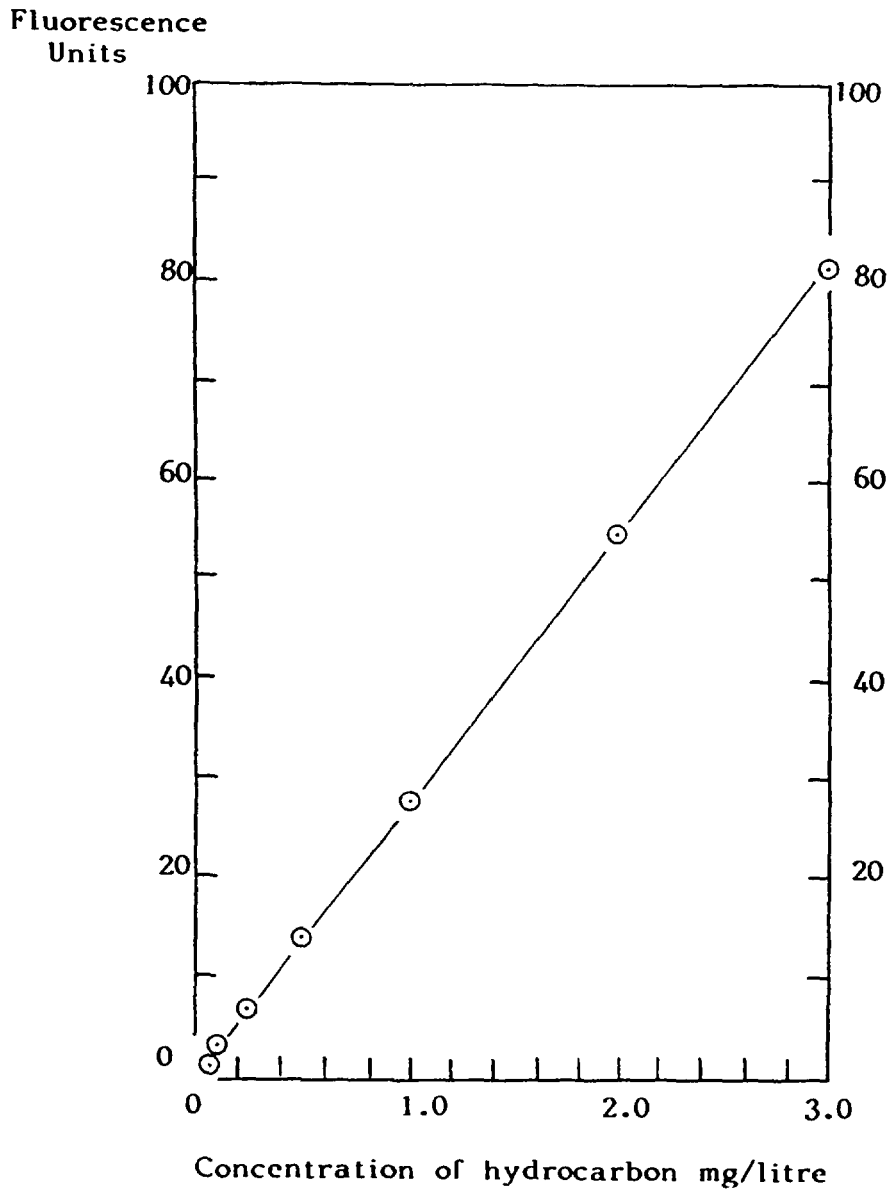


Fig. 1. Calibration of spectrophotometer hydrocarbon analysis.

(Note: Blank reading = 0.330 fluorescence units. 0.025 mg hydrocarbon/litre = 1.143 units, omitted for clarity)

ii. Analysis of Water Accommodated Fraction

An aliquot (100 ml) of WAF was extracted twice with 25 ml of a mixture of hexane and dichloromethane (7:3). The mixture was dried with anhydrous sodium sulphate. The volume was adjusted to 50 ml and then its fluorescence intensity was measured. The concnetration was determined from a calibration curve.

2.2. Biological

2.2.1. Sheim

A supply of sheim was provided by Dr. Khalid Abdul Ellah of the Mariculture and Fisheries Department of KISR. A representative sample of the fish was found to have a mean fork length of 4.6 cm, standard deviation (±1.6) cm. The mean weight was 2.82 g standard deviation (±1.05) g. The size range of the sample was in approximately Gaussian distribution, as shown in Fig. 2.

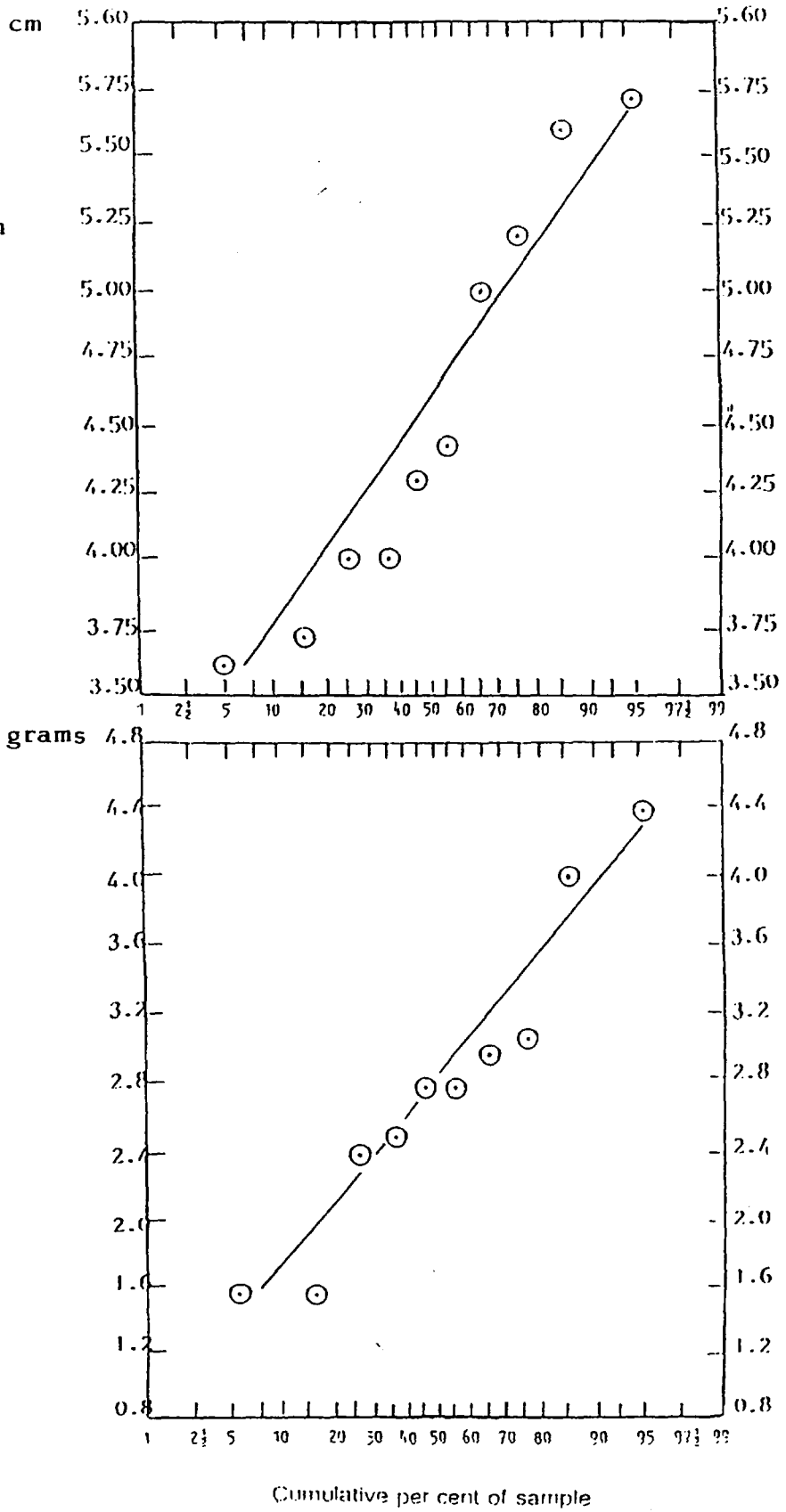
2.2.2. Dilution Water

The oil dispersions were prepared and the tests performed in natural seawater obtained from the Arabian Gulf by pumping from a near shore well, which provides sand filtration. The salinity was found to be 24.0 g/litre in chloride and 39.5 g/litre in NaCl. The specific gravity was 1.028, the pH value 8.0, and the concentration of dissolved oxygen was close to the air saturation value.

Mean length = 4.6 cm

Standard deviation = ±1.6 cm

95% Confidence limits = ±0.99 cm



Mean weight = 2.82 g

Standard deviation = ±1.05 g

95% Confidence limits = ±0.65 g

Fig. 2. Weight and lengths of fish.

2.3. Test Methods

As mentioned in the introduction, procedures were essentially as described in the EPA Protocol 40 CFR/300, II, (3) but the standard dispersions of oil and water were prepared by shaking in 2.5 litre glass bottles. The contents of the bottles were transferred to borosilicate glass beakers, in which toxicity tests were performed. The beakers were bubble aerated to maintain the concentration of dissolved oxygen closed to the air saturation volume. pH values were recorded at suitable intervals and were found to remain within the range pH 7.2 to 8.00. The mean value was pH 7.4. Temperature ranges from 19.5 to 22.2°C, mean value was 21.0°C. Concentrations of dissolved oxygen could not be obtained in the test dispersions of oil because the oil fouled the electrode. The lowest concentration recorded in the control was 82 per cent of the air saturation value. All data are recorded in Annex Tables 1, 2 and 3.

Six nominal concentrations of oil were set up, distributed at approximately equal geometric intervals over the range 0.10 to 1.0 per cent in volume of oil. An additional concentration of 2.0 per cent was also tested. A failure in the aeration system caused abandonment of the test in 0.10 per cent oil, but this does not appear to have affected the overall experiment.

Five two-liter volumes of oil water dispersion were prepared at each concentration and two fish were distributed at random into each beaker. The test was continued for a period of five days, one day longer than the four-day period in the protocol. A control was also set up containing fish and seawater. Thus ten fish were exposed at each concentration, plus ten controls.

2.4. Mathematical Methods

The periods of survival of individual fish were taken as being the interval from the first introduction of toxicant to the time when movement was ceased and could not be induced by gentle stimulation by a smooth glass rod.

A median period of survival was estimated as described by Bliss[5] . The four-day LC_{50} value was determined by the method of Granmo and Larsstuvold[6]. Individual periods of survival are recorded in Annex Table 4.

3. RESULTS

3.1. Chemical

It was seen that the majority of the oil formed a layer at the surface of the water in the test beakers. The fish avoided this layer and hence were exposed to the fraction dispersed within the water. These fractions were measured analytically as given in Table 1.

Table 1. Analysis of Hydrocarbon Content of Test Dispersions

Nominal Concentration of Oil% (Per cent by Volume)	Concentration of Hydrocarbons mg/litre	
	Start of Test	End of Test
2.00	0.416	0.105
1.00	0.588	0.079
0.56	0.696	0.031
0.32	0.845	0.029
0.18	0.433	0.052

3.2. Biological

Per cent mortalities following an exposure period of five days are shown in Table 2.

Table 2. Mortality of Sheim Following Five Days Exposure to Dispersed Oil

Nominal Concentration of Oil Per Cent by Volume	Concentration of Hydrocarbon Analysis at End of Test ppm	Per Cent Mortality After Five Days
2.00	0.105	50
1.00	0.079	30
0.56	0.031	10
0.32	0.029	10
0.18	0.052	20
Control	-	0

A median period of survival of 6000 minutes (95% confidence limits, 4532 to 8942 minutes) was obtained in the concentration of 2.00 per cent by volume as shown in Annex Fig. 1. A 4-day LC_{50} value of 2.55 per cent V/V (95% confidence limits 0.509 to 12.8 per cent) was obtained.

4. DISCUSSION

It is apparent that under the conditions of the test the acute lethal toxicity of the sample of oil evaluated here was comparatively low. Thus in a nominal concentration of two per cent by volume a median period of survival of 6,000 minutes (about 3½ days) was obtained. Also the relation of mortality to concentration was not greatly dependent on nominal levels of oil. Inspection of Table 2 indicates that mortality was recorded in all concentrations tested, but related to concentration found by analysis at the end of the test. It is considered that these analytical

values are the most reliable guide to the exposure conditions; inspection of the test beakers indicated that the massive fraction of the oil separated from the water shortly after the dispersions were removed from the shaking table. Therefore figures given in the central column of Table 1 probably give little indication of concentrations during the test.

This is confirmed by the 4-day LC_{50} value of 2.55% nominal which had very wide confidence limits namely from 0.500 to 12.8 per cent by volume. This shows that the response of the fish was not greatly concentration dependent. Taking the analytical value for 2.00% oil by volume (Table 1 -0.105 ppm) the corresponding hydrocarbon concentration at the 4-day LC_{50} point is calculated to be 0.134 ppm, 95% confidence limits 0.027 to 0.672 ppm.

The data suggest that the response of fish to this oil is as much dependent upon the state of distribution of the sample as the gross concentration; this factor probably applies equally under practical conditions in the Arabian Gulf.

5. CONCLUSIONS

5.1. The toxicity of a sample of Kuwait crude oil has been evaluated using sheim as the experimental fish.

5.2. Procedures were based on the EPA protocol 40, CFR/300 II.

5.3. Nominal concentrations tested were in the range of 0.178 to 2.00% of oil by volume.

5.4. Concentrations of hydrocarbons in test beakers were found analytically by the ROPME[4] Method.

5.5. A median period of survival of 6,000 minutes (3½ days) was obtained in a nominal concentration of 2.00% oil; about 0.105 ppm hydrocarbon by analysis.

5.6. A 4-day LC_{50} value of 2.55% oil was obtained, estimated to be 0.134 ppm hydrocarbon.

5.7. There was a relation between concentration of hydrocarbon found analytically at the end of test and per cent mortality.

5.8 The concentration of hydrocarbons at the highest concentration tested (2.00% by volume) was found to be 0.105 ppm.

REFERENCES

1. Metwally, M. Toxicity and uptake of crude oil and partially combusted oil by selected marine organisms in Kuwait. Proposal VR004P. Kuwait Institute for Scientific Research, 1994, 23 pp.

2. Dhaste, A.L. Petroleum Department, Kuwait Institute for Scientific Research. P.O. Box 24885, 13109 Safat, Kuwait, 1995.

3. Flaherty, L.M. National oil and hazardous substances pollution contingency plan. 40 CFR Part 300 II, Environmental Protection Agency 1984, Washington D.C. United States of America, 29192-29207.

4. ROPME Regional Organization for the Protection of the Marine Environment. P.O. Box 26388, 13124 Safat, Kuwait. 1989.

5. Bliss, C.I. Statistics in Biology: Statistical methods for Research Workers, Vol. I, McGraw Hill, New York, 1970.

6. Granmo, A.& Larsstuvold, M. Evaluation of results from short-term tests. The LC50 value and its confidence limits. Food and Agriculture Organization of the United Nations. Rome TF-INT, 1976, pp237.

ANNEX

Table 1. pH Values

Time in Minutes	Reference No and Concentration in Per cent Oil					
	I 2.0%	II 1.0%	III 0.56%	IV 0.31%	V 0.31%	VI Control
0	7.4	8.0	7.9	7.7	7.7	7.6
4220	7.2	7.3	7.3	7.3	7.3	7.2
5580	7.3	7.2	7.3	7.3	7.3	7.2
Mean Value	7.3	7.5	7.5	7.4	7.4	7.3

Overall mean value = pH 7.4 standard deviation = ±0.243

Table 2. Temperature, Degrees Celsius

Time in Minutes	Reference Number, Nominal Concentration Percent Oil and Temperature in Degrees Celsius					
	I 2.0%	II 1.0%	III 0.56%	IV 0.31%	V 0.178%	VI Control
0	21.5	21.2	21.1	21.4	21.5	20.8
2580	22.1	22.0	22.2	22.1	22.0	21.9
4220	21.1	20.5	20.7	20.9	20.8	20.9
5580	10.5	10.9	19.7	19.9	20.0	20.0
Mean Value	21.05	20.0	20.0	21.0	21.0	20.9

Overall mean value = 21.0°C, Standard deviation = ±0.82°C

Table 3. Concentration of Dissolved Oxygen, Percent of Air Saturation

Time in Minutes	Reference Number, Nominal Concentration Percent Oil and Oxygen Dissolve					
	I 2.0%	II 1.0%	III 0.56%	IV 0.31%	V 0.178%	VI Control
0	-	-	-	-	-	87%
5580	-	-	-	-	-	82%

Table 4. Periods of Survival of Test Fish

Time in Minutes	Reference Number, Nominal Concentration Percent Oil					
	I 2.0%	II 1.0%	III 0.56%	IV 0.31%	V 0.178%	VI Control
1410	0	0	10	0	10	0
2580	0	10	10	0	10	0
4260	20	10	10	0	10	0
5580	50	30	10	10	20	0
Mean Value	50	30	10	10	20	0

Toxicity of Material to Fish

LC50 Values. Test Substance = Kuwait Crude Oil , Test Species = Sheim

Period of Exposure = 4 days, 5,760 minutes

Conc. % v/v	Per cent mortality				X^2
	O	E	C	D	
2.00	50	45		5	0.010
1.00	30	33		3	0.504
0.56	10	24		14	0.080
0.31	10	16.5		6.5	0.022
0.18	20	11.2		8.8	0.055

ΣX^2 0.171+10 N= 10 N' = 20
= 1.71

LC
16 = 0.315
50 = 2.55 S = 8.095
84 = Exponent = 5.011

LC50 value = 2.55 per cent V/V

95% confidence limits
upper = 12.80
lower = 0.509

Period of Exposure =

Conc. % v/v	Per cent mortality				X^2
	O	E	C	D	

ΣX^2 N= N' =

LC
16 =
50 = S =
84 = Exponent =

LC50 value =

95% confidence limits
upper =
lower =

Period of Exposure =

Conc. % v/v	Per cent mortality				X^2
	O	E	C	D	

ΣX^2 N = N' =

LC
16 =
50 = S =
84 = Exponent =

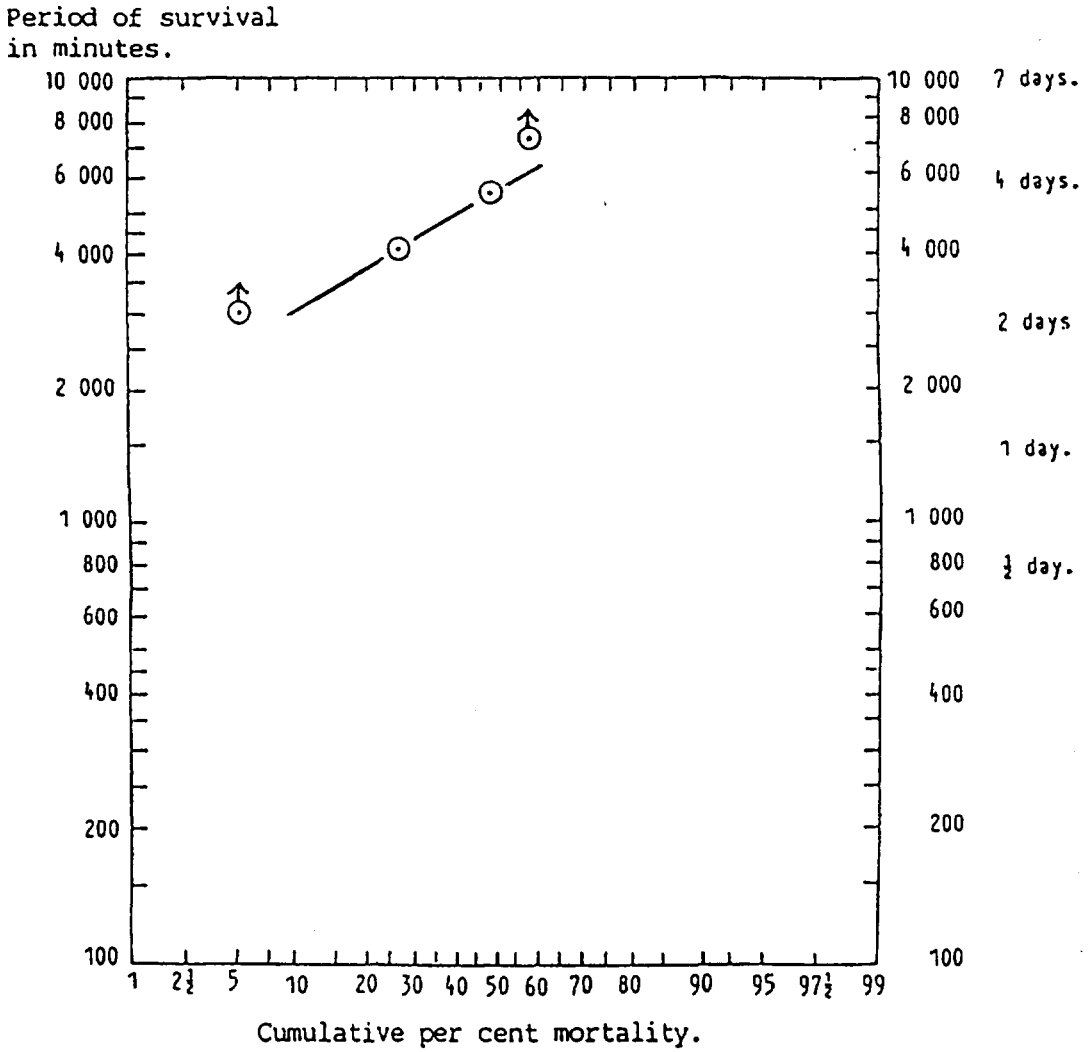
LC50 value =

95% confidence limits
upper =
lower =

TOXICITY OF MATERIALS TO FISH.

Test substance Kuwait crude oil Concentration 0.2% V/V Nominal

Client Company KISR Test species Sheim



Median period of survival = 6,000 minutes

Limits of standard deviation

upper = –

lower = 3,800 minutes

S = 1.579

N = 10 Factor = 1.324

95 per cent confidence limits

upper = 7942 minutes.

lower = 4532 minutes.

Fig. 1. Period of survival of sheim in kuwait crude oil.

REPORT

DYE DILUTION TEST

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DYE DILUTION TEST

SUMMARY

The Kuwait Institute for Scientific Research is investigating the aquatic toxicity of petroleum based hydrocarbons, in a programme[1] to assess the effects of such substances upon the biota of the Arabian Gulf. A specialized apparatus has been constructed for supplying mixtures of oil and water to test aquaria, and the evaluation reported here was made to evaluate the volumetric performance of this apparatus.

Standard solutions of the dye methylene blue were dosed through the equipment, and the optical absorbance of the resulting dilutions compared with values anticipated from the setting of the apparatus. Reproducibility was found to be good; variations between measurements made over several days showed only minor fluctuations. Thus figures from a nominal concentration of 22.5 mg methylene blue/litre ranged from 88.4 to 94.7 per cent of the nominal value. Hence the apparatus was found to dose precisely, but the overall accuracy was at a lower level. Data were found to form approximately Gaussian distributions but standard deviations were in the order of ±40 percent.

Hassan Al-Shemmari

Bioassay Laboratory

Khalid Al-Matrouk

10th October 1995

1. INTRODUCTION

Project VR004P[1] includes basically directed studies on the toxicity of oils to fish and aquatic invertebrates. A constant-flow apparatus has been constructed to facilitate this work, and the study reported here was made to assess the volumetric accuracy and precision of this apparatus. Standard solutions of methylene blue were dosed through the dilution system of the equipment, and the optical observance of the resulting concentrations of the dye were compared with values anticipated from the setting of the apparatus. The method was selected because of the ease of analysis of dye solutions.

2. MATERIALS AND METHODS

2.1. Apparatus

The apparatus is shown diagramatically in Fig. 1. Essentially, there are two parallel delivery systems, the first supplying a toxic dispersion of oil in water (Water Accommodated Fraction), and second uncontaminated water as a diluent for the WAF. These two supplies are mixed in set ratios by adjusting glass stopcocks (not shown in Fig. 1) and these mixtures delivered into the test aquaria.

For the present study, the apparatus was modified by disconnecting the oil contact assembly, and equipping the left-hand chamber of the tank (Fig. 1) with a device to maintain a constant level of methylene blue solution. This is shown in Fig. 2. The aspirator bottle was located above the tank, and the bubble tube adjusted vertically until the lower end corresponded to the desired constant level. Dye passed down the dip tube, with air rising up the bubble tube until the increasing level contacted the end of this tube, and thus prevented further inflow by causing an air lock.

2.2. Chemistry

2.2.1. Methylene Blue

A sample of methylene blue was obtained from Surechem Products Limited. It is understood that the dye was in a high state of purity suitable for microscopy. The molecular formula is given as $C_{16}H_{18}ClN_3S$. Molecular weight, 319.86.

2.2.2. Spectrophotometer

A Perkin-Elmer instrument, Lambda 3B (UV-visible) was used for the analysis, using 10 millimetre cells at a wavelength of 668 nanometres (λ maximum for methylene blue).

Standard solutions of methylene blue were prepared by weighting aliquots of the dye and dissolving in distilled water. Primary standards were diluted using bulb pipettes and volumetric flasks to obtain two ranges of concentration, namely

i. 0.0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0 mg methylene blue/litre.
ii. 0.0, 0.8, 1.6, 2.4, 4.8, 6.4, 8.0 mg methylene blue/litre.

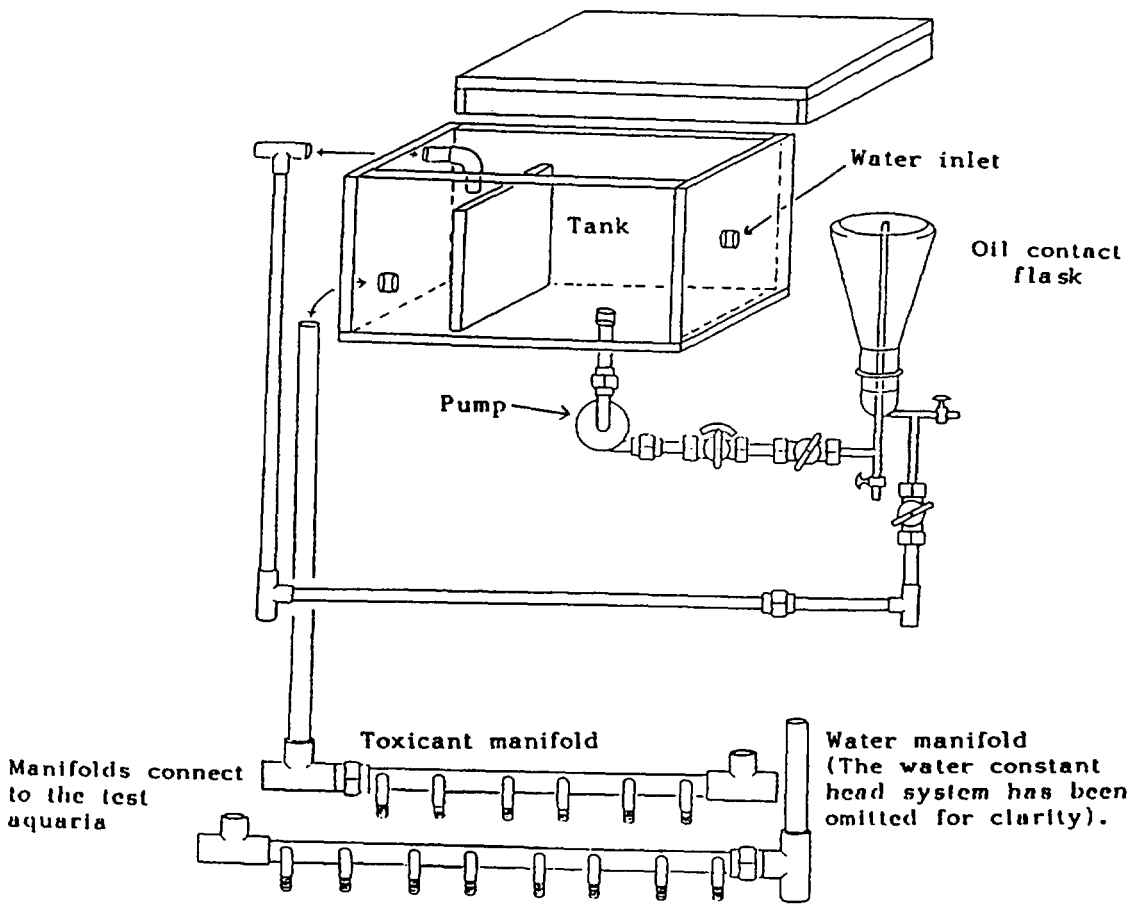


Fig. 1. Toxicity test apparatus (KISR, General Diagram).

This apparatus is designed to study the aquatic toxicology of oils or contaminated solid wastes. The principal is that water enters a constant head tank, and is withdrawn from there by a centrifugal pump, and passed through an oil contact flask, before returning to the left-hand chamber of the tank. Hence a saturated oil/water solution is obtained. This is distributed to test aquaria at a range of controlled rates by a manifold. Water is similarly delivered; the rates are adjusted to give the desired concentrations. Test fish are then introduced to the apparatus.

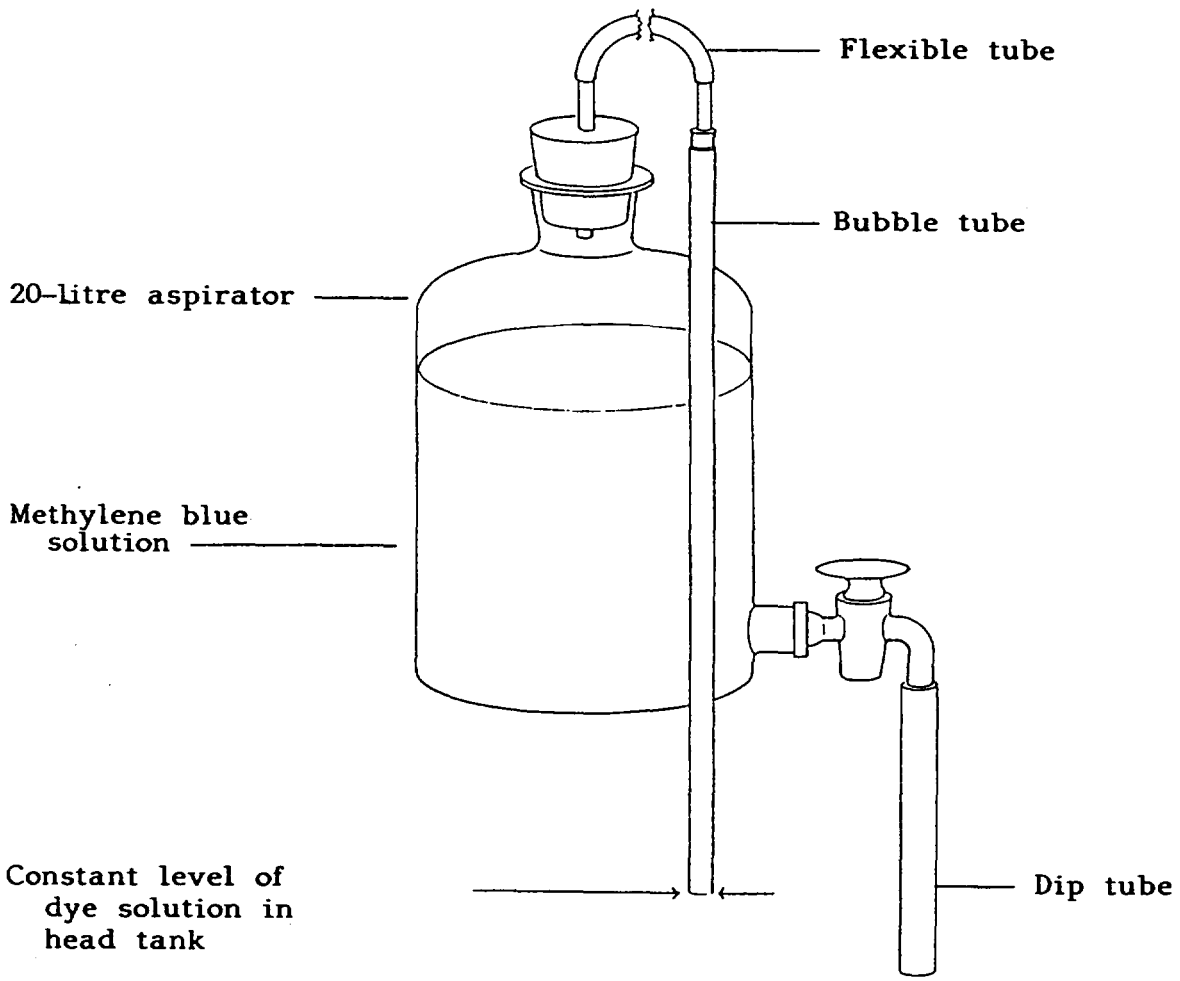


Fig. 2. Diagram of constant level system.

Linear calibrations of concentration against optical absorbance were obtained in both cases, and are shown in Figs. 3 and 4.

Samples of dye solutions from the apparatus were analyzed for methylene blue concentration, by measuring the absorbance and comparing these data with the calibration graphs (Figs. 3 and 4).

2.3. Statistical Evaluation

Individual concentrations found by analysis were expressed as percentages of their nominal concentrations. These were plotted as Gaussian distributions; with mean values, standard deviations, and 95 per confidence limits. Methods were essentially as described by Bliss[2].

3. RESULTS

All original data are recorded in Annex Tables 1 and 2. Mean values and the number of items for each group of analyses are shown in Tables 1 and 2.

Table 1. Analysis of Methylene Blue Solutions, 0-40 mg/litre

Date	Nominal Concentration	Mean Concentration Found	No. of Terms	Per Cent of Nominal
26 August	40.0	25.1	6	61.53
	22.5	21.6	6	93.73
	12.6	18.6	6	143.7
	7.1	8.8	6	116.8
	4.0	7.6	6	177.0
	0.0 (Blank)	0.48	6	-*
27 August	40.0	25.3	6	61.93
	22.5	21.3	6	92.59
	12.6	15.3	6	117.8
	7.1	8.2	6	116.1
	4.0	7.8	6	182.1
	0.0 (Blank)	0.49	6	-*
28 August	40.0	25.4	6	62.05
	22.5	21.9	6	94.7
	12.6	15.4	6	118.1
	7.1	8.8	6	116.9
	4.0	8.2	6	190.2
	0.0 (Blank)	0.54	6	-*

* Corrected for blank readings

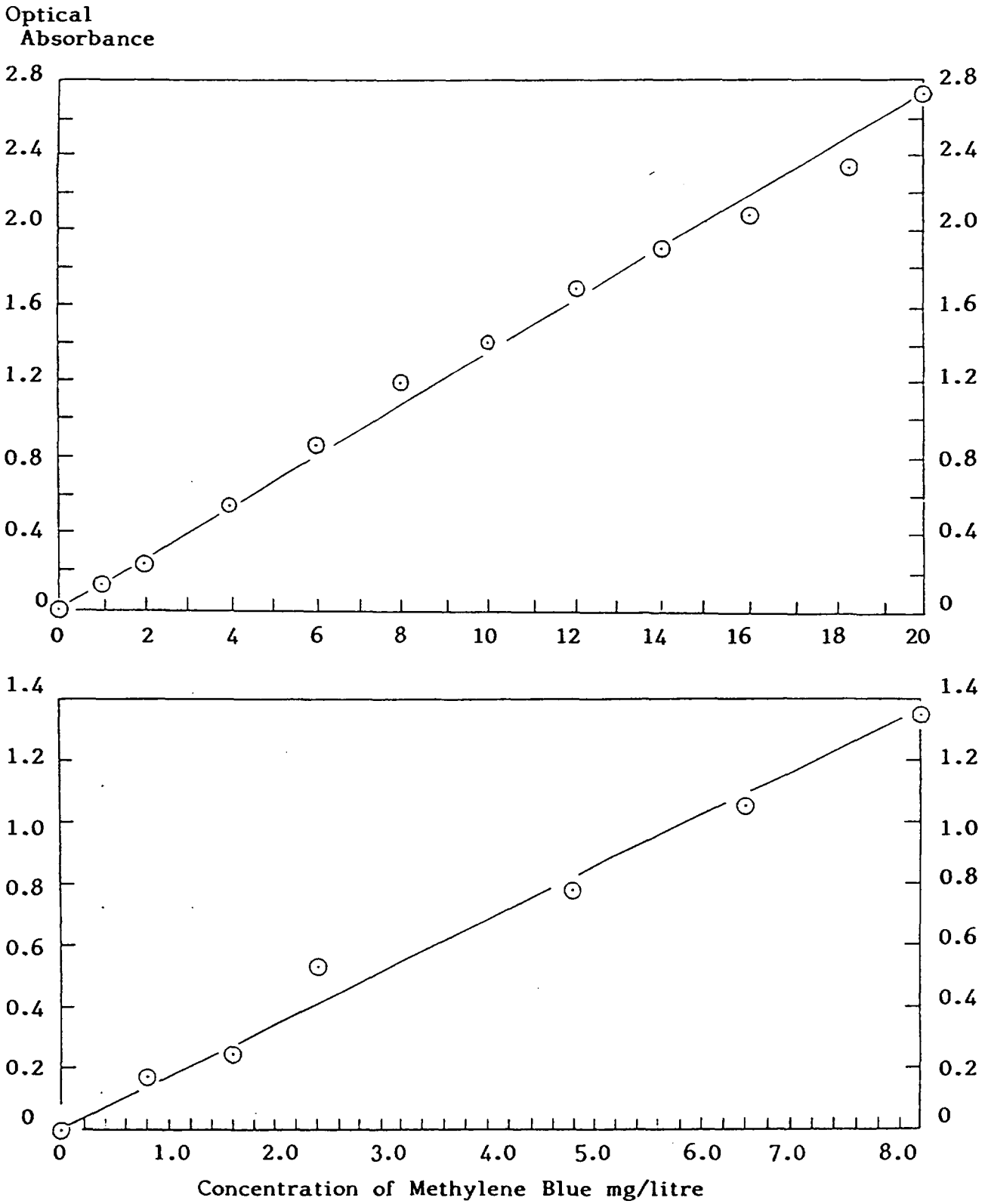


Fig. 3 & 4. Calibration of spectrophotometer.

Table 2. Analysis of Methylene Blue Solution; 0-8 mg/litre

Date	Nominal Concentration	Mean Concentration found	No. of Terms	Percent of Nominal
9 September	8.0	8.03	4	100.40
	4.49	0.98	4	88.40
	2.52	3.22	4	127.00
	1.42	2.68	4	188.70
	0.80	1.52	4	190.00
	0.00 (Blank)	0.00	4	-

The same data expressed graphically with mean values and 95 percent confidence limit are shown in Fig. 5 and 6.

4. DISCUSSION

The evaluation described here can be assessed for both precision and accuracy, and it is considered that in a background study for project VR004P both factors are important. (Precision is concerned with the reproducibility of results; accuracy refers to the degree of approximation to desired or target values). Thus in project VR004P, batches of fish are to be exposed to a range of concentrations of the Water Accommodated Fraction of Kuwaiti oils, and it is necessary to ensure that each concentration remains reasonably constant throughout the period of the test, and also that each level is sufficiently close to the desired value.

Inspection of the data shown in Tables 1 and 2 indicates that the reproducibility of the results was satisfactory; figures obtained for individual concentrations remained within the limits of confidence at the 95 percent level. Therefore it is reasonable to assume that a similar uniformity would be achieved for dilutions of WAF.

However, the accuracy of the dosing equipment was found to be less adequate. In the series of tests made over the concentration range 0-40 mg methylene blue/litre, mean values of 118.5, 114.1 and 116.4 per cent of nominal were obtained, but these figures are thought to have been distorted because of low percentages recorded for concentrations of 40.0 mg MB/litre at the upper end of the concentration range. These data were derived by extrapolation of the relation shown in Fig. 3, and it was later considered that this linear response failed to continue at concentrations much above those evaluated.

Inspection of Figs. 5 and 6 shows that considerable discrepancies were found between concentrations found by analysis and those predicted from the setting of the apparatus (Figs. 1 and 2). Standard deviations were in the order of ±40 percent about the mean values. Whether this level of accuracy is acceptable is a judgmental decision.

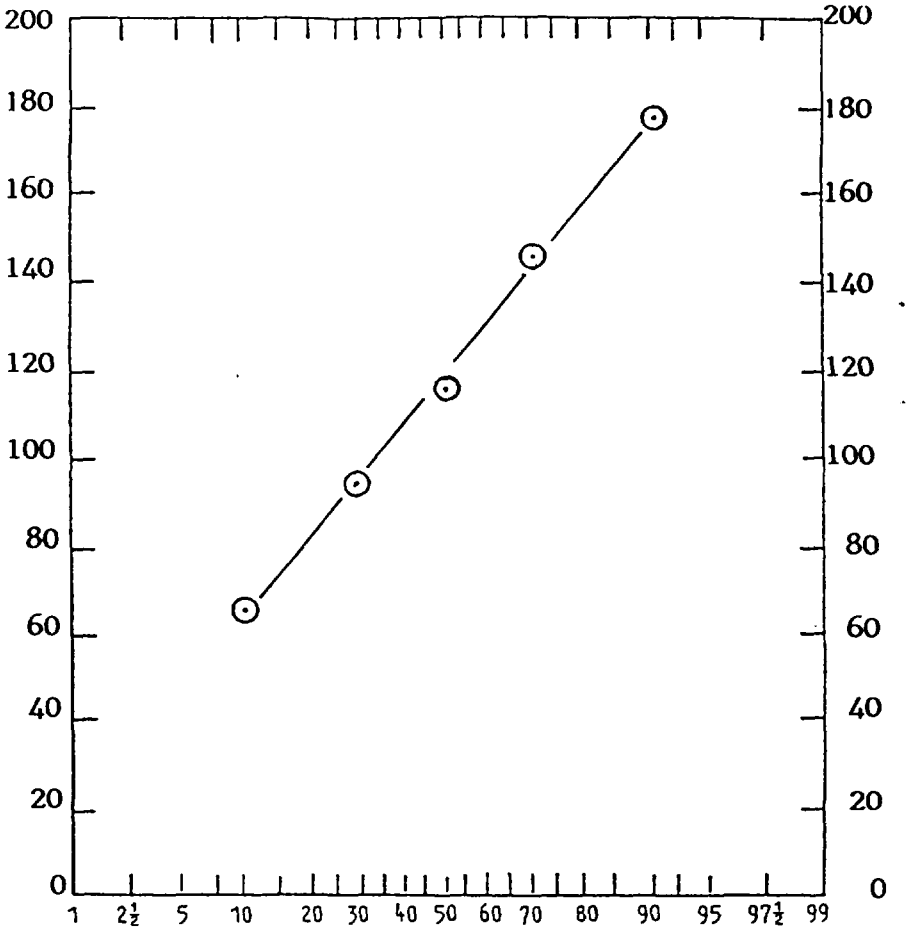
August 26, 1995

Concentration range
= 0.0–40.0 mg dye/litre

Mean value = 118.5 per cent

Standard deviation
= +39.8 per cent

95% Confidence limit
= ±34.9 per cent



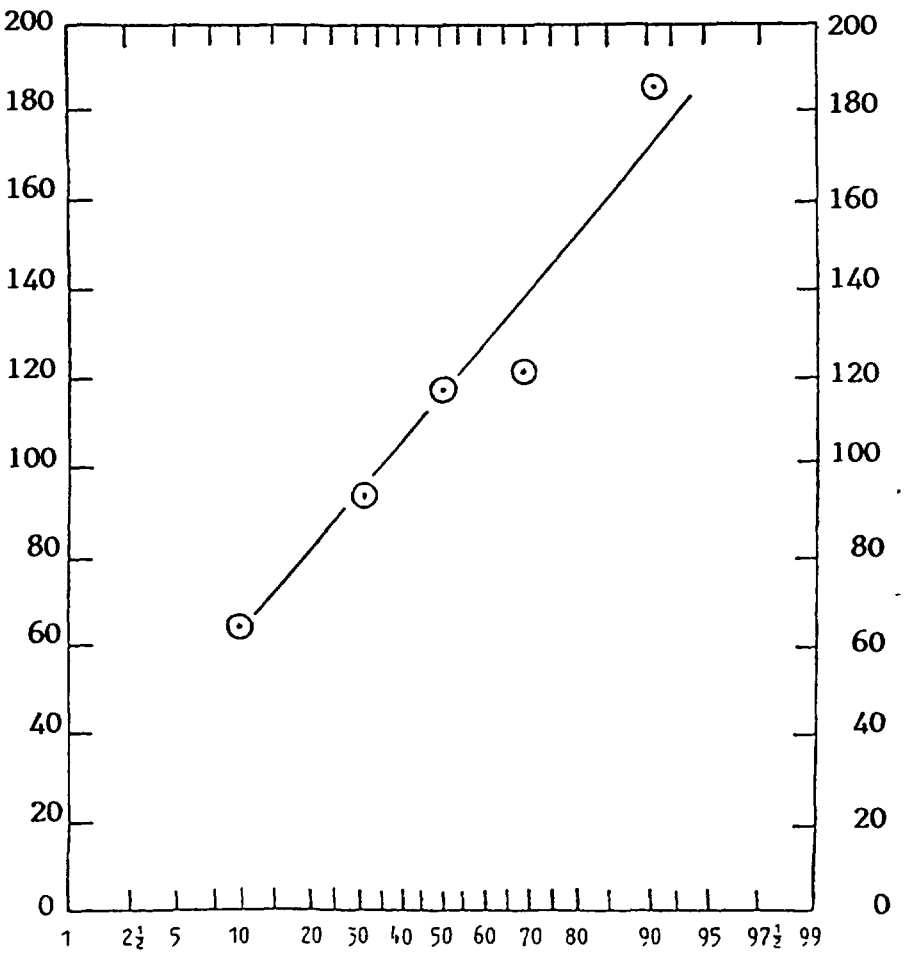
August 27, 1995

Concentration range
= 0.0–40 mg dye/litre

Mean value = 114.1 per cent

Standard deviation
= ±39.6 per cent

95% Confidence limit
= ±34.7 per cent



Cumulative per cent of sample

Fig. 5. Methylene blue dilution test.

(Data expressed as per cent of nominal concentration).

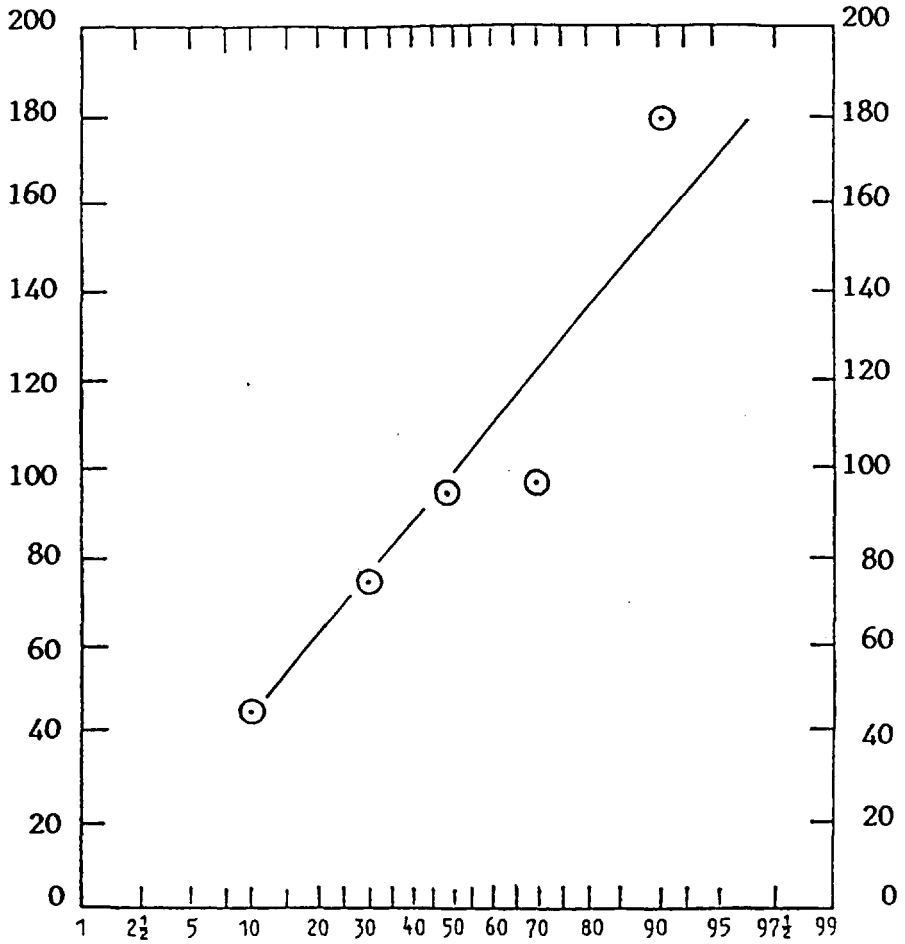
August 28, 1995

Concentration range
= 0.0–40.0 mg dye/litre

Mean value = 116.4 per cent

Standard deviation
= ±42.1 per cent

95% Confidence limit
= ±36.9 per cent



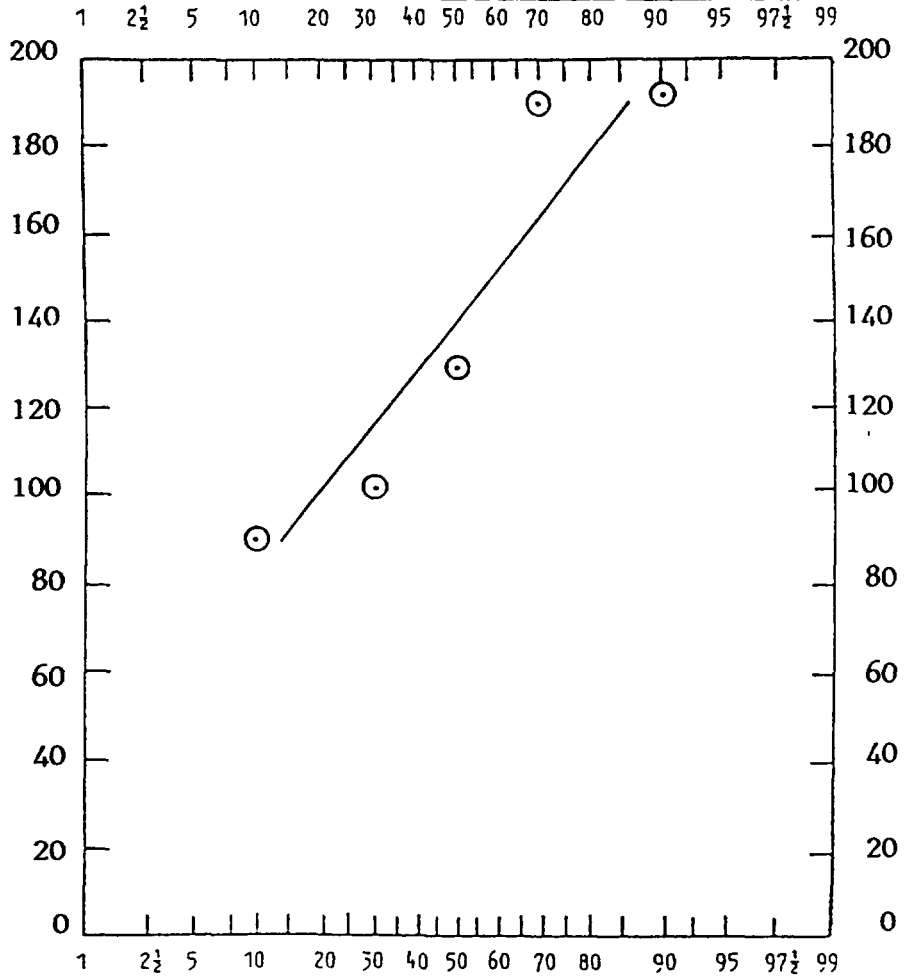
September 9, 1995

Concentration range
= 0.0–8.0 mg dye/litre

Mean value = 145 per cent

Standard deviation
= ±43.4 per cent

95% Confidence limit
= ±38.1 per cent



Cumulative per cent of sample

Fig. 6. Methylene blue dilution test.

(Data expressed as per cent of nominal concentration).

5. CONCLUSIONS

5.1. The precision and accuracy of an apparatus for Project VR004P have been assessed using a dye-dilution technique.

5.2. Methylene blue was selected as the dye. Two ranges of concentration were evaluated, 0-40 mg dye/litre and 0-8.0 mg dye/litre.

5.3. The reproducibility of the data was found to be high. Small variations were recorded between readings made from time to time on the same nominal.

5.4. Considerable scatter was found when comparing found concentrations with the nominal (desired) values. Standard deviations were in the order of ±40 percent of the nominal.

5.5. Whether or not the accuracy of the apparatus is sufficient to meet the objectives of project VR004P a judgmental decision.

REFERENCES

1. Metwally, M. Toxicity and uptake of crude oil and partially combusted oil by selected marine organisms in Kuwait. Proposal VR004P. Kuwait Institute for Scientific Research, 1994, 23 pp.

2. Bliss, C.I. Statistics in Biology: Statistical methods for Research Workers, Vol. I, McGraw Hill, New York, 1970.

ANNEX

Table 1. Optical Absorbance of Methylene Blue Solution Nominal Range 0-40 mg Methylene blue /litre

IA						
Time	**1:45**	**1:55**	**2:05**	**2:15**	**2:25**	**2:38**
1	3.494	3.494	3.455	3.455	3.515	3.515
2	2.971	2.978	2.997	2.959	3.053	3.038
3	2.122	2.143	2.192	2.132	2.225	2.192
4	1.176	1.235	1.211	1.186	1.245	1.258
5	1.003	1.098	1.042	1.023	1.073	1.067
6	0.050	0.076	0.061	0.061	0.077	0.079
IB						
Time	**10:06**	**10:20**	**10:30**	**10:40**	**10:45**	**11:00**
1	3.515	3.494	3.538	3.515	3.474	3.538
2	2.971	2.877	2.978	2.990	2.953	3.024
3	2.082	2.023	2.123	2.162	2.202	2.201
4	1.170	1.102	1.167	1.194	1.209	1.228
5	1.057	1.027	1.076	1.082	1.123	1.130
6	0.056	0.068	0.058	0.079	0.083	0.073
IC						
Time	**9:15**	**9:25**	**9:32**	**10:40**	**10:47**	**10:55**
1	3.515	3.515	3.494	3.538	3.561	3.538
2	3.003	2.997	3.031	3.053	3.076	3.076
3	2.130	2.117	2.157	2.182	2.118	2.175
4	1.236	1.208	1.228	1.242	1.227	1.238
5	1.107	1.106	1.144	1.151	1.139	1.160
6	0.057	0.070	0.085	0.081	0.078	0.083

Table 2.Concentrations of Methylene Blue Equivalent to Optical Absorbances Range 0-40 mg Methylene Blue/Litre (Table 1)

IA						
I	28.130	25.130	24.860	24.860	25.290	**25.290**
II	21.370	21.460	21.560	21.290	21.960	21.860
III	15.260	15.420	15.770	15.340	16.000	15.770
IV	8.460	8.880	8.710	8.530	8.960	9.050
V	7.220	7.890	7.500	7.360	7.730	7.680
VI	0.340	0.550	0.440	0.440	0.550	0.570
IB						
I	25.290	25.130	25.450	25.290	25.000	25.450
II	21.370	20.700	21.420	21.510	21.330	21.750
III	14.480	14.550	15.270	15.550	15.840	15.830
IV	8.420	7.430	8.400	8.590	8.700	8.830
V	7.610	7.380	7.740	7.780	8.080	8.120
VI	0.400	0.440	0.420	0.570	0.600	0.530
IC						
I	25.290	25.290	25.130	25.290	25.290	25.290
II	22.100	22.000	21.900	21.960	21.960	21.960
III	15.250	15.000	15.340	15.440	15.000	15.700
IV	9.050	8.960	9.050	9.100	9.050	9.150
V	7.900	9.870	9.750	8.200	8.120	8.210
VI	0.400	0.570	0.600	0.600	0.570	0.600

Table 3. Optical Absorbance of Methylene Blue Solution Nominal Range 0-8 mg Methylene Blue /Litre

	11:20	**12:13**	**9/9/95**	
1	1.388	1.375	1.366	1.359
2	0.727	0.730	0.660	0.696
3	0.620	0.525	0.514	0.559
4	0.493	0.449	0.446	0.463
5	0.270	0.285	0.255	0.242
6	0.006	0.004	0.000	0.000

Table 4. Concentration of Methylene Blue Equivalent to Optical Absorbance. Range 0-8 mg Methylene Blue/Litre (Tables 3)

I	8.15	8.0	8.0	8.0
II	4.20	3.87	4.20	3.68
III	3.60	3.01	3.00	3.28
IV	2.80	2.61	2.60	2.72
V	1.58	1.49	1.60	1.42
VI	0.00	0.00	0.02	0.00

Project VR004P **Master Plan Manager : Dr. M. Metwally**

Toxicity of Oils to Fish Note for a Study on Growth Rate Effects

1. INTRODUCTION

This study is designed to assess the effects of known concentrations of the Water Accomodated Fraction (W.A.F.) of Kuwait oils on the condition and rate of growth of experimental fish. Essentially, the procedure is to measure the weights and lengths of the fish before the start of each study, and compare these data with the same parameters as found either when fish are withdrawn from the test aquaria, or at the termination of the study. Overall comparision is based on data from the controls, which form a base line.

2. EXPERIMENTAL PROCEDURE

It is assumed that during the tests the fish will be fed on a standarised diet. In temperate waters, a suitable rate would be one percent of body weight per day, using a proprietary fish feed. Approximately two days before the start of a test, the fish are immobilised for weight/length measurements using a suitable aquatic anaesthetic. A formulated benzocaine such as MS 222 is suggested. It is also assumed that the concentrations of WAF will range from the acutely lethal to the sub-lethal, and that fish will be withdrawn for tissue analysis either on death, or at pre-determined intervals during the study. Weights and fork lengths are to be determined on these specimens on withdrawal, and on the survivors (including the controls) at the termination of the study.

3. STATISTICS & EXPRESSION OF RESULTS

3.1. Initial Assessment:

Before the start of the test, the mean and standard deviations are calculated for each aquarium (batch of fish), and for the entire test population. Also, a check is made to ensure that these data form reasonable approximations to normal (Gaussian) distributions, and that there are no significant differences (P=0.05) between the aquaria.

3.2. Condition Factor:

The condition factor of fish, K, is described by Carlander [1]. It is given by the equation :-

$$K = \frac{100 \times \text{weight in grams}}{(\text{Fork length in cm})^3}$$

For wild trout in good condition, the factor approximates to 1.0. For hatchery fed trout, about 1.2 is usual. Data may not be available for species from the Arabian Gulf, but this is not considered highly important, because in the context of the project the requirement is essentially comparitive. Alteration in the factor during the course of the experiment indicates variation in the condition of the fish.

3.3. Specific Growth Rates:

Growth rates of fish can be assessed using the Weatherley Index [2]. Provided that fish are fed on a consistant diet, and are free from interfering factors such as disease or spawning stress, the Index Linearises growth rate curves. The specific growth rate is given by :-

$$SGR = \frac{100 (\text{Log}_e F - \text{Log}_e I)}{D}$$

Where Log_e = Napierian Logarithm

F = Final weight of fish

I = Initial (starting) weight of fish

D = Period of test in days

Thus a growth rate regime is determined by taking data for the controls at the end of the test (F) and the corresponding data from the start (I), and then calculating a weight at any desired period (D). (It will be recalled from 3.1. that the batch parameter (I) has been checked to confirm comparability with the test population).

4. INTERPRETATION

It is clear that this study is of potential importance to fisheries in the Arabian Gulf. This factor should be considered in the interpretation of the data.

Toxic effects may be inhibitory, impairing the condition and/or growth of the fish, negative, with no significant differences between the test fish and the controls, or hormetic, when growth is stimulated by the toxicant.

Interpretation can be considered under three heads :

4.1. Increase/decrease in weight of samples of fish from the start of the experiment.

4.2. Relation of these data (4.1) to the estimated growth of the controls over the same period (Weatherley Index).

4.3. Gain/loss of condition (Carlander Factor), over the same period. Also, if the condition factor (K) of the controls is not significantly different at the end of the experiment from that at the start then (4.2) can be extended to relate growths in length as well as increases in weight.

A high condition factor (K) indicates that a fish is relatively heavy in relation to its length. Reduction in the factor shows that the animal is losing biomass; either by failing to assimilate food, or by expending excessive energy in a physiological response to the effects of the toxicant. Increase in the factor is favourable.

5. PRACTICAL APPLICATION

It is suggested that concentrations of WAF found to effect growth rates and condition factors be compared with hydrocarbon levels reported for the Gulf, particularly from areas where oil spillage is frequent, or natural seepage occurs.

Fish with a high condition factor are relatively fleshy and of greater commercial value than leaner specimens. Also, fair rates of growth are necessary to maintain populations of marketable animals. Therefore a study as described here could provide data to enumerate the financial effects of this aspect of chronic oil toxicity upon commercial fisheries in the Arabian Gulf.

REFERENCES

1. Carlander, K.D. Handbook of freshwater biology, Vol. 1. Iowa State University Press, 1969.

2. Weatherley, A.H. Growth and ecology of fish populations. Academic Press, London & New York, 1972.

PARTIAL COMBUSTION OF CRUDE OILS - TOXICITY OF FISH PROJECT VR004P

INTRODUCTION

During the Iraqi retreat from Kuwait wanton damage to oil fields caused the environmental release of up to 8,000 megalitres of crude oil. Some of this oil was ignited, and hence large volumes of both unburned and partly combusted oils were deposited into the surrounding areas. These areas include the Arabian Gulf, and a KISR Research Project, No VR004P, investigates the effects of such pollution upon the aquatic biota.

Samples of partly burned oils were obtained at the time of the fires, and while these are sufficient to permit detailed chemical analysis, the volumes are too small for an extensive research project in marine ecotoxicology. Therefore the apparatus described here has been designed for the partial combustion of oil under controlled conditions, so that fair volumes can be produced. Analytical data from samples of oil from this apparatus can then be compared with those from the existing field samples.

PRINCIPLE OF APPARATUS

In an oil well fire, oil is expelled vertically into the atmosphere, where vapourisation and combustion is maintained by the intrinsic heat of the oxidation reaction. So far as practicable, this principle is here copied on a laboratory scale by injecting oil into a controlled flame of either a hydrogen/air mixture or a mixture of air with vapourised liquid petroleum gas. The selected burner (Wilkens - Anderson Company) will accept gases with a wide range of heat values (from 600 to 3200 British Thermal Units) and by varying the gas to air ratio the flame temperature can be further controlled. For example, volumetrically hydrogen has a small calorific value, namely 320 BTU/cu ft, or 2849 calories/litre.

In the reaction $2H_2 + 0_2 = 2H_2 0$

two volumes of hydrogen combine with one volume of oxygen, but in the combustion of butane, calorific value, 3,300 BTU/cu ft, or 29378 calories/litre,

$$2C_4H_{10} + 13O_2 = 8CO_2 + 10H_2O$$

thirteen volumes of oxygen are required.

Ratios are greater for the higher molecular weight hydrocarbons occurring in crude oils. Therefore an additional admixture of air is required to support the combustion of injected oil, and by adjusting the temperature and the availability of air it is considered that differing degrees of oil combustion can be achieved. Following combustion, the partly burned oil is collected by a water cooled condenser.

DESIGN

The apparatus is shown in outline in Fig.1. It can be considered in three parts.

1. An array of variable orifice flow meters.

2. The burner assembly.

3. The combustion and condenser tubes.

1. Flow meters control the input of (1.1) air for the combustion of oil, (1.2) air for admixture within the gas burner, and (1.3) either LPG and/or hydrogen. The LPG and hydrogen meters are connected to the burner via a shuttle valve - a device which accepts inputs from two sources to a common output, but does not permit cross flow from one source to another. (i.e. a passive or gate).

2. The burner assembly is shown in Fig.2. It consists of a standard gas burner of the Meker type, but modified in two ways.

2.1. The air inlet port is replaced by a tubulure for connection to the flow meter, (2) in Fig. 1, so that the air input can be controlled.

2.2. A stainless steel tube is taken through the side of the burner tube to terminate in a jet protruding through the grid in the burner head. This tube connects to a micropump delivery for the required volume of test oil.

3. The combustion and condenser tube assembly is shown in Fig.3. It consists of a transparent quartz combustion tube, a water jacketed condenser tube in heavy wall Pyrex glass, two stainless steel plates and some pipe work.

The lower plate has a lip turned around the edge to accommodate the condenser tube, and a central hole to fit exactly around the barrel of the burner. A discharge tube is arranged to withdraw the partly burned oil draining from the condenser. Three tubes pass through the plate to carry combustion air to the upper plate. This plate has a central aperture with an internal lip to rest on the edge of the burner head. A lip round the edge of the plate supports the combustion tube, and three stainless steel rods centralize the plate within the condenser tube.

The length of the condenser tube is larger than as drawn; a one-metre standard length of Pyrex tubing is considered suitable.

FURTHER DEVELOPMENTS

1. Install pyrometer probes to monitor temperatures inside the combustion and condenser tubes.

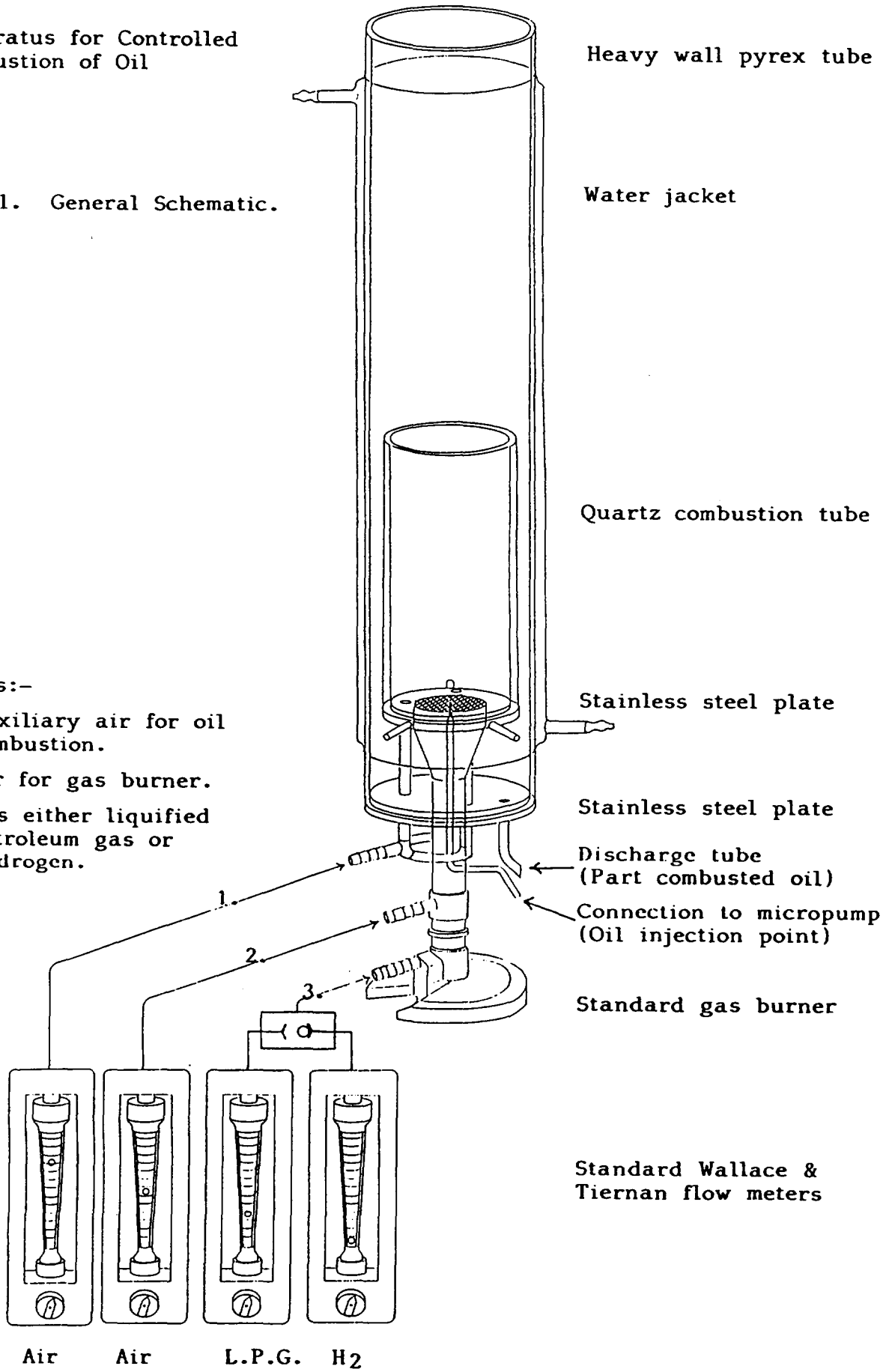
2. Increase the condensation capacity by fitting a cold finger condenser inside the lumen of the Pyrex tube.

3. Arrange a waterbath to preheat oil immediately before injection to the burner.

4. Install temperature control to the condenser water jacket to permit selective condensation of combustion products.

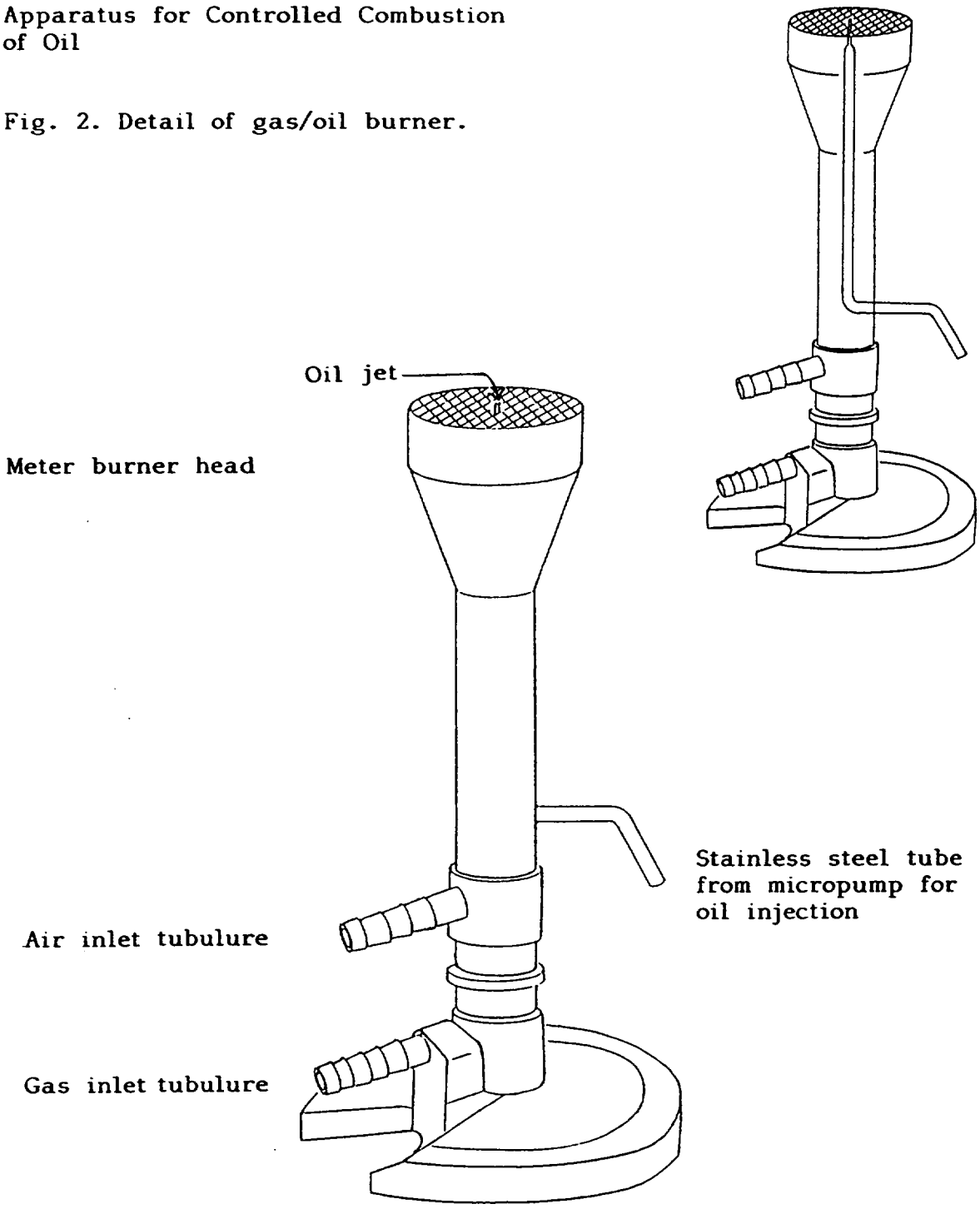
Apparatus for Controlled Combustion of Oil

Fig. 1. General Schematic.



Apparatus for Controlled Combustion of Oil

Fig. 2. Detail of gas/oil burner.



Apparatus for Controlled Combustion of Oil

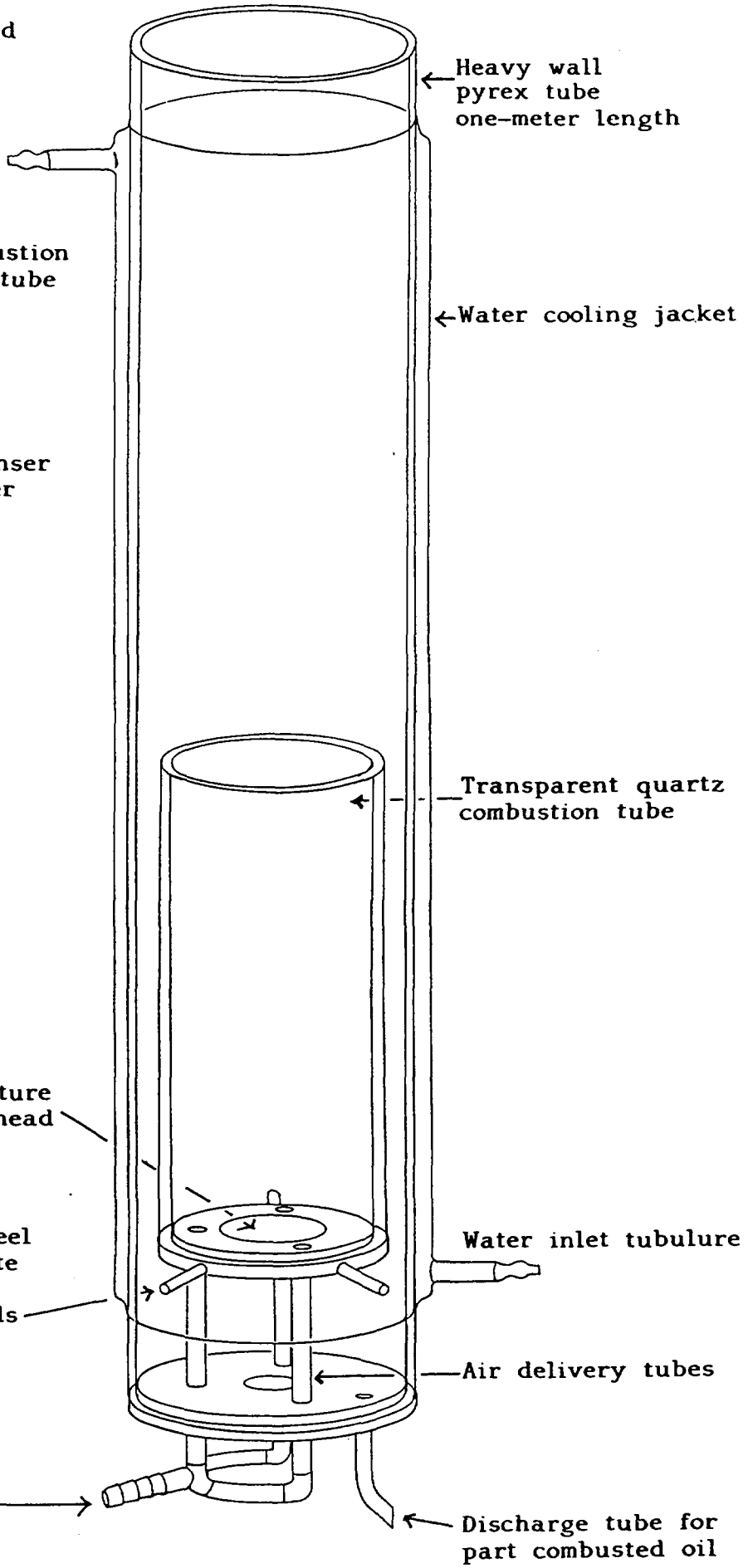


Fig. 3. Detail of combustion and condenser tube assembly.

(Note: The pyrex condenser tube is relatively longer than as shown).

ANNEX III

Documentation for Good Laboratory Practice, and Training

December 1995

بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للأبحاث العلمية

DRAFT

Date : التاريخ :
Ref. No. : مرجع رقم :

Standard Operating Procedures Provisional List

1. Maintenance

Routine maintenance is recorded in the experimental logs at suitable intervals, usually daily. Major maintenance, e.g. maker's servicing, is noted in a separate book.

1.1 Cleaning aquaria etc.

1.2 pH meters. Procedure for setting standard values and slope adjustment. Replacement of buffer solutions. Replacement of electrodes. Routine maker's service.

1.3 Dissolved oxygen meters. Standardization against air-saturated water, and zero dissolved oxygen concentration, using sulphite/cobalt chloride solution, or white spot nitrogen. Servicing of electrodes. Routine maker's service.

1.4 Electronic balances. Checks against standard weights. Routine maker's service.

1.5 Thermometer. Check against melting ice.

1.6 Peristaltic pumps. Checks on dosing rate. Replacement of flexible tubing.

1.7 Major analytical equipment. Consult maker's literature, and seek opinion of chemists with experience of the apparatus - HPLC and GLC, spectrophotometers etc.

2. Toxicology

Concentrations of dissolved oxygen, pH values and temperatures are recorded in all tests.

2.1 Toxicity to shrimps. 1,2,3 and 4 day LC50 values, Concentration/survival-time relation, as MAFF protocol AEP2, but modified to meet conditions in the Arabian Gulf.

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2.2 Toxicity to vertebrate fish. OECD protocol 203, but with modification to meet conditions in the Arabian Gulf.

2.3 Algal proliferation test. ISO/PARCOM procedure, possibly using an alternative species of (local) alga.

2.4 Oyster embryo test. PARCOM procedure.

2.5 Sediment worker. PARCOM procedure, if a suitable amphipod is available in the Gulf.

2.6 Daphnia immobilization. As OECD protocol 202.

3. Analysis - Wet Chemistry

3.1 Halide content of seawater by Mohr titration.

3.2 Concentration of dissolved oxygen by Winkler titration.

3.3 Chemical oxygen demand by acid dichromate, SCAS or ASTM method.

4. Analysis - Physical Methods

SOPs to be written by chemists using the apparatus, specific to analytical procedures used for the studies.

5. Biodegradability

(It is improbable that these studies will be made in the Salmiya laboratory).

5.1 Five-day BOD, SCAS or ASTM protocol.

5.2 Closed bottle test (28-day BOD). OECD protocol 301 D.

5.3 Shake-flask test. OECD protocol 301E, plus procedure for organic carbon analysis.

5.4 BODIS Test - (BOD of insoluble substances), ISO/AFNOR protocol.

5.5 Anaerobic degradation. ISO protocol.

بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للابحاث العلمية

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Date : التاريخ
Ref. No. : مرجع رقم

Standard Operating Procedures

Ref. No. KISR_____ Copy No. 1.

Date of Issuing: 25th December 1995

Dates of Revision:

Issued by: F.S.H. Abram (UNIDO) Revised by:

Original Copy, No. 1, kept in KISR archive.

Title: Preparation of Standard Operating Procedures.

PREPARATION OF SOPS

1. Preliminary All SOPs require:-

   1.1 A reference number.

   1.2 A copy number. (These relate to distribution. Outdated copies are recalled).

   1.3 Date of issuing, and date(s) of revision.

   1.4 The name of the person(s) issuing the procedure, and revising the same.

   1.5 The location of the original and revisions - usually the location of the archive.

   1.6 A title.

2. Range of SOPs Procedures for which SOPs are required include:-

   2.1 Toxicity test and analytical procedures.

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2.2 Maintenance of apparatus, including cleaning and standardization.

2.3 Maintenance of animal and algal stocks.

2.4 Sampling, including registration of samples.

2.5 Preparation of study plans.

2.6 Reporting procedures.

2.7 Quality assurance.

2.8 Archiving.

3. Contents

3.1 Source of methods. If the SOP is based on a standard method, a note of this is included. e.g. Protocol numbers and date for the guidelines of the Organization for Economic Co-operation and Development, the American Society for Testing and Materials, or the International Standards Organization.

3.2 Equipment. All necessary materials are listed. Scource of animals is indicated.

3.3 Methods. These are written out as concisely as practicable, in numbered paragraphs.

3.4 Study plans. In addition to (1.1-1.6) study plans indicate:-

i. The purpose of the study.

ii. The name of the sponsor. This must be a specific person. The name of say an Institution or Limited Company may be included, but will not serve in itself.

iii. The test material(s).

iv. The test species (if relevant).

v. The projected timescale. Starting date. Dates of completion of the laboratory work, the draft report, quality assurance, and final report.

vi. Methods. SOPs, standard protocols, statistical procedures.

vii. Replacement regime, if appropriate. Static test, batch replacement, or constant flow.

viii. Analyses

ix. Observations.

x. Personnel, including quality assurance officer(s).

Xi. Date of approval by sponsor.

3.5 Reporting. Format may vary with the nature of the study, but the usual arrangement is:

i. Title page.

ii. Summary.

iii. Statement of Quality Assurance.

iv. Contents page.

v. Introduction.

vi. Materials and Methods.

vii. Results.

viii. Conclusions.

(ix. Recommendations)

x. References. References are numbered, but the section itself is not numbered.

xi. Annex. <u>All</u> analytical data, recordings of mortality of animals, sizes of a representative sample of animals, and any other relevant figures are tabulated, with mean values and standard deviations. Calculations (e.g. of EC50 values) are also included, and any graphical analysis used to support the text. With a major report, an Annex Contents (separate from iv) is included.

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Kuwait Institute for Scientific Research معهد الكويت للأبحاث العلمية

Date : التاريخ
Ref. No. : مرجع رقم

DRAFT

STUDY PLAN

Purpose of study:-

Sponsor:-

Test material:-

Test species:-

Projected timescale:-

Test methods:-

Replacement regime:-

Analysis:-

Observations:-

Personnel:-

1._____ Study Director

2._____ Study Manager

3._____ Technical Assistants

4._____

Quality assurance:-

5._____ Independent Consultant

Archives of records and samples

Kuwait Institute for Scientific Research
Ecotoxicology Laboratory
Marine and Fisheries Department
Salmiya, Kuwait

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P. O. Box 24885 - 13109 Safat - Kuwait - Telephone: 4836100 - 4816988 - Cable: SCIENCE Kuwait - Fax: 4846891

Date of approval by sponsor:-

Signatures **Date**

Study Director:

Sponsor:

Quality Assurance Officer:

Amendments:

بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للابحاث العلمية

Date :
Ref. No. :

DRAFT

التاريخ :
مرجع رقم :

QUALITY ASSURANCE

STANDARD OPERATING PROCEDURE

Validation of Reports of Studies

Date effective:
Date suspended:

1. Obtain a copy of the draft report as soon as possible after the completion of the study.

2. Read the draft report and ensure that it accurately reflects all aspects of the study.

3. Ensure that data held by computer, if used, are a correct representation of the raw data as given on data sheets. This is to be done by checking random samples of physical data, such as temperature, pH, etc. A higher number of samples of LC50 calculations should be checked.

4. Check that data in tables/figures in the report are quoted correctly in the text, and that the report is internally consistent in the other ways.

5. Submit a written report audit to the Study Director with a copy to senior management. Discuss with the SD any problems encountered with a view to reaching agreement by way of explanation or amendment to the report.

6. Sign the statement attached to the report that the study was carried out using GLP principles, has been quality assured and that it is a true reflection of the conduct of the study. List the study phases inspected and the dates of inspections.

Distribution: SENIOR MANAGEMENT, STUDY DIRECTOR QUALITY ASSURANCE OFFICER, ARCHIVES

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Date : DRAFT التاريخ :
Ref. No. : مرجع رقم :

QUALITY ASSURANCE

STANDARD OPERATING PROCEDURE

Validation of Reports of Studies

Date effective:
Date suspended:

1. Obtain a copy of the draft report as soon as possible after the completion of the study.

2. Read the draft report and ensure that it accurately reflects all aspects of the study.

3. Ensure that data held by computer, if used, are a correct representation of the raw data as given on data sheets. This is to be done by checking random samples of physical data, such as temperature, pH, etc. A higher number of samples of LC50 calculations should be checked.

4. Check that data in tables/figures in the report are quoted correctly in the text, and that the report is internally consistent in the other ways.

5. Submit a written report audit to the Study Director with a copy to senior management. Discuss with the SD any problems encountered with a view to reaching agreement by way of explanation or amendment to the report.

6. Sign the statement attached to the report that the study was carried out using GLP principles, has been quality assured and that it is a true reflection of the conduct of the study. List the study phases inspected and the dates of inspections.

Distribution: SENIOR MANAGEMENT, STUDY DIRECTOR QUALITY ASSURANCE OFFICER, ARCHIVES

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DRAFT

Date :
Ref. No. :

التاريخ :
مرجع رقم :

STANDARD OPERATING PROCEDURE

Receipt of Test Samples and Use of Test Samples

Authorization: **Date effective:**
Date suspended:

Date:

This procedure relates to test substances as produced by manufacturers, and does not necessary apply to industrial effluents, sewages, or water samples.

PERSONNEL

1._____ Study Director

2._____ Study Manager

3._____ Technical Assistants

4._____

Quality Assurance Officer(s)

5._____ Independent Consultants

1. RECEIPT OF SAMPLES

1.1 On receipt substances for test will be labelled on the container with date and source of the sample. This information will also be entered into the sample record book together with other details given below.

1.2 The following information will be included in the record book entry.

1.2.1 The KISR project number.

1.2.2 The maker's name for the substance, and if known, the chemical name, and C.A.S. number.

1.2.3 The maker's code number and batch number.

1.2.4 Any relevant test protocol numbers. Usually these relate to the O.E.C.D., M.A.F.F., the I.M.O. or the E.P.A.

1.2.5 The date of receipt of the sample, and the gross weight of the unopened container.

1.2.6 The storage location of the sample.

1.2.7 Any other relevant information, particularly if there are handling hazards involved, and stability/storage time factors. (Most manufacturers provide hazard data sheets).

1.2.8 The name of the person making the entry.

2. **THE USE OF SAMPLES (WITHDRAWAL FROM ARCHIVE)**

2.1 A note will be kept of the weights or volumes of substances withdrawn from the container.

2.2 While the laboratory work is in progress, the sample may be kept in the sample preparation laboratory, provided that storage requirements are not infringed.

2.3 The withdrawal note will be kept on the container label initialled and dated.

2.4 Sub-samples for submission to a regulatory authority shall have the date, quantity and form of transmission entered into the samples record book.

2.5 Sub-samples will not be withdrawn under (2.4) without the consent of the sponsor. If this consent is arranged by telephone, the date of the call, name of person contracted, and any other relevant information will be entered into the sample record book.

2.6 Following the finalization of the study, the gross weight of the sample container will be entered into the record book, in addition to the withdrawal note.

Distribution:

KISR
BIOASSAY LAB

SAMPLE RECEIVED VOUCHER	VOUCHER NO.
TEST REQUIRED	DATE
SPONSOR NAME	

SERIAL NO.	DESCRIPTION	QTY	TEST CHARGES			
			UNIT PRICE		TOTAL AMOUNT	
			KD	Fils	KD	Fils

REMARKS:

1. ☐ SAMPLES RECEIVED IN GOOD CONDITIONS AS PER SPECIFICATIONS.
2. ☐ NOT AS SPECIFIED.

COMMENTS OF SPECIALIST FOR SUITABILITY TO CONDUCT TEST IN CASE OF NO. 2.

RECEIVER'S SIGNATURE

Distribution: Original - Sponsors; Green - Finance; Yellow - Specialist; Pink - Registration Record.

USAGE LABEL

TEST MATERIAL

DATE RECEIVED WEIGHT INITIALS

Date	Weight	Initials	Date	Weight	Initials

USAGE LABEL

TEST MATERIAL

DATE RECEIVED WEIGHT INITIALS

Date	Weight	Initials	Date	Weight	Initials

بسم الله الرحمن الرحيم

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Date : التاريخ :
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DRAFT

STANDARD OPERATING PROCEDURE

Cleaning of apparatus

Authorization: **Date effective:**
Date suspended:

Date:

It is important that all apparatus used in toxicity tests should be completely clean and free from previous test materials, which would invalidate the experimental results. This applies especially to the aquaria and associated apparatus used in toxicity tests.

Experimental Procedure

1. Laboratory coat and rubber gloves are to be worn.
2. Wash the apparatus thoroughly with a solution of approximately 1% "Pyroneg" in hand hot water. Fill completely with cold water and pour away. Rinse with hot water.
3. Allow apparatus to drain and then dry with a clean cloth.
4. Store tanks upside down when not in use.

Distribution:

SOP Cleaning of apparatus

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بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للابحاث العلمية

DRAFT

Date : التاريخ :
Ref. No. : مرجع رقم

STANDARD OPERATING PROCEDURE

Standardisation For pH Meters

Authorization: **Date effective:**
Date suspended:

A: Immerse probes in 7 pH buffer and set the value of the 7pH buffer.

B: Rinse probes in deionised water.

C: Immerse probes in 4pH buffer and set the value of 4pH buffer.

D: Repeat until no further adjustment is necessary.

E: Rinse probes in deionised water before each measurement.

NOTE: If the measurement to be made are mostly alkaline solution use of a 9 or 10pH buffer in preference to a 4pH buffer is recommended.

Distribution:

Standardisation For pH Meters

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بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للأبحاث العلمية

Date : 12th June, 1995 التاريخ :
Ref. No. : TOX SP0010 مرجع رقم

DRAFT

ACUTE TOXICITY OF OILS TO BRINE SHRIMP

STUDY PLAN

Purpose of Study:- To assess the toxicities of Kuwait crude oil and bunker oil using a standard method, to provide base-line data and practical experience for the applied research project VR004P. This is entitled 'Toxicity and Uptake of Crude Oil and Partially Combusted Oil by Selected Marine Organisms in Kuwait', - Drs Mohammed Metwally (Project Leader) and Lulwa Ali (Task Leader Toxicology). This study will also provide comparative data to support Mariculture Fisheries Department, KISR, in an evaluation of the acute toxicity of these oils to the planula larvae of corals in the Arabian Gulf.

Sponsor:- Environmental Sciences Department, Environmental and Earth Science Division, KISR.

Test material:- Samples of crude oil and bunker oil as provided by MFD, Salmiya Laboratory.

Test Species:- Brine shrimp, *Artemia salina.*

Projected time scale:- June/July 1995, subject to alteration to coincide with the spawning of Kuwait corals.

Test Methods:-

1. In general accordance with the Environmental Protection Agency's Protocol 40 CFR Part 300, Part II, (1984), except that the test substrate of oil accommodated in Kuwait seawater will be prepared in 10 litre aspirator bottles, using the same procedure as in MFD for tests on corals.

2. In accordance with the KISR Environmental Science Department's Standard Operating Procedure - Brine Shrimp Hatching Test, dated 6th June 1995, Ref. No. KISR TOX 0100.

Replacement regime:- No replacement - 48 hour static tests.

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Analysis:- Recordings of pH value, and concentration of dissolved oxygen at the start and end of each test in all concentrations. Recording of the incubator(s) temperature(s) at least twice daily.

Observations:-

1. Visual counting of the surviving numbers of nauplius larvae after 24 and 48 hours exposure, as indicated in protocol 40CFR.
2. Visual estimation of percent hatching of *A. salina* ova, as indicated in KISR TOX 0100.

Personnel:-

1.	Dr. Mohammed Metwally	Study Director (PL)
2.	Mr. F.S.H. Abram	Study Manager (Consultant)
3.	Dr. L. Ali	Task Leader
4.	Mr. H. Al-Shammari	Professional
5.	Mr. K. Al-Matrouk	Technician
6.	Mr. M. Bahlul	Technician
7.	Dr. Mirza Beg	Env. Science KISR.

Quality Assurance:-

Archive of records and samples:- Kuwait Institute for Scientific Research
Bioassay and Ecotoxicology Laboratory
Mariculture and Fisheries Department
P.O.Box 1638, Salmiya, Kuwait.

Date of approval by Sponsor:-

Signatures : **Date :**

Study Director

Sponsor

Quality Assurance Officer:

Amendments:

بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للابحاث العلمية

Date : 5 July, 1995
Ref. No. : TOX SP0020

ريخ :
ع رقم :

DRAFT

STANDARD OPERATING PROCEDURE

Toxicity of Oil in Water Dispersion (OWD) of Kuwait Crude Oil to Vertebrate Fish

Date effective :
Date suspended :

APPARATUS

Glass (or polyethylene) jar 4 to 5 liters with caps
Small mesh dip nets of soft material
Natural seawater
Sheim (Acanthropagrus spp)

METHOD

Test Organisms

Small *Sheim* are to be obtained from a single source for each series of toxicity tests.

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Preparation of Experimental water

Generally in accordance with the Environmental Protection Agency (EPA) protocol 40 CFR, part 300, II. Oil in Water Dispersion (OWD) is prepared by shaking seawater and Kuwait crude oil in a ratio of 1:20 oil to seawater in capped jar.

Shaking is performed on reciprocating or oscillating table for five minutes at approximately 315 to 333 strokes per minute. At the completion of shaking, remove the jar from the shaker to a constant temperature water bath or room, remove the lid and take water quality measurements.

An aliquot of OWD is to be analyzed for total hydrocarbon concentration by fluorometric method according to ROPME manual.

Toxicity Test

In an appropriate jars (or beakers) prepare series of five concentrations in a geometric progression defined by a sighting procedure from OWD stock at 20 ^{o}C +1. Place two fish in each jar with the aid of small-net mesh dip nets of soft material. Initiate aeration to provide dissolved oxygen (DO) and mixing after the fish added. The DO content of test solutions must not drop below 4 ppm. Record pH values, concentrations of dissolved oxygen and temperatures in the testing jars. A concurrent control test in exactly the same manner as other tests is performed. Each test consists of 5 replicates of each 5 concentration and the control.

Observe the number of dead fish in each test container and record at the end of each 24-hour period. Fish are considered dead upon cessation of respiratory and other overt movements whether spontaneous or in response to mild mechanical prodding. Remove dead fish as soon as observed.

At the end of 96-hour period, terminate the fish tests and determine the LC50 values and corresponding confidence limits.

Determination of lethal concentration

LC50 values and their 95 percent confidence intervals are determined by a recognized procedure such as that of Granmo and Larsstuvold.

بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للأبحاث العلمية

Date : 4th July, 1995 لتاريخ :
Ref. No. : TOX SP0020 مرجع رقم :

ACUTE TOXICITY OF OILS TO SHEIM

STUDY PLAN

Purpose of Study: To assess the acute lethal toxicities of Kuwait crude oil and bunker oil using a standard method to form a sighting procedure for the applied research project VR004P. this is entitled "Toxicity and Uptake of Crude Oil and Partially Combusted Oil by Selected Marine Organisms in Kuwait" - Drs. Mohammed Metwally (Project Leader, and Lulwa Ali (Task Leader - Toxicology).

Sponsor: Environmental Sciences Department, Environmental and Earth Sciences Division, KISR.

Test Material: Samples of crude oil and bunker oil, as used for study TOX SP0010, 12th June, 1995.

Test Species: Small specimens of sheim *Acanthopagrus* spp, provided by the Mariculture and Fisheries Department, KISR.

Projected Timescale: July/August 1995.

Test Methods: In general accordance with the Environmental Protection Agency's Protocol 40CFR Part 300, II (1984), and KISR Standard Operating Procedure No: TOX 0011 , other than that the EPA standard species will not be used.

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Analysis: Recordings of pH value, temperature, and concentration of dissolved oxygen at least daily in all concentrations.

Observations:
1. Recording of mortalities at frequent intervals during the first working day of the test, and thereafter at least twice daily for the remainder of the 4-day test period.
2. Recording of unusual or stressed behaviour by the test fish.
3. Weights and fork lengths of a representatiave sample of the test fish.

Mathematical Methods:
1. Estimation of LC50 values with 95 percent confidence limits by the methods of *Grammo* and *Larsstuvold.*
2. Estimation of median periods of survival with 95 per cent confidence limits by the method of *Litchfield.*
3. Expression of the relation between median period of survival and concentration of toxicant by plotting the variables on logarithmic axes.

Personnel:

1.	Dr. Mohammed Metwally	Study Director
2.	Dr. Lulwa Ali	Task Leader
3.	Mr. F.S.H. Abram	Consultant
4.	Mr. H. Al-Shemari	Professional
5.	Mr. K. Al-Matrouk	Technician
6.	Mr. M. Bahloul	Technician.

Quality Assurance: Dr. Mirza Beg — Environmental Science, KISR.

Archive of Records and Samples: Kuwait Institute for Scientific Research,
Bioassay and Ecotoxicology Laboratory,
Mariculture and Fisheries Department,
P. O. Box 1638, 22017 Salmiya, Kuwait.

Kuwait Institute for Scientific Research معهد الكويت للأبحاث العلمية

Date of Approval By Sponsor: 12/7/95

Signatures **Date**

Study Direction for Mohammed "Melwally LULWA ALI 12/7/95

Sponsor 12/7/95

Quality Assurance Officer 12-7-95

Amendments

Kuwait Institute for Scientific Research معهد الكويت للابحاث العلمية

Date : 2/5/95 التاريخ :

Ref. No. : الرقم :

Mr. Frederick S. H. Abram
Consultant Biologist
Hamilton Garrod
Babraham Hall
Babraham
Cambridge CB2 4AT

Fax No. 01223 830073

Dear Fred,

I hope you have reached England safe and well. I also hope that your wife is well.

I am sending you this fax to seek your help for gathering information about training courses/workshop or arranged training available in the UK in the field of Ectoxicity of Marine organisms.

Dr. Metwally spoke to Dr. Al-Muzaini about arranging a training course for me and Hassan in June or July in the UK, and Dr. Al-Muzaini suggested to write to you, since you are in England for such information.

You may provide our address to the apropriate organization to send the relevant information to us directly if you are busy and are unable to do so yourself.

Hoping to see you soon in Kuwait.

Yours sincerely,

Dr. Lulwa Ali
Associate Research Scientist
KISR

Fax : (965) 4845350

HAMILTON GARROD
Consultant Biologists

Babraham Hall
Babraham
Cambridge
CB2 4AT
Tel. 0223 832312 x255
Direct line 0223 830071
Fax 0223 830073.

BY FAX 00965 4845350

Dr Lulwa Ali,
Associate Research Scientist,
Kuwait Institute for Scientific Research.

9th May, 1995.

Dear Lulwa,

Training Courses.

Thank you for your fax of 2nd May. There has been some delay because of the V.E. Day anniversary celebrations in England and the associated public holidays. I am in touch with:-

Miss Rebecca Caldwell,
The Robens Institute,
University of Surrey,
Guildford,
United Kingdom.

It is probable that the Institute can help, but I am doubtful about the timescale which you indicate. The position should clarify in another day or two.

My wife is now convalescent, having been discharged from hospital last Thursday (4th May), and I should be returning to Kuwait on Monday 15th May, as provisionally arranged. Perhaps you will have a word with Dr Al-Muzaini to this effect.

With regards to all,

Yours sincerely,

Fred.

Fred Abram.

Telephoned Rebecca Caldwell 22nd May. Paperwork despatched, but will send a duplicate set to ensure contact FSHA.

بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للابحاث العلمية

22nd May, 1995

Date : التاريخ :
Ref. No. : مرجع رقم :

Mr. I.D. McGredy
Department of Trade and Industry
Westbrook Centre
Milton Road
Cambridge CB4 1YG
United Kingdom

Dear Mr. McGredy,

Sub: Kuwait.

Thank you for your letter No: IM/26/caf/2 of 26th April, 1995, sent to my Cambridge (Hamilton Garrod) address. There appears to be some slight misunderstanding because I have been working in Kuwait since November 1994; my partner Dr. John Garrod, forwarded your letter.

Nevertheless, we would be pleased to have contact with your Mr.Timothy Dearden. Perhaps he will get into touch with me in Kuwait (at K.I.S.R.). There are two or three topics which I would like to discuss, the principal item being the arrangement of training in the United Kingdom for Kuwaiti environmental scientists.

While writing, may I ask you to note that Hamilton Garrod is moving to larger laboratories in North Bedfordshire. The new address is:

Hamilton Garrod Limited
Edgeworth House
High Street
Arlesey, Bedfordshire SG15 6SX.
Telephone: 01462 731292.

With best wishes,

Yours sincerely,

F.S.H. Abram
Consultant Biologist, UNIDO

GOVERNMENT OFFICE
FOR EASTERN REGION

Overseas Trade Services

Mr Frederick Abram
Partner
Hamilton Garrod
Consultant Biologists
Babraham Hall
Babraham
Cambridge
Cambridgeshire CB2 4AT

Westbrook Centre
Milton Road
Cambridge CB4 1YG

Switchboard : (0223) 461939
Fax : (0223) 461941
Telex : 81582DTI EAO

Direct Line : (0223) 346718
Our Ref : IM/26/caf/2
Your Ref :
Date : 26 April, 1995

Dear Mr Abram

VISIT TO CAMBRIDGE

Our records show that you have expressed an interest in exporting to various markets around the world. Mr Tim Dearden, one of our Commercial Officers from the British Embassy in Kuwait, will be in the UK between 12 and 21 June 1995 to meet with businesses **specifically** dealing in the Power Generation, Water, Aviation & Ports and Construction sectors.

If you would like to meet this officer to discuss potential opportunities in the Kuwait market please let me know by Tuesday 9 April 1995. Please note that this is a preliminary enquiry to gauge the interest from businesses in the Eastern Region in meeting Mr Dearden as time will not allow him to visit every region.

If sufficient interest is shown and we are successful in bidding for his time, we will contact those companies who have responded to this letter with a view to arranging an appointment. I look forward to hearing from you and should you require any further information please do not hesitate to contact me.

Yours sincerely

I D McGREDY

بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للأبحاث العلمية

Date :June 4, 1995 التاريخ :
Ref. No. : مرجع رقم

Mr. A. Broderick
Director
British Council
P.O.Box 345
13004 Safat
Kuwait

Dear Mr. Broderick,

Sub : Technical Training in the United Kingdom

Thank you for your cordial reception during the recent visit to the British Council Offices of Dr. Lulwa Ali and myself. As discussed, my function in Kuwait is to provide technical advice to the Kuwait Institute for Scientific Research, under an assignment arranged by UNDP/UNIDO. Part of this remit is to arrange technical training for Kuwaiti scientists who are to staff a new bioassay laboratory recently completed by the Institute, at the Mariculture and Fisheries Department site, KISR Salmiya.

Essentially, what is needed is a brief tour of suitable laboratories and industrial premises in Europe, with a period of say three weeks 'hands on' experience in a laboratory already performing the type of bioassay work envisaged in Kuwait. As mentioned, the background is entirely aquatic. The new laboratory is to provide an environmental protection service for the Arabian Gulf, and it is hoped that this will extend beyond Kuwait national waters within the foreseeable future. No mammalian or avian studies are planned.

Coincidentally, my company in the United Kingdom (Hamilton Garrod Limited, Consultant Biologists) is familiar with my duties in Kuwait, and my Managing Director, Dr. J.F. Garrod would be pleased to assist, we have co-operated with the British Council previously along these lines.

....2/-

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Both KISR and myself will be very grateful for any help or advice which you are able to offer.

Dr. Ali joins me in sending our best wishes both to Dr. Omu and your goodself.

Yours sincerely,

Fred Abram.

F.S.H Abram
Consultant Biologist, UNIDO

FAX MESSAGE

No. of pages to follow this:

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Fax : (965) 2520069, 2551376

e-mail (X-400) : C=GB
A=TMAILUK O=A. Broderick
G=BC S=KUWAIT

To : Dr Mohammed Al-Attar
Deputy Director-General
Research, Life & Environmental Sciences
KISR

From : Director

Date : 22 7 95

Ref : KUW/ 608/8 & 2524/3

Your Ref : DDG/138/63/394

Fax : 4846891

Subject : UNDP/UNIDO Training Visit to UK

Dear Dr Mohammed Al-Attar

Thank you for your letter of 15 July 1995. I have referred your request to our headquarters in London whhere my colleagues have already taken the matter in hand. The will be looking first at the proposal to visit UK and will contact me soon with a possible itinerary and an estimate of the costs.

To help their enquiries could you give me the following information :

1. I have asked my colleagues to give estimates for good class hotels. I hope this is right.

4. Will the group need to be met on arrival at the airport ? Do they need to be guided/accompanied to their appointments or can they find their own way around the country ?

5. We would suggest hiring a mini bus to carry the group around to sites near London. This is probably more convenient than the train and possibly cheaper. Do you agree with this suggestion?

Aidan Broderick

Aidan Broderick
Director

To : Dr. Lulwa ENV
For Your Return
Dr Saleh Al-Muzaini
24/7/95

بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للابحاث العلمية

Date : 13/8/1995 التاريخ :
Ref. No. : DDG/148/63/483 الرقم :

Mr. Aidan Broderick
Director
The British Council
P O Box 345
13004 Safat
Kuwait

Dear Sir,

Thank you for your letter Ref. No.KUW/608/8&2524/3 dated 22/7/95 and appreciate your prompt action.

Please refer to our letter ref. No.DDG/138/63/394 dated 15/7/95 wherein we have emphasized that high priority to be given to locate two toxicologist to perform a training course in our Institute, preferably for a period of approximately two months.

Thanking you once again for your efforts, and hoping for your cooperation and assistance in future.

Yours sincerely,

Original signed by
Dr. Mohammad H. Al-Attar
DDG/L&ENV.

Dr. Mohammed H. Al-Attar
Deputy Director General of Research
Life and Environmental Sciences

CC: DG
DDG-L&ENV
DD/EES
DM/ENV
Central Registry

بسم الله الرحمن الرحيم

Kuwait Institute for Scientific Research معهد الكويت للابحاث العلمية

Date : 16th September, 1995 التاريخ :
Ref. No. : Fax: 2551376 مرجع رقم :

Mr. A. Jones
Deputy Director
The British Council
Kuwait.

Dear Mr. Jones,

Training for K.I.S.R. Scientists

Thank you for your remarks during our recent telephone conversation. May I confirm the position, as discussed:

1. Hotel expenses - absorbtion of costs by Hamilton Garrod Limited.

It is considered that a minimum per diem for the visiting scientist would be approximately STG 50 per head, equal to STG 4,200 for a three week stay for a party of four. This has been discussed by telephone with Dr. J. F. Garrod, and because the Company's quotation for the course is only STG 7,200, it is clearly impracticable to absorb this estimated STG 4,200. Therefore unless your council can indicate an alternative source of financial support it appears improbable that much can be done to contain these expenses.

2. Timetable for the proposed visit:

Hamilton Garrod will be pleased to arrange a tuition course at any time convenient for K.I.S.R., but some prior notice would be helpful because of laboratory programming. A period of two months would seem reasonable. (At present the company is heavily involved with studies mainly to do with the North Sea oil industry).

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Kuwait Institute for Scientific Research معهد الكويت للابحاث العلمية

3.UNDP/UNIDO assignment in Kuwait:

As mentioned, it is probable that I should be leaving Kuwait on the 12th December, following a 2-month extension of assignment. (Please note that this extension is not yet confirmed).

4. Training of scientists in Kuwait:

The proposal to bring expatriate scientist to K.I.S.R. to provide training in toxicology was designed as an alternative to sending a party to the United Kingdom.

5. Department of Trade and Industry:

Further to (1) it is possible that marginal financial support might be available from the D.T.I. A letter to Mr. I.D. McGredy at the Department's Cambridge office will be forwarded shortly, but it has to said that support is less than probable. The officer in the British Embassy is Mr. Timothy Dearden.

I hope that this is of some help; please do not hesitate to telephone if I can clarify further.

Yours sincerely,

Fred Abram.

F.S.H. Abram
Consultant Biologist, UNIDO/UNDP

المجلس الثقافي البريطاني The British Council

Our ref KI/W/608/8

Your ref

شعارنا : تشجيع التعاون بين بريطانيا وأقطار العالم الأخرى في الميادين الثقافية والتعليمية والفنية

ص . ب : ٣٤٥ صفاة ، 13004 كويت
٢ شارع العربي ، المنصورية ، الكويت
تليفون : ٢٥١٥٥١٢ ، ٢٥٣٢٢٠٤ ،
٢٥٢٠٠٦٧/٨ ، ٢٥٣٢٢٢٧
فاكس : (٩٦٥) ٢٥٥١٣٧٦/٢٥٢٠٠٦٩

Promoting cultural, educational and technical co-operation between Britain and other countries

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PO Box 345, 13004 Safat, Kuwait
2 Al Arabi St., Mansouriyah, Kuwait
Telephone 2533204, 2515512, 2533227, 2520067/8
Fax (965) 2551376/2520069

F S H Abram
Consultant Biologist
UNIDO/UNDP
MFD
KISR

FAX 5711293

18 September 1995

Dear Mr Abram

Thank you for your faxed letter of 16 September to my colleague Mr Jones.

I was pleased to see that matters had advanced during my absence on leave. However, I must make it clear that the British Council is not in a position to contribute financially to the costs of the training in Britain or to the visit to Kuwait by the British toxicologists.

In fact, nowadays we would normally charge a fee for the services we have provided so far . We shall not pursue this, of course.

I hope KISR will be able to find a source of funding for what is clearly a very important area of work.

With best wishes

Yours sincerely

Aidan Broderick

Aidan Broderick
Director

cc Dr Dhari Ali Ajmi, Director, Environmental & Earth Sciences Division, KISR

20th September, 1995 **DRAFT**
Fax: 0044 1223 461941

Mr. I.D. McGredy,
Overseas Trade Services
Westbrook Centre,
Cambridge, CB4 1YG
United Kingdom.
Your Ref: 1M/26/caf/2

Dear Mr. McGredy,

Training Visit to the United Kingdom

I write on the advice of Mr. F.S.H. Abram, Consultant Biologist UNDP/UNIDO, in connection with a proposed visit to the United Kingdom by KISR scientists.

The position is that the Government of Kuwait is making great efforts to improve environmental protection, and these include the establishment of a new KISR Bioassay and Ecotoxicology Laboratory. This is designed to provide a general service to the Gulf States, and has the United Nations' support. Initially work is to assess the aquatic toxicity of oils, effluents and other wastes; treatability studies will follow. This in turn leads to industrial consultancy on the installation of treatment plant to condition effluents to an environmentally acceptable standard.

KISR is a semi-academic institute, and at present we suffer from a shortage of funds to support overseas training, particularly to meet subsistence costs, but because of your Department's interest in supporting British industry (including water treatment companies) we feel that you may be able to make partial funding available in this context. The estimated hotel costs for the proposed party of four scientists are approximately 4,200 Sterling. Also, it might be possible to arrange technical literature and/or a works visit to see treatment plant in use and discuss practicalities of effluent disposal.

I shall be very obliged for any advice which you are able to offer.

Yours sincerely,

Dr. Dhari Al-Ajmi
Divisional Director
Environmental and Earth Sciences

Two pages inclusive.

ANNEX IV

Project Document - VR004P KISR4171R

December 1995

REVISED PROPOSAL

TOXICITY AND UPTAKE OF CRUDE OIL AND PARTIALLY COMBUSTED OIL BY SELECTED MARINE ORGANISMS IN KUWAIT

VR004P

M. METWALLY

ENVIRONMENTAL SCIENCES DEPARTMENT
ENVIRONMENTAL AND EARTH SCIENCES DIVISION

SUBMITTED TO

ENVIRONMENTAL PROTECTION COUNCIL

AND

KUWAIT FOUNDATION FOR ADVENCEMENT OF SCIENCES

KUWAIT INSTITUTE FOR SCIENTIFIC RESEARCH
P.O. BOX 24885
13109 - SAFAT - KUWAIT

AUGUST 1995

Toxicity and Uptake of Crude Oil and Partially Combusted Oil by Selected Marine Organisms in Kuwait

Dr. M. Metwally

VR004P

As above

ENV

EES

This project will:

1. Determine the short term (acute and subacute) toxicity of oil and partially combusted oil (PCO) from contaminated sites to fish and aquatic invertebrates. This will determine the toxicity of PCO relative to Kuwait crude oil.

2. Determine the bioconcentration and bioaccumulation of priority pollutants from oil and PCO in fish in the laboratory. This information is vital to understand the potential for contaminant transfer through food chain to humans.

3. Use biomarkers to monitor the responses of natural and cultured populations of aquatic animals to oil-derived contaminants.

4. Training KISR staff on the technology of bioassay techniques and toxicity testing employed in studies of oil and PCO on marine species.

The project will last for 3 years with an estimated budget of K.D. 160,000.

Crude oil - partially combusted oil - toxicity - bioaccumulation - aquatic ecosystem, fish, Kuwait

Contents

Introduction

As a result of the Iraqi invasion of Kuwait, massive quantities of petroleum oil were deliberately spilled into the Arabian Gulf, Kuwaiti soil and atmosphere. In addition to the direct hazardous impact of the crude oil, huge quantities were burned or partially burned which resulted in unpredicted disaster. The fall-out of smoke, mist, soot and particulates has left vast quantities of oil in the terrestrial environment and resulted in contamination of the air, soil and water of Kuwait. Preliminary investigations indicated that plants and soils in impacted areas contain high concentrations of both heavy metals and polyaromatic hydrocarbon components of petroleum oil (M. Abdal, personal communication). These compounds may be neurotoxic, cytotoxic and/or carcinogenic.

The Arabian Gulf and Kuwait Bay are major resources for Kuwait. Oil spilled during the Gulf War presents uncertain levels of risk to fish, shellfish and other biota in these waters as well as human and wildlife consumers. Potential impacts of smoke deposited during the oil field fires and wind-blown contaminated soils which may yet reach these waters are uncertain. Contaminant profiles in sediments and biota will provide one basis for assessing impacts when comparing laboratory and field toxicity studies. However, two major factors will limit the accurate prediction of ecological effects from chemical data alone. First; transport, fate and effects of contaminants will be jointly

determined by characteristics of contaminants themselves and by those of the ecosystems into which they are released. Therefore, relationships between contaminant concentrations in environmental samples and ecological effects will be highly site specific. Second; ecosystems span vast areas and distribution of contaminants will often be far from uniform in environmental samples (Burmaster et al., 1991). There will be a little likelihood that a sampling plan alone can adequately predict exposures for natural animals which move freely in their ecosystem.

Much is known about the toxicity of polycyclic aromatic hydrocarbons (PAHs) and heavy metals to fish and other aquatic life (Connell and Miller, 1984). However, one cannot accurately predict the toxicity of complex mixtures from information on their components. Useful information is available from case studies of other oil contamination incidents (Anderson et al., 1978; Bean et al., 1974) and laboratory experiments with water soluble oil fractions (Dauble et al., 1982).

Biological effects of PAHs were extensively reviewed by Molven and Goksøyr (1993) in a paper which includes 155 references, and covered field studies from the North Sea, the Baltic Sea and North America. These workers classified the biological effects of xenobiotics according to three levels of organization:-

(1) Biochemical effects (i.e. effects at the molecular and cellular level);

(2) Physiological/pathological effects (i.e. effects on tissues, organs, and organisms;

(3) Ecological effects (i.e. effects on populations, communities and ecosystems).

Practical studies are classified into two groups:-

(1) One or a few toxicants are investigated under laboratory conditions, the biota being exposed to the compounds at relatively high concentrations by injection, ingestion or from the ambient water.

(2) Xenobiotic material, as it occurs in nature, is investigated, the organisms being exposed at relatively low concentrations either in the laboratory or the field.

While the literature mentioned above provides general ideas of oil contaminant impacts on aquatic life, specific data are necessary for understanding the situation in Kuwait. This is particularly true given widespread contamination with partially combusted oil (PCO) from the oil fires. Kuwait Bay and the Arabian Gulf are major sites to which terrestrial deposits of contamination will be transported by the wind. Upon transport to these ecosystems, the long-term risks of toxicity and food chain transfer in aquatic life is unclear. It is necessary to directly assess the toxicity and accumulation of contaminant mixtures present in soils and sediments on fish and marine invertebrates.

In addition to issues concerning adverse impacts on natural populations of aquatic animals, the potential for contaminant accumulation in fish and shellfish will be carefully evaluated. Preliminary results for heavy metals indicate that lead, nickel, manganese and perhaps vanadium are present in parts per million

concentrations in contaminated soils surrounding oil fields (M. Abdal, personal communication). Fortunately, these particular heavy metals are not strongly bioaccumulated by aquatic animals (Connell and Miller, 1984). The PAHs are also present in parts per million concentrations in PCO and will be of more concern with regard to bioaccumulation (L.R. Curtis, personal communication). The chemical composition of PCO collected from oil lakes was investigated in a recent study conducted at KISR (Saeed et al., 1993). The study showed that the concentration of PAHs is higher in PCO compared to crude oil.

While some fish metabolize and excrete PAHs at reasonably rapid rates (Curtis et al., 1990; Dauble and Curtis, 1989) available information suggests shellfish are much less efficient in this regard (Little et al., 1985). Accumulation of PAHs by shellfish should receive priority in monitoring programs. Laboratory research proposed here will define the rates at which contaminants present in Kuwait are accumulated and eliminated by aquatic animals. Harkey et al. (1994) studied the assimilation of PAHs and polychlorobenzenes (PCB) by the sediment-dwelling amphipod *Diporeia* spp. They reported that organic contaminants with similar lipid solubilities (POW) associated differently with organic carbon, which resulted in differential bioavailability to *Diporeia*.

Laboratory research in this area will provide specific benefits for assessing risks posed by oil-derived contaminants and decision making on mitigation of contaminated sites in the following ways:

(1) Quantitative estimates of toxicity in established biological assay systems will provide essential data for the ecological risk assessment for natural resources.

(2) Determination of bioconcentration and bioaccumulation (food chain transfer) potential in fish and shellfish is an important component of human health risk assessment.

(3) Identification of sensitive biological responses to oil and PCO will provide cost-efficient technology suitable for monitoring contamination of aquatic ecosystems.

(4) Established biological assay systems provide meaningful and cost-efficient methods for assessing the effectiveness of remediation technologies. That is, a given reduction in material toxicity can serve as a benchmark for acceptable technology performance. Biological assay of contaminated soil and sediment can also be used to identify priority sites for remediation.

It is important to mention that there will be another study, in the field, complementing this project which will investigate the bioaccumulation and biomonitoring of crude oil and PCO in fish collected from Kuwaiti waters using the methodology adopted in this project. The proposed filed study will start at the end of this project.

Objectives

The general objectives for the proposed research in Aquatic Toxicology are:

1. To determine the short term (acute and subacute) toxicity of oil and PCO from contaminated sites to fish and aquatic invertebrates. This will determine the toxicity of PCO relative to Kuwait crude oil.
2. To determine the bioconcentration and bioaccumulation of priority pollutants (mainly PAHs) from oil and PCO in fish in the laboratory. This information is vital to understand the potential for contaminant transfer to animals (particularly humans) which consume fish and shellfish. Consumption of contaminated fish and shellfish is believed to be the primary route of exposure of several toxic chemicals in the industrialized world.
3. To screen recommended methodologies related to monitoring the responses of natural and cultured populations of aquatic animals to oil-derived contaminants. Laboratory work will provide new methods faster and answer questions more directly than field work alone.
4. Train KISR personnel on the technology of bioassay techniques and toxicity testing employed in studies of oil and PCO on marine species.

Scope of Work

Task 1: Mobilization

This task will comprise the first 3 months of the project and will ensure the availability and proper performance of all personnel and materials needed for the execution of the subsequent tasks. In particular, it will ensure the availability of test organisms, tanks, pumps, and mixers needed to execute other tasks.

The choice of species and size range of experimental fish will clearly need to be modified from those indicated in the standard methods (such as American Society for Testing and Materials, 1980a,b) to form a representative pattern for the Arabian Gulf. In general, the preferred length for fish in tests for acute lethality is around 5 centimeters. For bioconcentration, bioaccumulation and biomonitoring tests, larger animals (15-20 cm) may be necessary to provide sufficient biomass for the chemical analysis. *Epinephelus tauvina* (Hamoor) and *Pampus argenteus* (Zobaidy) will be the candidate fish species. In case of shellfish, local shrimp which is known as *Penaeus semisulcatus* (Roubyane Om-Nairah) will be suggested.

Task 2: Toxicity Testing (acute and chronic)

The amount (dose) of a chemical required to produce adverse effects defines its potency (Parrish, 1985). The dose an aquatic animal receives is determined by exposure concentration and duration. Exposure may be either from water or food. Standard

aquatic toxicity testing (American Society for Testing and Materials, 1980a,b) of actual contaminant mixtures present in Kuwaiti oil and PCO will provide a vital link between chemical analyses of these materials and literature on the chemical fate and toxicity of their constituents. Toxicity data will play the critical role of validation for extrapolation of previous research results to risk assessments for Kuwait.

Short term toxicity tests with fish and invertebrates (Parrish, 1985) will involve waterborne exposures. Exposure waters will be prepared by recirculating clean water through a column packed with a glass support matrix and oil or PCO. This design will provide solutions saturated with hydrophobic organic chemicals and metals (Curtis et al., 1985; Siddens et al., 1986).

The numbers of samples to be evaluated for toxicity will depend upon the number of replicate toxicity tests required per sample and the number of concentrations needed for each test. It is considered that a target of 20-30 toxicity tests using local fish and/or shell fish on contaminated soils, crude oils and oil partly combusted under laboratory conditions might be suitable. Each test will use five contaminant concentrations and dilution water controls. Each exposure condition will be duplicated with 10 individuals per replicate.

There will be two general experimental protocols for exposure of fish and an invertebrate species using available standard methods (American Society for Testing and Materials, 1980a,b). In the first, fish will be exposed to contaminant saturated water and

a logarithmic series of lower concentrations for 96 hours in flowing water systems. The cumulative mortality occurring at each concentration over the exposure interval will be determined. These data will define an exposure concentration- response (mortality) relationship. A modification of standard regression analysis (Litchfield and Wilcoxon, 1949) will permit estimation of the contaminant concentration required to kill 50% of the fish or shellfish within the course of the test (i.e., 96-hr LC50). Measurement of contaminant concentrations in exposure water will be required. Hexane extracts of water will be analyzed for PAHs by high pressure liquid chromatography with fluorescence detection and gas chromatography with flame ionization detection. Nitric acid extract will be analyzed for heavy metals by atomic absorption spectrophotometry.

The second biological assay technique will involve exposures to lower sublethal contaminant concentrations for longer periods (American Society for Testing and Materials, 1980a,b). The highest concentration will be a fraction of the 96-hr LC50 and exposures will continue for 28 days plus a depuration time. Growth rates of biota and mortality will be determined over this period. The conditions of these exposures will be more environmentally relevant than for 96-hr LC50 tests. Chemical analyses of fish exposed in these tests will estimate bioconcentration potentials for priority contaminants (detailed below).

Mortality tests for invertebrates will be conducted in a manner similar to those for fish using appropriate standard methods

(American Society for Testing and Materials, 1980a,b). Longer tests will be conducted to determine growth rate. Exposure concentrations will be based upon LC50 values.

Toxicity tests of the types described here will be of great value. That is, lethal and sublethal effect concentrations for biota exposed to water saturated with oil and PCO will be compared to literature values for its constituents. These data will allow a quantitative assessment as to whether unidentified constituents or chemical interactions pose toxic hazards to aquatic animals.

Task 3: Bioaccumulation

There is a concern that contaminants present in PCO and spilled oil may accumulate via the food chain to harmful levels in fish and shellfish of Kuwait Bay and the Arabian Gulf. Laboratory experiments can provide reliable estimates of the potential for these complex mixtures to be absorbed and eliminated by fish and shellfish. A good information base exists for individual PAHs (Curtis et al., 1990; Little et al., 1985) and nitrogen heterocycles (Dauble and Curtis, 1989), but the behavior of complex mixtures is unpredictable due to the potential synergistic or antagonistic impacts.

Chemical analyses of fish exposed in the growth and uptake from water described above (Task 2) will be an important step in assessing bioconcentration. Standard bioconcentration tests (American Society for Testing and Materials, 1980a,b) are the tests of choice to determine pollutant uptake from water only. Extracts

of whole fish, edible flesh and bile (the primary route of PAH excretion) will be analyzed by high pressure liquid chromatography (Freudenthal et al., 1975). More detailed analyses for fish will follow general methods described previously (Curtis et al., 1990; Dauble and Curtis, 1989). Hexane extracts of PCO and tracer amounts of radiolabelled PAH will be incorporated into fish food. This technique will assess uptake from water and food, simulating natural bioaccumulation. Standard bioaccumulation tests (American Society for Testing and Materials, 1980a,b) are the tests of choice to determine pollutant uptake from both water and food. Other bioaccumulation tests will be conducted with a diet containing tracer amounts of radiolabelled PAH alone. Labeling different chemicals with different radioisotopes (e.g., ^{14}C and ^{3}H) will permit their simultaneous measurement using dual label counting (Curtis and Hoyt, 1984). Chemical analysis will also be used to confirm the radiolabelled compounds. It is planned that 4 bioacocentration/bioaccumulation tests will be conducted in this task.

These studies will determine contaminant elimination rates important in risk assessment. They will also determine how elimination of toxic constituents is influenced by occurrence in a complex mixture. Understanding the potential of oil-derived PAHs to bioaccumulate in the aquatic life food chain is essential for setting rational policy to protect the resource and health of human and wildlife consumers of seafood.

Task 4. Biomonitoring Using Biomarkers

While laboratory research provides reliable data essential for risk assessment, environmental exposure scenarios are difficult if not impossible to predict (Curtis, et al., 1990). For this reason, monitoring natural biological resources is an essential component of a comprehensive risk assessment effort (Payne, 1976; Goksøyr and Forlin, 1992; Curtis et al., 1993). Analyses of animals for contaminant residues is one valuable approach to monitoring but is expensive and poorly detects exposures to substances which are rapidly eliminated. Methods which determine responses of organisms to contaminant exposures (biomarkers) can be substantially less expensive than analytical chemistry (Beyer and Goksøyr, 1993; Stegeman et al., 1992). This is an important consideration when entire ecosystems are in need of monitoring. Further, biomarkers which are sensitive to PAHs are particularly well-established. We intend to validate biomarkers with laboratory exposures to oil and PCO for monitoring in Kuwait (Beyer and Goksøyr, 1993).

The cytochrome P-450 system is composed of a family of enzymes which metabolize a variety of organic chemicals including PAHs. One important characteristic of this enzyme system is its adaptative capacity to increase activity after exposure to PAHs. The activity of a PAH-responsive enzyme can increase up to 100-fold after repeated exposures (Payne et al., 1987; Goksøyr, and Forlin, 1992). This phenomenon is termed induction and the underlying molecular mechanisms are well understood (DePierre et al., 1975; Prough et al., 1978; Nebert, 1989). Payne (1976) suggested

determinations of cytochrome P-450 activities in marine animals as a means for monitoring oil contamination. We propose to measure the cytochrome P-450 activities after laboratory exposures to oil and PCO following the Beyer and Goksoyr's procedure (Beyer and Goksφyr, 1993). These determinations will critically evaluate the sensitivity of these biomarkers for eventual monitoring efforts in Kuwait Bay and the Arabian Gulf. The exposure concentrations which induce cytochrome P-450 activities can then be directly compared to those which produce mortality or inhibit growth. It is planned that 4 biomarker tests will be conducted in this task.

Task 5: Data Analysis and Reporting

This task will coordinate calculation and interpretation of all the data generated by the other tasks and will submit progress reports and a final report at the end of the project. The latter will evaluate all findings and results.

Expected Output

The general outputs of this project will be:

1. To provide quantitative assessment of toxicity in established biological assay systems.
2. To determine the bioconcentration and bioaccumulation potential of oil and PCO in the marine ecosystem.
3. To identify sensitive biological responses to oil and PCO and

to establish biological assay systems that provide meaningful and cost-efficient methods for assessing the effectiveness of remediation technologies.

4. To have KISR scientists participate in various aspects of aquatic toxicology and in developing coordinated, comprehensive approaches to problem solving, as well as evolvement of expertise necessary for the continuing development of broadly based and flexible aquatic toxicological work over the long-term.

Project Team

Project Leader: Dr. Mohammed Metwally

Task Leaders	**M/M**
Task 1: Leader: Dr. Mohammed Metwally (R)	
Dr. M. Metwally (R)	0.29
Dr. Sami Al-Yakoob (R)	0.25
Dr. M. Beg (R)	0.25
Dr. L. Ali (R)	0.25
Task 2: Leader: Dr. Lulwa Ali (R)	
Dr. L. Ali (R)	3.00
Dr. M. Beg (R)	1.00
Dr. M. Metwally (R)	1.00
Dr. S. AL-Yakoob (R)	0.50
Dr. T. Saeed (R)	0.50

Mr. Hassan Al-Shemmari (P)	5.00
Mr. P.G. Jacob (P)	1.23
Mr. Ali El-Baz (P)	1.00
Mr. Majid Bahloul (T)	1.00
Mr. K. Al-Matrouk (T)	6.00
Mr. Ahmad Al-Khabbaz (T)	1.00
Task 3: Leader Dr. Mohammed Metwally (R)	
Dr. M. Metwally (R)	3.00
Dr. M. Beg (R)	0.75
Dr. T. Saeed (R)	1.25
Mr. Hassan Al-Shemmari (P)	0.50
Mr. P.G. Jacob (P)	3.50
Mr. Ali El-Baz (P)	0.50
Professional (CAL, P)	1.50
Mr. Majid Bahloul (T)	6.00
Mr. K. Al-Matrouk (T)	1.00
Mr. Ahmad Al-Khabbaz (T)	1.00
Task 4: Leader: Dr. Sami Al-Yakoob (R)	
Dr. S. AL-Yakoob (R)	3.00
Dr. M. Metwally (R)	0.75
Dr. M. Beg (R)	0.75
Dr. T. Saeed (R)	0.50
Mr. Ali El-Baz (P)	3.00
Mr. Hassan Al-Shemmari (P)	1.00
Mr. P.G. Jacob (P)	1.00
Mr. Majid Bahloul (T)	1.00

Mr. K. Al-Matrouk (T)	1.00
Mr. Ahmad Al-Khabbaz (T)	6.24

Task 5: Leader: Dr. M. Metwally

Dr. M. Metwally (R)	0.50
Dr. S. Al-Yakoob (R)	0.50
Dr. L. Ali (R)	0.50
Dr. M. Beg (R)	0.50

Organization and Management

The project will be carried out by the Environmental Sciences Department of the EES Division. Details of the project organization are shown in Fig. 1.

Project Requirements

A. Manpower

It is estimated that a total of 19.04 m/m Researchers, 18.23 m/m Professionals, and 24.24 m/m Technicians, will be required from KISR.

B. Materials and Operation Costs

For the performance of the various project activities it is

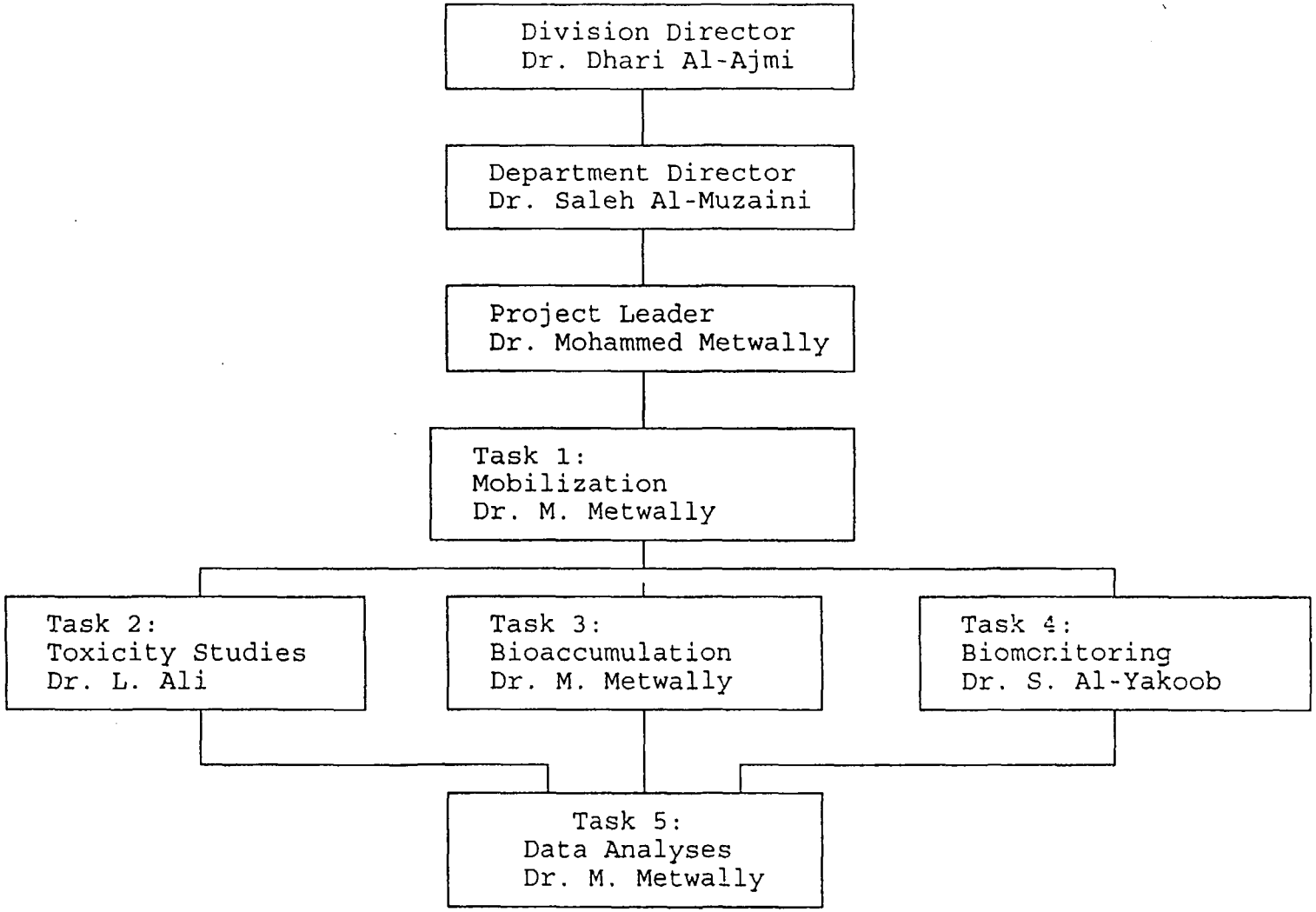


Fig. 1. Organization Chart

essential to put the foundation for an aquatic toxicology laboratory. Different fish and shrimp species, laboratory supplies including HPLC-solvents, glassware, and analytical-grade chemicals are required.

Project Duration

The duration of the project will be 3 years. Table 1 shows the project time schedule.

Training Plan

KISR staff participating in this project in Kuwait will receive a comprehensive on-the-job training through their par ticipation. Upon completion of the project, the trainees will be associated with:

1. State-of-the-art techniques of aquatic toxicology.
2. Sample analyses
3. Data handling and interpretation
4. Writing reports.

Budget

The total budget is estimated at 160,000 Kuwaiti Dinars. Table 2 summarizes the budget.

Table 1: Time Table for the Cooperative Research Project Between KISR and OSU

		O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S
		1995 1996 1997 1998
Task 1:	Mobilization	= = =
Task 2:	Toxicity studies	= =
Task 3:	Bioaccumulation	= =
Task 4:	Biomonitoring	= =
Task 5:	Data analyses and reporting	= = = = = = = = = = =

Table 2. Project budget (in K.D) sheet for the project.

Category		Cost	
Salaries, Wages & Benefits			
Researchers	19.04 (m/m)	41,500	
Professionals	18.23 (m/m)	17,600	
Technicians	24.24 (m/m)	15,900	

Subtotal			75,000
Operating expenses			
Experimental Laboratory Supplies		35,000	
Publication costs		1,000	
Operating Charges (Temporaries)		9,000	
Consultnts		5,000	
Services Charges (CAL, MFD)		8,000	
Travel		6,000	
Others (Car rental and Boat rental)		8,000	
Miscellaneous		3,000	

Subtotal			75,000
Capital Expenses			
Spectrophotometer			10,000

Total Cost			160,000
			=========

References

American Society for Testing and Materials. 1980a. Standard Practice for conducting toxicity tests with fishes, macroinvertebrate, and amphibians. ASTM E 729-80. Philadelphia.

American Society for Testing and Materials. 1980b. Standard Practice for conducting toxicity tests with larvae of four species of bivalve Molluscous. ASTM E 724-80. Philadelphia.

Anderson, J.W., R.G. Riley, and R.M. Bean. 1978. Recruitment of benthic animals as a function of petroleum hydrocarbon concentrations in the sediment. J. Fish. Res. Board Can. 35(5): 776-790.

Beyer, J. and A. Goksoyr. 1993. Cytochrome P450 observations in Gulf fish.Marine Poll. Bul. 27:293-296.

Bean, R.M., J.W. Blaylock, E.A. Sutton, R.E. Wildung, and F.M. Davidson. 1974. Characterization of sediments in the vicinity offshore petroleum production. Am. Chem. Soc. Div. Petrol. Chem. 19(4): 726-735.

Burmaster, D.E., C.A. Menzie, J.S. Freshman, J.A. Burris, M.I. Maxwell and S.R. Drew. 1991. Assessment of methods for estimating aquatic hazards at superfund-type sites: a cautionary tale. Environ. Toxicol. Chem. 10:827-842.

Connell, D.W. and G.J. Miller. 1984. Petroleum and related hydrocarbons. In, Chemistry and Ecotoxicology of Pollution. (R.L. Metcalf and W. Strum, Eds.). John Wiley and Sons New

York, NY. Pages 238-249.

Curtis, L.R. and D. Hoyt. 1984. Impaired biliary excretion of taurocholate associated with increased biliary tree permeability in mirex or chlordecone pretreated rats. J. Pharmacol. Exp. Ther. 231: 495-501.

Curtis, L.R., H.M., Carpenter, R.M. Donohoe, D.E. Williams, O.R. Hendstorm, M.L. Deinzer, M.A. Beilstein, E. Foster, and R. Gates. 1993. Sensitivity of cytochrome P-450 1A1 induction for distribution of TCDD and TCDF in Willamette River, Oregon, Env. Sci. Technol. 27(10):2149-2157.

Curtis, L.R., W.K. Seim and G.A. Chapman. 1985. Toxicity of fenvalerate to developing steelhead trout following continuous or intermittent exposure. J. Toxicol. Environ. Hlth. 15:445-457.

Curtis, L.R., L.K. Fredrickson, H.M. Carpenter. 1990. Biliary excretion appears rate limiting for hepatic elimination of benzo[a]pyrene by temperature acclimated rainbow trout. Fundam. Appl. Toxicol. 15: 420-428.

Dauble, D.D. and L.R. Curtis. 1989. Rapid bronchial excretion of dietary quinoline by rainbow trout (Salmo gairdneri). Can. J.Fish. Aqua. Sci. 46:705-713.

Dauble, D.D., W.E. Fallon, R.H. Gray, and R.M. Bean. 1982. Effects of coal liquid water soluble fractions on growth and survival of four aquatic organisms. Arch. Environ. Contam. Toxicol. 11: 553-560.

DePierre, J.W., M.S. Moron, K.A.M. Johannesen, and L. Ernster.

1975. A reliable, sensitive, and convenient radioactive assay for benzpyrene monooxygenase. Anal. Biochem. 63: 470-484.

Freudenthal, R.I., A.P. Leber, D. Emmerling and P. Clarke. 1975. The use of high pressure liquid chromatography to study chemically induced alterations in the pattern of benzo[a]pyrene metabolism. Chem. Biol. Interact. 11: 449-458.

Goksøyr, A and L. Forlin. 1992. The cytochrome P-450 in fish, aquatic toxicity and environmental monitoring. Aqua. Toxicol. 287:312-307.

Harkey, G.A., M.J. Lady, J. Kukkonen, and P.F. Landrum. 1994. Feeding selectivity and assimilation of PAH and PCB in *Diporeia* spp. *Environmental Toxicology and Chemistry*, 13,9, 1445-1455.

Litchfield, J.T. and F. Wilcoxon. 1949. Simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99-113.

Little, P.J., M.O. James, J.B. Pritchard, and J.R. Bend. 1985. Temperature-dependent disposition of(14C) benzo[a]pyrene in the spiny lobster, Panulirus argus. Toxicol. Appl. Pharmacol. 77: 325-333.

Molven, A. & Goksøyr, A. Biological effects and biomonitoring of organochlorines and polycyclic aromatic hydrocarbons in the marine environment. *Ecotoxicology Monitoring* (Ed. M.L. Richardson) 1993, *VCH Publishers Weirheim, Federal Republic of Germany*. pp 137-162 ISBN 1-56081-736-4.

Nebert, D.W. 1989. The Ah locus: genetic differences in

toxicity, cancer mutation, and birth defects. Crit. Rev. Toxicol. 20: 153-174.

Payne, J.F. 1976. Field evaluation of benzopyrene hydroxylase induction as a monitor for marine petroleum pollution. Science. 191: 945-946.

Payne, J.F., L.L. Fancey, A.D. Rahimatula, and E.L. Porter. 1987. Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. Comp. Biochem. Physiol. 86C:233-245.

Prough, R.A., M.D. Burke and R.T. Mayer. 1978. Direct fluorometric methods for measuring mixed-function oxidase activity. Meth. Enzymol. 520: 372-377.

P.R.Parish. 1985. Acute toxicity tests. In, Fundamentals of Aquatic Toxicology, (Rand G.G. and S.R. Petrocelli, Eds.). Hemisphere, New York, Pages 31-56).

Saeed, T., A. Al-Bloushi, and K. Al-Matrouk. 1993. Study of the chemical composition of oil in oil lakes and effect of weathering on aromatics. Kuwait Institute for Scientific Research, Report No. KISR4301, Kuwait.

Siddens, L.K., W.K. Seim, L.R. Curtis, and G.A. Chapman. 1986. Comparison of continuous and episodic exposure to acidic, aluminum-contaminated waters of brook trout (Salvelinus fontinalis). Can. J. Fish. Aquat. Sci. 43: 2036-2040.

Stegeman, J.J., M. Brouwer, T.D. Richard, L. Forlin, B.A. Fowler, B.M.Sanders, and P.A. Van Veld. 1992. Molecular responses to environmental contamination: Enzyme and protein systems as

ANNEX V

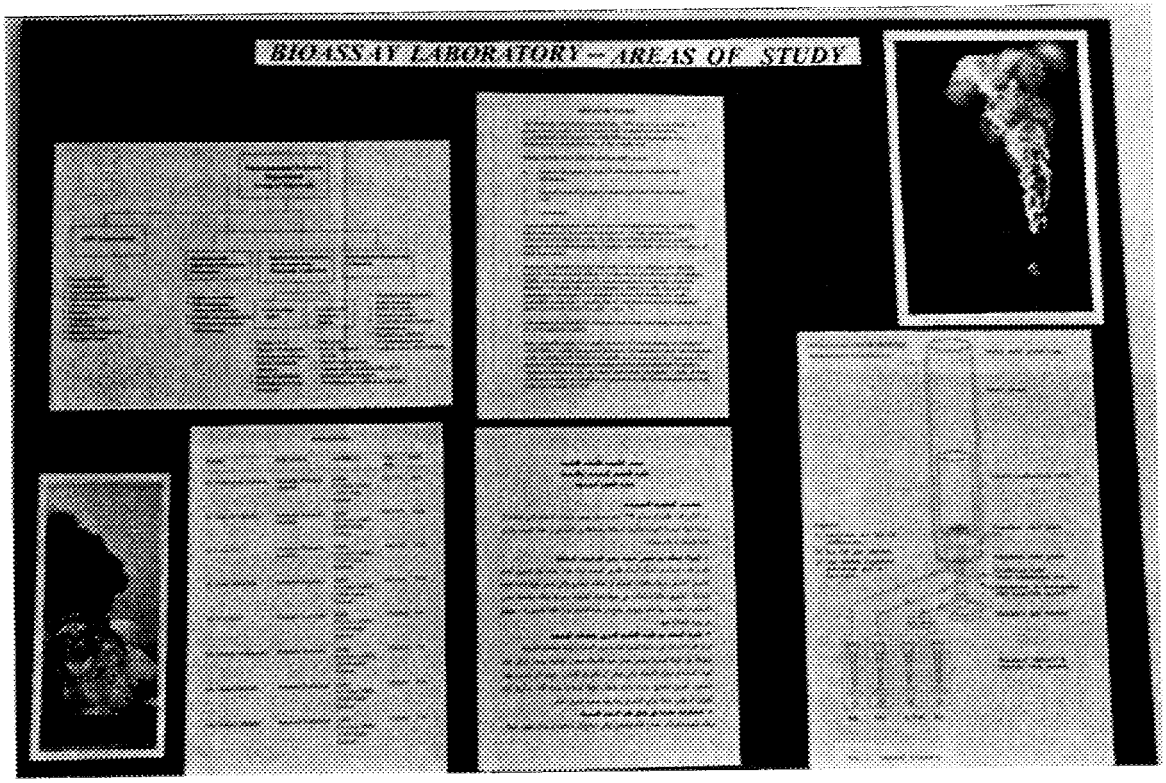
ANNEX V Photographs of the Inauguration Ceremony December 1995

Inauguration of the Bioassay Laboratory

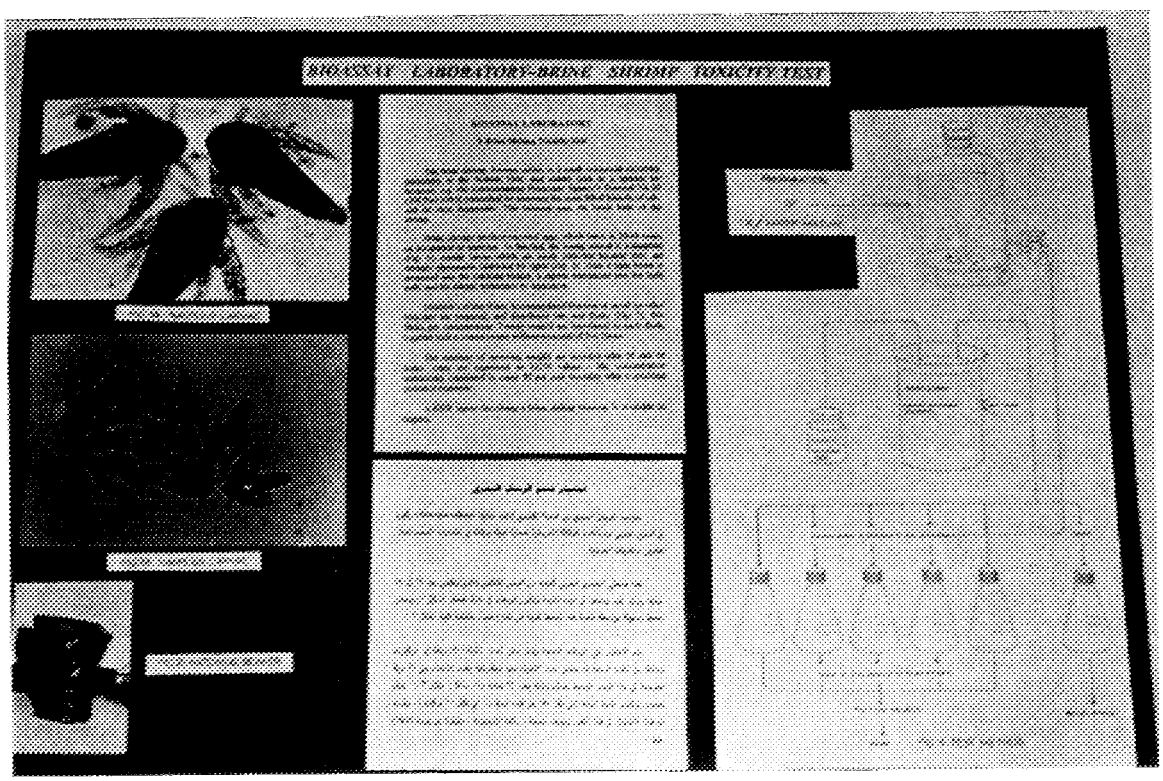
The new premises of the Mariculture and Fisheries Department, KISR, and the Bioassay Laboratory of the Environmental Sciences Department were opened officially on 20th November 1995 by Dr. Ahmed A. Al-Rubai, Chairman of the Board of Trustees.

Approximately 200 guests were present, plus members of staff, as shown in Plate I.

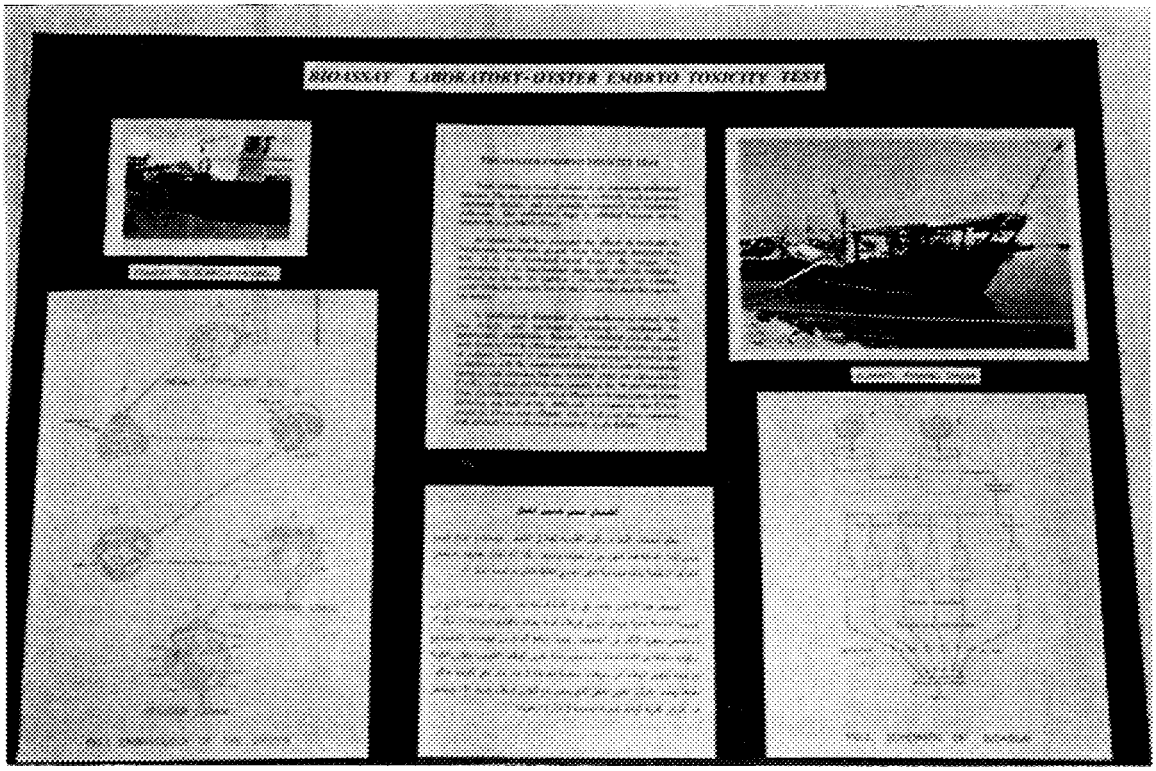
I. Opening Ceremony, Guests and members of staff.



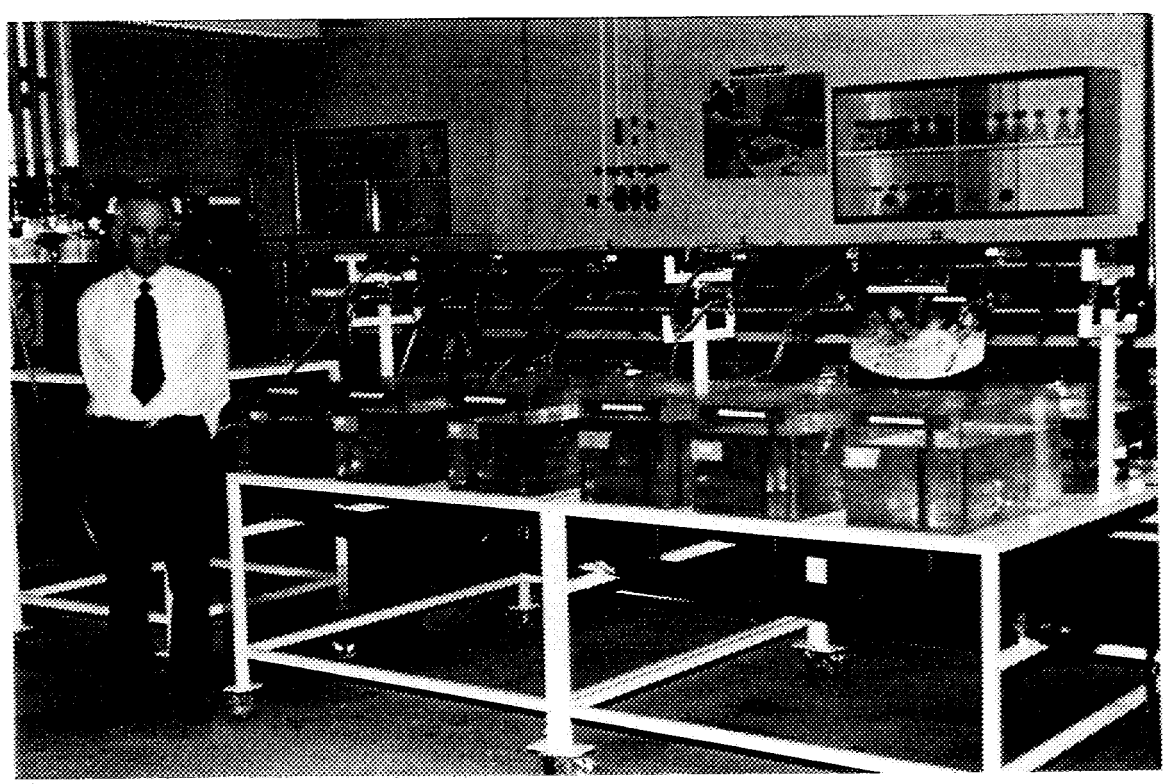
2. Introductory Poster to the Bioassay Laboratory.



3. Poster supporting a demonstration of the brine shrimp bioassay.



4. Poster showing the oyster embryo bioassay (in support – local pearl fishing).



5. Constant-flow toxicity apparatus. (Figs 1 and 2 page 7, refer).

ANNEX VI

UNIDO's Substantive Comment on the Report of Mr. F.S.H. Abram

The expert put his efforts to set up a new ecotoxicology laboratory in Kuwait for identifying agents causing degradation of marine resources. He succeeded in establishing toxicity testing system with aquatic invertebrates, fish, and larvae of coral. Much emphasis has been placed on constructing laboratory equipments, such as toxicity test apparatus and apparatus for controlled combustion of oil. Emphasis was also given to undertake the testings under GLP system. The initial step for GLP was taken by preparing necessary SOPs for the testings. Due to the constraints of a six-month delay in completion of the premises and lack of financial provision for the needed equipments, the expert could not proceed to give training on acute toxicity tests with algae and chronic tests with the three species.

Despite the achievement less than planned within the limited time frame and domestic situation of Kuwait, the impact of his achievement on every sector of Kuwait is deemed to be enormous. One of the indication of this conclusion came directly from the request of toxicity test of oil slick from a private company. There was also a request from academia to utilize the facility for thesis work for Ph.D. (letter of Mr. Abram to the back-stopping officer of UNIDO on 19 August 1995). It would be only the beginning of requests to KISR for services of these kind from all sectors of the Kuwait society, when we recollect from the previous UNIDO experiences in the implementations of the same projects in the Republic of Korea and Pakistan. Once the laboratory is under normal operation, those requests will be expected also from the neighbouring countries of the region and the world. Therefore, the facility would contribute to not only for understanding the impact of oil after the War of Kuwait, but also for managing toxic chemicals of environmental concern in the region where KISR should take a leading role.

The government and KISR need to be ready to support the laboratories to the stage of 'excellence' in techniques in both testings and related researches. More cooperative activities among concerned laboratories of KISR and technical assistance from developed countries will be needed to achieve the goal of excellence in the region, especially in the following areas: rearing of test organisms, chronic tests, environmental chemical tests, model ecosystem, in situ monitoring, and risk assessment.