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RESEARCH AND DEVELOPMENT IN PESTICIDES

DP/CPR/80/008

THE PEOPLES'S REPUBLIC OF CHINA

Technical report: Findings and recommendations*

Prepared for the Government
of the People's Republic of China
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Developme .t Programme

Based on the work of L. G. Copping,
bioassay specialist and technical adviser

Backstopping officer: B. Sugavanam
Chemical Industries Branch

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Vienna

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* This document has not been edited.

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Table of Contents

	Page
Abstract	1
Introduction	3
A. Screening Techniques	4
B. Safe Working Practice	13
C. Field Research	14
D. Mixtures	16
E. UNIDO/UNDP Equipment	16
F. Data Capture	18
G. Recommendations	19
Fig. 1: Existing Glasshouse Facility	21
Fig. 2: Proposed changes to Glasshouse	22
Job Description	23
UNIDO Comments	25

Abstract

The project funded by UNIDO/UNDP, the government of the United Kingdom of Great Britain and Northern Ireland and the Government of China to train staff at the Shenyang Research Institute of Chemical Industry in modern discovery research techniques and to construct a Bioassay Laboratory on a green field site next to the new Toxicological Laboratory (Project DP/CPR/80/008/11-73) is nearing its conclusion. The Bioassary Laboratory is complete, equipment purchased by UNIDO/UNDP is installed and four trainees were selected for training in Great Britain. The purpose of this visit was to ensure that screening techniques were relevant to the research targets and that new equipment and facilities were being used in an effective manner.

The purpose of the unit must be to discover new compounds or new mixtures of relevance to the pest problems of China's agricultural systems. This was not the case. Recommendations were made for improvements. SRICI's chemists should concentrate on analogue synthesis (not direct copies) of successful products and natural product chemistry. Compounds for empirical screening should be sought from other Institutes and Universities. Insufficient attention was being paid to personal safety in the Laboratory, spray areas and glasshouses. Improvements were suggested. The sprayers should be separated from growing areas. The design of the glasshouses is completely wrong for biological testing. Modifications to the internal fixtures were recommended. Much of the UNIDO/UNDP supplied equipment is working well but adjustments need to be made to the sprayer and the growth cabinets.

A more sophisticated data capture/retrieval system is needed. This will require funding. In addition a library/information service should be present at the Bioassay Laboratory.

UNIDO/UNDP should continue their support of this project to guarantee its success.

Report on Technical Visit to Shenyang Research Institute of Chemical Industry under UNIDO Project DP/CPR/80/008/11-73

by Leonard. G. Copping, 34 Saxon Way, Saffron Walden, Essex

Introduction - Programmes funded jointly by UNIDO/UNDP, ODA and the Government of China have established a Toxicology and a Bioassay Laboratory just outside Shenyang, Liaoning Province, China. In addition experts have spent time at the Institute reviewing their biological programmes and recommending advances in their procedures. Four young graduates, selected on the basis of their competence in English and their promise within the Institute, were sent to the United Kingdom for training in Discovery Research at appropriate Industrial and Government facilities.

In October 1990 UNIDO funded an International Conference on "Advances in Crop Protection in China and other Developing Countries of the Region" to celebrate the completion of the Bioassay Laboratory. The present visit was designed to strengthen fungicide, insecticide and herbicide research and development at the Shenyang Research Institute of Chemical Industry (SRICI). The opportunity was taken to :-

i) Discuss screening techniques with each section.

ii) Recommend the adoption of safe working techniques.

iii) View and discuss the methodology used in Field Research.

iv) Discuss the importance of mixtures and the evaluation of their importance and relevance to agriculture.

v) Review the use of UNIDO/UNDP supplied equipment and the laboratory complex.

vi) Discuss the storage and subsequent search of biological and chemical data.

A. Screening Techniques

Herbicide Group - There are six scientists in the Herbicide Group. These are:

Madam Cui Ji Fang	Group Heads
Madam Wei Xiao Li	
Mr Lin Chang Fu	
Mr Lang Zhao Guang	
Miss Sun Zhi Fang	
Mr Sun Bao Xiang	

Currently the primary screen consists of six plant species:-

Echinochloa crus-galli	Grasses
Avena fatua	
Digitaria sanguinalis	
Oil Seed Rape	Broad-leaved species
Flax	
Alfalfa	

Compounds are applied at 2.25 Kg/ha pre and post-emergence. Pre-emergence treatments are poured onto the soil and post-emergence treatments are applied by spray.

The development of these test species is a combination of important species and a concentration, since 1984, on the synthesis and evaluation of sulphonyl urea herbicides. The grasses are important weed species in China and the broad-leaved species are known to be susceptible to sulphonyl ureas. For this reason the species list is

unbalanced and does not represent the weeds which occur in the important food crops of China. After discussion, it was recommended that the list of primary specied be modified such that it did represent real targets as follows:-

Weed species	Crop
Echinochloa crus-galli	Rice, Soybeans, Corn, Cotton
	Total weed control.
Avena fatua	Wheat, Total weed control.
Cyperus rotundus	Rice, corn, cotton, soybeans
	Total weed control.
Potamogeton natans	Rice, Total weed control
Abutilon theophrasti	Cotton, Soybeans, Corn,
	Total weed control.
Amaranthus tricolor	Wheat, Corn, Soybeans, Cotton
	Total weed control

At present the compounds which show activity at the primary level are selected by the biologists and are evaluated further at reduced rates against the primary species and weed species which are related. Subsequent evaluation will examine the target weeds in crop situations. Again the selections for progression are based on biology.

The herbicide chemists have concentrated on the synthesis of sulphonyl ureas since 1984. For this reason two special tests have been established which compare all new sulphonyl ureas to chlorsulfuron. In both tests the standard is added at a rate of 10ppb whilst the new compounds are applied at a 100 fold higher rate of 1 ppm. The first

test is on corn seedlings in soil which are left in the dark for 5 days after treatment. The activity is assessed in terms of root growth in comparison to untreated and chlorsulfuron treated. The second test is similar but is conducted with chinese turnip seeds on filter paper. Again the assessment is on root growth. There was discussion on alternative assay methods for sulphonyl ureas and suggestions including single cell (either bacterial or higher plant) assays on defined medium, or, to be very accurate, the use of the cell free in vitro enzyme assay. Details and references to these alternatives were left at the Institute.

At the present time there is no biochemical support for the Herbicide (or any other!) project. This means that the adoption of in vitro assays are difficult. Additionally the identification of the mode of action of nearly synthesised compounds is not possible. It is the intention of the Institute to introduce a biochemical support group in the near future. The feeling is that this group would concentrate on metabolism of new compounds in target crops and in the environment rather than biochemical support of discovery research.

Worthy of special mention is the new rice herbicide discovered by the Institute and currently undergoing biological and toxicological investigation. To date it shows good field activity and no toxicological problems.

Insecticide Group - There are twelve scientists in the Insecticide Group. These are:-

Madam Tian Hui Zhi)
) Group Heads
Mr Zhoa Ri Jin *)

Mr Qiao Shi Xi

Mr Liu Tie Min

Mr Fen Deng Jin

Madam Wang Yia Jun

Mr Meng Qing Long

Madam Li Su Qing

Madam He Su Wen

Mr Shi Ji *

Mr Du Jing Yuan

Mr Shan

* UNIDO sponsored trainees

The primary screen is conducted on the following species:-

Musca domestica (susceptible) - sprayed at 500mg/l. Death is assessed after 24 hours.

Culex pipiens - 2nd to 3rd instar larvae are evaluated in a concentration of 10 Ug/ml. Activity is assessed after 24 hours.

Aphis laburnii - nymphs are grown on Vicia beans, dipped into chemical at a concentration of 500 mg/l. Assessment is made 72 hours later.

Tetranychus cinnabarinus - adult mites are placed into Phaseolus bean leaves and dipped into solutions containing 500 mg/l of the test compounds. Assessment is made 24 hours later.

It is intended to include 3rd instar larvae of the army worm Pseudoletia separata in the primary screen.

Secondary and tertiary laboratory screening varies with species. Against house flies activity is determined using a microapplicator applying 0.4 ml per fly; contact spray effects at reduced concentrations are also tested as is fumigant activity. Resistant flies are kept at Beijing Animal Research Institute and can be obtained when needed. In the microapplicator test per cent kill is assessed whilst in other tests it is knockdown which is important.

In the mosquito test the entire life cycle of the insect can be followed and on occasions activity against adults is assessed. Against mites lower rates are used and assessment times maybe extended to 72 hours. In addition an ovicide test is introduced and mobility in the plant is studied.

In all cases a standard is included which is in the same chemical class as the test compound. The point was made that it is important to keep a constant standard in each test. This enables comparisions to be made between tests.

Again discussions with the group identified important species of chinese agriculture. A possible list of species to be included in the primary screen was devised as follows -

Heliothis armigera (or Chilo suppressalis) - 3rd instar larvae

Pseudoletia separata - 3rd instar larvae

Aphis laburnii

Nilaparvata lugens - nymphs

Tetranychus cinnabarinus - adults and eggs

The inclusion of cockroaches, mosquitoes and house flies should only be included if the Institute has an interest in Public Health.

The emphasis in the Insecticide Group is on direct kill of the target pests. It should be emphasised that there are no alternative strategies using behaviour modifying chemicals and insect growth regulators. There are no tests in place to find this type of effect. In addition, modern insect pest management (IPM) strategies demand safety to insect predators. On the basis of this it would be of value to include important insect predators at least at the tertiary level.

In the later stages of screening the LC50 of each chemical is calculated. It was pointed out that the major factor is the slope of dose response curve. Often compounds with good LC50 values will have poor LC90 figures. For this reason it is important to establish the slope of the dose response curve.

Fungicide Group - There are nine scientists in the Fungicide Group. These are -

Madam Chui Dian Chong)
) Group Heads
Mr Sheng Zhong Yau)

Mr Zhou Liang Jia *

Mr Wang Kai

Madam Jai Xin Yong

Mr Shi Nai Gou

Miss Liou Jun Li

Mr Licu Wu Cheng

Mr Chui Zhing Xi

* UNIDO sponsored trainee

The current primary screen consists of a combination of in vitro and in vivo assays. In vitro assays are run at a concentration in agar of 50 Ug/ml whilst foliar in vivo tests are applied at 1000 Ug/ml and seed treatment assays at 0.1% of seed weight. All tests are replicated three times. The pathogans are -

in vivo	Erysiphe graminis	on wheat - spray
	Rhizoctonia solani	on cotton - seed treatment
in vitro	Botrytis cinerea	
	Fusasium oxysporum	
	Rhizoctonia solani	
	Pyricularia oryzae	
	Gibberella zeae	
	Verticillism albo-atrum	
	Colletotrichum gossypii	

Secondary testing is conducted by in vivo testing applying spray compounds at rates of 50, 75 and 100 Ug/ml with a hand held sprayer. Tests are protectant with inoculations 24 hours after spraying and eradicant with application 48 hours after inoculation. Additional

tests include a study of systemic activity and translaminar effects. Standard compounds are selected on the basis of chemistry related to the test compounds rather than on relevance of the pathogen.

After some discussion it was agreed that primary *in vivo* testing is the most important aspect of the fungicide screening with *in vitro* assays included to produce supportive data. For this reason the following *in vivo* pathogens were recommended for inclusion.

Pseudoperonospora cubenis	on cucumber
Erysiphe graminis	on wheat
Gibberella zeae	on wheat
Pyricularia oryzae	on rice
Rhizoctonia solani	on cotton
Botrytis cinerea	on tomato

In addition it is recommended that standards be selected for specfic pathogens and that these remain constant to allow comparisons to be made between tests. The preferred standards would be -

P. cubensis	metalaxyl
E. graminis	triadimefon
G. zeae	prochloraz (carbendazim)
P. oryzae	tricylazole
R. solani	quintozene
B. cimerea	iprodione

GENERAL POINTS - It is important in any innovative discovery research programme that a good supply of new chemicals is available for screening. At present the Institute synthesises less than 1,000 compounds a year which is no where near enough for a screening progamme. The reasons for this low chemical supply are many but key amongst them are the need for the originating chemist to prepare compounds shown to be active and selected for further testing in the laboratory and field together with a concentration of the group on the synthesis of new products using original synthesis methods. The strategy here is that patent law in China gives no compound protection but it does protect the method of synthesis/manufacture. Hence, a new method to make a commercial product will allow its manufacture in China. It is recommended that a group be established as a repreparation group thereby allowing the creative chemist to concentrate on the synthesis of new compounds. In addition the use of new processes to manufacture existing products is a strategy which often leads to less cost effective production and impure product as well as alienating the originating company leading to resistance regarding co-operation. The concept of synthesising analogues of products which fall outside patents, however, is a well recognised and acceptable research strategy. It is recommended that the group do not synthesise the products of other companies but that they initiate an analogue sythesis programme. In order to do this it is important to have access to the published patent literature.

A number of valuable text books have been donated to the Institute for the use of the scientists. Regrettably, there is no facility at the

Bioassay Laboratory to house these and other texts. It is important that all scientists have access to a good, up to date library.

The application and growing facilities at the Bioassay Laboratory offer opportunities for accurate and safe chemical spraying plus versatility in conditions under which tests are conducted. At present the Herbicide Group are not able to use the sprayer, for fear of contamination and consequently they have to rely on hand held sprayers and "pour on" application methods. Additionally the growth cabinets are not being used to their optimum. The flexibility offered by these sophisticated machines would provide ideal conditions for the development of disease where humidity, temperature and day length are critical. It is recommended that considerations be given to the intensive use of these cabinets.

B. Safe Working Practice

In any laboratory working on the evaluation of new potentially active chemicals it is important to adopt safe working methods and to wear effective protective clothing. Laboratory coats are worn by all scientists in the laboratory and it is preferred that these coats be worn only in the laboratory and not in offices or meeting rooms. In addition they should be laundered as least every two weeks. When handling technical material, concentrated solutions, spray dilutions or treated test material is essential that gloves be worn and a mask if relevant. Gloves must be washed before removal.

The Research Engineers sprayer is housed in the same working area as the growth rooms and in the vicinity of the glasshouses. The sprayer is not ideal as it allows the escape of spray droplets into the atmosphere and does not collect waste material for proper disposal. It is recommended that the sprayer be relocated to avoid possible contamination of the growing areas and that steps be taken to prevent the escape of spray droplets plus the installation of methods for the safe disposal of unused chemicals. An additional hazard in the spray area is the turntable sprayers used by the Herbicide Group. Whilst there is nothing wrong with such a piece of equipment it is important to ensure that is it made of material which is both resistant to chemical and solvent attack and which is easily cleaned. Far more important, however, is the need to surround it with an extraction hood to allow evacuation of spray droplets to the outside of the laboratory.

All equipment used in the preparation of spray solutions must be treated with caution and collected together safely for washing without risk of contaminating other laboratory fittings. The use of glass rods to stir concentrates is best avoided, the use of a mechanical mixer being preferred.

C. Field Research

The opportunity was taken to view field trials undertaken by the Plant Protection Service on behalf of the SRICI in both rice and top fruit crops. Trials in the field are conducted at National, Provincal, City and District levels and are essential for approval by the authorities prior to registration and commercial use.

The trials viewed in rice were herbicide mixture trials involving bensulfuron plus butachlor or quinclorac and pyrazosulfuron plus butachlor or quinclorac. Compounds were applied as "toxic sand" formulations, in which commercial products were added to sand which was then applied by hand to the experimental plot. Apparently this method of application is common amongst farmers, a point of relevance to the projected UNIDO/UNDP programme on pesticide formulation.

Plot size is either 20 or 100 sq.m with untreated plots left only in the smaller plots. Visual assessment of weed control is made 20 and 40 days after application and at harvest the uncontrolled weeds are counted and yield is measured together with 1000 grain weight and average head size. Weed control with all mixtures was excellent.

These trials were undertaken by Mr Dong Chang Quing and Madam Jin Zhi Wei.

Dalian is a major apple growing area of China. A visit was arranged to view a commercial/experimental orchard. The majority of the fruit harvested from 3,000 trees was sold commercially but areas were set aside for the evaluation of new compounds and mixtures from SRICI. A typical experimental plot would consist of 10 trees with only those at the centre of the row being evaluated. Standard treatments include organic arsenic for the control of canker (Valsa mali) and copper to control blotch of apple leaves (Marssomima mali) and fruit canker Physalospora piricola). Triadimefon has recently been introduced. Typical insecticide/acaricide treatments include parathion, synthetic pyrethroids, clofentezine, propargite and hexythiazox. Weed control is

by hand.

These trials were under the control of Mr. Fang Yong Wei and Mr. Wu Ming Qin.

D. Mixtures

The role of SRICI is to find answers to the problems of China's agriculture. The long term approach is to discover new chemicals with the necessary biological activity and environmental effects. In addition, a short term answer is to develop mixtures of commercially available compounds which have the necessary spectrum of activity. When developing mixtures there are two possible objectives. Firstly, to fill the gap in the spectrum of each compound thereby producing a mixture with broad spectrum activity and secondly to put two compounds together whose combined activity is greater than would have been expected; this is potentiation.

Laboratory and field trials are underway at present to develop both kinds of mixture. In the herbicide area broad spectrum weed control in rice with good crop selectivity is the objective whilst in the insecticide area mixtures of OP's and pyrethroids are being tested for potentiation effects. In the case of the insecticide mixtures, combinations have been discovered which give a surprisingly improved level of pest control; an effect which has been patented.

E. UNIDO/UNDP Equipment

Of the equipment supplied by UNIDO/UNDP, the Astell Incubators, Potter Tower, High Power Microscope, Microapplicator, Electronic Balance and Knock-down Chamber all work well and are in continuous use. The Stereo

Microscope is in great demand and at least one additional machine is needed by the Bioassay Group.

There are problems with the Growth Cabinets, one of the Fison 600H machines cannot keep the temperature down to 25 C if the outside temperature is high. This would suggest a fault in the refrigeration system. In addition, neither of the large Fison cabinets works without fault. One has an intermittent fault on the refrigeration and the other has a problem with the chart recorder. Fisons will be asked for advice. One of the large cabinets which was damaged in transit is still not functioning. It is difficult to determine what is needed to repair this instrument as it is still full of broken glass and damaged fittings. SRICI have agreed to clean it out and provide UNIDO with a list of fittings that are required. These include fluorescent tubes, tungsten bulbs plus sockets and starting units.

In neither Heraeus cabinet does the humidity control work.

The sprayer starter switch was damaged in transit. A new switch was given to SRICI when I arrived in Shenyang but it still had not been fitted by the time I left. In addition, the droplet size is so large that effective cover of plants passing through the sprayer is difficuilt to achieve. The spray solution is also exhausted very quickly. The nozzle size was found to be very large (model 02-F80) and smaller nozzles are probably required. Enquiries will be made in Great Britain.

The construction of the glasshouses is completely inappropriate for routine biological screening. There is inadequate artificial light, no

vents, poor cooling for the summer and inadequate heating for the winter. The design is such that natural light distribution is uneven and the absence of benching severely restricts utilisation of space.

In place of benching there are fixed troughs of soil at two different levels. This makes access to the glasshouse with a trolley very difficult. Suggestions for improvement were made (see Figs. 1 & 2). These included the removal of the soil bays to be replaced by mobile benching. It is important to test light receipts across this new benching and identify an area of regular light intensity for tests. This will not improve the access problems but should make better use of available space. The use of glasshouse screening paint in the summer should reduce the level of radiant heat input and plastic insulation (transparent "bubbles") will reduce heat loss in the winter. Better temperature control is needed. The high levels of humidity needed for fungicide testing can be achieved by the use of plastic covers placed over the test plants which are in standing water immediately after inoculation.

It was agreed that these changes would be tested in the Herbicide glasshouses.

F. Data Capture

Any discovery group generates much data. These results are of no value unless they are used not only to determine the value of a particular compound or mixture but also to provide a data base which is readily searchable by biological effect and chemical structure. Data should include:-

1. Biological effect.
2. Chemical structure.
3. Physicochemical properties.
4. Biochemical mode of action.
5. Quantity synthesised.
6. Toxicological effects.

These should be stored on computer which can be accessed routinely by chemist or biologist. Ideally data capture should be directly into the computer (with verification) to prevent transposition errors. In order to do this new equipment is needed by the Institute.

G. Recommendations

1. Modify all primary and secondary sources to reflect major Chinese pest problems.
2. Introduce biochemical support for all disciplines.
3. Introduce insect/mite predator species and other beneficial organisms.
4. Be aware of varietal difference of response to chemicals. Use more than one variety in tests.
5. Concentrate on establishing a group with nationally recognised excellence in agricultural crop protection.
6. Identify relevant standards and use the same ones in all tests at primary and secondary level.
7. Do not copy other companies products.
8. Concentrate on the synthesis of analogues and biologically active natural products. Seek compounds of different chemical structure from other Institutes and Universities.

9. Establish a library/information centre at the Bioassay Laboratory.

10. Use the controlled environment equipment. Identify faults and advise UNIDO of requirements.

11. Be aware of the risk from new compounds and always wear protective clothing.

12. Relocate sprayers and provide an evacuation hood for the turntable sprayer.

13. Continue mixture work as a short term solution to pest problems.

14. Modify the glasshouses to make them more appropriate for biological testing.

15. Improve data capture/retrieval capability.

16. UNIDO/UNDP must ensure that their support continues as and when needed.

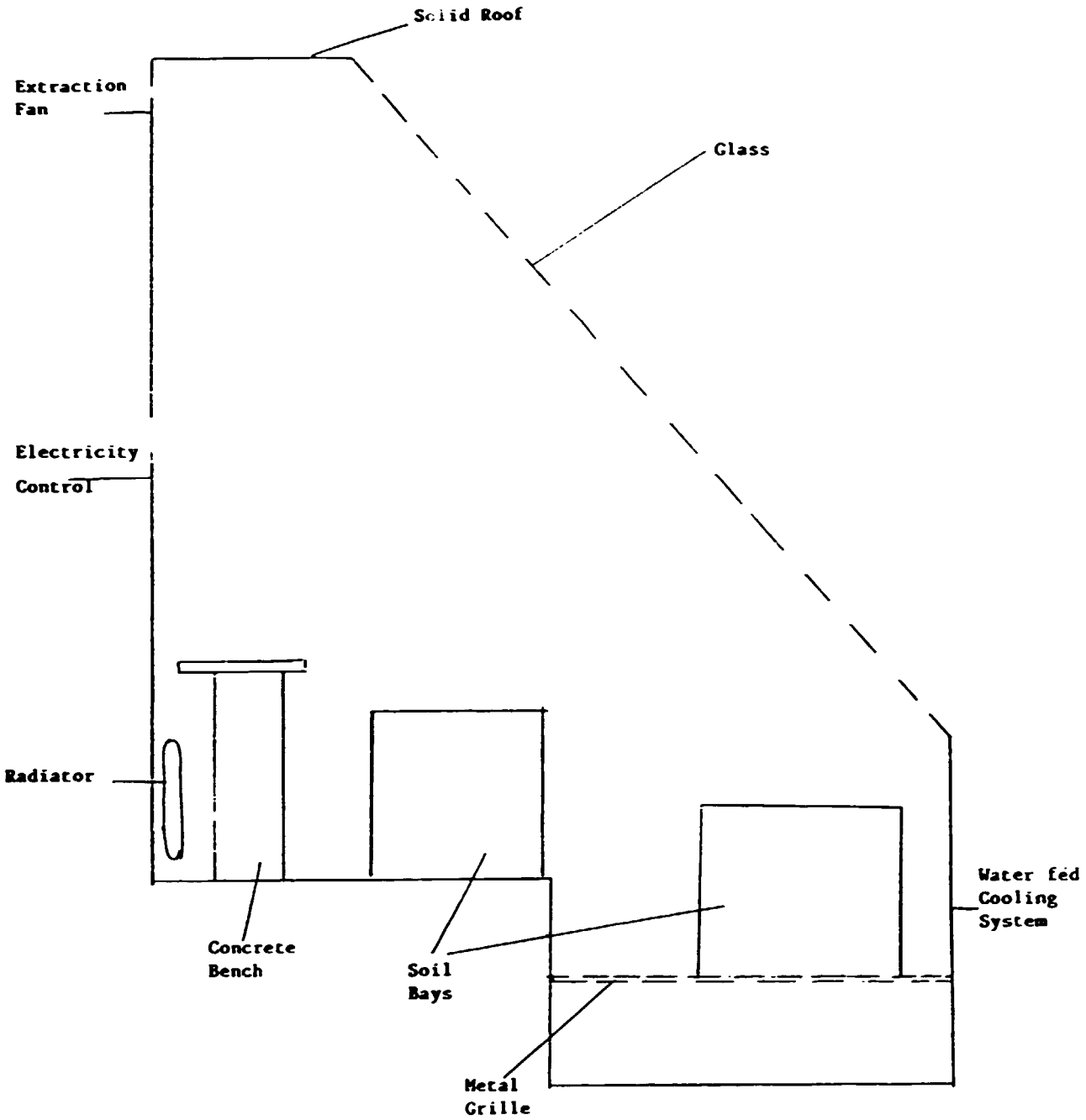

Fig. 1: Existing Glasshouse Facility.

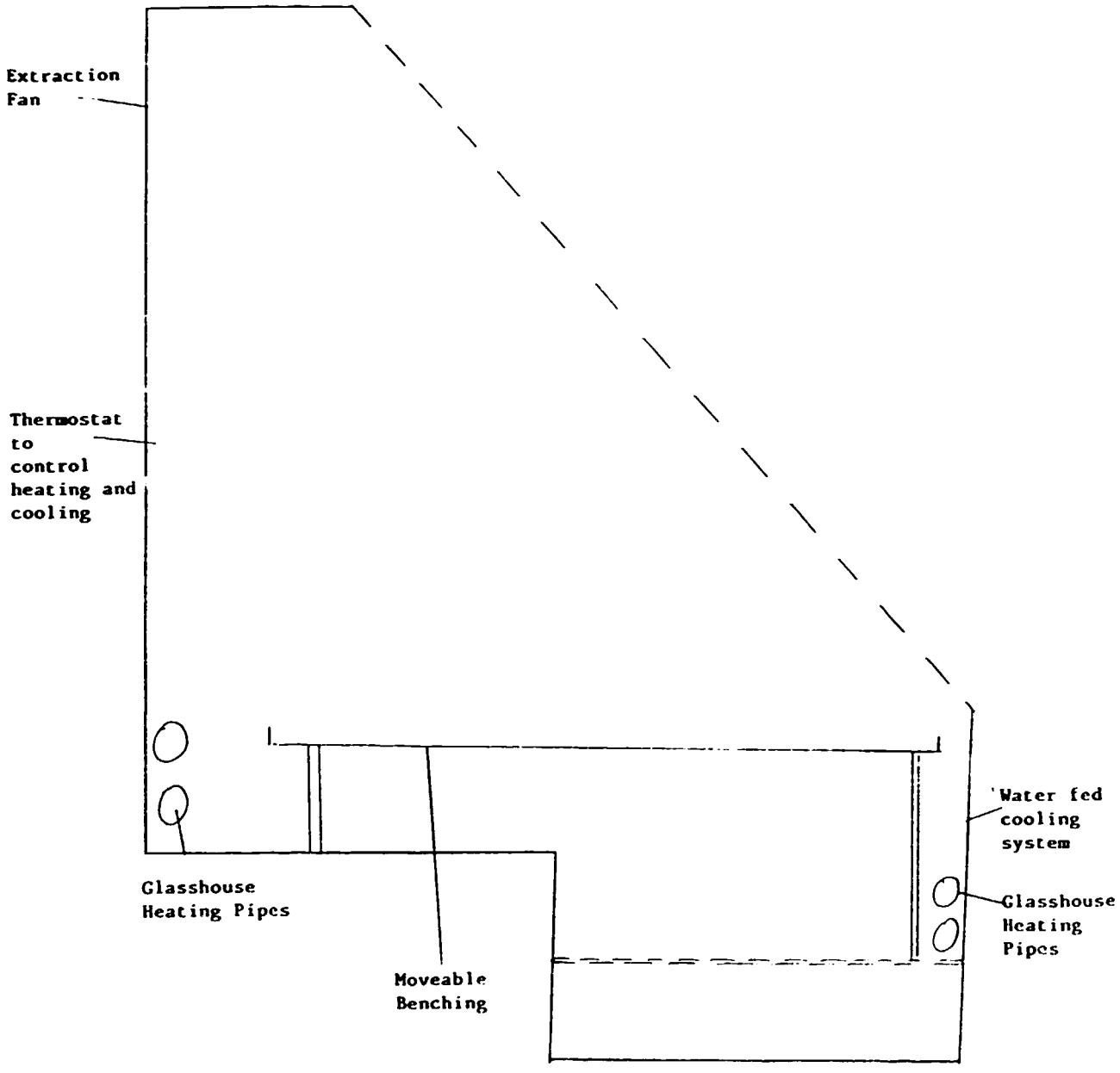

Fig. 2: Proposed changes to Glasshouse

B. Sugavanam/rp
3 July 1991

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

JOB DESCRIPTION

DP/CPR/80/008/11-73

Post title: Bio-assay Specialist (Fungicides, Insecticides, Herbicides and General)

Duration: 0.6 m/m

Date required: Aug/Sept 1991

Duty station: Shenyang, P. R. China

Purpose of project: To strengthen fungicide, insecticide herbicide and plant growth regulator research and development at Shenyang Research Institute of Chemical Industry.

Duties: The consultant is specifically expected to give advice:

i) in consolidation and improvements of the programme of regular screening of chemicals, their biological evaluation and selection of compounds for secondary and further screening in order to find compounds of good potential for agricultural outputs especially as fungicides and insecticides.

ii) to the staff in organizing their growth rooms to provide weeds and crop plants for screening purposes.

iii) in regular up keep of records and in proper interpretation of results against standards and also in selection of compounds for further development

He would also give necessary inputs to proper management of the whole station so that they follow standard operational procedure in bio-assay of compounds as potential pesticides.

During the consultant's asssignment, he is likely to overlap partly with another bio-assay specialist to discuss all aspects related to the proper management of the bio-assay laboratories.

Along with the other consultant, he is expected to submit a joint report giving his findings and recommendations

Qualification: A biologist, bio-chemist, plant physiology specialist with extensive experience in biological assay both in glass house screening and small scale field trials. Experience in pesticide screening, plant propagation and familiarity with problems associated with developing countries would be an added advantage.

Language: English

Background Information: UNIDO has been providing technical assistance to the People's Republic of China in the establishment of a toxicology evaluation and a bio-assay laboratory financially supported by UNDP and the Government of the United Kingdom of Great Britian and Northern Ireland. Both the facilities have been completed and are operational. In order to provide support for proper management of the bio-assay laboratories UNIDO is providing consultancy services to follow the acceptable norms to carry out bio-assay studies of chemicals as potential pesticides.

UNIDO COMMENTS

The report of the expert Mr. Copping will be the final on this project and he has given recommendations for further improving the screening techniques to increase the chances of finding active compounds. He has also made a valuable suggestions to include in the screen insect predators as they are important to be protected in the context of integrated pest management.

It is also important that in a project of this nature where technology is changing fast, the project authorities should keep a link with laboratories abroad and send fellows for training in specialized areas at periodic intervals. This would definitely provide the necessary impetus to introduce new techniques and also wherever possible reduce animals used in toxicology testing and in developing newer and reliable bio-assay techniques.