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AN ECOTOXICOLOGY RESEARCH CENTRE IN PAKISTAN

US/PAK/90/294

PAKISTAN

Technical report: Findings and recommendations*

Prepared for the Government of Pakistan
by the United Nations Industrial Development Organization

Based on the work of Erik Kirknel,
Consultant on Ecotoxicology

Backstopping Officer: Yong-Hwa Kim, Chemical Industries Branch

* This document has not been edited.

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Table of Contents

ABSTRACT

Title
Consultant for setting up Eco-toxicology Laboratory.

Objective
This fifth mission on the project consisted of a follow- up of the fourth mission, technical input in establishing the ecotoxicology laboratories and provide assistance in better understanding of the impact on ecosystem of especially pesticides, in order to implement working plans for the laboratory for the beneficial of Pakistan and RENPAP member countries. Collect information on further equipment necessary to be purchased and evaluation of needed training for staff members.

Conclusions

TPR meeting was held in Islamabad with a fruitful result.

Chem section is operating, needs routine.

Three plans for future activities for the chem section were made, annex V, VI and VII. Short term plan annex VIII. The plans are rough drafts for future activities. The economic needs are only for variable costs.

Terrestrial section has not proceeded as planned.
Microbiology section is completely without activity.
Internal (=local) disagreements seems to be the main cause of this.

Recommendations

The main recommendations are cited in annex IV.

Training of two scientists, Mr. Karam Ahad and Mr. Rauf Ahmed, chem. section, in one month in pesticide chemistry in Denmark.

Let Dr. S.A. Hassan, Darmstadt introduce working procedures (hands on) of impact of pesticides on beneficial arthropods in Islamabad for one week.

Hire Miss Tazeen Awan, chemist and quality assurance auditor. She seems qualified and has been working at Zeneca, Brixham, UK. Now returning to Pakistan. Very valuable in the chem section.

I. INTRODUCTION

This report is made by Erik Kirknel, Ministry of Agriculture, Danish Institute of Plant and Soil Science, Department of Weed Control and Pesticide Ecology, Flakkebjerg DK-4200 Slagelse. The job description is reproduced in Annex I.

The first part of the mission took place on 13-20 February, the second on 9 October to 6 November 1993, the third part from 14 March to 9 of April 1994 the fourth part from 19 December 1994 to 3 January 1995 and the fifth part from 3 July to 25 July 1995.

This fifth mission on the project consisted of a follow-up of the fourth mission, technical input in establishing the ecotoxicology laboratories and provide assistance in better understanding of the impact on ecosystem of especially pesticides, in order to implement working plans for the laboratory for the beneficial of Pakistan and RENPAP member countries. Collect infirmations on further equipment necessary to be purchased and evaluation of needed training for staff members.

The reports mentioned in Appendix III, describes the available reports done previously in the project.

II. ACCOMPLISHED SINCE LAST REPORT AT NARC

Chem section has fulfilled its obligations following the planned activities.

III. NOT ACCOMPLISHED SINCE LAST VISIT AT NARC

None of the equipment listed in last report of mine was arrived.

Generator not implemented.

Steering group not established.

Clear definitions of responsibility in the three sections still missing.

Terrestrial section has still not started raising a culture of parasites for testing the effect of pesticides, despite a complete training has been given to member of this group. No physical obstruction for not starting this project.

Still not enough space provided by NARC.

IV. FURTHER EQUIPMENT NEEDED

In agreement with Dr. Umar Khan Baloch, a list of further requested items needed was made during the visit.

Auto sampler vials w/screw cap. HP-type. 1000 pieces.
Screw cap. cat. no: 5182-0723, total 2300 Dkr
Vials, cat no 5182-0716, total 1380 Dkr
Hewlett-Packard
Birkerød Kongevej 25
3460 Birkerød
Phone +45 45.99.10.00
Fax. +45.42.81.58.10
Necessary for final sample storage.

Universal centrifuge 16
Inclusive 10 ml and 100 ml centrifuge glass ware
approximately price: 25.000 Dkr.
and

Pyrex 2 L water bottles, 50 pieces
Bottles, cat. no: 1-F-3010-2 L , total 3.500 Dkr
Red screw caps, cat no: 1-F-3010-A, total 1.050 Dkr
Bie and Berntsen A/S
Sandbækvej 7
2610 Rødovre
Phone +45.44.94.88.22
Fax: +45.44.97.27.09

V. CONCLUSIONS

TPR meeting was held in Islamabad with a fruitful result.

Chem section is operating, needs routine.

Three plans for future activities for the chem section were made, annex V, VI and VII. Short term plan annex VIII.

Terrestrial section has not proceeded as planned.
Microbiology section is completely without activity.
Internal (=local) disagreements seems to be the main cause of this.

VI. RECOMMENDATIONS

The main recommendations are cited in annex IV.

Training of two scientists, Mr. Karam Ahad and Mr. Rauf Ahmed, chem. section, in one month in pesticide chemistry in Denmark.

Let Dr. S.A. Hassan, Darmstadt introduce working procedures (hands on) of impact of pesticides on beneficial arthropods in Islamabad for one week.

Hire Miss Tazeen Awan, chemist and quality assurance auditor. She seems qualified and has been working at Zeneca, Brixham, UK. Now returning to Pakistan. Very valuable in the chem section.

ANNEXES

I. JOB DESCRIPTION

Purpose of project: To establish an eco-toxicology laboratory at the National Agricultural Research Centre (NARC) Islamabad, belonging to The Ministry of Agriculture and study the fate and effects of pesticides in the environment and also make Pakistan effectively interact as Technical coordinator giving inputs to eco-toxicology to the Regional Network on Pesticides for Asia and The Pacific (RENPAP).

Duration: 3 weeks in Islamabad, 1 week homebased july 1995.

Duty station: Islamabad, Pakistan.

Duties: The expert, during his return mission, is expected to assess the work carried out in the Centre with regard to the functioning of the various laboratories. He should also assess the progress made since the last expert group meeting (EGM), the type of project undertaken by the research groups and specially the type of linkages that should be maintained between the ecotoxicology centre and other laboratories.

The expert should:

-Assist in preparing a workplan for the next 12 months and also suggest a project concept for consideration beyond 1995 taking in consideration the utilization of facilities of manpower towards the benefit of government and industry in the field of pesticide and chemical safety.

-Take a major role in proposing agenda of discussions and reporting at the tripartite meeting scheduled in July 1995.

-At the end of the assignment he should submit a report giving his findings and recommendations.

Qualifications A chemist, biologist or environmentalist with extensive experience in analytical work related to pesticides and their residues in the ecosystem. Must have held senior position in Government laboratories or industries and supervision for group of analytical chemists. Must be familiar with GLP and MRL, ADI for pesticides and OECD guidelines with regarding to quality control and quality assurance. Experience in the developing countries would be an advantage.

During his/her homebased work he/she will identify the suppliers of equipment and also arrange any training to the counterparts on ecotoxicology and provide necessary advice for UNIDO to take action.

Language English

Background information:

The United Nations Industrial Development Organization (UNIDO) has been assisting 15 Asian countries through a network called Regional Network on Pesticides for Asia and the Pacific (RENPAP) mainly to promote safe development of pesticides. Under this project specific areas have been assigned to the different member countries to provide technical inputs as shown below:

Formulation technology:	India
Quality control:	Rep. of Korea
Bio-botanical pesticides and Residue analysis:	Thailand
Occupational safety:	Philippines
Operational safety, Waste management and Environment safety:	Thailand
Application technology:	Malaysia
Data collection/Dissemination:	India
Ecotoxicology:	Pakistan

Pakistan has taken up ecotoxicology based on the facilities available. These facilities need to be strengthened and from the contribution of Denmark to UNIDO Industrial Development Fund (IDF) a project has been approved to strengthen the ecotoxicology laboratory of the National Agricultural Research Centre of the Ministry of Agriculture. The main aim is to link pesticide industries with the ecotoxicology laboratory and make use of the facilities for carrying out experiments on the fate of pesticides they produce for Pakistan market. Self sustainability (partly or fully) of the project is also considered to be an important factor by providing services to industries, government institutions dealing directly or indirectly with pesticides.

ANNEX II

II. ITINERARY

ACTIVITIES IN ERC FROM 4 July 1994 to 25 July 1995.

Mainly stayed at NARC getting the analytical instruments operating for analysis.
Ordered spare parts for hydrogen generator and gel filtration unit.
Contacting the different suppliers in Europe for technical advise in order to correct malfunctions of instrumentation.
Made plans for future work for the Ecotox centre
Frequent meetings with the country director, Dr. Abd El-Rahim Marei.
Participating in the TPR meeting.

ANNEX III

III. REFERENCES AND REPORTS CONSULTED

1. **CALDERBANK, A.**
Unido report on environmental toxicology related to pesticides in Pakistan. DP/RAS/85/023. 1988.

2. **CALDERBANK, A.**
Unido report on an Ecotoxicolcgy Research Centre in Pakistan. DP/RAS/85/023. 1990.

3. **FLETCHER, K.**
Unido report on Establishment of an Ecotoxicology Centre. US/PAK/90/294. 1992.

4. **KIRKNEL, ERIK**
Unido report on findings and recommendations on establishing an Ecotoxicology Centre in Pakistan. DP/RAS/88/031. 1993a.

5. **KIRKNEL, ERIK**
Unido report on findings and recommendations on establishing an Ecotoxicology Centre in Pakistan. DP/RAS/88/031. 1993b.

6. **KIRKNEL, ERIK**
Unido report on findings and recommendations on establishing an Ecotoxicology Centre in Pakistan. US/PAK/90/294/11-01/B/J13426. 1995a

7. **KIRKNEL, ERIK**
Unido report on findings and recommendations on establishing an Ecotoxicology Centre in Pakistan. US/PAK/90/294/11-01/? 1995b

ANNEX IV

IV. REPORT OF TPR MEETING

Recommendations from the Chief Technical Adviser at the TPR meeting held in Islamabad, Pakistan, 17/07/1995.

(US/PAK/90/294/11-01).

The chemical analytical section of the Ecotox center is implemented to a degree of being able to detect residues of pesticides in ppm level of plant material.

Only routine is missing, and it is therefore a task to arrange funding for the future for this for Pakistani conditions, unusual but urgently needed activity. The section is equipped with the most modern analytical hardware. Intense training abroad for a couple of the staff members should ensure the section to be in a position of producing results very soon, of a high professional standard.

The Ecotox center has been started in cooperation with local and foreign professional and economic help. This initiating process is maybe over. I will **strongly recommend** local support, which is absolutely necessary in order to utilize this section for the benefit of the Pakistani need in this area.

It is necessary for producing high quality research in pesticide residue analysis, to introduce some sort of quality assuring system. The economic impact on the industry from the results of this kind of research, is often of great importance. If the industry and the national authorities are going to accept these results, they must have some sort of guarantee for high quality research. Too much incompetent research has been performed in this area all over the world in the past, with the consequences of mistrust from the industry.

Good Laboratory Practice (GLP) is such a system. It is implemented in the future in all competitive laboratories in order to survive. At the present moment it is not possible in Pakistan to get accreditation and accepted compliance with GLP.

Parallel to GLP is introducing ISO 9000 series of quality assurance system. ISO 9000 is a must for the industry in order to be able to export to the global market in the future.

But the section has been introduced to GLP and will be able to perform their research under the principles of GLP, if the discipline will be strengthened and the physically resources expanded. Under the prevailing conditions it is not possible to perform GLP like activities.

Because of this, I will **recommend** the local authorities to consider the ground floor of the building to be incorporated into the Ecotox center, because introduction of GLP in pesticide residue analysis is a must today.

It will also be of high beneficial value to demonstrate to others in your scientific community, what it means to work under a quality assuring system. The working conditions for the scientist are simply not satisfactorily to perform GLP on the chem section. More offices are needed and a conference room is essential. This has been pointed out in the previous reports. The traffic through the building, is introducing a high degree of contamination, reducing the quality of this low-level of detection.

Furthermore I will **appeal** to NARC to make eventually not operating gas chromatographs and other useful instruments in the possession of NARC, available for the chem. section in order to optimize all equipment locally available.

I will **recommend** to expand the Ecotox center with at least a couple of agricultural graduates, specialized in entomology, in order to strengthen the basic disciplines towards implementing Integrated Pest Management (IPM).

The terrestrial ecology section has been trained at the best places in Europe in the very important subject: The influence of pesticides on beneficial arthropods. This low cost research activity is expected to produce results of a very high international standard in the near future. The results are urgently needed in IPM-programs.

In the build-up phase of the Ecotox center, it was suggested from local side to form a section of microbiology. The discipline, the influence of pesticides on micro flora, is an extremely difficult research area to handle. Very few institutes on a global basis has come out with reproducible results to be utilized, not only by agricultural research and practice, but also by environmentalists and governmental agencies in registration processes. The parameters influencing the microbial community are so complex that it is difficult to find sound conclusion. I will **recommend**, also in the light of very limited resources, this section to be cancelled.

I will also **recommend** UNIDO to strengthen the cooperation with their consultants when receiving the report for consultancy. Better synchronous activity, and dialogue between Dept. of purchase and consultant. It is of course understood that UNIDO can not follow all recommendations made by consultant. But a dialogue would be beneficial for the priority in selection of hardware and training. Also it will be of extremely beneficial value if the consultant before a consultancy at least was informed what has been ordered and received in order to plan his activity. Better coordination between dates of consultancy, ordering and shipment of hardware will optimize the limited resources. These arguments are also valid for planning training and visits abroad. Well planned training is the basis for good result.

Finally I will **strongly recommend** to take a very important issue serious, mentioned in 3 out of 4 reports: Establishment of a steering group.

It must be understood, that this area of research, especially in Pakistan, is new and very few people are working with pesticide residue analysis. It will be fatal to the scientific community, and the invested resources in the chem section, if the best scientist in the country is not placed in a steering committee, influencing the decisions taken planning future activities, discussing with the section on difficulties in their research.

I have had the pleasure to have Dr. Syed Zafar Masood from Tropical Agricultural Research Institute, Karachi with me in Denmark recently. Dr Masood would be of great help in a steering committee consisting of 3-4 scientists, sitting together with scientists of the chem section. I will **recommend** his membership of the steering committee.

I have the greatest respect for politically influencing members of a steering group, but these members will have a political role to play as the scientists have their professional role to play: Chemical analysis, toxicology and ecotoxicology. These disciplines are the foundation on which the section should build sound, respected research for the benefit of the nation.

Erik Kirk, Kirknel

Islamabad 17/07/1995

UNIDO consultant.

Danish Institute of Soil Science.
Dept. of Weed Control and Ecotoxicology
Flakkebjerg
4200 Slagelse
Denmark

ANNEX V

V. FUTURE PLAN 1
- Monitoring Pesticides in the Indus River System

Monitoring The Indus river system and its four main tributaries, Jhelum, Chenab, Ravi and Sutlej, for concentration and transport of pesticides to the Arabian Sea.

Background

The background for this project is uses of vast amounts of pesticides in agricultural areas around the entire river system of Pakistan, going in north from the Himalayan mountains, south-west down to the Arabian sea.

The use of pesticide in Pakistan started in 1954. The pesticide consumption has increased from 906 metric tons a.i. in 1981, to 5296 metric tons a.i. in 1993-94, at the rate of 40% per annum. The sprayed area has increased from 1.8 mill. hectare to about 5.5 million hectare.This gives an average of more than 1 kg a.i. per hectare. Insecticide constituted 85% of the total pesticides used and herbicide as 6%. In all 91% goes directly to soil or plantation. The 75% of insecticides is used on cotton crop alone and rest is used on crops like rice, sugar cane, maize, fruits, vegetables etc.

In some areas of Pakistan, ground water is used for drinking water, but the rivers are mainly used as a source for drinking water. The water is not processed in such a way that it will remove any of the pesticides.

The MRL (Maximum Residue Limit) for drinking water in most developed countries is not toxicologically based but fixed from the point of view: We don't want pesticides in our drinking water.

MRL for drinking water is the quantitative limit of detection (LOQ) made 20-30 years ago. To day the LOQ is much lower due to better instruments than 0.1 ppb for a single pesticide or 0.5 for sum of all detected pesticides (EU).

It is a matter of national decision in what range the MRL should be. In Holland all drinking water is processed including removal of pesticides. The pollution of dutch raw water is sometimes so high that the toxicologically based MRL for vegetables is reached.

In the scandinavian countries, MRL for raw water (mainly ground water) is 0.1 and 0.5 ppb respectively. Wells are closed if MRL is exceeded. The pesticides found in drinking water, will by and by be removed from the market by the national authorities.

The basic argument for low MRL's in raw water is: We can not, as with vegetables, discard our pool of drinking water, if we one day find toxic effects of ppb-level of pesticides (Oestrogenic effects of this level of pesticides is a hot item in the occupational health camp today).

We have to live with a polluted water pool for decades maybe for centuries. The age of ground water is sometimes 100 years or more.

The nation must know what action is to be taken, either cleaning the raw water or banning the pesticides found in drinking water. Doing nothing may threaten public health.

Objective.

The objective of this study is to monitor the Indus river system for concentration and transport of pesticides from agricultural areas and hereby create knowledge of the potential risk of drinking water the population of Pakistan is exposed to. To investigate annual variations in the concentrations of pesticides in the river systems and find the major causes for leaking.

If pesticides are detected in the rivers, it will be a task for legislators to regulate the use of such pesticides, agronomists, the press and others to create awareness among farmers on the use of pesticides near the river systems and take countermeasures (spray techniques etc.) to avoid contamination of our potential drinking water.

Methods.

Water and sediment samples will be collected in the Indus river system and adjacent rivers, Jhelum, Chenab, Ravi and Sutlej. It is known that some pesticides released in rivers and lakes , soon after release is below limit of quantification in the water, but present in large quantities in sediments.

Kangas et al 1994 found this for cypermethrin in forest areas in Finland. Cypermethrin was leaking from treated areas, killing trouts in large quantities without leaving any residues in the water.

A quality assurance system will be established as far as to ensure validity of the data, well knowing that a full compliance with GLP (OECD) can not at the present moment be established in Pakistan. As a minimum, development of SOP's (Standard Operating Procedures) will take place.

Inter calibration will be established to an internationally accepted GLP laboratory.

1. Schedule of sampling

Sampling will be done along the Indus river from north to Karachi in south at a distance of about 100 km between sample sites. This gives 25 localities at the Indus river.

Each of the four main tributaries of the Indus river, Jhelum, Chenab, Ravi, and Sutlej will be monitored as far north as possible, with one sampling station and with another sampling station before joining the Indus river or one of the other rivers. The four rivers will in this way be covered by 8 sampling sites.

Chenab and Sutlej are regulated by India and will not carry water continuously.

Sampling will be performed after the main spraying season in cotton and when the monsoon have had some time to do run-off of the sprayed soils. This will probably be in the beginning of August. The winter sampling will be in February.

At Karachi, monthly sampling will be taken from the river Indus in order to reveal any variation within the year.

At each location, 8 water samples and 2 sediment samples will be taken. The study goes for 2 years.

Further studies, revealing the main sources of pesticide pollution, will consist of agronomic studies of the topography, possibilities of run-off, pattern of pesticide use and a finer mesh of sampling stations. This part of the project needs further planning.

Table 1 summarizes sampling of water analyses.

Table 1. Sampling of water and sediment samples at the Indus river and its four main tributaries.

River system	No of sample sites	No of sampl/ site / 2 years	Total no of samples
Water samples			
Indus	25	8 * 2	400
Four main tributaries	8	8 * 2	80
Karachi	1	8 * 2 (* 12)°	120

Total water samples			600
Sediment samples			
Indus	25	2 * 2	100
Four main tributaries	8	8 * 2	80
Karachi	1	10 * 2	20

			200
Total sediment samples			----
Total water and sediment samples			800

1.1 Water samples

2 L glass bottles (Pyrex) are needed for sampling. The bottles will be cleaned thoroughly with ethanol and suitable glass ware detergent, heated to a minimum of 450°C for 2 hours in order to evaporate/break down any organic substances.

Sampling will be performed by opening the 2.5 L bottle 0.5 m under water surface, at least at 1 m water depth. Close the bottle with cap under water.

1.2 Sediment samples

Sampling of sediments will be performed where water depth is about 0.5 m. Sample will consist of sampling 1 kg of sediment from 20-30 locations, at least 1 m apart from each other. Let sediment drip of on screen, before storage in polyethylene bags. Keep cool as possible when transport back to lab.

Dry sediment immediately after returning back to lab at 50°C until (send you details about this when returning home!) in a 1 cm layer. When dry mix well in polyethylene bag. Take 6 sub samples, each 50 gr. (maximum amount, check Soxhlet extractor). Store at 25°C.

2. Extraction of samples.

2.1 Water
Solid phase extraction

Solid phase extraction will be preferred because it can be done in situ and is cheap. The synthetic pyrethroids often have a low recovery in solid phase extraction and might present a problem. Recovery experiments will show this.

In case of preferring solid phase extraction, dichloro methane will **not** be added, but 5% methanol in order to get better performance of the C-chains.

Read SOP for Solid phase extraction.

Make sure the SPE discs are dry and cool after extraction, when transporting back to lab. Make sure field recovery (spiking in the field, and determine recovery in the lab.) is OK.

Liquid-liquid partitioning.

If recovery is low (Cypermethrin), liquid-liquid extraction shall be used.

Shipment of bottles will be performed in closed containers in order to avoid contamination. The sampling will only be performed by trained labor. In order to preserve the samples, dichloro methane will be added, 25 ml per 2.5 L.

Transport back to the analytical laboratory will take place within 10 days. Liquid-liquid partitioning with dichloro methane will be done immediately when the samples are arriving.

Read SOP for liquid-liquid partitioning.

2.2 Sediment extraction
Weigh 50 gr of sediment, transfer to Soxhlet. Extract with acetone 3 times. Dry over anhydr. sodium sulph. Read SOP for extraction.

Perform recovery experiment.

3. <u>Clean-up</u>

Depending upon the matrix, clean-up of the samples will be done by means of suitable procedure. Gel permeation chromatography alone or in combination with silica gel.

Detection will be done on GC with suitable detectors (ECD, NPD or eventually FPD). Quantification will be done by using external standards.

The methods will be validated at 0.1 ppb as the lowest level and up to the highest detected concentration reported.

Inter calibration will be established between an accredited GLP laboratory in Europe.

4. <u>Procedure for developing methods of analysis for all matrix</u>

4.1. Choose the standards of pesticides you want to incorporate in your study.

4.2. Make a mix of the different standards at 0.1 µg/ml..

4.3. Inject in GC. Note Rt.

4.4 Spike water sample, demineralized water.

4.5 Extract on SPE.

4.6 Make sure good recovery is established.

4.7 **Now** you have a good lab method.

4.8 Go sample in the field 4 * 2.5 liter of river water.

4.9 Extract one sample according to lab method.

4.10 **Check for peaks, either ghost or real pesticides!** This is your background chromatogram. Hopefully no peaks show up. Make spiking to river sample. Determine recovery.

Now you have a field method.

If there are peaks with the same Rt as standards, they might be pesticides **or** ghosts.

Then try to run standard from 4.2 on GPC, make elution profile. Make sure good recovery. **Then** run your river sample on GPC and detect again on GC. Compare chromatograms before and after GPC.

If peaks are still with the same retention time as pesticides, there are two possibilities. You either have pesticides in your river sample **or** bad clean up on GPC. Try run GPC-cleaned sample on other GC column.

If still the same Rt, the probability increases for pesticide identification.

The problem arise **if** you can't spike on a "clean sample". It is allowed to spike on a ghost peak, if the ghost peak is less the 20% of the spiked peak. Else contact me.

Estimated costs

Estimated costs of 1000 water samples for extraction and analysis is shown in table 2.

Table 2.

Estimated costs of 100 water samples for extraction and analysis (all items GC-grade).

Item	Total no of items	Cost	Total Cost
Dichloro methane	15 L		
Anhydr.Sod.Sulph.	1 kg		
Ethyl acetate	13 L		
Cyclo hexane	10		
Sol.ph.extr.discs.	100	70$	
Methyl alcohol	3 L		
Hexane	3 L		
		-----	-----
Total			

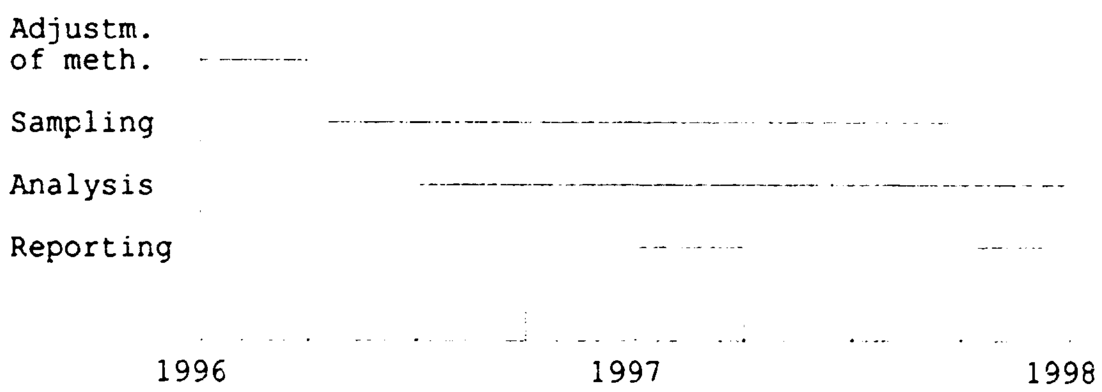
Table 3 shows estimated costs of 100 sediment samples

Table 3

Estimated costs of 100 sediment samples

Item	Total no of items	Cost	Total Cost
Ethyl acetate	10 L		
Cyclo hexane	10 L		
Acetone	15 L		
		-----	-----
Total			

Plan of activity



Adjustment of methods

A final validation and tuning of the analytical methods, inclusive inter calibration will take approximately 3 months.

Establishing cooperation with local laboratories for sampling and sub sampling will take place in this period.

Analysis

Analysis will start after final validation of methods and is scheduled to run for 18 months. This time span includes control measures of the method, e.i. repeated recovery experiments.

Reporting

Reporting will take place after one year and at the end of the project. The first report will consist of analytical results, only including a complete description of the sampling.

A mid-term report meeting will be held between sponsor and Ecotox center.

The final report will include all SOP's used, all raw data as logbooks, chromatograms, statistics etc.

Final meeting will be held at the latest 2 months after the project termination date, 1 January 1998.

The final report will be presented one month before the final meeting.

ANNEX VI

VI. FUTURE PLAN 2
- Maximum Residue Limit Control in Fruits, Vegetables etc., and Monitoring Drinking Water for Pesticides

Control lab. for MRL of pesticides in fruit, vegetables, cotton seed/oil, mustard and drinking water.

Background

The background for this project is a complete absence of an organized activity of this sort in Pakistan.

The MRL is a toxicologically based limit of pesticide concentration in human food. Different agencies around the world have their own MRL's (US FDA, EU, FAO/WHO), sometimes differing due to local points of view also other than toxicological reasons.

The MRL is made in order to avoid toxic effects in humans consuming pesticide treated crops. Pesticide are poisons directed towards organisms often with similar physiological structures as humans e.g. insects with their nerve system very similar to the human nerve system.

In order to avoid toxic substances in the pesticide treated food, a minimum of time must elapse between the last spraying of the crop and the harvest of the crop, in order to ensure proper metabolism of the pesticide. This is called the waiting period.

It is a well known fact among farmers, agronomists etc. that this time span between spraying and harvest, especially on vegetables, is very seldom established. Many farmers are not even aware of the importance of a waiting period. It is also a well known fact, that many vegetables are sprayed only a few days before the harvest, leaving unacceptable high dosages of pesticides on and in the food.

Seed treatment with pesticides presents a special problem. Too often it has been documented in developing countries, treated, not used seed has been used for human consumption, resulting in severe non reversible injuries and in many cases regrettably also in deaths.

Therefore it is a must to establish control measures in order to ensure MRL's to be respected and hereby prevent adverse effects in the public.

Drinking water is a subject for investigation in this project as well. The MRL for drinking water in most developed countries is not toxicologically based but fixed from the point of view: We don't want pesticides in our drinking water.

MRL for drinking water is the quantitative limit of detection (LOQ) made 20-30 years ago. To day the LOQ is much lower due to better instruments than 0.1 ppb for a single pesticide or 0.5 for sum of all detected pesticides (EU).

It is a matter of national decision in what range the MRL should be. In Holland all drinking water is processed including removal of pesticides. The pollution of dutch raw water is sometimes so high that the toxicologically based MRL for vegetables is reached.

In the Scandinavian countries, MRL for raw water (mainly ground water) is 0.1 and 0.5 ppb respectively. Wells are closed if MRL is exceeded. The pesticides found in drinking water, will by and by be removed from the market by the national authorities.

The basic argument for low MRL's in raw water is: We can not, as with vegetables, discard our pool of drinking water, if we one day find toxic effects of ppb-level of pesticides (Oestrogenic effects of this level of pesticides is a hot item in the occupational health camp today).

We have to live with a polluted water pool for decades maybe for centuries. The age of ground water is sometimes 100 years or more.

The nation must know what action is to be taken, either cleaning the raw water or banning the pesticides found in drinking water. Doing nothing may threaten public health.

Import of food is a special problem all over the world. It is a well known fact that developed countries with its control of MRL's often reject cargoes of food due to too high residues of pesticides or even banned pesticides. It is also a well known fact that these cargoes **always** find a consumer, especially a consumer with no MRL's or control of such. This potential risk for the Pakistani consumer, could very well turn out to be real.

There is a lack of systematic information on pattern and consequences of pesticides use on occupational hazard and pesticide residues in food in Pakistan. A number of studies have shown insecticide residues to be present in our daily food products, fruit and vegetables (NIH 1984, Illahi 1985).

Fruits and vegetables are grown in abundance in Pakistan. The country meets its own requirements besides exporting modest quantities of fruits and vegetables world wide and to the Gulf states in particular.

These crops receive insecticidal treatments for the control of different pests. While the application of pesticides is necessary in order to increase crop production and quality, its increasing use poses a serious threat to public health when not controlled for MRL.

Organo chlorine, organo phosphate and pyrethroid pesticides were reported in fruit and vegetable samples drawn from Karachi (Masud and Nusrat, 1992). Whereas fruit and vegetable samples collected from main selling point and grower fields of Karachi (Sind), NWFP, Islamabad, Quetta/Pashin districts of Baluchistan province, were detected pesticide residues of chlorinates, OP's and pyrethroids above the MRL proposed by FAO/WHO.

Milk supplied from Karachi Cattle Colony, were monitored for OC pesticide residues in the year 1984. 32 milk samples out of 79 (=40%), were found to be contaminated with either BHC isomers, p,p'-DDT, p,p'-DDE, heptachlor epoxide, aldrin and dieldrin.The most frequently occurring pesticide was gamma-BHC (Parveen and Masud, 1988).

Organo chlorine, organo phosphate and pyrethroid pesticides were monitored in samples of fruit and vegetables collected from the wholesale market of Karachi during July 1988 and June 1990. A total of 250 samples were analyzed. In 37% of the samples, variety of pesticides were detected. 26 % of total exceeded MRL (FAO/WHO), while 21% were reported below MRL. In remaining 16%, no pesticide residues could be detected (Masud 1989).

The only report on ground water contamination in Pakistan revealed that the shallow ground water in Samundri area drawn from the depth of 30-40 feet, is contaminated with pesticide residue. Monocrotophos is detected in the range of 0.04 to 0.06 ppm, cyhalothrin in the range of "traces" to 0.2 ppb (Jabbar et al 1990). Reports on drinking water contamination in Pakistan, focus on cattle drinking water in Karachi. This study revealed contamination with chlorinated pesticides and their metabolites (BHC, p.p'-DDT, p,p'-DDE, aldrin and dieldrin). 79 samples were analyzed for OC pesticides in 1984. Nearly 13% of the samples were found to be contaminated with different chlorinated pesticides and their metabolites. Recovery studies of 13 pesticides at different spiking levels, ranged between 76 and 107% (Parveen and Masud)

Objective.

The objective of this project is to establish knowledge on the risk of pesticide residue in home produced, for own consumption and for export, and imported food to public health in Pakistan.

To establish basic knowledge in order to perform a safer use of pesticides in food production in Pakistan. To establish knowledge on the contamination of drinking water in order to make necessary counter actions as processing of raw water, regulate the use of pesticides, inform farmers of proper use of pesticides or eventually ban the use of certain pesticides according to the national policy in this area.

Methods.

Introduction

Food and drinking water will be collected from the markets, rivers, ground water and faucets all over Pakistan.

The most frequently consumed food will have the highest priority. Selection of food items will tend to worst case scenarios, i.e. newly produced food, not stored for a long time, and then directly introduced to the market.

The pretreatment of samples will be representing worst case scenarios, but will represent normal practice. For example should bananas be peeled before analysis, but it is a question of local tradition whether or not the potatoes should be peeled and strawberries rinsed in water before the normal food processing in the kitchen.

All procedures will be described in detail from sampling to final report in order to ensure a retrospective case.

This includes naturally also safe cool transport of the samples to the analytical facilities in order to avoid decomposition of the pesticides.

A quality assurance system will therefore be established as far as to ensure validity of the data, well knowing that a full compliance with GLP (OECD) can not at the present moment be established in Pakistan. As a minimum, development of SOP's (Standard Operating Procedures) will take place.

The analytical facilities are established in Islamabad for modern pesticide residue analysis at state of the art. The analytical methods are developed for fruits and vegetables, namely multi residue methods used in Denmark. In Denmark these methods are subject to validation (Requirements from EU), and any change in the methods will immediately be reported to the chem lab.

These methods are cheap and fast ensuring reliable results down to 50% of the Danish MRL's, sufficient to control MRL's accepted in EU. Scientists have been trained in Denmark as part of a UNIDO program in residue analysis and capable of doing this type of analysis.

SOP's are widely developed for the analytical section of this project.

Inter calibration will be established to an internationally accepted GLP laboratory.

Fruits and vegetables

Sampling

Fruits and vegetables will be collected at the local markets according to table 1.

Table 1
Fruits and vegetables collected for pesticide MRL analysis at different locations.

Crop	Location	No of samples all locations	Total no of samples
Apple	Swat, Ziarat, Rawalakot.	2*3*3	18
Mango	Multan, Hyderabad	2*3*2	12
Melon	Karachi, D.I. Khan, Multan, Faisalabad	2*3*4	24
Banana	Hyderabad, Sakrand, Thatta	2*3*3	18
Orange	Faisalabad, Sargodha, Sheikhupura, Peshawar	2*3*4	24
Guava	Kohat, D.I. Khan, Rawalpindi, Faisala bad	2*3*4	24
Dates	Sukkur, Khairpur, Turbat,	2*3*3	18
Louqat	Kohat, Attock, Rawalpindi, Lahore	2*3*4	24
Pear	Peshawar, Mardan, Swat	2*3*3	18
Total no of fruit samples			180

The fruit samples are primarily analyzed for the following pesticides:
Azinphosmethyl, Dichlorvos, Phosphamidon, Monocrotophos, Cypermethrin, Malathion, Methamidophos and Fenvalerate.

Table 1 contd.

Caul.flow.	Karachi, Thatta, Hyderabad, Sukkur, D.I. Khan, Lahore, Rawalpindi, Quetta, Sialkot	2*3*9	54
Tomato	Same locations	2*3*9	54
Potato	Same locations	2*3*9	54
Cabbage	Same locations	2*3*9	54
Carrot	Same locations	2*3*9	54
Lettuce	Same locations	2*3*9	54
Spinach	Same locations	2*3*9	54
Cucumber	Same locations	2*3*9	54
Brinjal	Same locations	2*3*9	54
Onion	Same locations	2*3*9	54
Turnip	Same locations	2*3*9	54
Radish	Same locations	2*3*9 (°)	54
Total no of vegetable samples			648
Total no of fruit and vegetable samples			828

The vegetable samples will primarily be analyzed for the following pesticides:
Pirimicarb, Pirimiphos methyl, Oxydemeton methyl, Endosulfan, Malathion, Trichlorfon, Dichlorvos, Carbofuran, Triazophos, Methyl Parathion, Thiometon

(°: Double sample * no of samples per location * no of locations)

Cotton seed/oil and mustard

Sampling

Table 2
Cotton seed/oil for pesticide residue analysis

Sample	Location	No of samples all locations	Total no of samples
Cotton seed	D.I. Khan, Multan, Sakrand	2*10*3	60
Cotton oil	Same	2*10*3	60
Mustard	Attock, Rawalpindi, D.I. Khan, Multan, Hyderabad, Nawabshah, Quetta, Turbat	2*10*3 (°)	60
Total no of samples:			180

The cotton samples, and the mustard samples which are grown together with the cotton, will primarily be analyzed for the following pesticides:
Dimethoate, Methamidophos, Monocrotophos, Cypermethrin, Kelthane, Endosufan, Chlorpyrifos, Profenofos, Cyfluthrin, Methyl parathion, Fenpropathrin, Decamethrin, Fenitrothion, Mevinphos, Dichlorvos.

(°: Double sample * no of samples per location * no of locations)

Samples will be collected in duplicate. Depending upon the frequency of reported cases exceeding MRL, one can give more precisely a statistically valid number of samples per location to collect. Three samples per location is considered to be the minimum amounts of number per location.

It might be necessary later on in the study to increase this number in order to give a statistically sound picture of the situation. Sample size will vary according to the crop, but will be representative for sampling of such crops.

Transport from market to the laboratory doing the pretreatment, including sub sampling and freezing to -20 to -25°C, will be performed within 1-2 days. Sample size will consist of minimum 2 kg and as a minimum of 15 units (individual fruits or vegetables). The 15 units has first priority. The sample will be blended as one sample and mixed well. Sub sampling will consist of 15 sub samples, each 25 g. Only 2-4 sub samples will be needed for analysis, the remaining sub samples will be kept in case of losing the sample in analysis. Samples are not valid if storage life exceeds 9 months.

Transport of samples to laboratory for analysis will be done as fast as possible, making sure the samples are kept deep frozen. Analysis will be performed within 9 months after sampling.

Pesticide residue analysis

A multi residue method validated according to GLP and EN 45.000 quality assurance system will be used. The method is one of the multi residue methods used in the Danish control of pesticide MRL.

The method consists of extracting the 25 g sub sample with 50 ml acetone, and extract with 50 ml of ethyl acetate and cyclo hexane (1:1). Evaporation of an aliquot, filtering through High Flow Super Cell (Cellulose powder), and transferring the sample to GPC (gel filtration, Bio-Bead, SX-3, ethyl acetate: cyclo hexane)(1:1).

Quantification by GC (gas chromatography), equipped with NPD (nitrogen phosphorous detector), FPD (flame photometric detector) and ECD (electron capture detector). Quantification by use of internal standards and response factors between pesticides. Reporting limit will be 50% of MRL. If 50% of MRL is exceeded, reconfirmation on several chromatographic columns of different polarities will be performed.

Drinking water

Sampling

Drinking water will be collected at different localities distributed all over Pakistan, in urban as well as rural areas, as shown in table 3.

Table 3

Drinking water samples collected for pesticide MRL analysis at different locations.

Samples	Locations	No of samples all locations	Total no of samples
Water	120	2*2*120 (°)	480
Total no of samples:			480

The water samples will be analyzed for 10 most commonly used pesticides in Pakistan:
Profenophos, Cypermethrin, Monocrotophos,, Metamidophos, Cyfluthrin, Cyhalothrin, Fenpropathrin, Isoproturon, Fenvalerate, Biphenthrin. (To be confirmed later.)

(°: Double sample * no of samples per location * no of locations)

2.5 L glass bottles (Pyrex) are needed for sampling. The bottles will be cleaned thoroughly with ethanol and suitable glassware detergent, heated to a minimum of 450°C for 2 hours in order to evaporate/break down any residual organic substances.

Shipment of bottles will be performed in closed containers in order to avoid contamination. The sampling will only be performed by trained labor. In order to preserve the samples, dichloromethane will be added.

Transport back to the analytical laboratory will take place within 10 days. Liquid-liquid partitioning with dichloromethane will be done immediately when the samples are arrived.

Pesticide residue analysis

Depending upon the matrix, clean-up of the samples will done by means of suitable procedure: Gel permeation chromatography alone or in combination with silica gel.

Solid phase extraction done in situ might present a better and cheaper solution, in cases only pesticides with satisfactorily recovery for this type of extraction will occur in the sample. The synthetic pyrethroids often have a low recovery in solid phase extraction.

In case of preferring solid phase extraction, dichloromethane will not be added, but 2% methanol in order to get better performance of the C-chains.

Detection will be done on GC with suitable detectors (ECD, NPD or eventually FPD). Quantification will be done by using external standards.

The methods will be validated at 0.1 ppb as the lowest level and up to the highest concentration reported.

Inter-laboratory calibration system will be established with an accredited GLP laboratory in Europe.

Estimated costs of the pesticide residue program in fruits, vegetables, cotton seed/oil, mustard and drinking water.

Table 4
Estimated cost of 1500 analysis of fruits, vegetables and drinking water, inclusive validation of methods.

Item	Volume	Cost	Total cost
Sodium sulphate anhydr.	120 kg		
Acetone GC-grade	120 L		
Ethyl acetate GC-grade	330 L		
Cyclohexane GC-grade	220 L		
Dichloromethane GC-gr.	230 L		
Hexane GC-grade	100 L		
Ethanol techn. grade	350 L		
Sodium hydroxide	30 kg	-------------	
Sub Total:		-------------	-----------
Pesticide standards certified	50	12.000$	
Glassware, storage containers		10.000$	
Spare parts GC		10.000$	
Gel filtration		15.000$	
Small items		5.000$	

Table 4 contd

Hydrogen generator	1	7.000$	
Solid phase extraction		6.000$	
Oven,oper.temp. 450°C	1	10.000$	
2 L Pyrex red screw cap w/teflon lining	50	1.000$	
Freezer, 20-25°C, 300 L	2	1.500$	
Insulated transpor tation boxes for water analysis	10	1.500$	
Worst case repair		20.000$	
Est. sampling and transportation of plant material and drinking water to laboratory		30.000$	
Inter calibration		5.000$	

Sub total:		--------	---------
Total			
30% overhead:			---------
Total			---------

Plan of activity

Adjustm. of meth.			
Sampling			
Analysis			
Reporting			
	1996	1997	1998

Adjustment of methods

A final validation and tuning of the analytical methods, inclusive inter calibration will take approximately 3 months.

Establishing cooperation with local laboratories for sampling and sub sampling will take place in this period.

Analysis

Analysis will start after final validation of methods and is scheduled to run for 18 months. This time span includes control measures of the method, e.i. repeated recovery experiments.

Reporting

Reporting will take place after one year and at the end of the project. The first report will consist of analytical results, only including a complete description of the sampling.

A mid-term report meeting will be held between sponsor and Ecotox center.

The final report will include all SOP's used, all raw data as logbooks, chromatograms, statistics etc.

Final meeting will be held at the latest 2 months after the project termination date, 1 January 1998.

The final report will be presented one month before the final meeting.

ANNEX VII

VII. FUTURE PLAN 3
- Occupational Health in Agricultural Pesticide Sprayers, and Re-entry

Occupational pesticide exposure studies in agriculture.

Background

Operators of spraying equipment in agriculture, who are usually not wearing protective clothing during spray practice, are exposed to much higher dosages than ADI values (**A**cceptable **D**aily **I**ntake). It is not uncommon to find 1.000 to 5.000 times ADI values under European spray conditions.

The definition of AOEL values (**A**cceptable **O**perator **E**xposure **L**evel) has not yet been agreed upon internationally, but EU is at present trying to define the unit. The unit will be based on daily amount of exposure to pesticide, evaluation of the route of exposure (skin, inhalation, mouth), penetration factors, safety factor (probably 100), transformation factor from animal to human and NOEL (**NO E**ffect **L**evel) in long term experiments (chronic). Different AOEL's have been suggested, skin, inhalation and oral, but there is a tendency in favor of only one internal "blood stream level-AOEL"

One of the important parameters under discussion among dermatologists, is the penetration factor for human skin. It is actually unknown and a trigger parameter in the AOEL-unit. The most highly exposed body part in spraying pesticides in agriculture is the skin and usually hands are exposed sometimes up to 95% of the total body exposure. One very important parameter for skin penetration is the skin humidity. With increased skin humidity follows increased pesticide penetration, typically up to 10 fold increase for wet skin compared with dry skin.

Two parameters are important under climatic conditions in Pakistan for spraying personnel.

1) Often high humidity on the skin due to high temperature when spraying.

2) Light clothing often due to high temperatures when spraying.

and maybe a third parameter of importance:

3) Usually the sprayers in Pakistan are not aware of the risk of exposure to pesticides.

Re-entry of workers in sprayed areas is another group of workers with a very high risk of exposure. Cotton pickers, fruit pickers, collecting of vegetables etc. are usually in the high risk area. In Pakistan with no control of MRL (**M**aximum **R**esidue **L**imit) on food, the dosage remaining on the food at harvest might be much higher than expected.

Banana pickers in Nicaragua working in pesticide sprayed plantations, have shown a frightening example. Sterility among males was a very common picture.

Even if MRL is respected, say on oranges, apples etc., there is a big difference between consuming 100 g of apple per day and picking 2.000 kg of apples with residues of pesticides on the surface only.

Bystanders is a group of people normally not considered to be exposed to pesticides. Spraying of pesticides near villages might represent risk. They are surely exposed with pesticides. Wind drift within 20 m from a hydraulic spraying equipment at 3 nozzle pressure and wind speed of 5 m/sec, will deposit up to 5% of the field applied dosage/area. Often children in villages are subjects to this type of exposure.

There are numerous reports documenting decrease in acetyl choline esterase among pesticide sprayers using carbamates and organo phosphate insecticides. This is not new information. Epidemiologist are at present working on finding causes of reduced fertility in the population. Pesticides are one of the suspected causes, especially after discovering lack of normal male genital development in reptiles in Florida, probably due to DDT analogs or other oestrogen mimicking chemicals in the swamps.

Factory workers, pesticide sprayers, re-entry personnel and bystanders are with no doubt the most exposed group of the Pakistani population. Spray personnel and reentry workers are with no doubt the largest group of people exposed and will be preferred in this study. It is an urgent matter to produce solid evidence and facts on this matter in order to take counter measures and to avoid the very high risk for adverse effect on this group of the public.

Objective

The objective of this study is to produce evidence of the degree of exposure of pesticides to spray personnel and re-entry personnel, in order to create awareness among farmers on this issue and take counter measures as protective clothing, technical adjustments on equipment and education.

Methods

Introduction

Different methods have been used in the past. A review of methods and recommendation for future exposure studies have been made by the EUROPOEM (**Euro**pean **P**esticide **O**perator **E**xposure **M**odel) working group edited by Graham Chester, Zeneca.

Depending on the individual study, the workers are equipped with collecting media. One should select a method where **total exposure** is measured on the body and later consider the role of (protective) clothing.

Some studies are made with the usual clothing. The argument is that the person is protected on the covered area. This is to some extent true, but usually exposure studies are of short duration and penetration of the pesticides through the clothing normally takes some days. Furthermore, farmers do not wear special clothing when spraying and if they do, reports claim that normal washing procedures do not clean the clothing.

The problem is covering a test person with a full suit in 30°C and 90% humidity and let the person work with normal speed for 8 hours! The solution could be to cover as much as possible with light cotton cloth. A must is wearing light cotton gloves because the hands normally is the heaviest exposed part of the body. Field experiment has shown that the exposure by oral route and inhalatory route is of minor importance. If one should choose to cut down the amount of sample (and cost) this should be remembered.

The EUROPOEM document argues very strongly for the use of some sort of quality assurance system. An approach to GLP will be the best solution and satisfy most authorities. Special emphasis should be put on validation of the analytical methods, recovery on field spiking of samples and storage recovery.

Because the dosages are relatively high (in the mg range, residue analysis in µg range), the use of HPLC (photometric measurements) can be used. This technique give far more reproducible results than GC. Clean-up is in many cases reduced to filtering the sample before analysis.

The analytical techniques are developed and available at the Ecotox center chem. lab.

Exposure scenarios

Three scenarios of spraying:

1. Hydraulic tractor mounted sprayers. This technique is mainly used in cotton and wheat.

2. Carried knapsack sprayer, motor driven. Used in vegetables and cotton mainly.

3. Carried knapsack sprayer, hand driven. Usually used at small farmers in vegetables.

Three scenarios of exposure:

1. Mixing
2. Loading.
3. Spraying.

Body exposure, cotton gloves, long pants and long sleeve shirt, divided into:

1. 2 gloves
2. 2 lower legs
3. 2 thighs
4. 1 hip
5. 1 front torso
6. 1 rear torso
7. 2 arms

Totally 11 body parts.

Locations:

1. 2 provinces, Sind and Punjab.
2. 4 locations

Totally 8 locations.

Totally 792 samples.

Crops:

1. For hydraulic sprayers, cotton is the most sprayed crop (=1 crop)
2. Knapsack sprayer, motor driven, is mainly used in cotton and vegetables (=2 crops)
3. Knapsack sprayer, hand driven, is used among small farmers and used in vegetables (=1 crop)

This gives totally (792/3)*4 = 1056 samples

Two scenarios of re-entry:

1. Cotton picking

2. Fruit picking, apple and orange.

Totally 3 crops

Body exposure, cotton gloves, long cotton pants and long cotton sleeve shirt, divided into:

1. 2 gloves

2. 2 lower legs

3. 2 thighs

4. 1 hip

5. 1 front torso

6. 1 rear torso

7. 2 arms

Totally 11 body parts.

Locations:

1. 2 provinces, Sind and Punjab.

2. 4 locations

Totally 8 locations.

No of workers involved in re-entry per location:

1. 4-6

Total no of samples: 1320

The workers involved in the study will be the ones who used to work on the type of the work to be performed. The study will reflect a practical situation of spraying/re-entry.

SOP's (standard operating procedures) will be used all through the study from protocol, sampling, analysis to report. Full documented GLP will not be used.

Of special emphasis in the study will be.

1. Location with map
2. Name of land owner and workers
3. Name of study director
4. Crop to be sprayed or re-entry
5. Date of spraying or date of last spray (re-entry)
6. Name of pesticide, active ingredient, % active ingredient, batch no and deliverer.
7. Dosage of a.i per ha sprayed
8. Volume of water per ha
9. Nozzle pressure and type
10. Duration of spray/re-entry.
11. A complete description of working procedure correlated to hour. Climatic registrations as temperature, wind speed and direction and humidity at start and end of experiment.
12. A complete description of sampling of gloves and suits with coding, transport to lab and storage.
13. Field recovery on field spiking of gloves and suit.
14. Lab recovery on storage of spiked samples.

Chemical analysis.

Recovery experiments will be done on gloves and suits spiking at levels of 250 µg and 1000 µg respectively. Recovery should be above 60% and documented.

The suit is cut in pieces as indicated, wearing latex or polyethylene gloves in order to avoid cross contamination of the samples. Placed in 2.5 L Pyrex glass bottles, added 0.5 L ethanol (technical grade) and soaked overnight. Next day, 5 minutes of shaking, 50 ml filtered (anhydr. sod. sulph. and cotton plug) sub sample is transferred to evaporator and evaporated to dryness. Redissolved in 5 ml of ethyl acetate : cyclo hexane (1:1), 2 ml transferred to gel permeation chromatography.

The eluted sample evaporated to dryness, redissolved in appropriate solvent for HPLC. Lowest concentration for gloves will be 10 µg per glove which should be sufficient for detection of most pesticides on UV-VIS detector. If not, use GC with suitable detector.

Quantification will be done using linear regression calibration after Miller and Miller, at least 5 external standards. LOD and LOQ will be calculated inclusive statistical parameters for significance.

Results

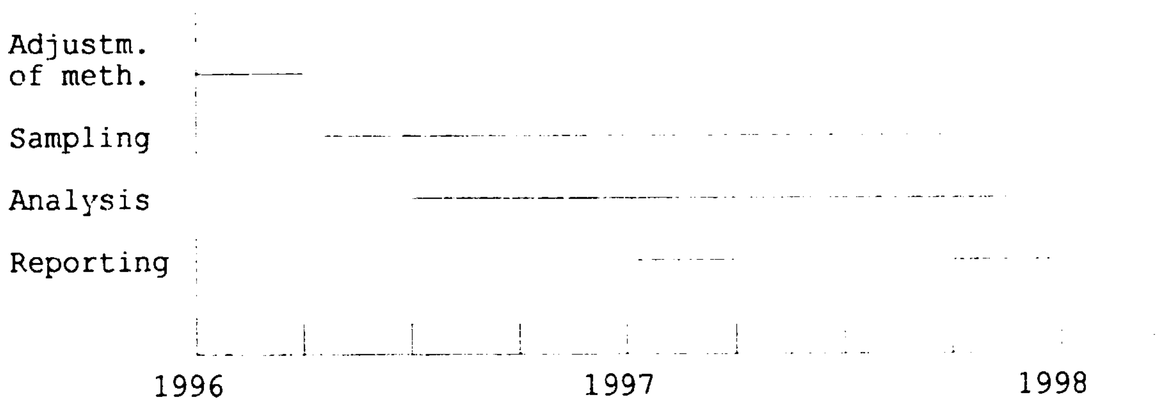
Results will be expressed as:

mg a.i. per worker * hour * body part, and

mg a.i. per worker * hour * total body exposure.

Full documentation inclusive raw data will be in report document.

Plan of activity



Adjustment of methods

A final validation and tuning of the analytical methods, inclusive inter calibration will take approximately 3 months.

Establishing cooperation with local laboratories for sampling and sub sampling will take place in this period.

Analysis

Analysis will start after final validation of methods and is scheduled to run for 18 months. This time span includes control measures of the method, e.i. repeated recovery experiments.

Reporting

Reporting will take place after one year and at the end of the project. The first report will consist of analytical results, only including a complete description of the sampling.

A mid-term report meeting will be held between sponsor and Ecotox center.

The final report will include all SOP's used, all raw data as log-books, chromatograms, statistics etc.

Final meeting will be held at the latest 2 months after the project termination date, 1 January 1998.

The final report will be presented one month before the final meeting.

ANNEX VIII

VIII. SHORT-TERM PLAN, Next 3-4 months

The chem section was given a common task for all members of the group during the following 3-4 months.

The three plans outlined in rough draft in appendix V, VI and VII, will be initiated after this period, partly or in full scale.

The common task will be plan no 2, MRL studies in fruits and vegetables.

The section has been instructed to perform the following:

1. Determine retention times and response factors for 10-15 pesticides on GC.

2. Make elution profile for these 10-15 pesticides on GPC.

3. Extract 4-6 crops from the market, according to the method of analysis given.

4. Using own and the Danish retention time table and response factor table from the food control, analyze all major peaks on the chromatograms of the crops. Come up with proposal for eventually identified pesticides and make quantification.

5. Validate the method with respect to spiking at level of MRL and 10 times the level of MRL with a pesticide you find place for at the chromatogram of the crops.

6. Inter calibration. I will send the chem section unknown pesticides to identify and quantify.

ANNEX IX

IX. UNIDO'S Substantive Comments on the Report of Mr.E.Kirknel - (US/PAK/90/294/11-01)

For the fifth mission, the expert successfully completed his mission on evaluating progresses in the Ecotoxicology Centre, preparation for the TPR meeting, and proposing the Work Plan for the laboratory. He noted that two laboratories at the Ecotoxicology is not functioning properly and supply of equipment had not been punctual. The TPR meeting was perceived as successful by the expert in persuading Pakistan government to support the Ecotoxicology Centre in the coming 2nd phase of the project based on his recommendations at the meeting.

His report, however, failed to analyze in depth the cause of the malfunction of the terrestrial ecology laboratory and the microbiology laboratory. He reasoned the cause as internal disagreements, but this point should have been more elaborated. Delay of equipment supply should have been technically analyzed and suggested substantial solutions. These point will be pursued by UNIDO and will be corrected for better implementation in the future.

The three Work Plans for the second phase of the project are technically detailed enough to be implemented as it is, but the second proposal on "control lab. for MRL of pesticides in fruit, vegetables, cotton seed/oil, mustard and drinking water" might overlap with FAO mandate and should be negotiated with the organization. The third proposal on "Occupational pesticide exposure studies in agriculture" might be overlapped with the effort of WHO and ILO and need further discussion. As the Work Plans have concentrated on chemical monitoring aspect and ignored other laboratories of the Ecotoxicology Centre, broadening of scope of the Work Plan to be inclusive of the other laboratories will be subject of practical discussion by the steering committee for the research projects.