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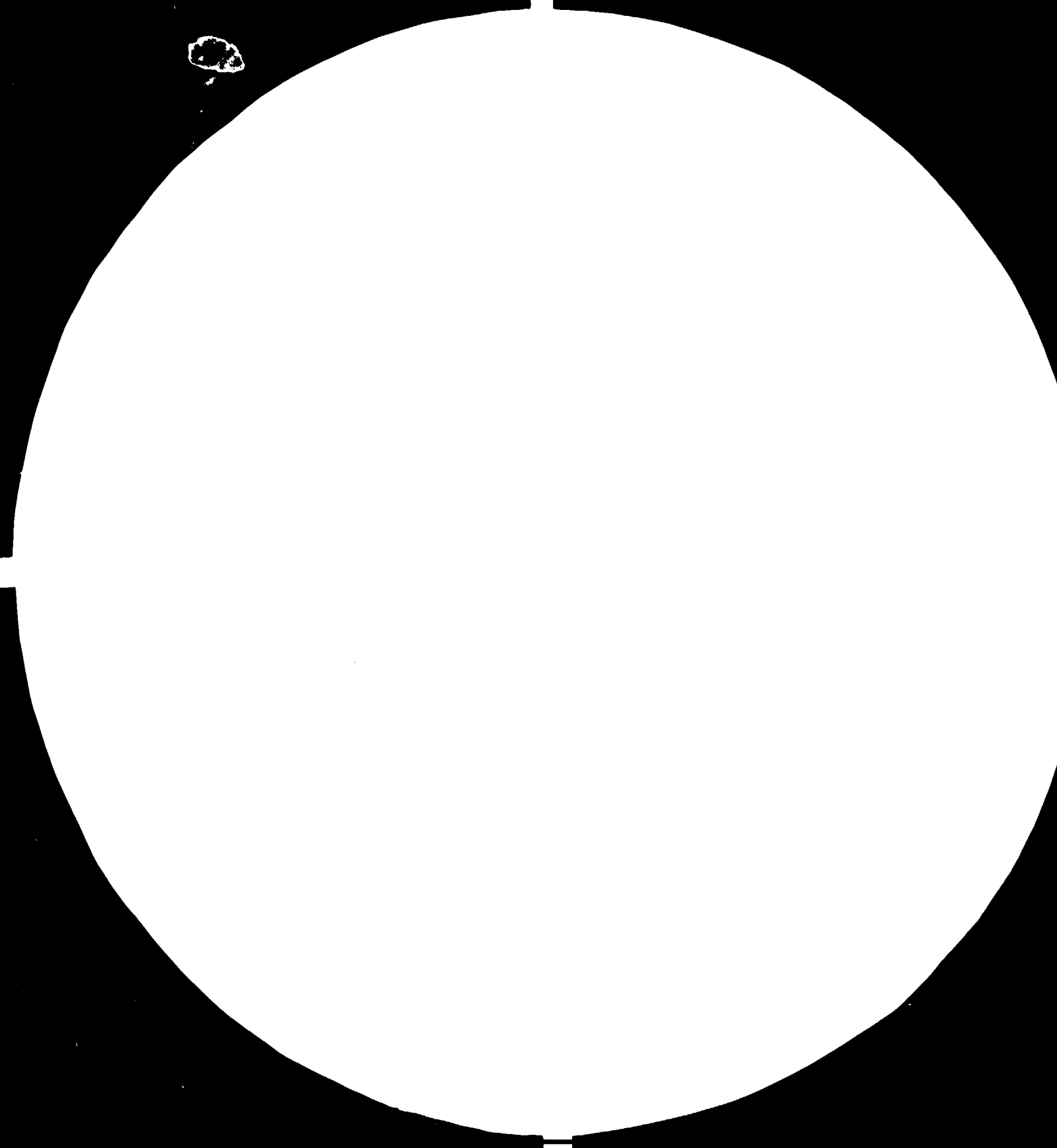

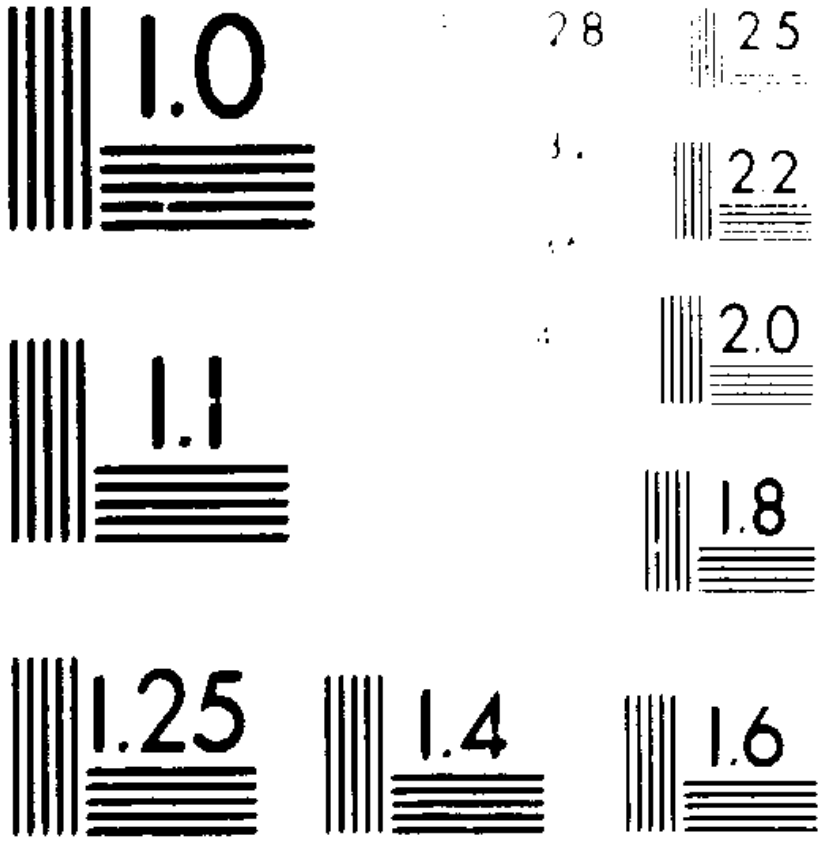

MICROCOPY RESOLUTION TEST CHART

RESTRICTED

13445

DP/ID/SER.B/442
7 February 1984
English

Nepal.

STRENGTHENING THE ROYAL DRUGS RESEARCH LABORATORY

DP/NEP/80/C03

NEPAL

Terminal report *

Prepared for His Majesty's Government of Nepal
by the United Nations Industrial Development Organization
acting as executing agency for the United Nations Development Programme

Based on the work of Mr. J.P.G. Williams,
pharmacologist

United Nations Industrial Development Organization

Vienna

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I. SUMMARY

The object of the project 'Strengthening the Royal Drugs Research Laboratory' is to help the RDRL to fulfill the objective of HMG Nepal that Nepal should be self-sufficient in essential drugs. The project document was signed in December 1981. Purchase of pharmacology equipment commenced in September 1982 and a technical expert in Pharmacology was fielded in December 1982 for a period of 12 months.

After arrival the pharmacology expert made an appraisal of the situation in RDRL and a number of areas of potential development were identified. During the expert's continued presence in the laboratory further areas became apparent. Of particular importance in any scientific drug development program is the existence of a well organized and efficient animal house. The expert has devoted much of his energy to emphasing this point which has been well received. Recommendations were made to the Director of the Laboratory and to the UNIDO CTA and many of these suggestions have been or are being incorporated into the animal house. The Director General of the Dept. of Medicinal Plants identified at meetings with the expert a number of particular areas in which it was felt the RDRL should work, accordingly the expert turned his attention to these areas advicing on protocols and techniques etc. Consideration of training aspect of his job led the expert to make a number of recommendations especially in relationship to the library and a substantial number of books on pharmacology have been or will be added to the department's library. Recommendations for training in specific areas eg PhD in toxicology have also been made.

The Director General has requested that the UNIDO experts provide a work plan for the laboratory to pursue after their departure. In response to this the pharmacology expert has presented a generalised flow chart for the investigation of biolcgically active materials from biological sources at the research level.

The necessity to purchase a generator and other equipment for the animal house together with certain other factors led to reappraisal of the original equipment list and the substitution of certain other items.

II. RECOMMENDATIONS

1. His Majesty's Government's aim of making Nepal self-sufficient in essential drugs is a laudable and enlightened objective which will take many years to achieve. The work reported here should be seen as the beginning of a open ended research and development programme aimed at achieving HMG's objective. Because of the infant nature of pharmacology and drug development in Nepal the RDRL will need continuious support in terms of expertise, instrumentation and training.

2. Continue strengthening and expanding the biology section in both space and personnel.

3. Appointment (and/or training) of a suitably senior person in toxicolcgy (Appendix 1)

4. All bioassay work be coordinated by the biology section and meaningful experiments be undertaken to provide statistically acceptable results (Appendix 1, Health Safety*).

5. Any agent to be applied into or onto humans or economic animals must be tested in/on experimental animals before it is used in/on humans - including the laboratory staff. - (Appendix 1, Health and Safety*).

6. Improve the standing of biology section by suitable training (Appendix 2) It is imperative that for biological and animal work suitably trained candidate be selected.

7. Improve animal house staff by training additional personnel and by more advanced training for one or more persons to allow for future expansion (Appendix 3 section on animal house personel).

8. Plant collection: - Voucher specimens must be taken with every plant collection, the samples should be housed in the same conditions as herbariun specimens. (Appendix 4)

9. Plant collection: - Adequate information must be recorded so that recollection can be made from the same population (Appendix 4)

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* Basic approach to Health and Safety in Laboratories - John G. Meredith NEP/80/003 Documentary output dated 1st September 1983.

10. Record keeping: - Be as uniform as possible throughout the laboratory, and that a central record storage system be initiated, these records should eventually be computerised. (Appendix 4)

11. Within the biology section expertise and instrumentation should be developed in pharmacokinetics, pharmacodynamics, biochemistry and toxicology.

12. For the hypoglycaemic assay glucose be measured by glucose oxidase and rats be used instead of rabbits.

13. Attempts to establish an anthelmitic assay be continued especially by contact with other laboratories in SE Asia.

14. That contact be maintained with the WHO Indigenous plants for antifertility programme.

III. BODY OF THE REPORT

A. Introduction

His Majesty's Government's (HMG) plans for health care in the Kingdom of Nepal includes self-reliance in essential drugs. The United Nations Development Programme (UNDP)/United Nations Industrial Development Organisation (UNIDO) project DP/NEP/80/003 is a program of support and development aimed to assist HMG in attaining this objective by "Strengthening the Royal Drugs Research Laboratory (RDRL)". The project includes the provision of equipment and technical experts in the areas of analytical chemistry, pilot plant development, economics and biology. The biology support includes two UNIDO appointed experts, one in microbiology and one in pharmacology. The analytical chemist also has extensive knowledge in pharmacy and medicinal plants. The need to strengthen the pharmacology section had been a recommendation of an earlier technical assistance programme (SI/NEP/78/801 1980).

A list of equipment dated 25 December 1981 was prepared by the staff of RDRL and after approval in April-May 1982 by the UNIDO consultant was accepted as part of the project DP/NEP/80/003. The purchase of pharmacological equipment from this list began in September 1982. The UNIDO appointed technical expert for pharmacology arrived at RDRL on 1st December 1982.

Original Job Description

The original job description (DP/NEP/80/003/11-02/32.1.D)* described the purpose of the project as "to develop further the existing facility for production of plant derived pharmaceuticals at the Royal Drugs Research Laboratory". The expert's duties were defined as assisting the Director of RDRL to set up a facility for the pharmacological screening of medicinal plants and conduct pharmacological evaluation on plant preparations produced. Specifically the expert was to:

(a) Build-up a suitable and adequate colony of laboratory animals for different pharmacological tests including bioassays.

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* see Appendix 13.

(b) Organise in collaboration with local counterparts laboratory biological screening programmes and procedures for the pharmacological and toxicological assessment of medicinal plants, and plant products.

(c) Train local counterparts in conducting bioassays and pharmacological tests; in the breeding and maintenance of laboratory animals, and in research in pharmacology.

B. Animal Colony

A well run, productive animal house is a cornerstone of drug research and development, without such an establishment any attempt by RDRL to develop drugs or to determine the efficacy of pharmaceuticals or assess safety of any substance coming in contact with humans or animals would be futile.

The animal house in December 1962 occupied the first floor of a three story building Access to the animal house was impeded at the north end by piles of tins resulting from the distillation of pine oil and to the south by trash from the Royal Drugs Limited, a disued cement mixer made access to the entrance of the animal house difficult and acted as a source of mosquitoes. The animal colony was housed in two rooms of 583.6 square feet and 712.8 square feet. The first of these housed about 80 rabbits and the second about 50 guinea pigs, 120 rats and 200 mice. A third room was used for cage storage and cleaning and for food preparation, which also took place in one of the animal rooms. A fourth room on the same floor had been set aside as a screening room. All rooms had insect netting in fair but not perfect condition, rodent barriers were present at exit doors. The entrance to the building is at the north and an escape door is provided at the south end of the building. No facilities existed for the safe disposal of animal bodies nor for waste (used bedding, faeces etc). The rooms were heated by electric fan blowers and kerosene burners. Temperatures observed in winter were minimum 11, maximum 25 and in summer minimum 19, maximum 35. Temperatures range in winter was influenced not only by the ambient temperature ($< 5^{\circ}C$) but also by power failures that meant the electric heaters did not work. Day length varies little at the latitude of Kathmandu and no recommendations were made concerning light control. Much later (in August) it was discovered that some lights were actually put on at night, this may have contributed to the inconsistent breeding performance. The animal technician was instructed that this should not be done.

The water supply was limited to two taps in the corridor outside the actual animal rooms. These taps are in constant use making the approach to the animal rooms slippery and hazardous. There was one small hand basin at the far south of the floor near the escape door. Cage cleaning was carried out with cold water as no hot water was available. There was no provision for investigators or animal house staff to wash after handling animals.

Record keeping was restricted to date of birth identified on cage, there were no records of individual animals, it was not possible to make an assessment of fertility rate, of lifespan, or of growth rates. The actual condition of the animals was fair with respect to the rodents but the rabbits deteriorated to the extent that several animals died and others had to be destroyed by the expert. The veterinary support at that time was inadequate. The disease condition is a complex situation also involving diet, ideally if the animals were fed on sterlized pelleted food, (this would stop reinfection,) the disease state could then be dealt with and this, associated with better caging would probably improve the colony, this solution is however not immediately achievable.

At the time of the original assessment the number of animals cages was insufficient. Since that time both cages and racks have been ordered and the situation is (or will be when all orders are filled) tolerable. (depending on the work load).

The animal house was staffed by an animal technician (Mr. Khan) and several animal "boys" who performed the actual cleaning and feeding. Mr. Khan had a period of compassionate leave during the earlier part of the year and his place was taken by two other people. The condition of the animal colony during Mr. Khan's absence indicated that a person of his experience and quality is indispensible to the running of the animal house and further that he has no adequate staff back up in the laboratory.

After the initial assessment of the laboratory animal facilities at Kathmandu the pharmacology expert visited Central Drug Research Institute, Lucknow, India, he was accompanied by his counterpart Mr. S.K. Joshe and microbiologist Mr. Tuladhar. The objectives of this trip were to visit an animal house in a comparable situation to RDRL, to obtain protocols and organisms to enable the RDRL to carry out anthelimintic and antidysentary screening (see below), to discuss the use of the Grass polygraph and to establish and enhance interlaboratory relations (Appendix 3). The visit was a success especially in showing what could be achieved in the terms of animal care and in demonstrating the importance of the animal house to such a laboratory. However parasites were not and have not been provided.

It should be apparent from the preceeding paragraphs that there was scope for improvement in the animal facilities. At the time of writing (Mid September) many of these improvements are well advanced.

The most important problem seen in the first few week of the expert's appointment was the temperature. Discussions were started with the CTA which led to the ordering of 6 Hitachi air conditioning units that could hold the interior environment constant either above or below ambient temperature. Since these units depended on the electrical supply for functioning a generator was also ordered. The air conditioning units arrived in May and are now installed. The generator has been ordered by UNIDO headquarters and should arrive in October. The Director General RDRL has indentified space for the generator and it is expected that it will be installed immediately on arrival thus protecting the animals from the fluctuation in temperature which can be anticipated will occur in the winter.

When the air conditioning had been ordered the expert drew up a plan for some structured alternations to be made to the animal house. During the discussions of this plan it became clear that more space might be made available within the building due to the moving out of Royal Drugs Limited (RDL). In response to this a new plan was drawn up and as additional HMG funds were available it was possible to introduce new plumbing including hot water from solar heaters. The new plan included two further rooms on the ground floor. One of these will be used for cage cleaning and storage while the second will be a kitchen with a storage area. The kitchen will contain a stainless steel sink and a second brick sink (or trough) which will be used to soak fresh vegetables in copper sulphate solution to reduce the worm infestation. The kitchen will house the heaters for the food and also a steam sterlizer. Since the cage cleaning etc. can now (or on the completion of the building works) be moved downstairs the room previously used for that purpose will be freed for use as an animal room, adding another 317 square feet of animal space. The space available in the large room has been divided to give small rooms. Small rooms are better for the isolation of experimental animals from stock animals, for the segregation of sick animals and those of different

species. Small rooms also restrict the spread of pathogens. The spread of pathogens will also be reduced if staff, - investigators as well as animal house workers wash their hands between handling different animals. This should be much easier with the increased number of sinks and the provision of hot water. Again hot watershould increase the efficiency of floor washing for the purposed cleanliness.

Further in relation to hygiene and sanitation the mounds of animal waste are being removed and a reguler system of disposal has been established. For the disposal of animal bodies an incinerator was designed by the CTA and constructed at the Balaju Yantra Shala(BYS) Engineering Works. Although it is essential that all animals containing pathogenic organisms should be destroyed by incineration it is not always convenient to do this if only a few animals are to be disposed of at one time. To enable safe storage of carcasses a freezer was ordered and has now been received. It will be placed in the kitchen.

A steam sterilizer was located and moved to the animal house where it will be used to sterilize the rice husks employed as animal bedding.

The approaches to the building are currently being cleaned up, the cement mixer which was a breeding site for mosquitoes has been removed as have most of the tin cans, it is now possible to gain access to the back of the building and the drainage in front of the building is being improved. These changes which may seem insignificant are significant in relation to the restriction of entry of pathogens into the animal colony.

The number of cages has been increased substantially. At the end of 1982 200 plastic cages with 100 tops were ordered and these arrived in February - additional racks were ordered to accomodate the increased number of cages.

Authority to have more cages manufactured locally has recently been granted, 50 rabbit cages, 4 rabbit breeding cages, 12 rat cages and 6 guinea pig pans are being fabricated by Balaju Yantra Shala Engineering Works. With the new space for cage cleaning which should become available it will

be possible to renovate many of the cages which are currently in a very delapidated condition and to maintain the new cages in a good condition. Additional cage tops will be fabricated to use with the plastic cage purchased in 1982.

A small number of the plastic cages have been modified to use in reproduction and in diarrhea testing. The bottom of the cages were cut out and new bottoms made, ideally these should have been made of stainless steel wire but there is no facility for welding stainless steel wire and a sheet of aluminum with a large number of slots stamped out of it was used. When used in determining mating the copulation plug should fell through, when testing for antidiarrheal plant extracts the fluid faeces fall through. A rack was designed to carry these altered cages which have to hang from supports rather than simply rest on shelves, this rack was fitted with wheels to demonstrate the usefulness of being able to move racks of cages easily.

The number of animals in the colony has been increased (table I), this increase has been achieved in spite of the enormous amount of disruption caused for the past 8-10 weeks (22/8/83) and in spite of increased use by the pharmacology section.

Table I.
Animal Number

Date	Rabbits	Guinea Pigs	Rats	Mice	Total
8/2/83	86	54	120	244	504
11/7/83	94	68	236	552	950
24/8/83	83	18	199	516	816

Weekly records of the state of the animal colony are now routinely available.

Mr. Khan now has two assistants but these people appear to have no training in animal care, it is essential that Mr. Khan has trained assistants. There is a tendency to believe that anybody can work with animals. This attitude must be altered, the care of animals is a skilled profession and drug development and product safety testing are totally dependent on animal studies.

C. Screening Procedures

A Objectives

B Materials for bioassay

- i. Animals
- ii. Plant collection and record keeping
- iii. Plant extraction

C Methods for bioassay

- i. Hypoglycaemic agents
- ii. Anthelmintic agents
- iii. Antidiarrhial agents
- iv. Antifertility
- v. Ayurveda
- vi. Toxicity

D Results of bioassay.

Objectives

The screening of plant materials for biological activity is a rational scientific procedure following normal experimental method, i.e. there is an objective, materials, methods and results. The broad objective is described in the project document as making Nepal selfreliant in essential drugs. The direct objectives were obtained at a meeting with Dr. Malla and senior officers of the RDRL in December 1982. In discussions with Mr. S.K. Joshi a somewhat broader picture was obtained clarifying the fact that RDRL work on a yearly program within the framework of an overall five year plan. The December discussions contributed to the preparation of the 1983/84 program.

The objectives of the screening programme were identified as an examination of plant materials for the following activities:

Lowering blood sugar (hypoglycaemic agents)
Antidysentary agents (this category includes antidiarrheal as the initial pharmacological testing is the same).
Anthelminitic agents
Antifertility agents

The first three of these objectives were identified by Dr. Malla in December 1982, while an interest in antifertility activity was expressed in June 1983.

Dr. Amatya was interested in testing for insecticidal activity, and after some discussion on insect breeding he contacted HMG Agricultural laboratorys.

An interest in antileach and antitick activity was discovered in informal conversations when the expert expressed some concern over the safety and statistical aspects of the testing currently going on.

In discussions with counterpart it became apparant that there was also a need for toxicology testing.

At several meetings the testing of Ayurvedic preparations was discussed.

Materials for Bioassay

i. Animals

The materials for bioassay are firstly the experimental animals and secondly the materials to be tested. The condition of the experimental animals has been briefly described, a good colony is highly desirable. At present the mice, rats and guinea pig stocks are quite healthy, the rabbits however are not healthy. The simplest action with the rabbits would be to destroy the entire colony and start again taking greet care to prevent infection. This action is quite inappropriate at the RDRL. With the increased space and number of rooms which will be available when the rebuilding is completed, it should be possible to segregate the breeding animals, to remove their worm and protozoan parasites and by great attention to cleanliness and sterilizing the food and the bedding it may be possible to produce a healthy colony. This is most desirable as these animals are used for quality control and safety assessment.

ii. Plant collection and record keeping

Plant material for testing is readily available in Nepal. The expert has been unable to observe actual collection in the field but the examination of collections brought to the laboratory together with discussions with various members of the laboratory have shown a number of

weaknesses in the collection methods. The most striking of these is the lack of voucher specimens (Appendix 5). In an effort to bring the importance of such matters and especially of record keeping to as many people as possible the microbiology and pharmacology experts sought a meeting with the operational staff. At this meeting general methods, especially record keeping using the pharmacologist's experiences with the WHO plants for antifertility program as an example, would have been discussed. This meeting was not possible. The record keeping documents which had been sent to Dr. Malla in April and included as an appendix in the pharmacologist's interim report in May were however discussed with Dr. P.M. Adhikari and Mr. S.K. Joshi in early July, these meetings led to acceptance of the idea of interelated forms for collection, extraction and bioassay of plant material. It was also agreed that such records should be centralized and that steps would be taken towards computerising the data. Appendix 4.

iii. Plant extraction

At RDRL plant material is routinely extracted in 50% ethanol for phytochemistry. The pharmacology expert's view is that if the objectives of the laboratory are to undertake academic chemistry then the extraction of plant material with 50% ethanol might be acceptable but if the laboratory's objectives are to discover what biological activity either efficacy or toxicity is present in any plant then the plant should be tested in a manner as near the human usage method as possible, in general this means some form of water treatment. These different views led to long debates which eventually ended in a compromise that the plants that were to be tested in a objective test eg. Anthelmintic, Antidysentry, could be extracted with water but material for which objective tests were not immediately available (coughs, colds, headache etc) but which were being examined for toxic effects could be extracted with 50% ethanol! In practice it was found later that there was no suitable equipment for drying water extracts, thus the preparation of dried water extracts has not been possible. A freeze-dryer has been ordered but is unlikely to arrive before December.

Methods for Bioassay

i. Hypoglycaemic agents

This assay has been in use in RDRL for some time but the results are described as 'unsatisfactory', the source of this 'unsatisfactory' description was a failure to get the expected response from a known hypoglycaemic agent, Tolbutamide. Although this could have been due to a "faulty" batch of Tolbutamide a perusal of a small amount of data available suggested a more fundamental problem. When the control data was plotted as percentage change it appeared that the controls in each experiment behaved differently from one another. An examination of the protocols revealed a number of other weaknesses.

A series of experiments soon demonstrated many other problems with the procedure.

a. Temperature - the animals were transported from a relatively warm room through the cold outdoor into a relatively cold laboratory - consequently periferal blood vessels constructed and were difficult to sample from, also as the operators were cold the manipulations were more difficult.

b. Electricity - several times the experiments had to be abondoned because of power cuts.

c. Technician had insufficient experience of taking blood - he had examined only 3 plants during the previous year.

d. Insufficient animals for reasonable level of work. We used six animals on Monday (experiment lost through power cut) and then four more on Tuesday, this exhausted the supply as the animals cannot be reused for two weeks. On Thursday we used females just to keep the momentum up, and this experiment was delayed for a day due to power failure.

A number of recommendations and actions were made. The temperature of the sampling laboratory was elevated, the technician's ability to take blood improved with further practice. It was suggested that a different method of glucose estimation be employed. The method in use was the Folin type reaction and it was suggested that this might be

replaced by the glucose oxidase method but counterpart thought it would be too complex with supplies etc. to use an enzyme test. Later another method, the ferricyanide method from the Indian Pharmacopea was used but suffered from the same disadvantages of the use of boiling water (Kathmandu's altitude means water boils below 100°C) and sensitivity to oxygen. For these reasons and for the needs of toxicology an 'Ames blood analyser' has been purchased. This instrument uses clinical reagent packs of enzymes and uses glucose oxidase for the estimation of blood glucose. This may solve the chemistry problem but the animal problem remains. Rabbits are highly susceptable to stress from different sources (at least two died in catatonic rigor due to the noise emitted by drills being used during the alterations to the animal house). The susceptability is accentuated by the manner of handling the animal. This bad handling is mainly due to poor training. Some of these problems may be overcome if the number of biologists in the laboratory is increased but at present the simplest solution may be to introduce a more resiliant animal as the test organism, it has been suggested that for initial screening the rat should be used (as in CDRL). The use of rats and the glucose oxidase reaction may be in use before the expert leaves.

ii. Anthelmintic agents

Extracts have previously been tested using an in vitro earthworm assay, which now all agree is inappropriate. In the expert's initial report the in vitro nematode assay was recommended but it seemed more easy to start by using the cestode Hymenolepis nana. This organism is in use for screening plant products at CDRI Lucknow, and the methods were described by J.C. Katiyar at the UNESCO-CDRI workshop held at Lucknow in October 1982. The UNIDO technical expert assumed that the CDRI would make these parasites available and predicted that the method would be in use of RDRL by April 1983. A visit to CDRI was arranged with the collection of parasites as one of the objectives. Dr. Katiyar however did not wish the expert or the counterpart to take parasites or infected animals from CDRI but wished to come to Kathmandu himself. On returning to Kathmandu arrangements for

Dr. Katiyar's visit were made. It was only after the expert has spent a day waiting at the airport that it became apparent that Dr. Katiyar was not coming to Kathmandu. This was in early May and after several attempts Dr. Katiyar has still not yet visited the RDRL and the assay is not being carried out. A number of other laboratories, School of Pharmacy, Singapore; National Drug Control Laboratory Bangkok; School of Pharmacy, Penang and the National Drug Laboratory, Kuala Lumpur have been contacted as possible sources of parasites (the initial mailing to these laboratorys was at the same time as other letters were lost in the post and as no replies were received, the laboratories have been contacted again).

Attempts have been made to develop an infected colony from naturaly occuring infections, this is progressing slowly.

iii. Antidiarrhea agents

In his initial comments Dr. Malla asked the expert to develop tests for Antidiarrhea and Antidysentary plant extracts. The terms Diarrhea and Dysentary are often used synonymously, diarrhea tending to be used in conjuction with certain organisms eg. Amoebic dysentary. The causes of diarrhea are varied but can be divided into two broad classes the first of these is that caused by a microorganisms or by the body's response to the parasite or its toxins eg Amoebic etc. and the second caused by "environmental" change rather then a specific organism. Both classes present with fluid feaces and the greatest hazard is the loss of fluid from the body, it is possible to device a model that can be used as a test for agents that might effect this sort of symptom. In the case of the classes of diarrhea produced by microorganisms it is possible to produce tests for the specific pathogen. Test methods for specific pathogens have been described by the UNIDO technical expert in Mircobiology Ms. Cordes and will not be discussed further.

An assay for antidiarrheal agents was deviced based on the work of Janssen using castor oil as the diarrheal agent, this was passed to the national counterpart in February, the same test can be used to test for plants with reported purgative actions. (Appendix 6). The assay was examined after cage modifications had been carried out and to date (August) a satisfactory dosage regimen is being sort.

iv. Antifertility

The greatest problem facing Nepal and for that matter the rest of the world is overpopulation. The search for plant materials with antifertility effect is therefore an activity of the greatest significance. Dr. Malla ask the expert to look into suitable methods of antifertility screening in May, this was apparently in responce to anticipated government action. The expert has identified a number of plants reportedly used in Nepal (Appendix 7), a method of testing (Appendix 8), trained an operative in certain aspects of the assay and placed the RDRL in contact with the WHO's programme in this area. Certain cage modifications have been carried out to facilitate the control of breeding. Serious experimental work is held up by the building work which is going on in the animal house.

v. Ayurveda

At several meetings the question of testing Ayurvedic formulations and the formulations proposed by Dr. Bojor (SI/NEP/78/80L 1980) has been raised. In response to these questions the expert has stated that anything can be tested for biological action provided an appropriate test exists, (for instance it is simple to test for an action on worms but not for a headache). The interpretation of the test however can be very difficult. One problem often mentioned in relation to Ayurvedic preparations is that they are complex mixtures, the expert's view here is that if the complex mixture is being used by humans then it should be examined, (the interaction of the various components being a source of future research). The most serious problem lies in the use of the result of the assay. Since Ayurvedic preparations are not rigorously controlled the effect on one batch of a given preparation may differ from the effect of another, thus making any prediction of efficacy or safety a nonsense, thus until some form of quality control can be introduced for Ayurvedic preparations any bioassay has little or no predictive value.

vi. Toxicity

Mr. Joshi has requested some help in preparing protocols for toxicology. (Appendix 9). A start was made on histopathology by training in histology, it was found however that certain items of essential equipment were

not available and that the microtomes available did not work. Attempts were made to borrow a microtome from the University and the Medical School but without success. A new microtome and accessories were ordered from India and are now in use. A histopathology programme was suggested involving the collection of normal and pathological material but this programme was delayed until the microtome was working. LD_{50} of preparations are routinely carried out using mice. Methods in toxicology and teratology have been discussed.

The protocols for toxicology involve the assay of a number of substances in the blood. There were no means by which these assays could be carried out, access to spectrophotometers is limited (the machine in quality control will be used increasingly for chemical analysis) and knowledge of enzyme estimations and kinetics seemed to be negligable. It was therefore decided to purchase an 'Ames Blood Analyser' (see also under bioassay for hypoglycaemic agents). This is a simple instrument designed for routine use in hospital laboratorys. The blood analysis is carried out using reagent kits which are provided by the manufacturer thus reducing the logistic problems of supplies. The use of these reagent kits requires little or no knowledge of biochemistry. The instrument has just been received and training will commence after the final report is completed.

The LD_{50}, the Hippocratic test, the blood analysis and the histopathology should provide a good grounding for any toxicological studies but once again it is necessary to identify the lack of trained personnel as a major problem. In the simple screening of plants for activity the LD_{50} is sufficient but once drug development begins not only must the whole collection of toxicological techniques be used but the results must be interpreted and understood. The need for a senior toxicologist has previsously been stressed in a special recommendation to Dr. Malla. (Appendix 1)

Result of Bioassay

The results of bioassay (for efficacy or toxicology) must be maintained in a central file, this file should contain all the information available on a specific plant. A start towards such a system has been made in recommendations passed to Dr. Malla in April and discussed with Dr. P.M. Adhikary and Mr. S.K. Joshi in July. (Appendix 4)

The interpretation of the results of bioassay requires an understanding of living systems, of pharmacology, and of toxicology. The use of the data in determining the laboratory's activities needs knowledge of the drug market (is it worth developing a drug for a specific action? or is there too much competition?), of drug development and costs (can the drug be made at a reasonable cost, are there the resources both financial and material) toxicology (are the side effects tolerable, will the drug be acceptable outside Nepal) and of the legal aspects (will the drug be allowed on the market, what insurance does the laboratory have if sued for damages from toxic effects). Recommendations for the training of suitable staff have been made to Dr. Malla. (Appendices 1 and 2).

D. Training

1. General
2. Literature, books
3. Animal care and handling
4. Microscopy
5. Polygraph
6. Microcomputer
7. Toxicology
8. Screening methods.

These activities should not be thought of as separate operations from the others of the expert's job description.

Training, General

Training has been imparted by discussion and demonstration in the laboratory and animal house rather than by any didactic teaching techniques. The expert is very conscious that his stay here is brief and that it is the local staff that must handle the equipment. The instruction technique has therefore been 'Low key', encouraging local activity at all times, with the emphasis on the essence of a technique, with stress on numerical values, and the objectives of the test.

Literature, books

Since this is a research laboratory training and learning are going on continually and hopefully the idea of continued selfeducation has been evident in the expert's approach. This concept of selfadvanced is intimately connected to everpresent need to 'keep up with literature', to know what is going on the rest of the world. Nepal is particularly badly situated in this respect, for instance the laboratory subscribes to the current awareness journal 'Current Contents' which comes out weekly and is an essential research aid. The laboratory Director kindly put the expert's name on the circulation list, to date in week 37 of Gregorian calender only 11 have been received. At present the delivery of journals seems to be a very difficult problem.

To encourage the idea of continual selfeducation the UNIDO experts drew up a list of books in their respective areas. (see CTA's report)

Animal care and handling

Methods for handling animals, administration of various drug forms, ways to disect, aspects of anatomy, histology, pathology and pharmacology have been demonstrated and discussed during the usual working regimen. Aspects of animal care have been discussed with the appropriate technician as have matters of sanitation and safety, especially in the animal house.

Microscopy

The study of the mechanism of action and of the safety of any plant product requires an examination of the changes that may be brought about in animals by the drug. A most important method is the examination of material microscopically, for this the material must be properly prepared - the whole process is called histology and when applied to pathological material(which may be from an animal that has died due to a plant extract) histopathology.

Standard protocols are available in many textbooks but the expert was able to add a number of practical aids and also to identify the lack of certain equipment. Material for microscopy has to be rendered stable

(fixed) and them has to be cut into very thin slices, to do this it has to be dehydrated and embedded in parafin wax. The instrument which actually cuts the slices or sections is called a microtome. A microtome is a robust precision instrument which works with a very sharp heavy razor or knife. Microtome knifes are jellously guarded and cared for by histology technicians as they are the centre of the whole technique. The process of keeping the knife sharp is a process of polishing the edge, very much in the way that a barber strops his razor. It was necessary to order a new strop and a new knife. Later it was found that both of the microtomes that were available had mechanical faults, a new instrument was ordered and has been installed in the laboratory and is functioning. When the sections are cut they are wrinkled and must be flattened by floating on a water bath, a thermostaticalley regulated waterbath with the very valuable refinement of a black lining has been ordered to facilitate the floating out.

With the augmentation of the specimen preparation methodology it was necessary to purchase a new microscope. An exactly similar microscope was requested by the microbiology expert. Both instruments can be used to take photographs and one photographic attachment to serve both instruments has been purchased. At the time of writing the microscope had not been passed to the counterpart's laboratory because of space limitations,this will be releived when the building work in the animal house is completed. This has meant that the expert has not yet been able to demonstrate the use of photomicrographic equipment. The expert hopes to demonstrate the use of this equipment before leaving.

Polygraph

The Grass Polygraph is a six channel instrument for measuring the type of biological activity which can be converted into a mechanical or electrical signals, in the Grass Polygraph mechanical signals are converted into electrical signals so that they may be recorded on a moving chart. This instrument is found in many hospitals and physiology laboratories where it records variables such as heart beat and brain waves. With the necessary transducers it can be used to measure the activity

of isolated organs. Although this is an instrument of formidable appearance it can be operated after a little practice by staff who are unaware of the electricalcircuitry needed to produce the recordings. The expert has worked together with the counterpart on the instrument.

The Polygraph is a sophisticated recording device but the recordings can only be as good as the "preparation" from which they are made. Several members of the laboratory have developed the necessary skill in setting up and using such preparations under the guidance of Mr. S.K. Joshi. The second level of skill is designing the experiments and in interpreting the results. These skills have been developed in the laboratory with the use of more elementary recording equipment before the Polygraph arrived.

Microcomputer

After some initial misgivings the concept of computerisation has been embraced by members of the RDRL.

To encourage the understanding of the fundamentals of drug action and of statistical methods as well as pharmacological calculations, a microcomputer with pharmacological calculation and filing software was requested by the pharmacology expert. The instrument arrived in February. A small number of RDRL staff were sent on a training course to learn 'BASIC' programming and these have taught other members of the laboratory the use of computer.

The experts (pharmacologist and analytical chemist) demonstrated the value of the available programs. The pharmacology section were not able to exploit the calculation capability due to the shortage of data due in term to the lack of animals. The chemical section of the laboratory was quick to see the value of the filing program and soon computer time became in great demand. The demand has become so great that, after discussion with the senior members of the RDRL a second computer has been ordered, this second machine - which arrived in Sept. will be used primarily by the chemical section, and has, through a hard disk attachment and some additional hardware, the capability to handle a greater volume of data more rapidly.

Toxicology

The concept of the LD_{50} has been discussed as has the number of animals that should be used in such tests. Earlier the numbers of animals available meant that the number used in the test was less than optimum but with the increase in the size of the stock larger numbers can be used.

The Hippocratic test was introduced to the laboratory by Prof. Sandburg. This procedure requires a considerable familarity with the normal behaviour of the animals, the test has been used successfully in the laboratory.

Practical training has been given in histology but there has been insufficient time since the introduction of the new microtome to engage in any histopathology.

It is hoped that there will be time to offer some experience with the enzymatic assay of blood constituents with the Ames blood analyser which has just arrived.

Screening methods

A protocol was produced for the antidiareheal test, cages were modified and the assay is in the development stage.

The anthelmintic assay has not begun (see bioassay) but experience with faecal analysis has been gained.

The antifertily assay has a long lag phase and breeding has been unsatisfactory due to the construction work in the animal house. However the idea of pair breeding, and the assessment of vaginal smears have been carried out. The background to the assay and the basis of reproductive toxicology and teratology have been discussed.

E. Equipment

A review of the condition existing in NDRL led to a discussion with the CTA on providing equipment not contained on the original equipment list, items such as air conditioning units, a generator and a microcomputer were included in these discussions. In March 1983 a a halt in spending was called by the CTA and the equipment list was then reexamined by the experts and certain items replaced. Lists of the equipment are contained in the CTA's report.

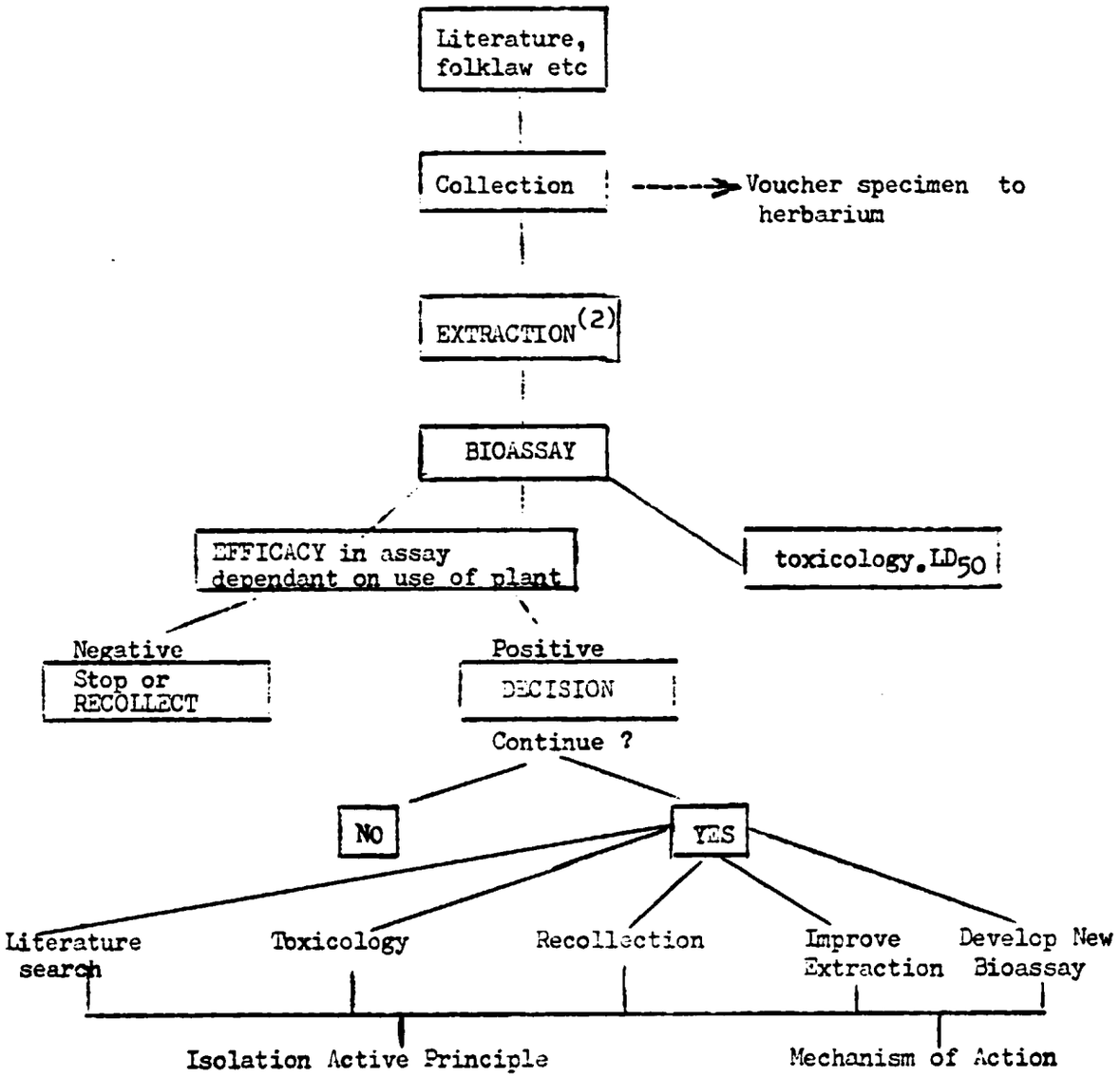
F. Work Plan

Dr. Malla (Director General, Dept. of Medicinal Plants) requested that the experts leave a work plan for the laboratory to follow after their departure. A generalised flow chart for the development of biologically active material from biological sources is presented. No attempt is made to indicate specific plants, the RDRL have this well in hand.

Scheme for examination of plant material for biological activity

One of the ways in which the RDRL is seeking to attain the objective of self-reliance in essential drugs is by the examination of Nepal's flora in a search for biologically active agents. There are two approaches to such a study, one involves the collection and testing of plants collected at random, while the other approach is to follow the indigenous ethological record i.e. to examine plants that have been reported to be used by the people. The RDRL is persueing the second path. Whatever plants are selected the next steps are the same, (as indeed they are for synthetic drugs). Two questions must be asked, are the plant extracts active, and is that activity toxic to humans ? It is ethically unacceptable to test extracts on human beings until there is a large volume of knowledge and then only if the benefits of the extract far outweight the dangers. Testing of plant material (or any other material) is therefore carried out in animals.

Scheme for the examination of plant materials for biological activity[1]



(1) See also 'Traditional Pharmacopoeias Revisited. UNIDO/10.511 25 Aug. 1982

(2) May not be needed, reports may show that the plant is eaten.

The identification of biologically active material from plant sources requires a range of skills and expertise, it is therefore a team function. The team should include a project leader, a botanist, a chemist and a biologist (these could be section leaders).

In consultation with other members of the group the project leader should select the plants to be tested in any particular year. Inputs into this selection should include availability, collectability, botanical description, distribution. When a number of plants have been selected their collection should be planned. Some plant materials can be collected at any time (eg bark) others (flower or fruits) may only be available at a specific time, some may keep (eg. seeds) others (especially flowers) may perish, some materials must be used fresh while other may be kept as dried material (leaves) all this information must be collated into a rational collecting programme which takes into account the availability of animals and space in the animal house as well as the staff limitation in the pharmacology section. The program must have some elasticity to accmodate public holidays etc. and unforseen problem (for instance rats tend to show an animal breeding cycle even when maintained in a stable isolated environment, this data is not available for the Kathmandu colony).

IF DECISION is YES then a wide range of activities must be initiated.

i. Recollection of plant material from same site and bioassay.

If bioassay is negative - repeat collection and assay again, reexamine documents and voucher specimens, if still no activity recollect again at same time of year as initial collection if still no activity, abandon.

If bioassay is positive - make collection of 50-100 kg. Make further collections at different times of year and from different localities. Examine distribution and abundance.

ii. Improve extraction

The first extraction should be carried out using a method similar to the usage style of humans. Once activity has been found then

other methods of extraction should be examined to endeavour to increase the yield of biologically active material. Yield must be measured, of course in the Bioassay. Activity from different plant parts should be determined.

iii. Toxicology

Lethal Dose 50 should be redetermined using larger number of animals and the Therapeutic Index determined. The determination of the TI should be performed at regualr intervals as the isolation of the active principle is carried out. It may not be possible to obtain an LD_{50} with crude material and in this case an especial watch must be made for the appearance of toxicity as purification and concentration continue.

Acute and long term toxicity should be carried out on an early extract and repeatedly as the extraction continues - this will be the major time delay in the whole project and other parts of the extraction program must not be allowed to progress too far ahead of the toxicology. It must always be remembered that if the toxicity increases and TI decreases the project should be abandoned.

iv. New Bioassay

Most in vivo primary screens are immensly demanding in time, animals and labour and since the chemical extraction is dependant on the bioassay it is necessary to develop a new test which will be fast and only use a small number of animals. It is impossible to predict what tests might be of use but the first thing to find out is if the plant extract works directly on an easily available tissue, this may be on isolate organ preparations or a simple in vitro survival of parasites. Once such an assay has been discovered then it will greatly accelerate the chemical extraction but the programme must not depend on the new bioassay nor for that matter on any chemical test that may be devised the extracts must be continually assayed on the whole animal test system.

v. Isolation of active principle

This is the final objective of the improved method of extraction and the new bioassay. Once the active species is identified a simple

assay should be devised for future work. This future work might involve chemical synthesis of analogues and the examination of a wide range of plants for the material.

vi. Mechanism of action

All of the other sections provide information for this part of the investigation. During the examination of the mechanism of action other actions (side effects) of the extract may be discovered. The toxicological data should then be examined and if necessary tests deviced to determine if the side effect is potentially hazardous. This determination of the mechanism of action will need an examination of the biochemistry, pharmaco dynamics and pharmacokinetics of the extract.

vii. Literature

Once the decision to investigate a plant is made it is desirable that a detailed examination of the world literatures on the plant is carried out. Such a search may save several years work by indicating the active component or it may save the waste of several years work by indicating reasons for the drug being rejected.

The DECISION to proceed will divert a large amount of resources from the general screening program into the examination of a single plant. If it is assumed tha Xn plants are to be examined during one year and that plant x is found to be active and that it is necessary to divert y % of the resources of the biology section to the examination of this plant, then the effect on the general screening program will be to reduce the number of plants examined by $(X_n - x)\ (\frac{Y}{100})$

for example:

if Xn = 30 , x = 4 Y = 50

substitute

$(30 - 4)\ (\frac{50}{100})$ = (26)(½) = 13.

To obtain the total number of plants

$$x + (Xn - x)\left(\frac{Y}{100}\right)$$

eg. $4 + \quad = 17$

In this example the fourth plant in the year program was active, 50 % of the resources were diverted to examine that plant and then only a total of 17 plant from the projected 30 could be tested.

This scheme is by no means exhaustive but it should show that the whole process of drug development and safety assessment is dependent on a strong active biology section backed up by a good animal facility.

APPENDIX - 1

Interoffice Memorandum

To: Dr. S.B. Malla
Director General
Department of Medicinal Plants, HMG.

Date: 2[illegible] July 1983
Ref.: NEP/80/003(12)E

Through: John G. Meredith
Project Co-ordinator
and CTA-NEP/80/003.

From: J.P.G. Williams
UNIDO Expert/Pharmacologist.

Subject: Toxicology.

In my final report I will make some recommendations concerning the organisation of the biology section. This section should be organised with the objectives of the RDRL clearly in mind. It is my belief that one of the objectives of the RDRL is to produce biologically active materials that will be used by humans, entering into or onto the body. This places an enormous moral and legal responsibility on the research staff, especially the person who is responsible for declaring a drug safe. Such a person should have adequate training not only in the technology and theory of toxicology but in their legal and moral responsibilities. Such a person should be of senior rank within the organisation of the RDRL because it will be on his or her professional decision that the development of a drug will depend. (In a pharmaceutical company this decision could trigger an expenditure of 5-10 million dollars). It is imperative that such a person have the integrity and status to stand against the wishes of the rest of the management team.

My immediate recommendation is that a suitably qualified person be sent abroad for training. It should be clear from the above that this person should receive adequate training. There are a number of Universities that offer M.Sc. courses in toxicology and I would recommend this as the most suitable course of action. Later a second person should receive similar training.

The person sent should have a good grounding in biology and chemistry with a good knowledge of biochemistry and elementary statistics. They should also be prepared to work with animals and to kill animals.

I believe that such a course is run at the University of Surrey, Guilford, Surrey, U.K.

Note: On further reflection especially on the subject status it is desirable that the person sent should persue a Ph.D. programme (if possible industrially related) after completion of the M.Sc. course.

APPENDIX 2

Suggested Fellowship Programme of the Royal Drugs Research Laboratory

It is our opinion that the fellowship programme should be related to the aims of the Royal Drugs Research Laboratory and to enhance its research capability. We recommend that staff be sent for MSc. or PhD. training and that this training be directly related to the projects of the Royal Drugs Research Laboratory as conceived in its long term planning. We see little benefit for RDRL (or Nepal) when a MSc. or a PhD. candidate on return to Kathmandu is faced with a situation where her/his experience and training cannot be utilized due to lack of facilities and relevant projects. Before the candidates leave there should be a clear understanding by the candidate, the supervisor at RDRL and the host university on the relationship of the training to the research activity of RDRL. It does not strengthen RDRL just to send people away for training without further specification. We believe that the most appropriate type of training for members of RDRL would be that which combines industrial experience with academic education (e.g. CASE awards and sandwich courses in UK).

Assuming that RDRL wishes to do research in the fields of medicinal plants/drug production we would recommend the following areas of training:

1. Chemist/Pharmacist - MSc.-PhD

   Instrumental analysis related to the stability testing of drugs with emphasis on chromatographic methods.

2. Pharmacist - MSc.-PhD

   Formulation of drugs and pharmacokinetics. The training must be linked to a pharmaceutical company.

3. Pharmacist - MSc.

   Formulation of drugs and pharmacokinetics. The training must be linked to a pharmaceutical company.

4. Chemist/Pharmacist/Biologist - MSc.-PhD

   Toxicology (general)

5. Chemist/Pharmacist/Biologist - MSc.

   Toxicology (specializing in techniques).

6. Pharmacology - MSc.-PhD

7. Research and Development Management in Pharmaceutical Industry - MIT-Courses, Amsterdam.

8. Microbiologist - MSc.-PhD

   Quality assessment of pharmaceutical products. The training should be linked to a Governmental Control Institute and a pharmaceutical company, preferably in the US..

9. Basic computer training course for at least one pilot plant operator.

10. An on-the-job training for approx. 6 months in an Indian firm working in the phytochemical sector for the technicians at the pilot plant (at present there are three).

11. An on-the-job training at WILSONS (possibly 3 months) for the boiler mechanic.

APPENDIX 3

Visit to Central Drug Research Institute, Lucknow
20/3/1983 - 25/3/1983

- by Dr. J.P. Williams
Pharmacologist

Purpose

To examine animal house with view to recommending improvements - especially in environmental control for RDRL.

To discuss various screening methods, in particular hypoglycaemic anthelmintic, antiamoebic and general toxicology.

To examine and discuss physiological recording equipment.

To determine the availability of pathogens for screening and to discuss possible training of RDRL personnel.

Accompanied by Mr. S.K.G. Joshi and Mr. B.R. Tuladhar.

Mr. Joshi accompanied me on the visit to the animal house and to meet with the groups examining antiamoebic activity and those carrying out the hypoglycaemic test. After visiting the Parasitology department Mr. Joshi remained there to examine the methods of helminth culture in greater detail. Mr. Tuladhar divided his time between the fermentation section and the amoeba group obtaining a good insight into both activities.

On arrival we were met by Dr. Bhattacharji and taken to Dr. Dhawan's office where we discussed the planned itinerary for the week. A little later we met with the director Dr. Nitya Nand who expressed his good wishes to Dr. Malla and RDRL which we reciprocated and extended invitations to Dr. Nand and Dr. Dhawan to visit RDRL. Dr. Nand also indicated his wish for closer collaboration with Nepal and his willingness to help RDRL in any way.

We were then introduced to Dr. K.R. Bhardwaj who is in charge of the animal house. Dr. Bhardwaj conducted us around the animal house which is composed of three major buildings one of these was in the last stages of completion and is expected to open in a few weeks time as a Primate centre. There are

currently some 400 primates and this number will be increased to 1000 when the facility is fully operational. Of the other buildings one housed only infected animals and we were not shown this building. The third building with an adjacent kennel area, a froggery and a support area for sterilizing, cage cleaning, cage painting and incinerator, housed the remainder of the colony.

The animal colony is of impressive magnitude in 1981 a total of 72,000 animals were used, at the time of our visit the colony held 51,800 animals including 23,000 mice and 14,500 rats. The numbers which are displayed on a panel in the entrance hall of the primate house are adjusted weekly indicating not only the magnitude of the operation but also excellent record keeping.

Some of the points arising from our extended discussion with Dr. Bhardwaj are listed below:

1. The facility is centrally air conditioned, heated in winter and cooled in summer (It was stressed by several people that only two areas in CDRI are air conditioned - the sophisticated instrumentation area and the animal house.)

2. All bedding is autoclaved before use (but not after use when it is distributed for use as fertilizer!)

3. All dead animals are incinerated, after postmortem when necessary.

4. For cage sterilizing and cleaning we were shown a "Jet Steam" - a steam generating machine such as is used to clean cars. Dr. Bhardwaj said that this equipment was so useful he was ordering 2 more units at I.Rs.34,000 each.

5. For cage maintenance three people do nothing but repaint cages.

6. The name of the supplier of polypropylene cages was provided.

7. Cages were labelled with cards attached to hooks. Dr. Bhardwaj said that in their climate small insects might inhabit the crevices of the more conventional label holder.

8. In the mouse, rat and hamster colony, record keeping was by cage number since it was not practical to earmark 10s of thousands of mice etc. However in the inbred colonies of special animals individual records were maintained. The index card for each cage indicated the fate of the offspring and the origin of the breeding animals occupying the cage

9. During discussion on staff training Dr. Bhardwaj recommend a training course at Hyderabad. The course is at two levels one for technical and other for graduate staff. He said that the Hyderabad course was a little weak on practical training and an ideal combination would be to take the Hyderabad course and then a period of practical training at CDRI. (4)

10. Dr. Bhardwaj drew our attention to the seniority of the person in charge of the animal house and pointed out that this seniority was necessary in such an important section of the Institute. Dr. Bhardwaj is a grade E. Scientist.

11. During discussion of the budget arrangements Dr. Bhardwaj showed us a monthly return (Appendix 4) which displayed the number, type and cost of the animals used by each project, thus allowing good cost accounting in both the animal house and the projects.

12. With reference to staff safety Dr. Bhardwaj said that all staff received tetanus shots and regular physical check-ups.

APPENDIX 4

Recommendations for Primary Record Keeping in the Examination of Plant Materials for Biological Activity

It is assumed that a research group consisting a project director, a field botanist, a chemist, a pharmacologist and a microbiologist has been established. It is further assumed that a series of objectives (specific plants to be examined) has been established and that a programme of plant collection relating priority to availability has also been established. It is desirable that a numerical target is set for the group, this target will be dependent on the facilities available eg. plants, number of extractors, availability of enough animals and sufficient staff. It is also highly desirable that the research group should meet frequently and formally (i.e. that all members should be present at a specified place and time).

It has been clearly stated by Dr. Malla that the object of this project is to determine the activity of medicinal plants and not to isolate active compounds. It is with this objective in mind that the following documentation has been designed.

It is anticipated that the project director will keep a file for each plant species which will include an indication of the reasons for the choice of the particular species, the types of activities which have been reported and a few key references. These data will be of use when evaluating the plant and planning any future action.

P1

Plant Collection Form to be filled in by Field Collector as far as possible IN THE FIELD

1. Plant name: _____ Nepali name: _____

2. Plant part: _____ 3. Species no. _____

4. Collection No. _____ 5. Date collected: _____

6. Voucher specimen No.: F/SSS/CC/DD,DD,DD/V/P

7. Site of collection:

8. Condition of plant when collected:

9. Condition of field storage:

10. Amount of material available at collection site:

11. Comments:

12. Field note book ref:

13. Plant collected by: _____ Signature _____

14. Plant delivered to: _____ Signature _____

15. Date of delivery: _____

Copies to:

Project Director
Chemist
Pharmacologist
Microbiologist

Chemical Extraction Form

1. Plant name: _____ Nepali name: _____

2. Plant part: _____ 3. Species No. _____

4. Collection No.: _____ 5. Date collected: _____

6. Specimen No.: C/SSS/CC/DD,DD,DD/EE

7. Date received: _____

8. Condition of material on date of extraction: _____

9. Drying method: _____ 10. Weight Dried Plant _____

11. Date extraction commenced: _____ 12. Date extraction completed _____

13. Solvent: _____ 14. Time: _____ 15. Temperature _____

16. Deionized Y or N 17. Concentration procedure: _____

18. Residual weight: _____ 19. Dry Weight Equivalent: _____

20. Aqueous solubility: _____ 21. Appearence: _____

22. Amount sent for bioassay: _____ 23. Date sent for bioassay _____

24. Amount sent for microbiological testing: _____

25. Date sent for microbiological testing: _____

26. Comments:

27. Note book no. page no.: _____

28. Extraction carried out by: _____ 29. Signature: _____

30. Extract delivered to: _____ 31. Signature: _____

32. Date: _____

Copies to:

Project Director
Botanist
Pharmacologist
Microbiologist

P3

Bioassay 1.

Pharmacological testing

1. Plant name: _____ Nepali Name: _____

2. Plant part: _____ 3. Species No.: _____

4. Collection No. _____ 5. Date collected: _____

6. Extract No.: B/SSS/CC/DD,DD,DD/EE/AA

7. Date received: _____ 9. Dry Weight Equivalent: _____

9. Solubility/Vehicle _____

10. Reported activity: _____

11. Appearence: _____ 12. LD_{50} _____

13. Assay Type _____ 14. Date commenced _____

15. Active Y,N,E _____ 16. ED_{50} _____

17. Comments (record or refer to any other bioassay and toxicity data)

18. Note book no. page no. _____

19. Carried out by: _____ Signature _____

20. Date _____

21. Comment and recommendation by Senior Pharmacologist.

Copies to:

Project Director
Botanist
Chemist
Microbiologist

Bioassay 2.

Microbiological testing

1. Plant name: _____ Nepali name: _____

2. Plant part: _____ 3. Species No. _____

4. Collection no. _____ 5. Date collected _____

6. Extract No.: M/SSS/CC/DD,DD,DD/EE/TT

7. Date received: _____ 8. Dry Weight Equivalent _____

9. Solubility/Vehicle _____

10. Reported activity: _____

11. Appearance _____

12. Test type _____ 13. Date commenced _____

14. Active: Y, N, E _____

15. Comments:

16. Note book no. page no. _____

17. Carried out by _____ Date _____ Signature _____

18. Comments and recommendation by Senior Microbiologist:

Copies to:

Project Director
Botanist
Chemist
Pharmacologist

Key to Form

Form P1. Field Collection

1. Plant name, both binomial and local.

2. Plant part, see appended list.

3. Species no., this should be allocated from the master list of plants SSS

4. Collection no. this is allocated by the botanist in the field and refers to his collection, this should also be recorded in the field note book.

5. Date collected. The date the specimen was collected not the dates of the collecting trip.

6. Voucher number.

F=Field collection, SSS from 3, CC from 4, DD,DD,DD date from 5.
V=Voucher specimen, P is the place at which the voucher specimen is lodged eg. T, Thapathali, G, Godavari (Not yet fixed).

7. Site of collection.

This should be described in sufficient detail to allow a recollection of a sample of the same population from the same site - was it in heavy shade, on N or S facing slopes etc.

8. Condition of plant, (was the plant in bud, in full flower etc. was the plant infested with fungi or ants etc., had the plant been damaged by browsing animals.)

9. Condition of field storage (burlap bags, plastic, loose, bottom or top of large pile of material.)

10. Amount available at collection site, was the plant abundant or sparse.

11. Comments - can be used to expand 7-10

12. Field Note Book reference, page no.

13.} Name, printed and signature of collector.
14.}

15.} Name, printed and signature of person taking over the plant
16.} from the field collector.

17. Date plant material passed to next person (presumably the chemist who is responsible for extraction).

Form P2. Chemical Extraction Form

1-5 as P1.

6. Specimen No. C indicates chemistry, SSS/CC/DD,DD,DD as P1. EE extraction No. allocated by chemist.

7. Date received by chemist.

8. Condition of material on date of extraction.

9. Drying method

10. Weight of dried plant - if only part of the sample is used for extraction then this should be indicated here and that figure used in the estimation of the Dry Weight Equivalent (19).

11-12 Dates extraction commenced and completed.

13-15 Conditions of extraction

16. Has the extract been deionised? Yes or No

17. Concentration procedure.

18. Residual Weight

19. DWE, this is the yield of extract from one kilogram of dry plant 18/10 g/kg
eg. if 500 grams of dried plant gives rise to 150 grams then
DWE = 300 g/kg.
if 1.4 kg of dried plant gives rise to 15 grams then
DWE = 10.7 g/kg.

20. Aqueous solubility: Yes or No.

21. Appearence

22. Amount sent for bioassay

23. Date sent

24. Amount sent for microbiological testing

25. Date sent

26. Comments

27. Note book no. and page no.

28-29 Name and signature of chemist

30-31 Name and signature of pharmacologist and microbiologist

32. Date material passed to pharmacologist and microbiologist

Form P3.

1-5 as P1.

6. Extract No. B=Bioassay, SSS,CC,D,D,D,D,DD as P1,2.
EE extract number as P2, AA bioassay number.

7. Date received from chemist

8. Dry weight equivalent from chemist's form.

9. Solubility/Vehicle (if not sol. in water vehicle should be recorded here).

10. Activity reported in literature. e.g. Anthelminthic.

11. Appearence of extract at <u>time of bioassay</u>

12. LD_{50}

13. Assay type performed. eg. Antidysentery

14. Date assay commenced

15. Active, Yes, No. Equivocal.

16. If active, ED_{50}

17. Comments - refer here to any other bioassay and toxicity data.

18. Note book ref. no. page no.

19-20 Name, Signature, of person who carried out assay and date.

21. Comment and recommendation by senior pharmacologist.

Form P4.

1-5 as P1.

6. Extract No. M=Microbiological test SSS/CC/DD,DD,DD, as P1,P2,P3.
EE extract number as P2. TT Microbiological test number.

7. Date received from chemist.

8. Dry weight equivalent from chemist's form.

9. Solubility/vehicle (if not sol. in water vehicle should be recorded here).

10. Activity reported in literature

11. Appearence of extract at time of microbiological testing.

12. Test type performed e.g. antibacterial- <u>E. coli</u>

13. Date test commenced

14. Active, Yes, No, Equivocal.

15. Comments

16. Note book ref. no. page no.

17. Name signature of person who carried out test and date.

18. Comment and recommendation by senior microbiologist.

Bioassay of Plant Material

The bioassay results may be negative, positive or equivocal.

If the results are equivocal then a higher dose range should be used if possible - or a different route of administration or a different vehicle.

If the results are negative then no further action need be taken, however there may be reasons to believe that the negative test is not a good representation of the plants' activity and a higher dose range may be tried.

If the results are positive then no further action need be taken, it would however be valuable to know if the activity was always the same.

Thus given any result it could be argued that a second collection should be made at a different time of the year and the activity of that extract compared with the first.

Note: Certain modifications have been suggested,

1. Specimen number ...SS not be included.
2. V and P not be included.
3. That the ethnic group using the "Nepali" name be included.
4. That when possible photographs of the material be made and a question. Photograph: Y, N be added to the Field Collection form.

APPENDIX - 5

Caesalpinia decapetala

The protestations of the experts in relation to record keeping, correct naming of plants and the collection of voucher specimens have not been universally accepted as significant.

The species Caesalpinia sepiaria is mentioned in 'Medicinal plants of Nepal' and Caesalpinia bendue is mentioned in the plant list in appendix '12'. The description of the distribution given in the Flora of British India suggests the species occuring in the Himalaya was C. bonducella. When reviewing the description of these species the expert came across a passage which underlines the difficulties of plant identification and the need for voucher specimens with every collection.

APPENDIX 6

Primary Screen for Anti-Diarrhea Agents

Agents will be tested at a single high dose.

Dose level will depend on available information, eg. LD_{50}, human usage level or will be the maximum dose that can be administered in two millilitres.

Vehicle, wherever possible this will be water, then ½% agar, then acacia.

Route, Oral.

Animals, 36 Rats 220 ± 20 grams, six per day for six days.

Special items. Cages with grids.

Procedures

The evening before the test six rats should be placed in individual cages in the screening laboratory. Food should be withheld but water must be freely available. (The room temperature should not fall below 18 nor rise above 24°c.)

The following morning the rats should be weighed and treated. Animal Nos. 1-5, five different extracts, No. 6, vehicle only. The dose level should be as high as possible* and administered in 2 ml of vehicle. (If sufficient material is available the dose should be determined earlier to save time on the morning of dosing.)

One hour later all rats should receive 1 ml of commercial castor oil by gavage. This is time zero.

The animals are then observed hourly and the appearance of diarrhea noted.

Failure to find diarrhea is a positive response.

A positive response at the end of one hour only means the dose should be increased.

A positive result at the end of two hours indicates an active extract.

Note: *If this dose proves fatal reduce by 50%.

APPENDIX 7

Plants reported to be used as antifertility agents in Nepal

ALSTONIA SCHOLARIS
AMARANTHUS SPINOSUS
ARECA CATECHU
ARTEMISIA VULGARIS
BUDDLEJA ASIATICA
CAESALPINIA BONDUC
CARDIOSPERMUM HALICACABUM
CYPERUS ROTUNDUS
EMBELIA RIBES
JATROPHA CURCAS
JUNIPERUS COMMUNIS
LEPIDIUM SATIVUM
MENTHA ARVENSIS
MORINGA PTERYGOSPERM
MARDOSTACHYS JATAMANSI
NIGELLA SATIVA
PIPER LONGUM
RAUWOLFIA SERPENTINA
RICINUS COMMUNIS
RUBUS MOLUCCANUS
SARACA INDICA
URTICA DIOICA

As Revised: JULY 1982

APPENDIX 8

WHO's antifertility protocol

A METHOD FOR EXAMINING THE EFFECT OF PLANT EXTRACTS ADMINISTERED ORALLY/SUBCUTANEOUSLY DURING THE FIRST 10 DAYS OF PREGNANCY IN THE RAT

1. Rationale

The antifertility activity of plant extracts is examined in female rats after mating. This assay is a non-specific preliminary test aimed at identifying extracts, compounds, etc. that exert their activity during the first 10 days of pregnancy in the rat, i.e. agents that interfere with the process of implantation or disrupt early pregnancy. MB-30 refers to oral administration and MB-31 to subcutaneous administration of plant extracts (or other test material/compounds). MB-30 is the test of first priority (see flow sheet attached).

2. Procedure

2.1 Synopsis

Animals will be mated and then dosed orally (MB-30) with the plant extract (or test material/compound) for ten days. On Day 16 of gestation, the animals will be autopsied. The number of pregnant animals, the number of implantation sites, the number of normal fetuses and the number of corpora lutea of pregnancy will be reported.

2.2 Animals

Virgin female Sprague-Dawley rats will be used. The animals should be eight weeks of age and 180-200 grams in weight[a]. The animals may be housed up to 4 per cage according to cage size. It is important that the number of animals per cage should not vary, i.e. the population density in each cage/group should be the same. Furthermore, animals on different treatment groups should not be caged together and litter mates should be distributed throughout all of the groups in an experiment. All animals should be serially numbered using the ear punch[b] or another suitable technique.

NB: Footnotes are to be found at end of protocol.

2.3 Pairing

A proven male[c] should be used once per experiment and placed overnight with one or a maximum of 2 females between 1600 and 1800 hours. Beginning the following morning, vaginal smears[d] should be observed and recorded daily until positive mating occurs. When spermatozoa are found in the smear, mating has occurred. After mating, the female enters the experiment. The male may be kept with the female(s) or removed in the morning and reintroduced in the evening until mating of the female(s) is completed. Females that do not mate during two estrous cycles are not used. If animals were obtained from outside sources, a seven day acclimatisation period, followed by daily vaginal smears over two estrous cycles, should be completed before pairing with a male.

2.4 Grouping

Animals are sequentially (randomly) assigned to control and experimental (treated) groups immediately after positive mating. The first female with a positive sperm smear is assigned to the control group, the second to the first treatment group, the third to the second treatment group ... the fifth to the control group again, etc. Each group should contain ten females[e]. If there is more than one plant extract to be tested then the number of experimental groups may be increased to three[f], provided that all groups use the same vehicle. If different vehicles are used, different control groups must be used for each vehicle.

2.5(i) MB-30 Dosing: Day 1 - Day 10

The day on which a positive mating smear is found is Day 1 of gestation. The animals should receive extract, test compound, or vehicle[g] for ten consecutive days beginning on Day 1 of gestation. The material should be administered orally (intragastrically) by flexible stomach tube or metal feeding needle immediately following the completion of the weighing[h]. The animals should be dosed on a constant g/kg body weight basis according to their daily weights. Extracts should be administered

at the highest possible dose; the limiting factor usually is the viscosity of the extract solution[g] in a volume not exceeding 1.0 ml. The dose should be reported in grams of extract per kilogram of body weight (not in terms of dry weight equivalent). The dosage should be made up daily, or every other day, the remainder being kept in the refrigerator at 3-5°C[i].

2.5(ii) MB-31 Dosing: Day 1 - Day 10

The day on which a positive mating smear is found is Day 1 of gestation. The animals should receive extract, test compound, or vehicle[g] for ten consecutive days beginning on Day 1 of gestation. The material should be administered subcutaneously in a different site each day (beginning at the head region of the back and moving toward the tail) immediately following completion of the weighing[h]. The animals should be dosed on a constant g/kg body weight basis according to their daily weights. Preferably an abbreviated assay for determining the maximum non-lethal dose of the extract should be used (e.g. MB-60) or the extracts should be administered at about 25% of the highest possible dose, the limiting factor usually is the viscosity of the extract solution in a volume not exceeding 0.5 ml. The dose should be reported in grams of extract per kilogram of body weight (not in terms of dry weight equivalent). The dosage should be made up daily, or every other day, the remainder being kept in the refrigerator at 3-5°C[i].

2.6 Autopsy Day 16

On Day 16 of gestation, the animals should be weighed, sacrificed and autopsied. The uteri and ovaries should be removed, the number of corpora lutea of pregnancy on the ovaries should be counted, the uteri should be opened longitudinally and the number of pregnant animals and the number of normal (and abnormal - dead and/or degenerate fetuses) recorded. The placenta, fetus and membranes should be removed and the number of implantation sites recorded. At autopsy, other organs such as liver, kidneys, lungs, adrenals and thyroid should be grossly examined and described.

3. Report

The report and procedures outlined here assume a ≥ 90% pregnancy rate in the control animals[(j)]. Levels A-D indicate the importance of results in order of priority. Data should be included for both control and treated groups in each case; however, only pregnant animals are used in reporting results and calculating standard deviations under Levels B, C and D. As indicated in the flow sheet, positive MB-30 → MB-40 (hamster confirmatory assay)is the line of first priority. A copy of the RECORD SHEET for reporting results (section IV) is attached. Under "Comments" in section IV of the RECORD SHEET include, for example, mention of body weights, weight gain/loss and toxicity (results of gross examination of organs).

Result	Criteria for Decision Making	
Level A[(j)]		
Number of animals pregnant / Number of animals dosed	70% pregnant	= equivocal and retest at higher dose
	60% pregnant	= positive and proceed to MB-40 or MB-41 as appropriate
	> 70% pregnant	= negative
Level B		
Number of implantation sites per pregnant animal ± SD	40% reduction	= equivocal and retest at higher dose
	60% reduction	= positive and proceed to MB-40/41
	< 40% reduction	= negative
Level C		
Number of normal fetuses per pregnant animal ± SD	40% normal	= equivocal and retest at higher dose
	20% normal	= positive and proceed to MB-40/41
	> 40% normal	= negative

Level D

Number of corpora lutea ± SD	40% reduction	= equivocal and retest at higher dose.
	60% reduction	= positive and proceed to MB-40/41.
	< 40% reduction	= negative

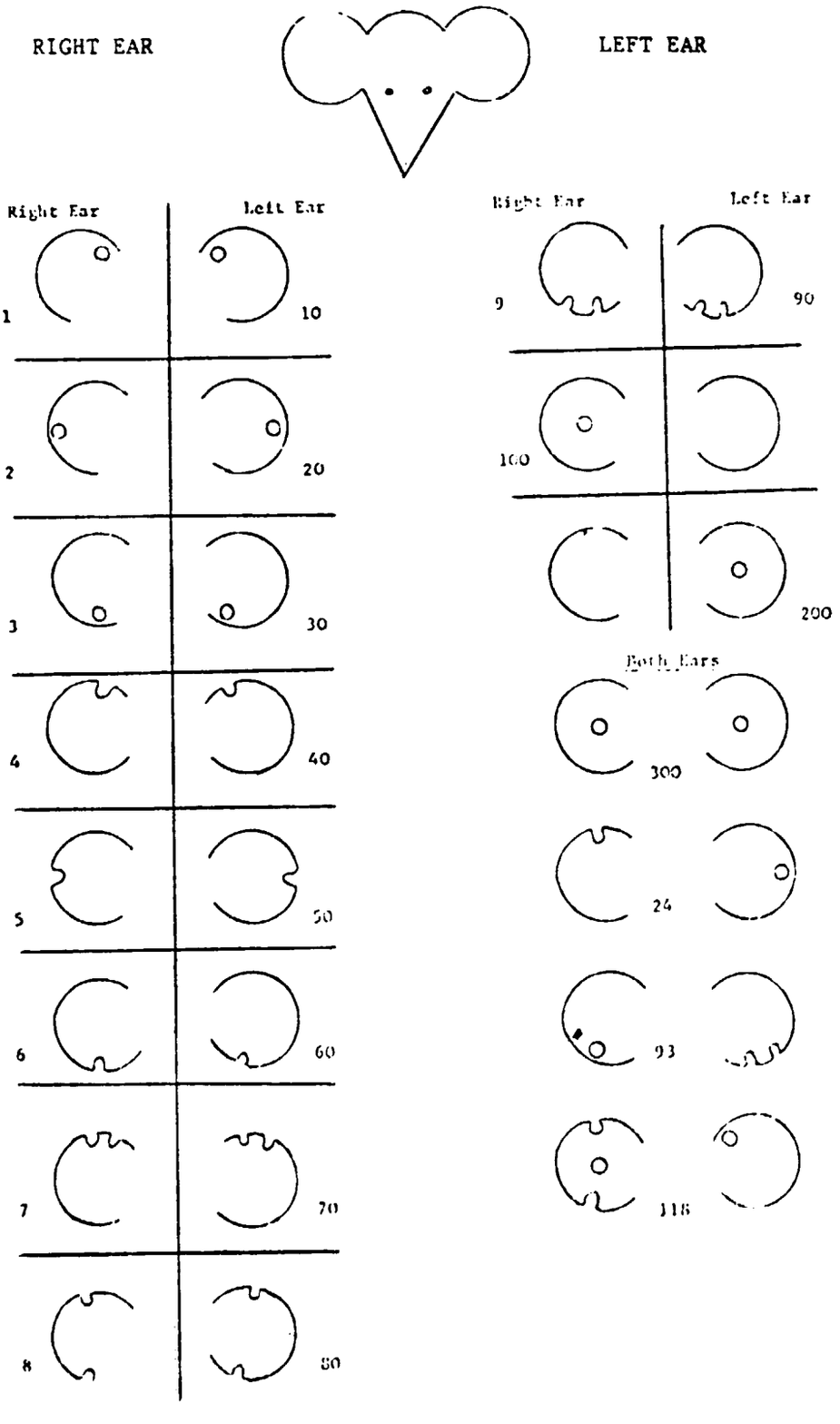
N.B. Only pregnant animals should be included in the numerator and denominator when counting the number of implantation sites, normal fetuses and number of corpora lutea, i.e. levels B, C and D respectively.

Notes

(a) It is recognised that centres breeding animals may be less able to conform to this specification. In this case, the range of ages and weights of the animals should be included in the reported data kept by the centre and mentioned under comments on lines 38-41 on the RECORD SHEET.

(b) EAR PUNCH

ANIMAL IDENTIFICATION SYSTEM



(c) Proven males

When a given female appears to be in proestrus in the morning, place a male in her cage between 1600 and 1800 hours. Remove the male the next morning, regardless of whether or not they have mated; weigh the female daily from this time on. As additional females, including ones that did not mate and/or did not become pregnant, come into proestrus, pair them with males, using the latter in sequence. Males not mating the first time that they are paired with a female will eventually be paired with another female or perhaps with the same one. Once the entire group of females have been successfully impregnated, each by a different male, a colony of proven breeder males will have been established. The proven males should be replaced at regular intervals to be determined by each centre, and appropriate records kept.

(d) Preparation of vaginal smears

The animals should be weighed and vaginal smears taken early in the morning of each day (7 days/week).

A few drops of saline are taken up into an eye-dropper. Holding the rat with one hand, insert the tip of the eye-dropper into the vagina with the other hand, taking care not to touch the cervix. Expel the saline into and withdraw it from the vagina 2-3 times. Expel the contents onto a microscope slide and examine while still wet. The same dropper may be used repeatedly if adequately washed between smears; if mating has occurred, great care must be taken to wash the dropper between smears or, preferably, a fresh dropper should be used for each animal. The cells of the smear are observed using a microscope. There are three cell types in the vaginal smear: leukocytes, round epithelial cells with easily distibguishable nuclei, and cornified cells in which nuclei are difficult to discern or are absent. The proportion of different cell types is recorded daily.

Below are indicated the relative proportions of N (nucleated epithelial cells), (cornified epithelial cells) and L (leukocytes) found in typical morning vaginal smears taken from 4 and 5 day cyclic rats.

Day of cycle	4-day cycle Rat		
	N	C	L
Proestrus*	+++	++	±
Estrus°	±	+++	-
Metestrus	++	±	+++
Diestrus	++	+	+++

Day of cycle	5-day cycle Rat		
	N	C	L
Proestrus*	++	+++	-
Estrus°	±	+++	-
Metestrus	+++	±	+++
Diestrus I	++	+	+++
Diestrus II	+++	++	±

* Mating normally occurs on the night between the days of proestrus and estrus.

° Freshly ovulated ova are found in the oviducts on the morning of estrus.

Smearing prior to mating and through the days of dosing is optional; however, if the pregnancy rate in the control group drops below 90% the rats must be smeared before mating to ensure that pairing occurs around proestrus.

(e) Ten animals are sufficient if the pregnancy rate of the control group is 90% or higher. If the pregnancy rate is lower, the number of animals in each group must be increased; if the pregnancy rate of the control group is 80%, sixteen animals should be used in each group.

(f) Up to a maximum of five experimental groups can be used with one control group if the pregnancy rate of the control group is consistantly greater than or equal to 90%

(g) No suitable vehicle other than water has so far been identified. If the extract is not soluable in water, it should be solubilized in a small amount of polyvinylpyrrolidone (PVP) or suspended with gum acacia (see Appendix 11).

(h) Immediately following weighing and before dosing the investigator may wish to record the vaginal smear as an optional exercise.

(i) The total quantity of the extract may be made up in several aliquots and frozen at -20°C; an aliquot being thawed to room temperature each day for dosing. If this procedure is adopted, aliquots should be flash (snap) frozen using liquid nitrogen or an acetone-dry ice mixture.

(j) In order to maintain statistical significance between the pregnancy rates of the treated groups versus the control group at the $P < 0.05$ level the following criteria should be used:

(i) If the control group has a 100% pregnancy rate, the treatment group is positive (active) with a 60% or less pregnancy rate.

(ii) If the control group has a 90% pregnancy rate, the treatment group is positive (active) with a 50% or less pregnancy rate.

If the pregnancy rate of a control group having only 10 animals is less than 90%, the entire experiment may have to be repeated; especially for extracts showing equivocal or positive results in the experiment.

APPENDIX 9

Toxicity test

1. LD_{50}

It is quite likely that an LD_{50} will not be obtainable with many plant materials. In this case the Maximum Tolerated Dose MTD may be used for calculating doses. The MTD is the largest amount of a material that can conveniently be administered and will depend on the vehicle, 2g in a 200 gram animal is a rough guide.

2. Chronic Toxicology

Animals At least 24 single sex animals of same age in 4 groups of 6 animals (at least)

If it is necessary to use animals of both sexes then 4 of each sex is the absolute minimum. If sex differences appear (after considering weight/age differences) then numbers should be increased to at least 6 of each sex.

Dose 4 Dose levels $\frac{LD_{50}}{5}$, $\frac{LD_{50}}{10}$, $\frac{LD_{50}}{100}$, 0

if no LD_{50} , $\frac{MTD}{5}$, $\frac{MTD}{10}$, $\frac{MTD}{100}$, 0

0 = Vehicle only , MTD = Maximum tolerated dose.

Animals should be dosed daily at the same time of day, by the oral route for six weeks.

If there is insufficient material to dose 24 animals for 42 days then the lower dose levels should be dropped and the group size maintained at the high dose levels.

Blood tests should be conducted on all animals before doseing starts. Blood tests may also be conducted at two week intervals. The following blood tests should be carried out.

Cytology
- White cell count (total
- Differential white cell count
- Platelet count
- Reticulocyte count
- Retoutocyt count
- Packed cell volume.

Chemistry

Albumim
Alkaline Phosphatase
Bilirbin
Cholesterol
Creatinine
Glucose
Glutamate oxaloacetate transaminase SGOT
Haemaglobin
Total protein
Urea

Histopathology

The following tissues should be examined:

Adrenal
Bone marrow
Brain
Heart
Intestine small
" large
Kidney
Liver
Lung
Ovary
Spleen
Stomach
Testes
Thyriod
Uterus.

The actual number of the slide must be included in the histologists report.

Reproduction testing

Dated pregnant females (see antifertility protocol) should receive MTD/5 from day 1 of gestation through till day 20. On day 20 the female

should be killed and the conditions of the contents of the uterus examined. The ovary should also be examined. Any fetuses should be recorded as alive or not, weight and numbers of fetuses should be recorded. The condition of the placentas and the number of resorption sites should be noted, as should the number of corpora lutea.

APPENDIX - 10

A number of formulation of rodent diets are given

Species: Rats and Mice

Developed at Porton by Dr. Paterson and Christopher Hill in the 60's.
Ideally suitable for breeding and stock holding.

On quality and performance, PMD has a proven record with leading Accredited Breeders, Pharmaceuticals, Universities, etc.

Labsure PMD achievements remain as a tribute to Dr. Paterson's original formulation.

Approximate daily feeding rates: Adult Rat 15 – 25 g
Adult Mouse 5 g

PMD is manufactured in a 3/8" (9.6 mm) Pellet size.

Proximate Analysis

Crude Oil	2.7%
Crude Protein	19.8%
Crude Fibre	5.3%
Calcium (as Ca)	0.6%
Phosphorus (as P)	0.7%
Salt (as Na Cl)	0.5%
Metabolisable Energy	2583 kcal/kg
Carbohydrate (as %)	53.82

Amino Acids (as percentage of feed)

Threonine	0.7
Glycine	0.9
Valine	1.0
Cystine	0.2
Methionine	0.3
Isoleucine	0.8
Leucine	1.5
Tyrosine	0.7
Phenylalanine	0.9
Lysine	1.0
Histidine	0.5
Arginine	1.2
Tryptophan	0.2

Trace Elements Added

Manganese	25 ppm
Copper	7 ppm
Cobalt	0.4 ppm
Iron	30 ppm
Iodine	1.3 ppm
Magnesium	102 ppm

Vitamins Added per kg

Vitamin A	8,000 iu
Vitamin D_3	1,000 iu
Vitamin B_2	8 mg
Nicotinic Acid	50 mg
Pantothenic Acid	12 mg
Vitamin B_{12}	12 µg
Vitamin E	60 iu
Vitamin K	10 mg
Folic Acid	10 mg
Choline Chloride	200 mg
Vitamin B_1	4 mg
Vitamin B_6	6 mg

An expanded form of CRM

Proximate Analysis	
Crude Oil	2.9%
Crude Protein	18.3%
Crude Fibre	3.5%
Calcium (as Ca)	0.8%
Phosphorus (as P)	0.6%
Salt	0.7%
Metabolisable Energy	2916 kcal/kg
Carbohydrate (as %)	56.33

Trace Elements Added	
Manganese	25 ppm
Copper	7 ppm
Cobalt	0.4 ppm
Iron	30 ppm
Iodine	1.3 ppm
Magnesium	102 ppm

Amino Acids (as percentage of feed)	
Threonine	0.6
Glycine	0.9
Valine	0.8
Cystine	0.2
Methionine	0.3
Isoleucine	0.7
Leucine	1.4
Tyrosine	0.6
Phenylalanine	0.8
Lysine	1.0
Histidine	0.4
Arginine	1.2
Tryptophan	0.2

Vitamins Added per kg	
Vitamin A	8,000 iu
Vitamin D_3	1,000 iu
Vitamin B_2	8 mg
Nicotinic Acid	50 mg
Pantothenic Acid	12 mg
Vitamin B_{12}	12 μg
Vitamin E	60 iu
Vitamin K	10 mg
Folic Acid	10 mg
Choline Chloride	200 mg
Vitamin B_1	4 mg
Vitamin B_6	6 mg

Species: Rats and Mice

Developed at Porton by Dr. Paterson and Christopher Hill in the 60's. Ideally suitable for breeding and stock holding.

On quality and performance, PRD has a proven record with leading Accredited Breeders, Pharmaceutical's, Universities, etc.

Labsure PRD achievements remain as a tribute to Dr. Paterson's original formulation.

Approximate daily feeding rates: Adult Rat 15 - 20 g
Adult Mouse 5 g

PRD is manufactured in a 3/8" (9.6 mm) Pellet size.

Proximate Analysis	
Crude Oil	2.7%
Crude Protein	19.7%
Crude Fibre	5.3%
Calcium (as Ca)	0.9%
Phosphorus (as P)	0.7%
Salt (as Na Cl)	1.0%
Metabolisable Energy	2568 kcal/kg
Carbohydrate (as %)	53.48

Trace Elements Added	
Manganese	25 ppm
Copper	7 ppm
Cobalt	0.4 ppm
Iron	30 ppm
Iodine	1.3 ppm
Magnesium	102 ppm

Amino Acids (as percentage of feed)	
Threonine	0.7
Glycine	0.9
Valine	1.0
Cystine	0.2
Methionine	0.3
Isoleucine	0.8
Leucine	1.5
Tyrosine	0.7
Phenylalanine	0.9
Lysine	1.0
Histidine	0.5
Arginine	1.2
Tryptophan	0.2

Vitamins Added per kg	
Vitamin A	8,000 iu
Vitamin D_3	1,000 iu
Vitamin B_2	8 mg
Nicotinic Acid	50 mg
Pantothenic Acid	12 mg
Vitamin B_{12}	12 μg
Vitamin E	60 iu
Vitamin K	10 mg
Folic Acid	10 mg
Choline Chloride	200 mg
Vitamin B_1	4 mg
Vitamin B_6	6 mg

DIETS FOR SCIENTIFIC RESEARCH ANIMALS

FORMULAE (%)

	S.G.I	18	RGP	418	86	PRM	RAT CAKE	FFC(M)	FPI	FGCP
Wheat				44	48	19	17	x	x	
Barley		20	40		24	5	7	x	x	54
Oats, Finely Ground	12		12	38		19	17			
Maize, Yellow						9	7	x	x	27
Middlings	18		15			19				
Bran	40	15					17			5
Linseed Cake, Expeller		10	10							9
Soya Bean Meal, Extracted						9		x	x	
Groundnut Cake, Expeller		15								
Meat Meal (63% Protein)		8			6		9			
Fish Meal, White (66% Protein)	10		7	8	6	5	5	x		
Grass Meal (17% Protein)	20	30	15		5				x	
Dried Yeast, Unextracted				1	5	2	1		x	
Dried Skimmed Milk				3		7	14		x	
Molasses				5	5	5	5	x	x	5
Chalk		1								
Salt	1	1	1	1	1	1	1	x	x	
Vitamin Supplement			x	x	x	x	x	x	x	x
Mineral Supplement			x	x	x	x	x	x	x	x

CALCULATED ANALYSES

Oil	3.20	3.78	3.29	2.86	2.12	2.68	2.96	2.23	1.70	2.61
Protein	18.10	25.00	17.98	14.90	18.10	18.66	22.40	18.40	14.39	11.33
Fibre	8.68	11.02	8.61	4.98	3.70	5.09	4.51	3.22	3.63	4.68
Calcium (Ca)	0.99	1.05	1.05	1.03	0.81	0.86	1.27	1.12	0.67	0.41
Phosphorus (P)	0.79	0.68	0.70	0.53	0.67	0.67	0.91	0.66	0.51	0.43
Salt (NaCl)	1.39	1.40	1.25	1.24	1.28	1.27	1.25	1.32	1.27	0.59

APPENDIX 11

SOLUBILIZATION OF WATER-INSOLUBLE EXTRACTS, FRACTIONS OR ISOLATES FOR DOSING BY PVP CO-PRECIPITATION

1. INTRODUCTION

Water-insoluble chemical compounds or plant extracts can readily be rendered soluble in most cases as their polyvinylpyrrolidone (PVP) co-precipitates. The efficacy of the PVP co--precipitate as a dosage form for the evaluation of gossypol as a spermicidal agent has been reported (1).

2. METHOD

2.1. Dissolve 1 part of the water-insoluble fraction in reagent grade ANHYDROUS methanol or acetone[a] ($CHCl_3$ or benzene cannot be used, except as noted below[b])

2.2. Dissolve 4 parts[c] of polyvinylpyrrolidone 10,000 (PVP 10) in a minimum volume of the same solvent.

2.3. The two solutions are mixed and the solvent removed by rota-vapor, initially at half speed, then at full speed when the volume is minimal so as to insure a fluffy product.

2.4. The product is now water soluble or partially soluble, and should be stored in air-tight, amber bottles kept in a dessicator in room temperature, and dispensed as needed.

---

[a] The volume of solvent used to dissolve the fraction is not important. One can use one-liter of methanol to dissolve 10 mg of the fraction if necessary.

[b] In the case where it is very difficult to dissolve the $CHCl_3$ or petroleum ether extract in methanol, one can use 10-20 ml of $CHCl_3$ to dissolve the insoluble residue. The $CHCl_3$ solution is combined with the methanol soluble portion of the extract, such that the total volume of $CHCl_3$ in the mixture is not more than 1%. This 1% $CHCl_3$ in methanol solution is then added to the PVP solution in methanol.

[c] Occasionally it is necessary to increase the portion of PVP 10,000 to 5 or more parts in order to solubilize a substance.

2.5. The PVP-coprecipitate should be divided in daily dosing aliquots and stored in individual vials. The contents of each vial are dissolved in water just prior to dosing daily[d].

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[d] Since the length of time that a PVP co-precipitate will remain in solution varies from substance to substance, the general rule of preparing a full dosing complement in aliquots, freezing and thawing (CG-02, section 4) is not applicable.

3. LITERATURE CITED

1. Waller, D. P., L. J. Zaneveld and H. H. S. Fong, Contraception, 22: 183-187 (1980)

APPENDIX - 13

JOB DESCRIPTION

DP/NEP/8?/C03/11-02/32.1.D

Post title	Pharmacologist
Duration	12 months
Date required	ASAP
Duty station	Kathmandu (Nepal)
Purpose of project	To develop further the existing facility for production of plant derived pharmaceuticals at the Royal Drugs Research Laboratory, Kathmandu/Nepal
Duties	The expert will assist the Director of the Royal Drugs Research Laboratory, Kathmandu, to set up a facility for the pharmacological screening of medicinal plants, and conduct pharmacological evaluations on plant preparations produced. Specifically the expert will do the following: (a) Build-up a suitable and adequate colony of laboratory animals for different Pharmacological tests including bioassays. (b) Organise in collaboration with local counterparts laboratory biological screening programmes and procedures for the pharmacological and toxicological assessment of medicinal plants, and plant products. (c) Train local counterparts in conducting bioassays and pharmacological tests; in the breeding and maintenance of laboratory animals, and in research in pharmacology. The expert will also be expected to prepare a final report, setting out the findings of his mission and his recommendations to the Government for further action which might be taken.
Qualifications	Ph.D. or quivalent research qualifications in Laboratory Research in Pharmacology; Experience in the pharmacological testing of plant extracts and natural products. Proven ability in pharmacological research on medicinal plants or plant-derived natural products.
Language	English