

THE INNOVATION FUND

Project 31313

**ANTIMALARIAL MEDICINES FROM MEDICINAL PLANTS OF
SOUTHERN AFRICA**

QUARTERLY REPORT

01 JULY 2003 – 30 SEPTEMBER 2003



1. General Information

Project Number	31313
Focus Area	Fauna and Flora
Abbreviated Project Title	Antimalarial medicines
Quarterly Report Number	11
Reporting period start date	01 July 2003
Reporting period end date	30 September 2003
Report Author's name and position in project	Ms Sindiswa Luwaca, Research Assistant
Report Author's Tel no., e-mail	(021) 938 0560; sindiswa.luwaca@mrc.ac.za
Project Start Date	01 January 2001
Expected Project End Date	31 December 2003
Total Project Budget	R6.7 m
Project Leader's Name	Prof. Peter Folb
Project Leader's Tel no., e-mail	(021) 406 6286; pfolb@uctgsh1.uct.ac.za
Consortium Members	Medical Research Council (MRC) University of Cape Town (UCT) National Botanical Institute (NBI) CSIR University of Pretoria (UP)
Technical Evaluator (1)	Prof Michelle Ramsay
Technical Evaluator (2)	Mr Edward Mabogo
Report Author's signature, date	
Project Leaders signature, date	

2. Aims and Objectives of the Project

The aim of this project is to develop novel, effective and non-cytotoxic plant based medicines for the treatment and prevention of malaria from medicinal plants of southern Africa. The objectives of this project are as follows:

- Transformation of indigenous plants and knowledge into new, proven safe and effective anti-malarial treatment.
- Sharing of benefits with communities, genetic resource holders and with individuals that provided source information.
- Capacity development (developing young research scientist within the programme).
- Develop a comprehensive pharmacology database on South African traditional medicines with antimalarial activity.
- Creation of jobs in farming communities through commercial cultivation of indigenous plants that have antimalarial properties.

It is expected by the end of the project (i.e. 31 December 2003), Consortium would achieved their main objective, viz. discover 2-3 novel antimalarial compounds that could be further developed into a drug, channelling benefits via a national trust fund.

3. Project Progress

3.1 Project Meetings

Meeting Date	Meeting Venue	Present	Meeting Type (Steering Committee/progress/etc)	Meeting objectives	Meeting Outcome
06 Aug 2003	NBI, Pretoria	Prof P Folb Dr C Clarkson Dr N Bhagwandin Mr M Matsabisa Dr B Bredenkamp Ms R Gayram Dr V Maharaj Ms P Pillay Mr X Mthembu Dr N Crouch Dr M Wolfson Ms O Grace	Progress/ scientific meeting	<p>Report on scientific progress of the project for the past 6 months.</p> <p>Discuss agro-processing proposal.</p> <p>Discuss publication guidelines</p> <p>Convergence with NDDP.</p> <p>Feedback on quarterly and financial report.</p> <p>Discuss workplan for completion of the project.</p> <p>Feedback on Trust deed and consortium document.</p> <p>Discuss second phase plant selection.</p>	<p>Agreed to formulate a work plan to complete the project.</p> <p>Proposal for the agro-processing programme will be submitted to Dr Bhagwandin. MRC will fund half of the costs.</p> <p>Decision made that consortium will transfer its intellectual property to the current programme (NDDP).</p> <p>Consensus on publishing scientific results.</p> <p>Consortium awaits for the approval of the trust deed from SARS.</p> <p>Consortium agreement to be signed by members.</p>
16 September 2003	MRC, Cape Town	Prof P Folb Dr N Bhagwandin Ms S Luwaca	Administrative meeting	<p>Feedback on the proposal for agro processing.</p> <p>Feedback on quarterly report.</p> <p>Template of the final report.</p>	<p>Decision made that MRC will fund half of the expenses for NBI and CSIR. MRC-IKS will be funded based on submission of an approved workplan.</p> <p>Template of the quarterly report sent to consortium members and inputs will be invited until 03 Oct 2003.</p>

					<p>Template of final report received from NRF. Decision made that all consortium members should submit report including work that they have achieved, what they have not achieved and the reason why based on project objectives.</p>
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3.2 Progress for reporting period

3.2.1 Work Planned for Completion

- NBI to continue recollections and botanical profiling of promising taxa.
- Further fieldtrips to collect extra chemotypes in support of agroprocessing activities.
- Submission of extracts of Ref 2, Ref 11, Ref 25, Ref 26 and Ref 29 for antiplasmodial *in vivo* assaying.
- Continue with structure elucidation of pure compounds from active fractions of P01609-1A (Ref 2). Targeted isolation and identification of other compounds from active fractions.
- Reverse-phase preparative liquid chromatography of active band (MAL-II-22K) of P00601-2B (Ref 11) to isolate and identify active compounds.
- Continue with purification and identification of active compounds from dichloromethane fraction of P05644-1B (Ref 25).
- Continue with targeted purification of active bands/compounds from P05633-1B (Ref 26).
- Continue with purification of P02424-2B (Ref 29) to isolate and identify active bands/compounds.
- Two crude extracts, P02424-2B and P00601-2B, were identified for *in vivo* testing. These crude extracts have been prepared in sufficient quantities by the CSIR and have are currently being tested for *in vivo* activity at UCT. Six promising extracts were prioritised for the fractionation and isolation of pure compounds.

The *in vitro* antiplasmodial and cytotoxicity testing of these fractions is ongoing in collaboration with CSIR.

- Identification of the few remaining differentially expressed gene transcript from the reverse subtracted library (down-regulated transcripts).
- Confirmation of differential expression of identified sequences from both the forward and reverse subtracted libraries using virtual Northern blotting.
- Identification of encoded proteins (where possible), cluster analysis and interpretation of gene expression results.
- Determination of the IC_{50} of MAL-II-24A in comparison to pyrimethamine on *P. falciparum* DHFR gene-complemented yeast strains.
- Determine if MAL-II-24A and pyrimethamine act synergistically using *P. falciparum* DHFR gene-complemented yeast assay system

3.2.2 Work Completed

- Agroprocessing plan (submitted on 25 April 2003, approved 25 August 2003).
- Recent collections and recollections of target taxa were undertaken by the NBI in KwaZulu-Natal and Limpopo Province.
- Crude extracts of Ref 11 and Ref 29 were “defatted” to improve antiplasmodial activity and submitted for *in vivo* assaying.
- Targeted isolation of five compounds from active fractions of P01609-1A (Ref 2) completed. Structure elucidation and bioassaying of these compounds ongoing.
- Reverse-phase preparative liquid chromatography of active band (MAL-II-22K) of P00601-2B (Ref 11) completed. Alternative reverse phase column/s and purification techniques are being investigated for further fractionation work as pure compounds were not obtained.
- Active fraction of P05644-1B (Ref 25) was identified. New bulk extract (P05644-2B) from a second recollection was prepared and fractionation process was repeated. Active fraction has been reproduced successfully.
- Primary fractionation of P02424-2B (Ref 29, recollected) completed. Secondary fractionation of those bands with improved antiplasmodial activity is ongoing.

- To date a total of 615 crude extracts and 536 purified fractions have been tested for in vitro antiplasmodial activity. The most promising purified fractions have also been tested for in vitro cytotoxicity.
- Differentially expressed transcripts from the reverse subtracted library (down-regulated transcripts) were sequenced and some of the proteins which they encode were identified by interrogation of sequences deposited in GenBank using the BlastX program. The sequences, for which no proteins could be identified, were further analyzed using the BlastN program and PlasmoDB database of *P. falciparum* deposited sequences.

Table 1 represents the sequences from the reverse subtracted library obtained thus far. These sequences would then correspond to those transcripts whose expression is potentially suppressed or limited within the DFMO inhibited (polyamine-depleted) parasites.

Table 1 Results of sequences from reverse substrate library

Clone	Length blasted	Protein/Sequence identified within <i>P.falciparum</i>	E-value
R1A1	441bp	rRNA-1-28s-A	4.4e-95
R1A10	391bp	high molecular weight rhoptry protein 2	2e-65
R1B1	268bp	rhoptry complex polypeptide RhopH3	4e-47
R1B5	391bp	cytoadherence linked asexual protein 2	7e-36
R1C5	591bp	rRNA-1-28s-S	1.1e-67
R1C7	285bp	similar to sequence on chromosome 7	2.1e-57
R1D2	343bp	Hypothetical <i>P.falciparum</i> protein	5e-40
R1D11	439bp	rRNA-1-28s-S	4e-56
R1E3	285bp	similar to sequence on chromosome 7	1.4e-51
R1E7	417bp	high molecular weight rhoptry protein 2	7e-76
R1E10	335bp	merozoite surface protein 1 precursor	5e-57
R1G1	425bp	similar to sequence on chromosome 3	1.1e-71
R1H3	410bp	Hypothetical <i>P.falciparum</i> protein	4e-45
R1H8	109bp	similar to sequence on chromosome 14	1.4e-18
R2A2	350bp	serine-repeat antigen protein	1e-51
R2A5	398bp	high molecular weight rhoptry protein 2	2e-63
R2B2	358bp	Hypothetical <i>P.falciparum</i> protein	7e-45
R2B11	315bp	similar to sequence on chromosome 5	8.8e-64
R2C10	361bp	putative rhoptry protein	9e-63
R2C12	189bp	Hypothetical <i>P.falciparum</i> protein	3e-29
R2D9	362bp	Hypothetical <i>P.falciparum</i> protein	9.3e-46
R2D11	253bp	similar to sequence on chromosome 2	4.5e-40
R2F8	337bp	putative cysteine protease	9e-63
R2H5	336bp	ADP ribosylation factor	1e-59
R2H10	58bp	similar to sequence on chromosome 13_1	3.1e-07
R3A2	406bp	putative cysteine protease	6e-80
R3A6	396bp	similar to sequence on chromosome 7	7.5e-74
R3C5	232bp	Hypothetical <i>P.falciparum</i> protein	9e-41
R3C6	359bp	Hypothetical <i>P.falciparum</i> protein	2e-29
R3D5	368bp	protective antigen	5e-51
R3E6	370bp	erythrocyte membrane protein 1	8e-69

The pure compound MAL-I-59C obtained from the CSIR was assayed for antifolate activity with the YEPD plate assay of the wildtype and resistant phenotypes of *P. falciparum* DHFR gene-complemented yeast strains. As shown in the Table 2 below, the compound tested at a concentration of 300ug per 50cm² plate, had neither anti-folate nor cytotoxic effects on any of the yeast strains.

Table 2. The effect of pure compound MAL-I-59C on *P. falciparum* DHFR gene-complemented yeast strains.

Compound	Inhibition (Yes/No)	No of experiments	Remarks
MAL-I-59C	No	3 (each strain)	Not antifolate/Not cytotoxic

The aim of the yeast liquid culture assay is to determine the IC₅₀ of the pure compound (MAL-II-24A) that was derived from plant extract P00631C. The positive control pyrimethamine was dissolved in DMSO (10mg/ml), serially diluted and 100 μ l of each dilution was assayed in duplicate in 1 ml liquid yeast cultures. MAL-II-24A (10mg/ml) was similarly diluted and assayed in duplicate experiments.

Yeast cells were grown overnight in YEPD liquid media at 30°C to mid-log phase to ensure cells are actively growing. The optical density was read the following day at 660nm and cultures were diluted to an optical density of between 0.1- 0.2 before addition of the inhibitor. Absorbance readings were taken each hour to obtain semi-logarithmic plots for determination of the IC₅₀'s. These experiments have been repeated several times now, mostly in triplicate or quadruplicate, and proven to be exceptionally variable between experiments conducted on different days. Within the same experiment little variation is apparent between the IC₅₀s obtained. However, the trends of the IC₅₀s obtained are as expected: the IC₅₀s increase for both pyrimethamine and Mal-II24A with the number of mutations associated with the anti-folate resistant phenotype. This observation serves to validate the assay method in showing that this compound acts directly on DHFR to inhibit folate biosynthesis.

Serious attention is being given to the cause(s) for the variable results and a number of possible contributing factors have been eliminated already. Since the compound inhibits the quadruple-mutated wild type DHFR strain (no other drug has been shown to be able to do this), it is important to also establish whether it acts synergistically with pyrimethamine. These assays would serve several purposes: if it acts synergistically, MAL-II-24A could be

used as an adjunct to pyrimethamine, possibly at a concentration below the toxicity shown with malaria parasite cultures and improve its pharmaceutical index; it would be indicative of a mechanism of action not involving the active site centre of DHFR where pyrimethamine acts (a novel finding); it could serve as a starting compound for a combinatorial chemistry approach to improve its specificity, anti-plasmodial effect and to reduce its cytotoxicity. Arrangements have been made with Dr Peter Smith and Dr C Clarkson to determine if there is any synergism between MAL-II-24A and pyrimethamine on malaria parasite cultures.

- A total of 381 extracts were received from the CSIR during the months, November 2001 and December 2002. Initially, 115 of these extracts were specified to be of priority. Screening assays were conducted on these extracts by means of Adulticidal, Larvicidal and Repellency studies. No potential positives have been noted for both the Adulticidal and Repellency trials using the prioritized extracts. Due to the completion of the 115 extracts recommended for screening, the Malaria Programme has recently concluded initial screening of all 266 extracts remaining for Adulticidal and Larvicidal activity. No positive extracts have been identified for Adulticidal assays. Activity against the aquatic larval stages have proved to be very effective, since thirty nine extracts have been identified as being potential “hits”, with mortality ranging from 80 - 100 %. Of these 39 extracts, 21 produced 100% mortality. These results are currently being validated, by conducting repeat trials.

3.2.3 Work not completed

- Extracts of Ref 2, Ref 25 and Ref 26 were not submitted for *in-vivo* antiplasmodial assaying as a decision was reached to proceed only with *in vivo* work on Ref 11 and Ref 29 extract.
- Could not continue with targeted purification of active bands/compounds from P05633-1B (Ref 26) as material from original extract was exhausted and new extract from recollected material was inactive. Active bands/compounds identified in original extract were not evident in new extract.

- The gene expression profiling assays are on schedule. Sequencing and analysis of the clones from the reverse subtracted library is almost complete. Once this process is completed and clones selected, virtual Northern blot analysis will be performed using all the relevant inserts from both forward and reverse subtraction to confirm the results. Further bioinformatics analysis will be performed on the confirmed results in an attempt to annotate some of the sequences with unidentified functions and to provide a coherent explanation for the drug-induced alterations in gene expression.
- The yeast liquid culture assays are taking longer than anticipated due to the unexpected variability between experiments as stated above. Once this issue is resolved, the outstanding studies (synergistic and specificity assays) could be completed in a relatively short time.

3.2.4 Work completed but not planned

- One active compound from P01609-1A (Ref 2), which showed evidence of cytotoxicity in the CHO assay, has been submitted to UP for their biochemical evaluation.
- Further investigation of the chloroform extract from sequential extraction of P00599-1A (Ref 15) using different solvents with increasing polarity. Reproduced sufficient quantity of this extract for further fractionation work to see if observed activity improves.

3.2.5 Issues

The following issues have not yet been resolved or finished:

- Finalisation of consortium agreement.

3.2.6 Issue Resolution

N/A

3.2.7 Work planned for the next quarter

- Complete structure elucidation of those compounds isolated from P01609-1A (Ref 2), which show good antiplasmodial activity. Those compounds that show cytotoxicity as well will be considered for SAR (structure-activity relationship) studies.

- Continue with fractionation of P00601-2B (Ref 11) to isolate and identify active compounds.
- Continue with further purification of the reproduced active band from P05644-2B (Ref 25) to isolate and identify active compound/s.
- Continue with targeted purification of active bands/compounds from P05633-1B (Ref 26) once a new source of plant material has been identified and the antiplasmodial activity of the original extract can be reproduced.
- Continue with purification of active bands of P02424-2B (Ref 29) to isolate and identify active compound/s.
- Continue with purification of chloroform extract of P00599-1A (Ref 29) to isolate and identify possible active compounds.
- The above-mentioned in vitro work and in vivo studies will continue, prioritising the most promising candidates to bring the project to completion.

3.3 Milestone status

Milestone	Expected Completion Date	Actual Completion Date	Status
Output 1. Selection criteria for plant species	End of month 1	End of month 2	Completed
Output 2. Protocol for collection, preparation, extraction & fractionation of plants	End of month 1	End of month 2	Completed
Output 3. Agreed screening methods & screening workload of consortium members	End of month 3	End of month 2	Completed
Output 4. A shortlist of plants and localities for collection.	End of month 4	End of month 4	Completed
Output 5. Initial plant collection and debugging and screening methods.	End of month 6	End of month 8	Completed
Output 6. Collection, extraction and screening reaches required throughput level.	End of month 9	On-going activity	On-going activity
Output 7. First potential anti-malaria compound/standardized fraction produced.	End of month 15	In Progress	In Progress
Output 8. Agro-processing based	End of month 18	End of month 32	Final Proposal submitted

production of quantities of promising substance for advanced pharmacology and chemistry.			
Output 9. Pharmacological profiling of promising substance	End of month 24	On going	In Progress
Output 10. Chemical profiling of promising substances.	End of month 24	On going	In Progress
Output 11. Provisional patenting of new anti-malarial substance.	End of month 27	N/A	N/A
Output 12. Negotiations on technology transfer to industry partner.	End of month 36	N/A	N/A

4. Financial Information

See Appendices A-C.

Declaration: **I hereby declare that the information supplied is a true reflection of the financial records of Project 31313**

Financial Statements prepared by:

Signature:

Date:

4.2 Cash Flow Projection for the next Quarter

See Appendices A-B

5. Requirements from the NRF

N/A

6. Follow up: Recommendations by Technical Evaluators

N/A

7. Summary by Project Leader

A work plan has been completed for conclusion of the programme by the last working day, 31st December 2003. An agroprocessing plan has been drawn up, within the terms of the original programme submission, and work on this has commenced. Funding has been allocated for the purpose. It will be noted from the content of this report that to date altogether 615 crude extracts and 536 purified fractions have been tested for *in vitro* antiplasmodial activity and the most active of these have also been

submitted to cytotoxicity testing to determine their selectivity index. 39 compounds have been identified with promising larvicidal activity against aquatic larval stages. A comprehensive account of the results obtained over the three years of the project is in the final stage of compilation, and it will be submitted for publication before completion of the project.

8. **Submission date of next report:**

31 January 2004

**APPENDIX A
FUNDS RECEIVED**

Year 1		Year 2		Year 3	
Receipt Date	Amount	Receipt Date	Amount	Receipt Date	Amount
02/01/2001	R1 195 425	07/05/2002	R562 125	06/06/03	R686 250
15/03/2001	R512 325	07/07/2002	R686 250		
02/07/2001	R562 125	01/10/2002	R686 250		
01/10/2001	R562 125	12/12/2002	R686 250		
18/12/2001	R562 125				
Total Yr1	R 3 394 125	Total Yr2	R2 620 875	Total Yr 3	R686 250

**APPENDIX B
CONSORTIUM DISBURSEMENTS**

MRC Malaria Research Programme						
Instalments	Date Due	Amount	Payment	Balance	Reference	Date paid
1	By agreement	36,602.75	36,602.75	0.00	FJ1928/1	31/03/01
2	15/03/01	36,602.75	36,602.75	0.00	FJ1928/1	31/03/01
3	30/06/01	36,602.75	36,602.75	0.00	FJ 805/1	29/08/01
4	30/09/01	36,602.75	36,602.75	0.00	FJ 592	29/07/02
5	15/12/01	47,603.75	47,603.75	0.00	FJ 592	29/07/02
6	15/03/02	47,603.75	47,603.75	0.00	FJ 592	29/07/02
7	30/06/02	47,603.75	47,603.75	0.00	FJ 592	29/07/02
8	30/09/02	47,603.75	47,603.75	0.00	FJ1125	30/09/02
9	15/12/02	52,961.50	52,961.50	0.00	FJ2062	19/02/03
10	15/03/03	52,961.50	52,961.50	0.00	FJ2318	31/03/03
11	30/06/03	52,961.50	52,961.50	0.00	FJ376	30/07/03
12	30/09/03	52,961.50	0.00	52,961.50		
		548,672.00	495,710.50	52,961.50		
CSIR						
Instalments	Date Due	Amount	Payment	Balance	Reference	Date paid
1	On signature	169,212.00	169,212.00	0.00	#0010330	30/03/01
2	15/03/01	169,212.00	169,212.00	0.00	#0010409	09/04/01
3	30/06/01	169,212.00	169,212.00	0.00	#0010807	10/08/01
4	30/09/01	169,212.00	169,212.00	0.00	# 73129	17/10/01
5	15/12/01	245,322.25	245,322.25	0.00	# 73770	17/01/02
6	15/03/02	245,322.25	245,322.25	0.00	#0020330	30/03/02
7	30/06/02	245,322.25	245,322.25	0.00	#0020831	31/08/02
8	30/09/02	245,322.25	245,322.25	0.00	#75959	30/09/02
9	15/12/02	300,581.50	300,581.50	0.00	#76924	08/01/03
10	15/03/03	300,581.50	300,581.50	0.00	#10002839	14/03/03
11	30/06/03	300,581.50	300,581.50	0.00	#10004660	30/06/03
12	30/09/03	300,581.50	300,581.50	0.00	#10007787	28/10/03
		2,860,463.00	2,860,463.00	0.00		
National Botanical Institute (NBI)						
Instalments	Date Due	Amount	Payment	Balance	Reference	Date paid
1	On signature	55,879.25	55,879.25	0.00	#0010409	09/04/01
2	15/03/01	55,879.25	55,879.25	0.00	#0010409	09/04/01
3	30/06/01	55,879.25	55,879.25	0.00	#001087	10/08/01
4	30/09/01	55,879.25	55,879.25	0.00	# 73125	16/10/01
5	15/12/01	51,613.00	51,613.00	0.00	# 73818	30/01/02
6	15/03/02	51,613.00	51,613.00	0.00	#74113	14/03/02
7	30/06/02	51,613.00	51,613.00	0.00	#75036	25/06/02
8	30/09/02	51,613.00	51,613.00	0.00	#75692	30/09/02

9	15/12/02	58,534.00	58,534.00	0.00	#10002216	28/01/03
10	15/03/03	58,534.00	58,534.00	0.00	#10002842	14/03/03
11	30/06/03	58,534.00	58,534.00	0.00	#10004762	30/06/03
12	30/09/03	58,534.00	58,534.00	0.00	#10007792	28/10/03
		664,105.00	664,105.00	0.00		
University of Cape Town (UCT)						
Instalments	Date Due	Amount	Payment	Balance	Reference	Date paid
1	On signature	91,694.75	91,694.75	0.00	# 71349	27/03/01
2	15/03/01	91,694.75	91,694.75	0.00	# 71349	27/03/01
3	30/06/01	91,694.75	91,694.75	0.00	#0010831	31/08/01
4	30/09/01	91,694.75	91,694.75	0.00	# 73133	18/10/01
5	15/12/01	118,941.50	118,941.50	0.00	#0020131	31/01/02
6	15/03/02	118,941.50	118,941.50	0.00	#74115	14/0302
7	30/06/02	118,941.50	118,941.50	0.00	#0020705	05/07/02
8	30/09/02	118,941.50	118,941.50	0.00	#0021014	14/10/02
9	15/12/02	141,062.75	141,062.75	0.00	#76946	09/01/03
10	15/03/03	141,062.75	141,062.75	0.00	#10003373	11/04/03
11	30/06/03	141,062.75	141,062.75	0.00	#10005010	30/06/03
12	30/09/03	141,062.75	0.00	117,080.47		
		1,406,796.00	1,265,733.25	117,080.47		
University of Pretoria (UP)						
Instalments	Date Due	Amount	Payment	Balance	Reference	Date paid
1	On signature	44,430.75	44,430.75	0.00	#0010514	14/05/01
2	15/03/01	44,430.75	44,430.75	0.00	#0010514	14/05/01
3	30/06/01	44,430.75	44,430.75	0.00	#0010807	10/08/01
4	30/09/01	44,430.75	44,430.75	0.00	#0011109	09/11/01
5	15/12/01	57,525.75	57,525.75	0.00	# 73877	07/02/02
6	15/03/02	57,525.75	57,525.75	0.00	#0020416	16/04/02
7	30/06/02	57,525.75	57,525.75	0.00	#75040	25/06/02
8	30/09/02	57,525.75	57,525.75	0.00	#75966	30/09/02
9	15/12/02	67,192.25	67,192.25	0.00	#76935	08/01/03
10	15/03/03	67,192.25	67,192.25	0.00	#10003273	07/04/03
11	30/06/03	67,192.25	67,192.25	0.00	#10005194	09/07/03
12	30/09/03	67,192.25	67,192.25	0.00	#10007798	28/10/03
		676,595.00	676,595.00	0.00		
MRC Business Management						
Instalments	Date Due	Amount	Trf to 5664	Expenses covered by Bus.Manag.	Reference	Date paid
1	On signature	30,825.00	30,825.00	0.00	FJ2203	26/03/03
2	15/03/01	30,825.00	20,623.50	10,201.50	FJ2203	26/03/03
3	30/06/01	30,825.00	12,305.91	18,519.09	FJ2203	26/03/03
4	30/09/01	30,825.00	14,085.68	16,739.32	FJ2203	26/03/03
5	15/12/01	34,425.00	19,040.26	15,384.74	FJ2203	26/03/03

6	15/03/02	34,425.00	8,316.02	26,108.98	FJ2203	26/03/03
7	30/06/02	34,425.00	20,124.01	14,300.99	FJ2203	26/03/03
8	30/09/02	34,425.00	14,105.46	20,319.54	FJ2203	26/03/03
9	15/12/02	43,368.75	17,453.73	25,915.02	FJ2203	26/03/03
10	15/03/03	43,368.75	14,193.27	29,175.48	FJ2320	31/03/03
11	30/06/03	43,368.75	29142.28	14226.47	FJ516	12/08/03
12	30/09/03	43,368.75	0.00	43,368.75		
		434,475.00	200215.12	234259.88		
MRC Corporate						
Instalments	Date Due	Amount	Payment	Balance	Reference	Date
1	On signature	7,517.00	7517	0.00	FJ1971/3	31/03/02
2	15/03/01	7,517.00	7517	0.00	FJ1971/4	31/03/02
3	30/06/01	7,517.00	7517	0.00	FJ601/3	30/07/01
4	30/09/01	7,517.00	7517	0.00	FJ1134/2	29/01/01
5	15/12/01	9,139.25	9139.25	0.00	FJ1591&2165	28/01 & 31/03/02
6	15/03/02	9,139.25	9139.25	0.00	FJ2165	31/03/02
7	30/06/02	9,139.25	9,139.25	0.00	FJ739	29/07/02
8	30/09/02	9,139.25	9,139.25	0.00	FJ1543	25/11/02
9	15/12/02	10,828.25	10,828.25	0.00	FJ1762	27/02/03
10	15/03/03	10,828.25	10,828.25	0.00	FJ516	12/08/03
11	30/06/03	10,828.25	10,828.25	0.00	FJ516	12/08/03
12	30/09/03	10,828.25	10,828.25	0.00	FJ850	28/10/03
		109,938.00	109,938.00	0.00		
GRAND TOTAL		6,701,044.00				

APPENDIX C
INCOME AND EXPENDITURE STATEMENT FOR REPORTING PERIOD

	<u>R</u>	
INCOME	678,154.63	
Balance b/f (April - June 2003)		678,154.63
EXPENSES	650,546.72	
Overhead Costs	10,828.25	
Payments :	605,041.50	
- CSIR (12th instalment)	300,581.50	
- Malaria Unit (12th instalment)	52,961.50	
- Business Management (12th instal.)	8,691.78	
- UCT (12th instalment)	117,080.47	
- UP (12th instalment)	67,192.25	
- NBI (12th instalment)	58,534.00	
Motor rental	830.05	
Insurance on motor vehicles	40.00	
Accommodation - RSA	492.93	
Local travel : toll fees/petrol	60.00	
Airtravel - RSA	6,174.00	
Staff Allowance	130.00	
Staff MV Allowance	113.40	
Temporary workers	12,750.12	
Salary (S Luwaca)	14,086.47	
NETT INCOME OVER EXPENSES	27,607.91	

The income & expenditure statement is a true reflection of the MRC's financial records.

PREPARED BY: TASNIM MOHSAM
7 October 2003
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