

UNITED STATES GOVERNMENT

Memorandum

93/0001034901
PD-AP6-724

TO : AA/TA, Dr. Erven Long
THRU : TA/RES, Dr. Miloslav Rechcigl
FROM : TA/H, Lee H. Howard, M.D.

DATE: MAR 8 1977

SUBJECT: Research to Characterize the Biochemical Constituents of the Polar Organelles of Erythrocytic Merozoites of Malaria

Introduction: Research will be conducted to characterize the biochemical constituents of the polar organelles of erythrocytic merozoites of malaria. The research will be conducted by Araxie Kilejian of the Rockefeller University. It will take about five months and cost about \$28,680.

Discussion: The purpose of this study is to follow-up on a promising line of research that may assist in the development of a vaccine against malaria. This work is particularly pertinent due to the early success of Dr. William Trager, Rockefeller University, New York, under contract with AID (AID/ta-C-1200).

Investigator: Dr. Araxie Kilejian, is an associate professor at Rockefeller University. She received the Bachelor and Masters Degree in Science from the American University in Beirut, Lebanon and a Doctorate in Philosophy from Rice University, Houston, Texas. She is the author of numerous scientific papers and has research experience in the following: basic analytical, chromatographic, and electrophoretic techniques used in isolation and characterization of all major biochemical classes - polysaccharide, lipid, nucleic acid, and protein; membrane transport; electron microscopy, culture of malaria merozoites, and isolation of subcellular organelles from malaria parasites.

Objectives: The main objective of the proposed study is to purify and characterize a potential antigen for a malaria vaccine. In some animal models the use of crude malaria antigen for vaccination has been shown to have undesirable side effects, such as anemia. Therefore, any antigen that can be used safely for human vaccination should be free of undesirable contaminants. From the available literature on malaria immunity, the polar organelles of merozoites appear to be a logical source for a pure antigen.



Significance to AID Objectives: According to recent figures published by WHO, there are about 1.2 billion people living in areas where malaria is not yet controlled. In the past, malaria programs have been successful in removing the threat of this scourge from millions of people and opened vast tracts of land for development. Recently dramatic increases in the reported cases of malaria in Asia, Latin America and Africa caused by resistance of the vectors to insecticides and resistance of the plasmodia to drugs forced scientists to look for alternative methods of control. The development of a malaria vaccine could be the answer to one facet of this control methodology.

This small research project is a direct corollary to the work being pursued by several AID-supported research teams investigating malaria immunity and the development of a malaria vaccine. Therefore, the success of this study would help expedite the development of a malaria vaccine contributing to the protection of the investment in other AID-supported projects in health, agriculture and economic development in LDCs where malaria abounds.

External Review: This proposal has been reviewed by well-known authorities in research and public health programs. The reviewers strongly recommend that AID fund this project as it will be worth its reasonable cost. "I strongly recommend funding; it is a bargain for the investment," one reviewer wrote.

Recommendation: That you approve this project for funding in the Small Research Program, in the amount of \$28,680 for a period of approximately five (5) months.

Approved: *Ernest J. Long*

Disapproved: _____

Date : *Apr 7, 1977*

Attachments:

- a. Project Statement
- b. Research Proposal

Clearances:

TA/H:RDNewman *R.D.N.*
 TA/PPU:RSimpson *W. Simpson*
Richard J.

PROJECT STATEMENT

Project Title: Chemical and Immunological Characterization of Polar Organelles of Erythrocytic Merozoites of Malaria

Status: New

Contractor: Rockefeller University, New York

Principal Investigator: Dr. Araxie Kilejian

Duration: 5 months - June 1, 1977 to November 1, 1977

Estimated Cost: \$28,680

Project Manager: Mr. Edgar A. Smith/Mr. Jalil S. Karam

Narrative:

The purpose of this project is to support research on the characterization of the biochemical constituents of the anterior electron dense organelles (polar organelles) of erythrocytic merozoites from Plasmodium lophurae.

This research is well underway and is complimentary to the research being done at the same institution under an AID contract (AID/ta-C-1200).

This proposal is a straightforward well-defined study and the results will be relevant to the work on P. falciparum and could help lead to identification of antigens responsible for protective immunity in human malaria.

Project Description and Background

The specific aim of this study will be:

- a) collection of isolated polar organelles of malaria merozoites,
- b) chemical characterization of their constituents,
- c) testing whether hyperimmune host sera contain specific antibody to the contents of these organelles.

Recent studies by Cohen and coworkers (WHO Technical Report Series No. 579, 1975) indicate that erythrocytic merozoites are exceptionally effective as immunogenic agents for malaria. A unique feature of merozoites of all species of malaria is the presence of polar organelles that are absent from other erythrocytic stages. Some of these organelles have ducts that terminate at the anterior end of merozoites and their contents have been implicated in having a function during merozoite penetration of the red blood cell. It seems reasonable to hypothesize that antibodies against any substance important for entry of merozoites into host cells could interrupt penetration.

Methods

Erythrocytic merozoites of Plasmodium lophurae will be collected from in vitro cultures, and fractions enriched in polar organelles will be prepared by procedures employed presently. Recovery from each experiment is very small, as would be expected when the minute size and small number of these organelles per merozoite is considered. Concentrated effort will be directed toward performing several isolation experiments to collect sufficient amounts of material for direct chemical analysis. The only contaminants of collected fractions appear to be membrane vesicles. As a control, a fraction that is enriched in these vesicles and bands at lower density than the organelles in sucrose gradients will also be collected and analyzed. There is some evidence that a histidine-rich protein that was initially isolated and characterized from erythrocytic stages is also a component of polar organelles. Special effort will be invested in proving conclusively the presence or absence of this protein.

To determine whether the contents of these organelles are immunogenic, hyperimmune duck sera and normal duck sera will be iodinated with ^{125}I and their binding to isolated organelles tested. The

specificity of the reaction will be evaluated by electron microscope auto-radiography. The reason for this indirect approach is to overcome the non-specific precipitin reaction in avian sera and the solubility problems that may result from high salt concentration required in avian precipitin reactions.

Significance to AID Objectives

The worldwide malaria program has been a large and generally successful AID investment in many countries. However, due to various factors, i.e. withdrawal of funds, insecticide resistance, etc., there has been a resurgence of malaria in many parts of the world. An effective malaria vaccine would go a long way toward protecting populations affected by malaria and would contribute to the protection of the investment in AID-supported projects in health, agriculture and economic development in those LDCs where malaria occurs. This project can be carried to a conclusion in the five months requested and will help to guide related work carried on with P. falciparum by Trager and associates (Contract No. AID/ta-C-1200). If the polar organelles of P. lophurae are found to contain immunogenic antigens, the methods developed with this species will be directly applicable to merozoites of the human malaria, P. falciparum from cultures.

External Review

This proposal was reviewed in December, 1976 by Drs. E. Sherman and R. L. Beaudoin. The external reviewers were impressed by the project proposal and thought that it has high scientific merit and that it was a logical extension of Dr. Kilejian's work on biochemical characterization of the malaria parasite.

A Name and Address of Organization:

The Rockefeller University
1230 York Avenue
New York, New York 10021

Date of Submission:

October 20, 1976

B. Type of Organization:

Private, Non-Profit

C. Title of Proposal:

"Chemical and immunological characterization of polar organelles of erythrocytic merozoites of malaria."

D. Desired Starting Date and Duration:

June 1, 1977 to November 1, 1977

E. Research Plan and Objectives:

1. Support is requested to continue our efforts in characterizing the biochemical constituents of the anterior electron dense organelles (polar organelles) of erythrocytic merozoites from Plasmodium lophurae. Recent studies by Cohen and co-workers (WHO Technical Report Series No. 579, 1975) indicate that erythrocytic merozoites are exceptionally effective as immunogenic agents for malaria. A unique feature of merozoites of all species of malaria is the presence of polar organelles that are absent from other erythrocytic stages. Some of these organelles have ducts that terminate at the anterior end of merozoites and their contents have been implicated in having a function during merozoite penetration. It seems reasonable to hypothesize that antibody against any substance important for entry of merozoites into host cells could interrupt penetration. Aside from our studies (summarized in the enclosed manuscript), there is a complete void in the literature on the chemical nature of polar organelles. A major limitation to such studies has been the unavailability of large numbers of merozoites that are required for standardization of any new isolation procedure. In this respect, Plasmodium lophurae provides an ideal, relatively inexpensive system for initial studies on merozoites. Once procedures are developed they could be applied to the less readily available human malaria parasites.

2. The specific aim of this study will be:

- a) Collection of sufficient amounts of isolated polar organelles.
- b) Chemical characterization of their constituents.
- c) Testing whether hyperimmune host sera contain specific antibody to contents of these organelles.

3. Erythrocytic merozoites of Plasmodium lophurae will be collected from in vitro cultures and fractions enriched in polar organelles will be prepared by procedures employed presently. Recovery from each experiment is very small (as would be expected when the minute size and small number of these organelles per merozoite is considered). Concentrated effort will be directed to perform several isolation experiments to collect sufficient amounts of material for direct chemical analysis. The only contaminants of collected fractions appear to be membrane vesicles; as a control a fraction that is enriched in these vesicles and bands at a lower density than the organelles in sucrose gradients will also be collected and analyzed. We have some evidence that a histidine-rich protein that was initially isolated and characterized from erythrocytic stages is also a component of polar organelles. Special effort will be invested in proving conclusively the presence or absence of this protein.

To determine whether the contents of these organelles are immunogenic, hyperimmune duck sera and normal duck sera will be iodinated with ^{125}I and their binding to isolated organelles tested. The specificity of the reaction will be evaluated by electron microscope autoradiography. The reason for this indirect approach is to overcome the non-specific precipitin reaction in avian sera and the solubility problems that may result from high salt concentration required in avian precipitin reactions.

4. The importance of malaria as a public health problem is well established. Characterization of the chemical nature of contents of organelles of merozoites that most likely function in penetration might allow the design of rational antagonists to prevent host cell invasion. If they were shown to be immunogenic, they could also serve as the antigen for a vaccine. Since these organelles are present in all species of malaria, findings of this study on an avian species could form the basis for application to human species.

5. This is a well defined project that can be carried to a conclusion in the 6-month period requested and that will help to guide related work carried on with P. falciparum by Trager and associates. If the polar organelles of P. lophurae are found to contain immunogenic antigens the methods developed with this species will be directly applicable to merozoites of P. falciparum from cultures.

F. Facilities Available:

As our past work indicates, we have adequate facilities and equipment to carry out the proposed research.

G. Personnel:

Araxie Kilejian, Principal Investigator; 563-62-4965
Ellen Kracauer, Assistant for Research; 074-46-4623

Biographical Sketch of Principal Investigator:

Education:

American Univ. of Beirut, Lebanon; B.S.; 1957
American Univ. of Beirut, Lebanon; M.S.; 1959
Rice Univ., Houston, Texas; Ph.D.; 1965

Research and/or Professional Experience:

Associate Professor, Rockefeller University, 1976-
Assistant Professor, Rockefeller University, 1972-1976.
Visiting Assistant Professor, University of California, 1971-72.
Post-doctoral Research Fellow, University of California, 1969-71.
Assistant Professor of Biology, Haigazian College, Beirut, Lebanon
1968-69.
Assistant Professor of Parasitology, University of California,
1966-68.
NIH Post-doctoral Research Fellow, University of Massachusetts,
1965-66.
Graduate Teaching Assistant in Biology & Parasitology, Rice
University, 1962-64.
Trainee in Medical Technology, Community Hospital, Fresno, Calif.
1961-62.
Research Assistant, American University of Beirut, 1959-61.

Research Experience: Basic analytical, chromatographic and electrophoretic techniques used in isolation and characterization of all major biochemical classes - polysaccharide, lipid, nucleic acid and protein; membrane transport; electron microscopy, culture of malaria merozoites, isolation of sub-cellular organelles from malaria parasites.

Publications of Principal Investigator:

- Kilejian, A. 1976. Studies on a histidine-rich protein from Plasmodium lophurae. Proceedings of the 2nd International Symposium on the Biochemistry of Parasites. (In Press).
- Kilejian, A. 1976. Does a histidine-rich protein from Plasmodium lophurae have a function in merozoite penetration? J. Protozool. 23:272.
- Kilejian, A., Liao, T.-H., & Trager, W. 1975. Studies on the primary structure and biosynthesis of a histidine-rich polypeptide from the malaria parasite, Plasmodium lophurae. Proc. Nat. Acad. Sci. USA 72:3057
- Kilejian, A. 1975. Circular mitochondrial DNA from the avian malarial parasite, Plasmodium lophurae. Biochim. Biophys. Acta 390:276.
- Kilejian, A. 1974. A unique histidine-rich polypeptide from the malaria parasite, Plasmodium lophurae. J. Biol. Chem. 249:4650.
- Simmons, J.E., Buteau, G., MacInnis, A., & Kilejian, A. 1972. Characterization and hybridization of DNAs from gyrocotylidean parasites of chimaeroid fishes. Internat. J. Parasit. 2:273.
- Kilejian, A., & Schwabe, C.W. 1971. Studies on the polysaccharides of the Echinococcus granulosus cyst, with observations on a possible mechanism for laminated membrane formation. Comp. Biochem. Phys. 40B:25.
- Schwabe, C.W., Kilejian, A., & Lainas, G. 1970. The propagation of secondary cysts of Echinococcus granulosus in the Mongolian jird, Meriones unguiculatus. J. Parasit. 56:80.
- Kilejian, A. 1970. Flame Cell. IN: Gray, P. (ed.) The Encyclopedia of the Biological Sciences. Van Nostrand Reinhold Company.
- Read, C.P. & Kilejian, A. 1969. Circadian migratory behavior of a cestode symbiote in the rat host. J. Parasit. 55:574.
- Schwabe, C.W., & Kilejian, A. 1968. Chemical aspects of the ecology of Platyhelminthes. IN: Florkin, M. and Scheer, B. (eds.) Chemical Zoology, Vol. 2, Academic Press.
- Kilejian, A., Ginger, C., & Fairbairn, D. 1968. Origins of intestinal lipids available for absorption by Hymenolepis diminuta (Cestoda). J. Parasit. 54:69.
- Kilejian, A. 1966. Permeation of L-proline in the cestode, Hymenolepis diminuta. J. Parasit. 52:1108.
- Kilejian, A. 1966. Formation of the L-proline pool of the cestode, Hymenolepis diminuta. Exptl. Parasit. 19:358.
- Kilejian, A. 1963. The effect of carbon dioxide on glycogenesis in Moniliformis dubius. J. Parasit. 49:862.
- Kilejian, A., Sauer, K., & Schwabe, C.W. 1962. Infrared spectra and chemical composition of the hydatid cyst. Exptl. Parasit. 12:377.
- Kilejian, A., Schinazi, L., & Schwabe, C.W. 1961. Histochemical observations on Echinococcus granulosus. J. Parasit. 47:181.
- Schwabe, C.W., Schinazi, L., & Kilejian, A. 1958. Age resistance to secondary Echinococcosis in the white mouse. Amer. J. Trop. Med. Hyg. 8:29.

I. Budget Information:

Estimated Total Cost of Project - \$28,680

J. Budget Categories:

Salaries with Fringe Benefits:

Kilejian, Araxie (Principal Investigator)	\$ 9,740
Kracauer, Ellen (Assistant for Research)	<u>5,340</u>
Total Salaries	\$15,080
Overhead of 76.4% for University	\$ 9,200
Animals, animal board, supplies	\$ 4,000
Travel - to A.S.P. Meeting	<u>\$ 400</u>
	\$28,680

K. Other Research Projects:

Supported by Grant awarded to Prof. W. Trager.-
 RO1 AI 10640; N.I.H.; Physiology of intracellular parasitism.
 Support ends May 30, 1977.

AIB 1350-19C (7-71)	DEPARTMENT OF STATE AGENCY FOR INTERNATIONAL DEVELOPMENT	1. Cooperating Country TAB	Page 1 of 5 Pages
		2. PIO/T No. 931-0001-3177708	3. <input checked="" type="checkbox"/> Original or Amendment No. _____
PIO/T	PROJECT IMPLEMENTATION ORDER/TECHNICAL SERVICES	4. Project/Activity No. and Title 931-0001 Small Research Program (Biochemical Analysis of Merozoites)	

DISTRIBUTION	5. Appropriation Symbol 72-11X1026	6.A. Allotment Symbol and Charge 426-31-099-00-22-71	6.B. Funds Allotted to: <input checked="" type="checkbox"/> A.I.D./W <input type="checkbox"/> Mission	
	7. Obligation Status <input checked="" type="checkbox"/> Administrative Reservation <input type="checkbox"/> Implementing Document		8. Funding Period (Mo., Day, Yr.) From 6/1/77 To 12/1/77	
	9.A. Services to Start (Mo., Day, Yr.) Between 5/15/77 and 6/15/77		9.B. Completion date of Services (Mo., Day, Yr.) 12/1/77	
	10.A. Type of Action <input checked="" type="checkbox"/> A.I.D. Contract <input type="checkbox"/> Cooperating Country Contract <input type="checkbox"/> Participating Agency Service Agreement <input type="checkbox"/> Other			
	10.B. Authorized Agent CM/COD/TA AID/W			
	Estimated Financing		(1) Previous Total	(2) Increase
\$1.00=				(4) Total to Date
11. Maximum A.I.D. Financing	A. Dollars		28,680	28,680
	B. U.S.-Owned Local Currency		FUNDS RESERVED BY	
12. Cooperating Country Contributions	A. Counterpart		<u>ALW</u>	
	B. Other		<u>POSTED 5/24/77</u> SER/EM/CSD	

13. Mission References	14. Instructions to Authorized Agent Prepare and enter into a five-month contract with the Rockefeller University for the performance of the services noted herein. The funds authorized by this PIO/T are budgeted to support the full five months of activities.
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15. Clearances - Show Office Symbol, Signature and Date for all Necessary Clearances.	
A. The specifications in the scope of work are technically adequate <i>EAS</i> TA/H: EASmith	B. Funds for the services requested are available <i>MAC 5/3/77</i> TA/PPU: MZozynski
C. The scope of work lies within the purview of the initiating and approved Agency Programs <i>for Newman 4/24/77</i> TA/H: IMHoward	D. TA/RES: JERickson <i>JER</i> 5/2/77 TA/RES: MRchcigl <i>MR</i> 5/2/77
E. <i>RD</i> TA/H: RDNewman	F. TA/PPU: LWak field <i>LW</i> 4/29/77

16. For the cooperating country: The terms and conditions set forth herein are hereby agreed to	17. For the Agency for International Development <i>J. N. Gunning</i> Signature: John N. Gunning Title: Chief, TA/PPU/PPA	18. Date of Signature 5/1/77
Signature and date:		
Title:		

AID 1350-JX (9-70)	Cooperating Country TA/H, RDA-13 Research	PIO/T No.	Page 3 of 5 Pages
PIO/T	Project/Activity No. and Title 931-0001 Small Research Program (Biochemical Analysis of Merozoites)		

20. Equipment and Supplies (Related to the services described in Block 19 and to be procured outside the Cooperating Country by the supplier of these services)

A. (1) Quantity (2) Description

(3) Estimated Cost

(4) Special Instructions

See Budget attached.

B. Financing of Equipment and Supplies

(1) By AID - \$

(2) By Cooperating Country -

21. Special Provisions

- A. This PIO/T is subject to AID (contracting) (PASA implementation) regulations.
- B. Except as specifically authorized by AID, or when local hire is authorized under the terms of a contract with a U.S. Supplier, services authorized under this PIO/T must be obtained from U.S. sources.
- C. Except as specifically authorized by AID/W, the purchase of commodities authorized under this PIO/T will be limited to the U.S. under Geographic Code 000.
- D. Other (specify):

AID 1350-1X (9-70)	Cooperating Country TA/H, RDA-13, Research	PIO/T No.	Page 4 of 5 Pages
PIO/T	Project/Activity No. and Title 931-0001 Small Research Program (Biochemical Analysis of Merozoites)		

22. Reports by Contractor or Participating Agency (Indicate type, content and format of reports required, including language to be used if other than English, frequency or timing of reports, and any special requirements)

The contractor will provide, to the Office of Health, Technical Assistance Bureau, AID: a) 20 copies of the final report within 3 months after the expiration date of the contract in which she will incorporate detailed presentation of methods, significant observations and/or findings and recommendations; b) 50 copies of reprints of any scientific publication based on work under the terms of this contract. Such publication should acknowledge AID research contract support. Investigators are expected to make the results of their research promptly available to the scientific public.

23. Background Information (Additional information useful to Authorized Agent and Prospective Contractors or Participating Agency; if necessary cross reference Block 19.C(4) above.)

If the polar organelles are proven to contain immunogenic antigens, this method will be applicable to merozoites of Plasmodium falciparum and therefore will be incorporated in the research for an effective malaria vaccine.

24. Relationship of Contractor or Participating Agency to Cooperating Country and to AID.

A. Relationships and Responsibilities

B. Cooperating Country Liaison Official

C. AID Liaison Officials

E. A. Smith/J. S. Karam

CONTINUATION
SHEET

FORM SYMBOL

DEPARTMENT OF STATE
AGENCY FOR
INTERNATIONAL DEVELOPMENT

TITLE OF FORM

 Worksheet IssuancePAGE 5 OF 5 PAGES1. Cooperating County
TA/H, RDA 13, Research

2.o. Code No.

2.b. Effective Date

2.c. Amendment
 Original OR No: _____

3. Project/Activity No. and Title 931-0001

Small Research Program (Biochemical
Analysis of Merozoites)Indicate block
numbers.

Use this form to complete the information required in any block of a PIO or PA/PR form.

BUDGET CATEGORIES

1. Salaries with fringe benefits	
a. Principal Investigator	\$9,740
b. Assistant	5,340
	<hr/>
	15,080
2. Overhead of 61% for University	9,200
3. Animals, feed and supplies	4,000
4. Travel	400
	<hr/>
Grand Total	\$28,680

Initial Environmental Examination

Project Location: Rockefeller University, New York City

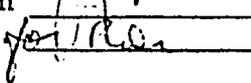
Project Title: Chemical and Immunological Characteristics of Polar Organelles of Erythrocytic Merozoites of Malaria

Funding: FY 1977 - \$28,680

Life of the Project: Five months

IEE Prepared by: Jalil S. Karam

Environmental Action Recommended: It is recommended that this project receive a negative determination and that no additional environmental examination be carried out.

Concurrence: TA/H/EH, James F. Thomson 
TA/H, Lee M. Howard, M.D. 

Examination of Nature, Scope and Magnitude of Environmental Impacts

Description of Project

This project is a small research project to characterize the biochemical constituents of the polar organelles of erythrocytic merozoites of malaria. It is sponsored by the Rockefeller University as a small research project and is a direct corollary to the work being pursued by several AID-supported research teams investigating malaria immunity and the development of a malaria vaccine.

This is a small research project that is carefully monitored by competent and well trained personnel and therefore does not require an environmental impact statement under the provision of AID Reg. 16, Part 216.2 (b).

The AID project manager will be in contact with the investigator during the life of the project to insure that the interest and purpose of this project are being faithfully carried out.

IMPACT IDENTIFICATION AND EVALUATION FORM

Impact
Identification
and
Evaluation 2/

Impact Areas and Sub-areas 1/

A. LAND USE

- | | |
|--|---------------|
| 1. Changing the character of the land through: | |
| a. Increasing the population ----- | _____ N _____ |
| b. Extracting natural resources ----- | _____ N _____ |
| c. Land clearing ----- | _____ N _____ |
| d. Changing soil character ----- | _____ N _____ |
| 2. Altering natural defenses ----- | _____ N _____ |
| 3. Foreclosing important uses ----- | _____ N _____ |
| 4. Jeopardizing man or his works ----- | _____ N _____ |
| 5. Other factors | |
| _____ | _____ |
| _____ | _____ |

B. WATER QUALITY

- | | |
|---|---------------|
| 1. Physical state of water ----- | _____ N _____ |
| 2. Chemical and biological states ----- | _____ N _____ |
| 3. Ecological balance ----- | _____ N _____ |
| 4. Other factors | |
| _____ | _____ |
| _____ | _____ |

1/ See Explanatory Notes for this form.

2/ Use the following symbols: N - No environmental impact
 L - Little environmental impact
 M - Moderate environmental impact
 H - High environmental impact
 U - Unknown environmental impact

C. ATMOSPHERIC

- 1. Air additives ----- N
 - 2. Air pollution ----- N
 - 3. Noise pollution ----- N
 - 4. Other factors
-
-

D. NATURAL RESOURCES

- 1. Diversion, altered use of water ----- N
 - 2. Irreversible, inefficient commitments ----- N
 - 3. Other factors
-
-

E. CULTURAL

- 1. Altering physical symbols ----- N
 - 2. Dilution of cultural traditions ----- N
 - 3. Other factors
-
-

F. SOCIOECONOMIC

- 1. Changes in economic/employment patterns ----- N
 - 2. Changes in population ----- N
 - 3. Changes in cultural patterns ----- N
 - 4. Other factors
-
-

G. HEALTH

- | | |
|---|-------|
| 1. Changing a natural environment ----- | N |
| 2. Eliminating an ecosystem element ----- | U |
| 3. Other factors | |
| _____ | _____ |
| _____ | _____ |

H. GENERAL

- | | |
|---------------------------------|-------|
| 1. International impacts ----- | U |
| 2. Controversial impacts ----- | N |
| 3. Larger program impacts ----- | N |
| 4. Other factors | |
| _____ | _____ |
| _____ | _____ |

I. OTHER POSSIBLE IMPACTS (not listed above)

_____	_____
_____	_____
_____	_____