1996 Project Abstract for the Period Ending June 30, 1997
This project was supported by the Minnesota Future Resources Fund

Project Title:	A Thousand Friends of Frogs
Project Manager:	Tracy Fredin, Director
Organization:	Hamline University, Center for Global Environmental Education (CGEE)
Address:	1536 Hewitt Avenue, St. Paul, MN 55104
Web Site:	cgee.hamline.edu/frogs
Legal Citation:	ML 1996, Chap 407, Sec. 8 Subd. 7
Appropriation:	$28,000

Statement of Objectives

This project focused on an investigation of the health of frog populations in the state of Minnesota and development of a prototype environmental education project looking at frogs as indicators of environmental health. There are three objectives of the project:

1. Assist the Minnesota Pollution Control Agency (MPCA) in monitoring the known sites of deformed frogs in Le Sueur County and the lower Minnesota River basin.
2. Coordinate an informal, state-wide frog monitoring project that involves youth from schools and other organizations gathering information on the health of frog populations.
3. Develop a prototype, state-wide environmental education project for youth in formal education settings.

Overall Project Results

AThousand Friends of Frogs has been a successful project. The combination of citizen monitoring and public education/outreach has had synergistic effect. The citizens and scientists of Minnesota are leading the nation in creating an awareness of amphibians as bio-indicators and problems associated with thier decline and deformity. Frogs have proven themselves to be key in engaging the public, educators, and youth in environmental education and awareness.

- The Ney Frog Project successfully monitored the Le Seuer County site in coordination with researchers from the MPCA. Deformed frogs and toads were found at these sites and the young people were valuable in determining when these frogs with deformities appeared.
- 10,000 newsletters and frog monitoring surveys were distributed through schools and organizations to youth statewide.
- Over five hundred data reports were received by CGEE.
- CGEE acted as a data gathering area and answered thousands of questions from a concerned public. Over five thousand people called in response to the citizen survey and related media outreach.
- Over 100 reports of deformed frog sites were forwarded to the MPCA. Twenty-two sites of deformed frogs were investigated by the MPCA and it was determined that 54 of Minnesota's 87 counties had reports of deformed frogs. The gathering of this data would have been very difficult and expensive for the MPCA to do without the assistance of the citizen volunteers who reported the deformities.
- 1,000 copies of an eight-page study guide were developed and distributed throughout the state affecting 25,000 students statewide.
- Conducted Frog Week, and on-line education experience from October 21-25 and continued project for several weeks due to popular demand.
- Presentations were made to over 25 schools and many community groups about frogs as bioindicators and the deformity issue. This provided direct contact with over 1,000 students and citizens.

Project Results Use and Dissemination

The project has been used by formal and nonformal educators in Minnesota and beyond. We have made presentations ranging from first graders to environmental law students. Materials from the study guide will be incorporated next year in the National Wildlife Federation's Ranger Rick NatureScope. Enclosed are copies of the newsletter/survey, study guide, video tapes of the program being features on national TV, newspaper coverage, student art work, and hard copy of some of our Web site. That address is cgee.hamline.edu/frogs.

LCMR 1996

July 18, 1997

LCMR Work Program 1996

I. Project Title and Project Number: Investigation of Deformed Frogs in Minnesota

Program Manager: Dr. Judy Helgen
Agency Affiliation: Minnesota Pollution Control Agency (MPCA), Water Quality Division
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A. Legal Citation: ML 96, Chp.407, Sec.8,Subd.7b
Appropriation Language: This appropriation is from the future resources fund to the commissioner of the pollution control agency to investigate the health of frog populations and evaluate the causes of frog deformities.
$28,000 of this appropriation is for a grant to the Center for Global Environmental Education at Hamline University to be used to work with schools and other organizations, including the study of frogs as environmental indicators.

B. Total Annual Project Budget: $151,000
Budget: $123,000 (PCA)
Balance: $32,927
Budget: $28,000 (Hamline)--on separate workprogram
Balance: $0

C. Status of Match Requirement: None required.
Amount committed to date: $151,000

II. Project Summary: In 1995, the MPCA received a number of reports of deformed frogs in several locations in Minnesota. Collections from four sites and necropsies have confirmed the serious nature of these deformities, both external and internal. Gross contortions and reductions in rear legs, extra legs, missing eye, as well as possible reduced testis size and abnormal bladder, were some of the abnormalities seen. For this project, an investigation of sites which had deformed frogs, including reconnaissance of reported sites and chemical analysis of sediments, water and frog tissue will be done. On-site and laboratory rearing of eggs from reproducing adults using site and control sperm will determine whether the site conditions are causing deformities or they are the result of something carried within the eggs or sperm. To determine whether there has been genetic damage in the abnormal vs. control frogs, DNA flow cytometry for cell DNA content and chromosome analysis will be done. Long-term breeding experiments will be initiated to determine if the defects are transmitted to offspring. This work needs to begin in spring of 1996 to allow study of reproducing populations of leopard frogs in May and larval stages in June. To determine whether the agent is causing damage to the ovaries or testes, necropsy combined with histopathological analysis will be done.

III. Twelve Month Work Program Update Summary:
(Hamline University Thousand Friend of Frogs 1996 Project abstract and Final Work Plan Update Report July 1, 1997 are attached separately along with a copy of the looseleaf binder titles A Thousand Friends of Frogs Educator Activity Guide).

The work outlined in this work program in near to completion. The chemical analysis planned by MPCA in A2 and C3 on sediments and frog tissues were unexpectedly delayed when the analytical contract laboratory went into bankruptcy and new contracts had to be formed. These are in place, and frog tissues and sediment samples are at the contract analytical laboratories now and the results should be returned to MPCA by end of August, 1997. This data will be very important in directing future analysis of tissues and sediments in the next round of work for 1997 under separate funding to MPCA. Samples of both normal and abnormal frogs taken from sites having abnormal frogs are being analyzed as well as samples of normal frogs from control sites. Most of the samples are the young of the year frogs, but some samples of adults are being analyzed also. Comparisions of tissue level concentrations in the juvenile vs the adult frogs may be informative: previous work on the frogs of the Granite Falls area showed differential concentrations of certain metals between young and adults, in some cases the young had lower levels of metals, in other cases the levels were higher in the juveniles.

Necropsies were carried out on 157 leopard frogs from five of the study sites by Dr. McKinnell and Dr. Helgen at MPCA. Examinations of internal organs and lesions were made by Dr. McKinnell (see his summary below). Data need to be compiled. These frogs are included in the frogs sent for tissue analysis in June, 1997.

The frequency of abnormalities in the frogs collected by MPCA (LeSueur, Meeker, Polk, Becker sites and one visit to Crow Wing sites), McKinnell (Ottertail County, one Meeker visit) and Hoppe (Crow Wing County and see his work summary below for other sites) showed a wide range of abnormalities in the leopard frog populations. Dr. Hoppe has found abnormal frogs in five species of frogs and toads. In 1996 there were important seasonal differences in the frequencies of abnormalities such that in the Le Sueur County site (Ney farm) there was only 1% abnormals in July, and 46% abnormals in late September. At the Meeker County site, the opposite was true: 66% abnormal frogs in early July, dropping to 20% in October. In other sites the frequencies were not very different during the season (Polk, Crow Wing) or dropped from 43% to 24% (Becker). This information is very useful in planning future frog investigations. It means that one site visit is not enough to confirm whether a site has abnormal frogs, that in some sites the deformed frogs may be late developing, in other sites there may be a large number at first and significant drop as the season progresses. We are recommending that a minimum number of 100 frogs be examined and returned to the site at each visit. One curious observation was that, apparently, frogs with extra legs did not appear at the Le Sueur County site (Ney) until October. MPCA ceased sampling that site Sept. 30. The report was confirmed only verbally with the teacher Cindy Reinitz.

The extent of frog deformities in 1996 was much greater and more widespread than expected at the time of the proposal in the spring of 1996 by MPCA. Citizen, scientific and media interest

was overwhelming and continues even now, with national and international interest in Minnesota's deformed frogs.

Reports of deformed frogs in Minnesota have been received from MPCA from DNR and USFW staff, biologists, teachers and numerous citizens from approximately 54 of Minnesota's 87 counties. Of the 164 reports of abnormal frogs for 1996, 102 were reports of more than one abnormal frog observed. Twenty-one sites in 14 counties have been confirmed to have deformed frogs. The distribution of abnormal frogs appears to be statewide, with the exception of the far northwest and far southwestern areas.

During the summer and fall of 1996, MPCA established four focal areas in the state for research on deformed frogs. Each focal area consists of one or two sites with deformed frogs and one 'reference' site, as defined by having normal frogs. The focal areas are in northwestern MN, i.e., Polk and Becker Counties, and in Crow Wing County, Meeker County and LeSueur County. There was some difficulty in finding reference sites using the operational definition that reference sites had normal frogs.

At each site the frog populations were surveyed, abnormalities were recorded, and frogs were measured. Collection was done until a minimum of 100 frogs was sampled. The affected sites were resurveyed in September in order to establish frequencies of abnormalities at the end of the season, some sites were surveyed three or more times through the season.

Both normal and abnormal frogs were collected for the purposes of the DNA and chromosome studies, for histopathology, necropsy and parasitology. These frogs were delivered by MPCA to Dr. McKinnell's laboratory. Frogs totaling at least 30 g wet weight were collected in chemically clean jars and preserved on dry ice and kept frozen for tissue analysis at MPCA. In addition, MPCA archived frogs in 10% formalin from each site.

From each site, sediments were collected by taking 3 cores, mixing and pooling to one sample using appropriate clean techniques and equipment for metals (plastic core tube) and organic chemicals (metal core tube). The samples were frozen in chemically clean jars at MPCA for analysis. Water was collected in jars provided by the MN Department of Agriculture from each site, and analyzed for the standard base neutral herbicides and acid herbicide lists of chemicals.

In 1996, deformed frogs were observed in six species of frogs and toads, primarily *Rana pipiens,* the Leopard Frog, but also in *Rana septentrionalis,* the Mink Frog, *Rana sylvatica,* the Wood Frog, *Bufo americanus,* the American Toad, *Pseudacris crucifer,* the Spring Peeper, and one of the Grey Tree Frogs, *Hyla chrysoscelis* or *Hyla versicolor*. The majority of the abnormalities were in the rear limbs, leg missing, leg a short stump, leg shortened or distorted, foot abnormal, but a few had other abnormalities such as abnormal front leg, eye abnormalities, and extra limbs or branches in limbs. The latter abnormalities, seen more commonly in 1995 in Leopard Frogs, were rare in Leopard Frogs in 1996, but did occur in one population of Mink Frogs.

The field component of this project was completed in 1996. Analysis is in progress on the DNA flow cytometry (Dr. McKinnell) and the frog chromosomes (Dr. Carlson). The contract is being formed for the sediment and frog tissue analysis (MPCA). Data has been received on the water

chemistry analysis by Dept. of Agriculture and is being analyzed. The *in situ* reproduction caging experiments were done and results are being analyzed (Dr. Hoppe). Necropsy is planned on the frozen frogs prior to their shipment for tissue analysis (McKinnell and MPCA).

Some parasitology analysis has been done by Dr. Steven Goldberg for Dr. McKinnell, frogs from three of the sites have been analyzed. Results are being processed. The remaining focal area sites need to be analyzed.

To date, some histopathology examination has been done, but the frogs from the focal area sites of 1996 remain to be examined. These results will be of critical importance in that the particular kinds of internal abnormalities may offer clues as to the causative agent(s).

In addition to the analyses above, MPCA has presented preliminary findings to a variety of meetings, including the September conference on the frog problem in Duluth organized by EPA, EPA Region 5 and other staff in Chicago, MN Environment Partnership, MN Department of Agriculture, Hatfield Marine Science Center (Oregon State U.), MN Department of Health, DNR Nongame Program staff and others. There have been uncountable interviews with news media, such as Channel 2 Almanac, Nightly News, Newton's Apple and Lehrer News Hour, the BBC TV news, Dan Rather and Tom Brokaw news, Good Morning America and Today Show, Washington Post, AP and other newspapers, radio interviews, e.g., Public Radio Living on Earth and local MPR, and recently, the Voice of America.

MPCA has provided information to citizens and media people, has received thoughtful suggestions and made contact with a wide number of scientists nationally. Numerous talks have been given at meetings, such as the Environmental Mutagenesis Society in April (MPCA, Dr. Carlson). MPCA hosted several staff people from the National Institute of Environmental Health Sciences (NIEHS) for background on Minnesota's frog problem. MPCA has established a cooperative relationship with the USGS/National Biological Health Service (NBS) National Wildlife Health Center Laboratory in Madison, WI. They became involved when deformed frogs were found at a national wildlife refuge. They are working up some frogs from MN in their diagnostic lab and their results will assist this investigation. An overview of their results showed that, of the few frogs examined from MN, there was no evidence of bacterial or viral disease and no clear evidence that parasites were implicated. Their histological examination suggested possible problems with bone formation, not just in the limbs but possibly in the vertebral column.

Dr. Hoppe, U. Minnesota Morris work program update June 30, 1997
A.1 Site visits: 1996 visits of sites where deformed frogs had been reported in 1995.

Surveys were done in 4 counties at 5 sites where deformed leopard frogs had been reported in 1995. Each site was visited from 2-7 times at dates encompassing pre-metamorphosis (spring, early summer) and post-metamorphosis (late summer, fall). Data obtained include description of site, surrounding land use, interviews with residents, and frogs captured and/or observed. Data on captured frogs include species, sex, age, color and pattern phenotype, and both descriptions and photos of any deformities noted. Some frogs were saved for DNA analyses, karyotyping, or

parasite surveys; most were released on site. Data reveal that significant numbers of deformed frogs were found in all but the Meeker Co. site.

County	Site	Dates visited
Le Sueuer	LSN	4/30, 5/3, 5/10, 6/10, 6/26, 7/1
Le Sueuer	LSB	4/30, 5/3, 5/10, 6/20, 7/1, 7/24, 8/27
Pope	PTB	5/4, 5/13, 5/15, 5/22, 6/6, 7/16, 8/13
Crow Wing	CWB	5/21, 6/19, 7/6, 7/11, 7/19, 8/1, 9/12
Meeker	MRR	5/28, 6/26

Frog population surveys:
Frog populations were surveyed through 52 site visits to 21 sites in 12 counties. Five sites were populations where deformed frogs had been reported in 1995 (see A.1), 8 sites were populations for which investigators Hoppe and McKinnell had substantial pre-1995 data showing no deformities, 2 sites were selected for their close proximity to "focal" deformity sites (see A.1), 3 sites were follow-up visits to 1996 citizen reports of abnormal frogs, and 3 sites were selected at random. Five sites were surveyed both soon after metamorphosis (July) and later in the season (Sept. or Oct.).

Surveys were done by "search-and-seize" methods, 2-4 individuals walking shorelines or feeding habitat and capturing frogs with nets. Data were recorded on site, including species, age, sex, phenotypic information specific to species, length, and whether various abnormalities were noted. All abnormal frogs were photographed. All frogs except as noted in A.1 were released on site.

Data reveal considerable variation in deformity frequencies, ranging from 0 to 50% deformed 1997 metamorphs. Deformed frogs were found at 1 to 5% in several populations for which pre-1996 data recorded no deformities.

In situ egg analysis
Egg mass analysis and rearing trials were done on 8 egg masses from 4 sites, 2 masses each from deformed frog sites in Le Sueuer (leopard frog), Pope (leopard frog), and Crow Wing (American toad) counties, and 2 from a control site in Douglas county (leopard frog). Masses were located by wading the breeding ponds and about 50 early embryos from each mass were left to develop in enclosures in the ponds. The remainder of each mass was taken to the lab and scored for % fertilization, % abnormal embryos, and hatching success. Then about 40 hatchling tadpoles from each mass were reared to metamorphosis in the lab while the remaining tadpoles were released back in the ponds where they were collected. Juveniles metamorphosing in the pond enclosures and lab aquaria were subsequently scored for presence of externally visible abnormalities and preserved for future analyses.

Data reveal considerable variation in egg mass quality. Formal statistical analyses need to be done yet, but variances are likely too high to detect significantly different masses or sites with this sample size.

In vitro ovulation and fertilization

These trials were not completed. Mature frogs would have had to be collected and in vitro matings set up early in the breeding season, well before the May 20 funding of this research. A few trials were attempted with late-collected frogs and frogs that had been refrigerated since mid-April, with very poor fertilization success. Unused funds allocated for these trials went to increased field survey efforts (section A.3 above).

Heritability tests
Deformed frogs were collected and are currently being lab-reared/maintained for these trials. Two mildly deformed adult leopard frogs were lab-maintained for mating trials. Twelve mild to moderately deformed juvenile leopard frogs were collected for lab-rearing for mating trials to be attempted in March, 1998, or as soon as they achieve reproductive maturity in captivity. The more severely deformed of these have died, and 8 deformed subadults remain to be reared to maturity.
One round of matings (normal male x normal female, normal male x deformed female, deformed male x normal female, and deformed male x deformed female is in progress. Tadpoles currently have early hind limb formation (ca. 1mm length) and show no visible deformities. They will be reared through metamorphosis. It should be noted that these are adults collected in 1996, which show very mild deformities.

Dr. McKinnell twelve month workprogram update, June 30, 1997
Dr. McKinnell collected frogs with Dr. Hoppe, with MPCA and by himself in 1996. Some of the frogs were used in the study of anatomical abnormalities, flow cytometry for DNA content, chromosomes (Dr. Carlson) and parasites. Parasite work was part of a collaborative effort with Dr. Steven Goldberg, a parasitologist at Whittier College in California. This effort involved surveys of the parasites of the Dakotas and Minnesota. Other frogs collected and frozen unfixed by MPCA were dissected and necropsied at MPCA by Dr. McKinnell with Dr. Helgen. Some samples from these frogs were sent for parasite analysis, most were sent by MPCA for tissue analysis.

A total of 513 frogs have been examined. The frogs were collected at various sites from the following counties: Becker, Otter Tail, Crow Wing, Polk, Le Sueur, Clearwater and Meeker. Internal abnormalities were catalogued. A thinning of the intestinal wall extending from the stomach to the cloaca was the most common condition detected. Small and transparent or nearly transparent testes were observed. A peculira condition, never seen before by Dr. McKinnell, was urinary bladders full of intact insects or insect parts. The bladder condition was found exclusively among the Henderson frogs with but one exception which was a Crow Wing County frog which seemed to have the condition but to a much reduced extent. Representative tissue fragments were preserved for histological analysis.

Flow cytometric analysis was undertaken on blood samples from 60 frogs. Fresh blood was taken in a syringe containing a small amount of heparin. This was centrifuged and washed with an isotonic electrolyte solution. The washed cells were suspended in propidium iodide and then mixed with the enzyme RNAse. These cells were examined in a flow cytometer. Data from this study is not clear, and there are no plans to continue this aspect of genetic analysis.

Chromosome studies were done by collaboration of Dr. McKinnell with Dr. Debra L. Carlson, a cytogeneticist at Augustana College in Sioux Falls, SD.

Dr. Debra L. Carlson, twelve month workplan update, June 30, 1997

Dr. Debra L. Carlson, twelve month workplan update, June 30, 1997

The physical abnormalities exhibited by frogs in Minnesota may result from genetic damage. To ascertain whether this is likely, the chromosomes of both normal and abnormal frogs of two frog species were examined. Peripheral blood was collected from juvenile and adult northern leopard frogs (Rana pipiens) and mink frogs (Rana septentrionalis). The blood was cultured in the presence of colcemid to block white blood cells in mitosis, and the cultures were treated with hypotonic solution, fixed in methanol:acetic acid (3:1) and dropped onto slides. The slides were treated with Giemsa to stain the chromosomes and with silver to show regions of ribosomal DNA (N-bands). Chromosome spreads were examined in terms of number of chromosomes per spread, morphology of chromosomes and number of silver-stained chromosomes per spread.

Frogs for chromosomal analysis were selected by and obtained from Robert G. McKinnell of the University of Minnesota. These animals were collected from eight sites (at least ten frogs per site with the exception of the Vermont collection, which consisted of three frogs): Nye, Bock, Meeker, Shawn-Hyde, Reiger, Doran, Roigren-Dassel, and Vermont. The Bock site actually served as three sites since ten normal mink frogs and ten grossly deformed mink frogs were analyzed in addition to the leopard frogs. Thus far, countable metaphase spreads have been obtained from forty animals, of which twenty-one displayed some sort of deformity (mild to severe). Up to 100 countable spreads have been recorded for each animal. The chromosomes from eleven (mostly deformed) frogs have yet to be analyzed. A report of that additional data will be submitted in the near future.

To date, the chromosomes from deformed Minnesota frogs do not exhibit any deviation from the normal number (2N = 26), the normal morphology (metacentric and submetacentric chromosomes) or the normal number of N-banded chromosomes (2 per 2N set). Not surprisingly, the chromosomes of normal-appearing frogs taken from the same sites are also normal in terms of number, morphology and N-bands. These data, however, do not preclude the occurrence of more subtle genetic alterations which are not discernible at this level of analysis.

IV. Statement of Objectives:

A. Site analysis: Reconnaissance of sites where deformed frogs have been reported, analysis of frog populations, and testing of sediment and water chemistry in selected sites will help rule out or suggest potential causes and will show if there is recurrence of deformities in 1996.

B. Reproductive health: Analysis of reproductive health in this year's reproducing frogs both in the breeding pond and laboratory with sperm from males from the site and from control areas will show if the site itself is causing the abnormalities.

C. Deformity analysis: Analysis by necropsy will show the pattern of external and internal deformities, whether there are tumors presence, and parasitic infections that might relate to the deformities. Analysis of frog tissues by histopathology, especially of the internal organs, e.g., testes will show if there is both reduced testis size and sperm production. The patterns of the deformities may offer clues as to causes.

D. Genetic analysis: Analysis of changes in DNA content and of the shapes of the cell chromosomes will show if there has been gross damage in the genetic material. Longer term breeding experiments will be initiated to determine eventually if there is genetic transmission of the abnormalities.

E. Data analysis, synthesis and reporting. A review and synthesis of the findings will be incorporated into a report.

Time Line for Completion of Objectives:

	5/96	7/96	9/96	1/97	6/97
A. Site analysis	**********				
B. Reproduction	**********	**********	**********		
C. Deformity		**********	**********	**********	
D. Genetic		**********	**********	**********	**********
E. Data synthesis/report				**********	**********

V. Objectives/Outcome:

A. Site analysis

A.1 Site visits: sites where deformed frogs will be visited; a preliminary evaluation of the site will be made; surrounding land use and general habitat will be recorded; overwintering sites within reach frogs migrating from the site will be sought; there will be discussion with citizens who reported the deformed frogs and permission will be obtained for further work.

A.2 Site analysis: water and sediment chemistry will be analyzed at the site and at some of the overwintering sites. This will be done at the time during or after the frog larvae have hatched from the egg masses at sites that have had high numbers of deformed frogs. About four affected sites and two control sites will be analyzed for heavy metals, PCBs and pesticides.

A.3 Frog population surveys: frogs coming to the site will be surveyed in late spring; young newly emerged frogs will be surveyed in summer to verify deformities are occurring at the site. At major sites, sufficient numbers of deformed frogs will be collected into clean jars and frozen on dry ice for tissue analysis for contaminants. Frogs will be collected and preserved in formalin for deformity analysis (Objective C). Frogs of similar species and age will be collected from control areas known to have no history of deformities. These may need to be from outside the state of Minnesota.

B. Reproduction analysis

B.1 In affected sites, egg masses laid by frogs in the site will be divided and one portion will be caged at the site, the other portion will be placed in clean water in the laboratory. This can show whether the breeding site water causes deformities.

B.2 Some females will be removed from the site to the laboratory before they have laid their eggs, so their eggs can be fertilized by site sperm and control sperm to test for sperm effects. Control females will be analyzed at the same time. The same reproduction endpoints given in the second sentence above will be measured.

B.3 Examination of gonads (see C.2 below).

C. Deformity analysis

C.1 Necropsies will be done to evaluate the extent and nature of the deformities, both internal and external, including morphological abnormalities, tumors and parasites or other infections.

C.2 Histopathological analysis will be done to confirm these are developmental deformities, to assess whether the ovary or testes are reduced in size or production of eggs or sperm in relation to control, normal frogs of the same age. Histopathological analysis will assess other internal deformities, for instance an apparent bladder deformity.

C.3. Frog tissue analysis of contaminants in deformed frogs compared with normal frogs will show what might have caused the deformities.

D. Genetic damage analysis

D.1 DNA content per cell is constant in normal cells. DNA content will be analyzed by flow cytometry to determine if there are gross changes, a sign of genetic damage. This is done on the blood cells from the frogs.

D.2 Chromosome analysis will be done on of frogs from sites including some control sites. Analysis of the shapes of the chromosomes for aberrations will show if gross genetic damage has occurred.

D.3 Heritability of the abnormalities will be determined eventually by breeding experiments. These will be set up as cross-matings of deformed females and males from affected and control sites. In this project time period, the frogs will be reared to sexual maturity. The breeding experiments will take place beyond the project end date.

V. Objectives/Outcome

A. Site analysis

A.1 Activity: Site visits. Reconnaissance of sites where deformed frogs have been reported will be done.

A.1.a Context: This will describe the sites that had deformed frogs and help establish the common features and distribution pattern of sites with deformed frogs in the state.

A.1.b Methods: Sites reported to MPCA as having deformed frogs in 1995 or 1996 will be visited and a preliminary analysis of the site will be made: habitat and land use, type of wetland, if excavated or created. Information on historical land use and previous frog populations will be obtained from local residents. Potential frog overwintering sites will be investigated. Permission to sample and collect frogs, water and sediments will be obtained from landowners. Wetland classification will be based on Cowardin et al, 1979, Classification of Wetlands and Deep Water Habitats of the United States, vegetation will be referenced to Ownbey and Morley, 1991, Vascular Plants of MN. Reference or control wetlands will be selected using the US EPA's criteria for selection of reference sites (minimal disturbance, no nonpoint pollution, in publicly owned land) plus the criterion that deformed frogs are not found at the control site.

A.1.c Materials: Film, diskettes, maps, air photos $800.00

A.1.d Budget: Materials MPCA $800.00, Travel MPCA $400, film $100, MPCA staff $3,911, 4 weeks; Salary DH $500, Travel DH $700 Total: ~~$6311~~ $6411

A.1.e Time Line:

	May 96	Sept	Jan 97	May
Product 1, visit 1995 sites	**********			
Product 2, visit 1996 sites		**********		
Report of site features			**********	

A.2 Site analysis:

A.2a Activity: Site analysis. Selected sites which had deformed frogs and control sites will be analyzed for water and sediment chemistry for pesticides, PCBs and heavy metals. This will show which contaminants the frogs or larvae were exposed to from the sites.

A.2b Methods: Field collections of sediments for analysis of metals will be made with properly clean plastic core tubes. The top 5 cm of three cores taken in the sediment of each wetland will be pooled and analyzed. Standard EPA approved protocols will be used by a certified analytical laboratory for analysis of mercury, selenium, cadmium and arsenic and total analyte list of heavy metals. Field collections of sediments for pesticides and PCBs will be taken with a properly clean metal core tube with pooling of three cores as above. Water samples for pesticide analysis will be taken with properly clean bottles provided by the testing laboratory. At the time the samples are taken, routine field measurements of pH, temperature, conductivity,

dissolved oxygen, time of day, and calcium will be taken. Four sites with deformed frogs and two control sites will be analyzed. If other sources of funding becomes available, chemical analysis will be done on overwintering sites.

A.2c Materials: MPCA $700

A.2d Budget: Materials $700, Travel MPCA $400, Salary MPCA $3911 (4 weeks), Chemical analysis, 6 sites $12,135

Total $17,146

A.2e Time Line:

	May 96	Sept	Jan 97	May
Collect samples	**********			
Chemical analysis		**********		
Data analysis			**********	

A.3 Frog population surveys:

A.3a Activity: Surveys of frog populations for deformities will show whether the deformities are recurring in sites reported in 1995 and will show the extent and nature of deformities appearing in 1996, if they recur. Populations near the sites where deformities were reported along the Minnesota River and in west-central MN will also be surveyed. This will establish the extent of deformities in 1996.

A.3b Methods: A minimum of 100 frogs will be collected, examined, and released at about six sites where deformities were reported in 1995, and a minimum of 100 frogs from 15 nearby and reference sites will be similarly assessed. Deformities will be recorded, frogs will be measured, weighed and photographed. Normal frog populations will be measured for comparison. Because the deformed frogs are slower and more erratic, there will likely be an unavoidable bias towards their capture. Likewise, the extent of mortality that has occurred before the site is surveyed cannot be avoided. The investigators will use consistent methods and effort in each site, with the understanding that a true population census will be difficult.

A.3c Materials: Nets $100, Calipers $50, Film $100, Field balance $200

A.3d Budget: Materials $450, Salary DH $5000, UMM student $1000, travel $1200

Total $7650

A.3e Time Line:

	May 96	Sept	Jan 97	May
Frog surveys	**********			
Write-up results			**********	

B. Reproduction analysis

B.1 *In situ* egg analysis

B.1a *In situ* egg analysis will be done by caging portions of eggs at the site and removing other portions to the laboratory for rearing. This will show if the deformities are caused by the conditions in the breeding pond.

B.1.b Methods: Four egg masses each will be studied from at least one affected and one control site in cages on site and in the laboratory. Each mass will be divided into a small sample of about 50 eggs to develop on site in screened enclosures. If it appears that supplemental feeding will be necessary, material from the site will be used. Another portion of the egg mass will be transported to UMM. The laboratory masses will be completely scored for percent fertilization, percent and type of abnormal embryos, and hatching success. From each set of

hatchling larvae, 25 will be raised through metamorphosis in clean lab water with clean pre-tested commercial food, the others will be released back on site. The lab-reared larvae will be scored for normal vs. abnormal leg and eye development and appearance of metamorphosed juveniles. The site-reared larvae will be observed periodically by netting samples to score for leg and eye developments, and appearance of metamorphosed juveniles. All larvae and metamorphs will be preserved for later necropsy.

B.1c Materials: Pond enclosures $300, Aquaria $300, Rearing supplies (food, aerators, etc.) $150

B.1d Budget: Materials $750, Salary DH $6500, UMM student $1500, travel $900
Total: $9650

B.1e Time Line:

	May 96	Sept	Jan 97	May
Cage egg masses	*******			
Analyze caged larvae	********			
Lab rear egg masses	**********			
Analyze lab-reared larvae	**********			
Data analysis, write-up			**********	

B2. Reproduction analysis:

B.2a Laboratory egg and sperm analysis: This work will demonstrate whether the deformities are carried by the sperm or eggs.

B.2b Methods: A minimum of four mature females will be taken to the laboratory from one site that had deformed frogs and induced to ovulate. It is not expected that mature deformed females will be found, but if they are they will be used in the study. As many females as can be managed will be induced, given the late spring startup of this project. Because Leopard Frogs lay eggs in early May, this work may need to be carried out also in May 1997, in which case additional funding may be sought to complete the work. Egg masses will be divided and fertilized with sperm from males from the affected site and males from a control site. Four females from one control area will similarly be fertilized with sperm from four males from the affected site as well as four control males. Eggs will be scored for % fertilized, % and types of abnormal embryos, hatching success, and % and types of abnormal hatchlings. Twenty-five larvae from each set will be reared to metamorphosis to score for deformities.

B.2c Materials: Aquaria $200, Rearing supplies $200

B.2d Budget: Materials $400, Salary DH $2500, UMM students $2000, travel $200
Total: $5100

B.2e Time Line:

	May 96	Sept	Jan 97	May 97
Induce females	**********			
Score larvae	**********			
Analyze data		**********		

C. Deformity analysis:

C.1 Necropsies

C.1a Necropsies of deformed and normal frogs will evaluate the nature and extent of the deformities, both internal and external and show whether tumors, parasites or other infections are present.

C.1b Methods: Necropsies will be done with standard visual inspection of external and internal tissues of the frogs. This will be done at the time that frog tissues are dissected for contaminant analysis. It is anticipated that all preserved frogs of this study will be subjected to postmortem dissection.

C.1c None needed.

C.1d Budget: Salary McKinnell, $3225, Junior Scientist, $2635 Total $5860

	May 96	Sept	Jan 97	May
Necropsy 1995 frogs	********			
Necropsy 1996 frogs		**********		
Data analysis, writeup			**********	

C.2. Histopathological analysis:

C.2a Histopathological analysis will show whether the deformities are not injuries, and whether the apparent reduction in testis size seen in many of the deformed frogs is real. Internal organs, especially the liver, will be studied for pathological changes. If liver or some other organ is revealed to be a target of toxic insult, more attention to that organ in a greater number of animals will be paid.

C.2b Methods: Tissues will be fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with H&E. This will be done at University of Minnesota School of Veterinary Medicine. The histological analysis will be performed by Dr. McKinnell and the Junior Scientist under his supervision. In addition, the testes will be examined to see if the apparent smaller size in deformed male frogs is reflected in reduced sperm numbers in comparison to work done on normal frogs of the same age class. Ovaries will be similarly analyzed. Parasites will be identified. Other abnormalities will be examined.

C.2c Materials: None.

C.2d Budget: Histology, $2,500; Junior Scientist, $3,815; McKinnell, $3225.
Total: $9,540

C.2e Time Line:

	May 96	Sept	Jan 97	May
Tissue preparation		**********		
Tissue examination			**********	
Data analysis				********

C.3 Frog tissue analysis

C.3a Frog tissue analysis of deformed frogs compared with normal frogs will show what contaminants are elevated in deformed frogs and might have caused the deformities.

C.3b Methods: Frogs will be collected and measured in the field, placed in chemically clean jars and frozen on dry ice in the field. Frog livers will be analyzed for heavy metals and frog carcasses for pesticides and PCBs. This will be done on pooled samples of deformed frogs from at least four affected sites and frogs from at least two normal sites. Tissues will be sent frozen in chemically clean jars to a certified laboratory for testing pesticides, PCBs and heavy metals with separate analysis for mercury, selenium, cadmium and arsenic.

C.3c Materials: $400

C.3d Budget: Materials, $400; Salary MPCA $1954 (2 weeks); Travel MPCA $800; Tissue chemistry, $13,255 (9 composite samples) Total: $16,409

C.3e Time Line:

	May 96	Sept	Jan 97	May
Frog collection	**********			
Frog dissection		*********		
Tissue analysis			**********	
Data analysis				**********

D. Genetic damage analysis

D.1 Cell DNA content.

D.1a The DNA content of normal postmitotic cells is constant for a species. Quantitative variation of DNA in non-cycling cells is an indication of genetic damage. The effects of toxic chemicals which affect genomic DNA, i.e., genoclastic substances, can be detected with flow cytometry.

D.1b Methods: Frog red blood cells are nucleated and are suitable for characterization with flow cytometry. Washed blood cells are stained in the dark with propidium iodide, mixed with RNase, and analyzed in a flow cytometer. Dr. McKinnell has experience with the EPICS 753 flow cytometer located in the School of Veterinary Medicine where most of the work will be done. Up to 10 frogs can be characterized per hour with this procedure. We expect to study 20 frogs per site where confirmed abnormal frogs have been taken, and the same number at sites not known to have abnormal frogs. No less than 10 sites, including normal and control, will be studied.

D.1c Materials: None

D.1d Budget: McKinnell salary $1,450; DNA flow cytometry, $3,000; Student assistant, frog care $3,120; McKinnell travel, $2,160 Total $9,730

D.1e Time Line:

	May 96	Sept	Jan 97	May
Sample collection	**********			
DNA flow cytometry	**********			
Data analysis		**********		

D.2 Chromosome analysis

D.2a Chromosome analysis will show if there is gross damage to the genetic material, i.e. additions or losses of chromosome parts, other major changes.

D.2b Methods: Cytogenetic (chromosome) analysis will be done on 10 frogs from each of the 10 sites as per D.1 above. Our laboratory developed a short term culture procedure used for the detection of mutagen-induced cytogenetic aberrations. The procedure to be used consists of treating cultured frog cells with a hypotonic solution of KCl. The cells are fixed in cold methanol-acetic acid, dropped onto glass microscope slides and flamed. The dried slides are stained with Giemsa and examined with the light microscope. Dr. McKinnell showed in earlier studies that chromosomes of frogs exposed to a genotoxic substance were characterized by rings, dicentrics, acentric fragments, and complex exchange figures. The chromosome aberrations were dose dependent. Frogs are exquisitely sensitive to genetic damage. In the present study, we will compare the cytogenetic profile of both normal and abnormal frogs.

D.2c Materials: $7,000 (culture supplies, photography, chemicals)

D.2d Budget: Materials, $7,000; Salary DC $4,810; Asst. $3,850; Travel $750
Total $16,410

D.2e Time Line:

	May 96	Sept	Jan 97	May
Sample collection	**********			
Cell culture	**********			
Cell staining	**********			
Chromosome analysis	**********			
Data analysis			**********	

D.3 Heritability test

D.3a Heritability test: Long-term breeding experiments will show if the deformities are genetically transmitted to offspring.

D.3b Methods: Six each of male and female juvenile frogs showing abnormalities will be collected from affected sites during the summer of 1996. They will be reared under laboratory conditions until sexual maturity, then mated *in vitro* both among themselves and cross-mated with normal frogs purchased out-of-state. Because sexual maturity normally takes 2 years, this grant period includes only rearing and hibernating the frogs from summer 1996 through June 1997. The matings will occur in April 1998.

D.3.c Materials: Rearing cages $150, Live food, insect culture media, and supplements $250

D.3.d Budget: Materials $400, Salary DH $500, UMM student $500

D.3e Time Line:

	May 96	Sept	Jan 97	May 97
Rear young frogs	**********	**********	**********	**********

E. Data analysis, synthesis, reporting

E.1 Data analysis, synthesis: Deformity occurrence data will be prepared as a map showing locations of confirmed findings of deformed frogs, with frequencies of deformities where available. Water and sediment chemistry will be analyzed in relation to levels expected as background and control sites data. Tissue chemistry data will be analyzed in relation to control frog tissue chemistry and to the presence or absence or extent of deformities at each site. The DNA content data and the cell chromosome damage data will be related. Lab-reared tadpoles and froglets will be compared to site-reared individuals with respect to occurrence of deformities. Egg mass quality of affected sites will be compared to control sites as well as to egg mass data from a variety of sites done in previous studies.

E.1b Methods: A comprehensive report will be written with analysis and interpretation of all the results. Standard Quality Assurance plans will be followed for chemical analysis, and standard statistical procedures will be used. A statistician at the University will be consulted on all data gathering protocols and on the results of flow cytometry. Data will be integrated in standard format at MPCA.

E.1c Materials: $100

E.1d Budget: Materials $100; Salary MPCA $15,644; Print, rent $2,500; Travel MPCA $700. Total ~~$18,944~~ $17,694

E.1e Time Line:

	May 96	Sept	Jan 97	May
Results of site surveys			**********	
Chemistry, tissue results			**********	
Histopathology, necropsy			**********	
Reproduction analysis			**********	
Overall report				**********

VI. Evaluation: The results of this project will report the extent and locations of deformed frog populations in Minnesota in 1996, the nature of internal deformities and whether there are effects on the gonads, whether the genetic material is abnormal, and which contaminants are in a set of affected sites and in frog tissues. This information will suggest which agents may have caused the deformities.

VII. Context within field: This project will represent the most thorough analysis of deformities in frog populations in the U.S. In Canada there is an ongoing investigation of the incidence of deformed frogs in agricultural and 'cultural' areas in Ontario and Quebec. There is a need for understanding causes of the global decline in amphibian populations and this project will contribute to that. This work adds to the ongoing research by Dr. Hoppe and Dr. McKinnell on the condition and status of frog populations in Minnesota. It adds to the work of Dr. Helgen and MPCA in developing biological indicators of wetlands and river condition.

VIII. Budget context: No funds were spent on this project during the period ending June 30, 1995. In the 2-year period beginning July 1, 1995, federal funding to MPCA of $24,300 has supported staff time and limited tissue analysis. The work done in 1995 on the preliminary investigation done by Dr. McKinnell was on a voluntary basis. Sediment and water chemistry analyses done in 1995 were donated.

IX. Dissemination: Project results will be disseminated through scientific meetings, through publications and distribution of the final report, by presentations to a variety of organizations, and communications to media, citizens, school students and scientists by telephone and email.

XI. Cooperation: Dr. Robert G. McKinnell is responsible as cooperator with MPCA for the overall work done through the University of Minnesota, the deformity analysis, reproductive capacity and genetics analysis. Dr. David Hoppe is responsible for the population surveys and the reproductive capacity analysis in laboratory and field. Dr. McKinnell will spend 11% of time for one year, Dr. Hoppe 25% time in one year.

XII. Reporting requirements: Semiannual six-month work program update reports will be submitted not later than January 1, 1997, and a final work program update and final report by June 30, 1997.

XIII. Attachments: Vitae of Dr. Helgen, Dr. McKinnell and Dr. Hoppe are attached.

1996 Project Abstract for the Period Ending June 30, 1997
This project was supported by the Minnesota Future Resources Fund

Project Title:	A Thousand Friends of Frogs
Project Manager:	Tracy Fredin, Director
Organization:	Hamline University, Center for Global Environmental Education (CGEE)
Address:	1536 Hewitt Avenue, St. Paul, MN 55104
Web Site:	cgee.hamline.edu/frogs
Legal Citation:	ML 1996, Chap 407, Sec. 8 Subd. 7
Appropriation:	$28,000

Statement of Objectives

This project focused on an investigation of the health of frog populations in the state of Minnesota and development of a prototype environmental education project looking at frogs as indicators of environmental health. There are three objectives of the project:

1. Assist the Minnesota Pollution Control Agency (MPCA) in monitoring the known sites of deformed frogs in Le Sueur County and the lower Minnesota River basin.
2. Coordinate an informal, state-wide frog monitoring project that involves youth from schools and other organizations gathering information on the health of frog populations.
3. Develop a prototype, state-wide environmental education project for youth in formal education settings.

Overall Project Results

AThousand Friends of Frogs has been a successful project. The combination of citizen monitoring and public education/outreach has had synergistic effect. The citizens and scientists of Minnesota are leading the nation in creating an awareness of amphibians as bio-indicators and problems associated with thier decline and deformity. Frogs have proven themselves to be key in engaging the public, educators, and youth in environmental education and awareness.

- The Ney Frog Project successfully monitored the Le Seuer County site in coordination with researchers from the MPCA. Deformed frogs and toads were found at these sites and the young people were valuable in determining when these frogs with deformities appeared.
- 10,000 newsletters and frog monitoring surveys were distributed through schools and organizations to youth statewide.
- Over five hundred data reports were received by CGEE.
- CGEE acted as a data gathering area and answered thousands of questions from a concerned public. Over five thousand people called in response to the citizen survey and related media outreach.
- Over 100 reports of deformed frog sites were forwarded to the MPCA. Twenty-two sites of deformed frogs were investigated by the MPCA and it was determined that 54 of Minnesota's 87 counties had reports of deformed frogs. The gathering of this data would have been very difficult and expensive for the MPCA to do without the assistance of the citizen volunteers who reported the deformities.
- 1,000 copies of an eight-page study guide were developed and distributed throughout the state affecting 25,000 students statewide.
- Conducted Frog Week, and on-line education experience from October 21-25 and continued project for several weeks due to popular demand.
- Presentations were made to over 25 schools and many community groups about frogs as bioindicators and the deformity issue. This provided direct contact with over 1,000 students and citizens.

Project Results Use and Dissemination

The project has been used by formal and nonformal educators in Minnesota and beyond. We have made presentations ranging from first graders to environmental law students. Materials from the study guide will be incorporated next year in the National Wildlife Federation's Ranger Rick NatureScope. Enclosed are copies of the newsletter/survey, study guide, video tapes of the program being features on national TV, newspaper coverage, student art work, and hard copy of some of our Web site. That address is cgee.hamline.edu/frogs.

Date of Report: July 1, 1997 1,000 Friends of Frogs LCMR Final Work Plan Update Report

Date of Work Plan Approval: May 20, 1996
Project Completion Date: June 30, 1997
Next Status Report Due: June 30, 1997

I. PROJECT TITLE: A Thousand Friends of Frogs

Project Manager: Tracy Fredin, Director
Affiliation: Hamline University, Center for Global Environmental Education (CGEE)
Address: 1536 Hewitt Avenue, St. Paul, MN 55104
Phone: (612) 523-3105 **E-Mail:** tfredin@seq.hamline.edu **Fax:** (612) 523-2489

Total Biennial Proj. Budget: **LCMR:** $28,000
Balance: $0

A. Legal Citation: ML 1996, Chap 407, Sec. 8 Subd. 7

Appropriate Language: (b) Investigation of deformed frogs in Minnesota. This appropriation is from the future resources fund to the commissioner of the pollution control agency to investigate the health of frog populations and evaluate the causes of frog deformities. $28,000 of this appropriation is for a grant to the Center for Global Environmental Education at Hamline University to be used to work with schools and other organizations, including the study of frogs as environmental indicators.

B. Status of Match Requirement: No requirement

II. PROJECT SUMMARY AND RESULTS:

This project focused on an investigation of the health of the frog population in the state of Minnesota and developed a prototype environmental education project looking at frogs as indicators of environmental health. There are three components to the program:

1. The Minnesota New Country School (MNCS) assisted the Minnesota Pollution Control Agency (MPCA) in monitoring the known sites of deformed frogs in LeSueur County and the lower Minnesota River basin. The outcome for this component is to provide the MPCA valuable information about the status of frogs in these sites that were discovered last year by the students of MNCS.

2. Hamline University and MNCS coordinated an informal, state-wide frog monitoring project that involved youth from schools and other organizations gathering information on the health of our frog population. The outcome of this component is to provide information that MPCA researchers can use in the investigation of deformities in the frog population.

3. Hamline University developed a prototype, state-wide environmental education project for youth in formal education settings with assistance from MNCS. The outcome of this component is to provide students in the state the opportunity learn about the frog as bioindicators and discuss the results of the frog monitoring program and MPCA's research on the deformed frogs found in LeSueur County and the lower Minnesota River basin.

Hamline University's Center for Global Environmental Education (CGEE) has been active in developing large scale public environmental education programs, monitoring projects, and on-line education and curriculum projects.

III. FINAL REPORT WORK PROGRAM SUMMARY:

A Thousand Friends of Frogs has been a successful project. We have made many positive impacts on the community at large and the K-12 education system. All aspects of A Thousand Friends of Frogs has been completed.

Overview of results:

Result 1. MNCS monitoring of LeSueur County and the lower Minnesota River basin deformed frog sites.

The Ney Frog Project successfully monitored the Le Sueur County sites in coordination with researchers from the MPCA. All of the objectives have been successfully met as described in the work plan. Deformed frogs and toads were found at these sites and the youth were valuable in determining when these frogs with deformities appeared. These students and their teachers—Chip Brown, Tom Fish, and Cindy Reinitz—have done a wonderful job in raising the public's awareness about frog deformities. This past summer they also worked very closely with the MPCA to assist them in their monitoring of the Ney pond. During this time they spent hundreds of hours at the pond and have contributed to the research efforts. They will continue to work with Cindy Reinitz to monitor the original site and others in the Le Sueur area.

Result 2. Develop, promote, and implement frog monitoring project through schools and organizations to youth statewide.

This was the most successful component of this project. 10,000 newsletters and surveys were distributed throughout the state. Over five hundred data reports were received by the Center and thousands people called in response to the citizen survey. Reports of more than 100 deformed frogs sites were forwarded to the MPCA. The Thousand Friends of Frogs hotline received over 500 calls a week. CGEE acted as a data gathering center and answered many questions from a concerned public. Twenty-two sites of deformed frogs were investigated by the MPCA and it was determined that 54 of Minnesota's 87 counties had reports of deformed frogs. The gathering of this data would have been very difficult and expensive for the MPCA without the assistance of the citizen volunteers who reported the deformities. All of the objectives have been successfully accomplished for this result. The public's response indicates that many citizens of the state are concerned about the frogs and the related environmental issues. The toll-free number was especially effective and the newsletter served to give a broader perspective on frog issues. This program will be continued for the next two and a half years through support from the LCMR appropriation, corporations, and the public.

Result 3. Implement week-long prototype environmental education project that disseminated and discussed the results of MPCA's research, results of frog monitoring program, and the role of amphibians as bioindicators.

The Frogs as Bioindicators environmental education project was also very successful. We developed an eight-page study guide and distributed 1,000 of these throughout the state reaching approximately 25,000 students statewide. Frog Week, an on-line education experience, was held from October 21-25 and continued one for several weeks due to popular demand. We are still receiving and answering questions about amphibians, deformed frogs, and environmental issues. The web site for this project is cgee.hamine.edu/frogs. CGEE staff and other presenters (several universities had their student use this as a service learning program in their biology classes) have given presentations about frogs as bioindicators and the deformity issue to over 25 schools and numerous community groups. This provided direct contact with over 1,000 students and citizens. Frogs as Bioindicators will be continued for the next two and a half years through support from the LCMR appropriation, corporations and the public. We plan on lengthening the duration of frog week to a month and also plan on offering it in both the fall and the spring. We are planning to further develop the study guide and offer teacher training for the program on a

continuing basis. We also will bring a global perspective and connections to the project by working with DAPTF (Declining Amphibian Population Task Force).

IV. OUTLINE OF PROJECT RESULTS:

a. • **Result 1** MNCS monitoring of LeSueur County and the lower Minnesota River basin deformed frog sites.

- Our goal is to involve youth and educators from MNCS in this component
- Students who discovered the deformed frog phenomena will monitor deformed frog sites
- This study will be ongoing throughout the summer
- Throughout the study, information will be forwarded to MPCA researchers and then distributed statewide during the environmental education project

Budget: $10,800 Balance: $0

Completion Date: September 30, 1996

• **Result 2** Develop, promote, and implement frog monitoring project through schools and organizations to youth statewide:

- Our goal is to involve over 500 youth
- Develop survey protocol
- Develop and distribute posters describing monitoring program and environmental education project
- Promote program to schools and organizations throughout state
- Youth will be guided to observe and monitor frogs state-wide
- Coordination of the data collection by MNCS

Budget: $13,850 Balance: $0

Completion Date: September 30, 1996

• **Result 3** Implement week-long prototype environmental education project that disseminates and discusses the results of MPCA's research, results of frog monitoring program, and the role of amphibians as bioindicators.

- Our goal is to impact 10,000 youth
- Distribute 5,000 educational posters state-wide to schools
- Create and manage WWW Web Page for public information exchange
- Deliver an Internet link to MPCA researchers and other experts to students
- Implement week-long prototype environmental education program in schools in late fall

Budget: $3,350 Balance: $0

Completion Date: December 30, 1996

b. Dissemination: The educational part of this program will inform Minnesotans of the results of MPCA's most recent findings about the deformed frogs, the results of the summer frog survey and the value of frogs as bioindicators. The MNCS will have a web site that focused on this project.

V. CONTEXT:

A. Significance: It is believed that the world-wide population of amphibians is declining. Amphibians function as an early warning system for environmental problems. The deformed frogs that students found in LeSueur County in 1995 served as a wake-up call to the condition of Minnesota's frogs and environment. The 1,000 Friends of Frogs project is an excellent opportunity to further explore the deformed frogs in LeSueur County, get youth involved in a

hands-on, state-wide monitoring project, and bring a prototype environmental education program utilizing amphibians as bioindicators into our schools.

It is important to remember that the student monitoring of frogs will give us excellent information based on physical observations of frogs, but is not a scientifically accurate study on the population of frogs in the state. The data collected will be extremely useful to the MPCA as it examines amphibians as bioindicators of water quality in local environments.

B. Time: The project would start the spring of 1996 and run through June 30, 1997.

C. Budget Context: Hamline University has not currently received funding for this project other than the current allocation of $28,000 from LCMR. We feel this is an excellent project and are in the process of looking for additional funding sources to further its development. We have submitted a grant to Dayton Hudson Foundation for $100,000 to support this program. We have also submitted a proposal for a much more in-depth Minnesota Frog Watch project for $300,000 to the LCMR that has been approved for the 1997-99 funding cycle. The cooperators in this program are contributing $12,000 in inkind services to this project.

BUDGET:

Personnel	$15,600	
Equipment	$4,100	(e.g. water testing kits, waders, nets, collecting jars, camera/marco lens, and tripod)
Acquisition	$0	
Development	$0	
Printing/Promotion	$5,800	
Travel	$700	
Other	$1,800	(e.g. postage, supplies, etc.)
TOTAL	**$28,000**	

VI. COOPERATION:

Minnesota Frog and Toad Survey Organization--John Moriarty
Minnesota Pollution Control Agency--Dr. Judy Helgen
Minnesota New Country School--Cindy Reinitz

VII. LOCATION:

Ecological classification location: S (St. Paul) R (LeSueur Co. and the lower Minnesota River basin) We are also encouraging youth from statewide to report to us about the frog population in their area and will work with students statewide in the fall education program.

VIII. Reporting Requirements: Semiannual six-month work program update report will be submitted not later than Jan. 1, 1997 and a final report by June 30, 1997.