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For TCU/RCAF Use Only:
Action _____
Amount _____
Project Code _____

TCU RESEARCH AND CREATIVE ACTIVITIES FUND

GRANT APPLICATION

Principal Investigator: Marlo K. Jeffries

Academic Rank: Assistant Professor

Department: Biology

College or School: College of Science and Engineering

Date of Appointment to TCU: August 2013	**Degree:** PhD	**Date Conferred:** August 2010

Project Title: Development and validation of a small fish model for assessing the effects of emerging contaminants on immune function

Amount Requested: $9988	**Project Period:** June 1, 2014 to May 31, 2015

Authorizing Signatures:

Principal Investigator: _____

Department Chair: _____

Dean of School/College: _____

ABSTRACT (200 WORDS OR LESS; NOTE 5 POINTS POSSIBLE ON EVALUATION SHEET)

Emerging contaminants (ECs) are chemicals that are present in aquatic systems and that can have adverse health effects, but are not subject to regulation. The effects of ECs on fish immune function have gone largely uninvestigated due to the lack of an appropriate model organism by which to assess EC-induced alterations in immunity. To remedy this, I propose developing the fathead minnow (FHM) as a model organism for evaluating EC-induced alterations in fish immunity. First, immune parameters, including white blood cell counts, immune gene expression and pathogen resistance, will be measured in FHMs with no history of exposures to ECs or infectious agents, so that normal immune function in this species can be characterized. Next, the utility of the FHM model will be validated by exposing groups of FHMs to polybrominated diphenyl ethers (PBDEs, a commonly-occurring EC). PBDE exposures are expected to alter the aforementioned immune parameters establishing that the immune system of the FHM is sensitive to ECs. Data generated in this study is expected to: 1) show the utility of the FHM as a model for immunotoxicity, 2) lead to the publication of two research articles, and 3) serve as pilot data for future grant submissions.

Does this proposed research:

☐ **Yes** ☒ **No** **Involve human subjects? If yes, date of Committee review:**

☒ **Yes** ☐ **No** **Involve live animals? If yes, date of Committee review:** Pending

☐ **Yes** ☒ **No** **Involve radioactive substances?**

☐ **Yes** ☒ **No** **Involve scheduled drugs?**

Type of Application:

☒ **New Project/SEED Project**

☐ **Continuing Project**

Renewal of TCU/RCAF Grant No.:

Supplement to other grant application:

Source:

Amount Requested:

Funding Period Requested:

Proposal Status:

☐ **Awarded** ☐ **Denied** ☐ **Pending**

☐ **Ongoing project for which external funding is not possible.**

The proposal must include explanation for the lack of external funding applications.

Previous TCU/RCAF Grants:

Grant No.: **Year:**

Grant Title: : _____

Grant No.: **Year:**

Grant Title: : _____

Grant No.: **Year:**

Grant Title: : _____

Grant No.: **Year:**

Grant Title: : _____

PROJECT NARRATIVE

Development and validation of a small fish model for assessing the effects of emerging contaminants on immune function

1. Abstract: Please see the cover application page.

2. Purpose: Emerging contaminants (ECs) are chemicals that are present in aquatic systems and that have the potential to cause adverse biological effects, but for which there are no regulations limiting their presence in the environment.[1] Evidence suggests that ECs can pose a threat to aquatic wildlife by altering immune function and as a result, there have been calls for further research in this area.[2,3] Despite this, little progress has been made in uncovering the immunotoxic effects (*i.e.*, adverse effects on the immune system caused by chemicals) of ECs in fish. This lag in progress can be partially attributed to the lack of a well-developed fish model for use in immunotoxicity assessments. With this in mind, **the overall purpose of the proposed study is to develop and validate the utility of the fathead minnow (FHM, *Pimephales promelas*) as a model organism for assessing the effects of ECs on immune function.** Two objectives will be met: 1) to characterize basic aspects of immune function in FHMs and 2) to demonstrate that the FHM immune system is sensitive to EC-induced alterations.

3. Project Background: Chemicals that have been classified as ECs include pharmaceuticals, personal care products (*e.g.*, detergents, fragrances, etc.), perfluorinated compounds (PFCs, used in non-stick surfaces) and polybrominated diphenyl ethers (PBDEs, used in flame retardants).[1] The presence of ECs, such as these, in aquatic systems poses a significant environmental threat because: 1) monitoring programs and regulatory standards are not in place to quantify and limit their presence in aquatic environments, 2) ECs are ubiquitous in aquatic systems and thus, nearly

all aquatic organisms are exposed to ECs 3) exposures to some ECs have been associated with adverse biological outcomes.

A multitude of studies have shown that EC exposures can directly alter the survival and reproductive potential of fishes.[4-6] However, few studies of fish have examined whether ECs can alter other important biological process like immune function. Proper immune function is vital to survival (as it allows organisms to fight infections from bacteria, viruses and parasites); therefore, efforts to determine the effects of ECs on immunity are critical if the consequences of EC exposures are to be fully uncovered. A growing body of evidence indicates that several types of ECs, including nonlyphenol (a component of detergents), ethinylestradiol (an active ingredient in birth control pills), PFCs and PBDEs, can suppress immune function and reduce the ability of an organism to fight infection.[7-12] However, the vast majority of these studies have focused on aquatic mammals (*e.g.*, dolphins, seals, etc.) or small rodents (*e.g.*, rats and mice).[7-9] Studies have uncovered similar effects in fish, [10-12] yet these studies have been limited in number and scope leaving a substantial gap in our knowledge of how ECs alter fish immunity. With that said, it is important to recognize that these studies, while limited, still provide key evidence that some ECs can alter immune processes in fish suggesting that the fish immune system is indeed a target for disruption by EC exposures.

A major obstacle in elucidating the effects of ECs on fish immune function is the lack of an appropriate model organism. Model organisms are species that are widely studied and are considered to be representative of other similar species. In toxicological research, the effect of a contaminant on a specific model organism is used to predict the effect of that same contaminant on other related organisms. If the effects of ECs on fish immune function are to be elucidated, an appropriate model organism must be developed. Unfortunately, an adequate model for assessing

the effects of contaminants on fish immunity has yet to be developed. Salmon and rainbow trout have been used in immune function assessments;[10,11] however, neither of these species are practical models due to their large sizes (often > 100 kg) and housing requirements. In contrast, the FHM possesses several key features that make it an ideal model organism for assessing the effects of ECs on immune function. FHMs are relatively small (~2-4 g at adulthood), easy to maintain in the laboratory, have short life cycles, and are representative of the Cyprinid (minnow) family of fishes (one of the most wide spread, ecologically important groups of fish). Most importantly, they are currently utilized as an environmental sentinel organism by the US Environmental Protection Agency and as a result, they are the most widely-used fish species in environmental toxicology.[13] As such, a great deal is known regarding several aspects of their biology (*i.e.*, growth, development, reproduction, etc.). However, immune function in FHMs has gone largely uninvestigated and as a result, basic aspects of immunity in this species must be characterized before it can be utilized as a model for immune function assessments. My lab has already begun to investigate basic aspects of immune function in FHMs and thus far, we have been able to: 1) characterize and quantify immune cells in blood samples and 2) characterize the ability of FHMs to resist infection by a common fish bacterium (*Yersinia ruckeri*). These efforts demonstrate that measures of immunity commonly used in other organisms can be made in FHMs. Once basic aspects of FHM immunity have been characterized, the utility of the FHM as a model organism for immunotoxicity will be validated by demonstrating that the FHM immune system is sensitive to EC-induced alterations.

4. Project Need/Significance: The presence of ECs in the aquatic environment, coupled with evidence that the immune system is a target for disruption by ECs, creates a clear need to uncover the effects of ECs on immune function in fish. The project proposed here seeks to aid in

the fulfillment of this need by developing and validating the FHM as a model organism for assessing the effects of ECs on immune function in fish. The development of the FHM as a model is poised to have a significant impact upon aquatic toxicology, as it will provide researchers with a tool by which to assess the impacts of various contaminants (not just ECs) on immune function. The availability of this tool would undoubtedly widen the scope of investigations aimed at characterizing the effects of exposures to contaminants and ultimately lead to a more comprehensive understanding of how contaminants affect aquatic organisms.

5. Project Potential: My research focuses on assessing the environmental risks associated with exposures to ECs and my previous research efforts have focused primarily on uncovering the effects of contaminants on parameters such as growth, sexual development and reproduction in fish. Through the experiments proposed here, I will be able to expand the depth of my research program to include assessments of immune function. I fully anticipate that ***the proposed project will result in at least two peer-reviewed publications and several presentations*** at scientific conferences. In addition, the data generated here is necessary to show the feasibility of projects that will be proposed to extramural funding agencies. Specifically, ***the results of this research will be used as pilot data in support of grant applications*** for submission to NIH and NSF.

6. Methods: To develop and validate the utility of the FHM as a model organism for assessing the effects of ECs on immune function, two primary objectives will be addressed: 1) to characterize basic aspects of immunity in FHMs and 2) to demonstrate that FHM immune system is subject to EC-induced alterations. The first objective will be satisfied by evaluating various immune parameters (outlined in Table 1) in adult FHMs with no known history of exposures to contaminants or pathogens (*e.g.*, bacteria, viruses, etc.). This will provide baseline data describing "normal" immune function in FHMs. Accomplishing the second objective will require

that adult FHMs be divided into three groups: 1) a control (unexposed) group, 2) a low-dose PBDE group, and 3) a high-dose PBDE group. FHMs will be exposed to PBDEs for 14 days and then the immune parameters in Table 1 will be measured. PBDE has been selected for use in this study as previous work has shown that it alters immune parameters in a variety of species.[9,12,14] Differences in these parameters between exposure groups and the control group will be evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test.

Table 1. Immune function parameters to be measured in the proposed study.

Immune Parameter	Biological Function/Significance
White blood cell (WBC) counts	WBCs help eliminate infectious agents from the body; alterations in WBC counts can impair an organisms ability to fight infections
Expression of immune genes	Several genes (*i.e.,* portions of DNA) encode for biological molecules that control immunity; alterations (*i.e.,* degree to which the gene is turned on) in these genes can alter the ability of an organism to fight infection
Pathogen Resistance	Pathogen resistance refers to the ability of an organism to fight and survive infection; alterations can lead to reductions in organism survival

References: [1]Richardson SD, Ternes TA. 2011. *Anal Chem* 83:4614-48; [2]Ahmed SA. 2000. *Toxicol* 15:191-206; [3]Casanova-Nakayama A et al. 2011. *Mar Poll Bullet* 63:412-416; [4]Hornung MW et al. 1996. *Toxicol Appl Pharmacol* 140:227-34; [5]Lange R et al. 2001. *Environ Toxicol Chem* 20:1216-27; [6]Parrot JL, Bennie DT. 2009. *J Toxicol Environ Health* 72A:633-41; [7]Ross PS. 2002. *Human Ecol Risk Assess* 8:277-92; [8]Neale JCC et al. 2002. *Develop Immunol* 9:215-221; [9] Thuvander A, Darnerud PO. 1999. *Toxicol Environ Chem* 70:229-42; [10]Watanuki H et al. 2002. *Comp Biochem Physiol* 132C:407-13; [11]Arkoosh MR et al. 2010. *Aquat Toxicol* 98:51-9; [12]Ye RR et al. 2012. *Environ Sci Pollut Res* 19:2477-87; [13]Ankley GT, Villeneuve DL. 2006. *Aquat Toxicol* 78:91-102; [14]Fernie KJ et al. 2005. *Environ Pollut* 138:485-93.

7. Budget and Budget Justification:

BUDGET FORM

Account Code		Amount	Total
A. SALARIES – Student Assistants/Research Assistants/Junior Faculty Summer Pay (State projected period and number of hours for which assistant will be employed. **Rate of pay:** Be sure to consult the External Grants information page for the current minimum wage			
6104	**1. Junior Faculty Summer Research Program Salary**	**$6000**	
	2.	**$**	**$6000**
B. TRAVEL (Itemize on separate sheet; do not include funds for presentation of research papers.)			
6220	**1. Staff -** Not required	**$0**	
6222	**2. Consultant -** Not required	**$0**	
6221	**3. Foreign -** Not required	**$0**	**$0**
C. PERMANENT EQUIPMENT (If requested equipment is presently available on campus, please explain, on separate sheet, why the available equipment cannot be used.)			
6340	**1.** Single channel syringe pump w/ foot pedal	**$545**	
	2.	**$**	
	3.	**$**	**$545**
D. OTHER EXPENSES (Itemize on separate sheet, include costs.)			
6430	**1. Supplies –** Not required	$0	
6437	**2. Research Supplies -** See next page for justification	**$3413**	
6341	**3. Computer -** Not required	**$0**	
6365	**4. Printing Services –** Not required	**$0**	
6360	**5. Mail Services –** Not required	**$0**	
6445	**6. Other –** None	**$ 0**	**$3443**
		TOTAL BUDGET REQUEST	**$9988**

BUDGET JUSTIFICATION

A. Salary: $6000 of salary support for the PI is requested through the Junior Faculty Summer Research Program and will allow the PI to dedicate her full effort towards the proposed research during the summer months.

B. Travel: Travel funds are not requested.

C. Permanent Equipment: A single channel syringe pump with a foot pedal ($545) is necessary to carry out the proposed project. The syringe pump will allow us to inject minnows with small volumes of bacteria as part of the pathogen resistance challenges. Currently, a syringe pump that suits the needs of the project does not exist elsewhere campus. At the end of the project period, the syringe pump will remain in the PIs lab where it will be utilized in follow up experiments.

D. Other Expenses

1. Supplies: None requested.

2. Research Supplies: A total of $3443 is being requested to provide supplies and reagents necessary to conduct the proposed research as detailed below.

Item	**Justification**	**Cost**
2,2',4,4'-Tetrabromodiphenyl Ether (BPDE-47)	For exposing fish to BPDE-47	$96
Brine shrimp eggs	For exposing fish to BPDE-47 via dietary uptake	$99
Yersina ruckeri (bacteria)	For pathogen resistance assays	$295
Microbiology Supplies (nutrient agar, nutrient broth, petri dishes, syringes, fish saline solution)	For growing *Y. ruckeri* to be used in evaluations of pathogen resistance	$694
Hematology Supplies (microscope slides, stain)	For counting white blood cells	$364
Gene Expression Analysis Supplies (RNA extraction kits, qPCR reaction reagents and consumables, primer sets)	For quantifying the expression of immune function genes	$1895

3. Computer: None requested.

4. Printing Services: None requested.

5. Mail Services: None requested.

6. Other: None.

Appendix A: Record of Scholarly Activity.

Peer-reviewed publications:

Kolok AS, **Sellin Jeffries MK**, Knight L, Snow DD, Bartelt-Hunt, SL. *In press.* The hourglass: A conceptual framework for the transport of biologically active compounds from agricultural landscapes. *Journal of the American Water Resources Association.*

Sellin Jeffries MK, Claytor C, Stubblefield W, Pearson WH, Oris JT. 2013. Modeling the risk of PAH photo-induced toxicity in Pacific herring following the *Exxon Valdez* oil spill. *Environmental Science and Technology* 47:5450-5458.

Sellin Jeffries MK, Mehinto AC, Carter BJ, Denslow ND, Kolok AS. 2012. Taking microarrays to the field: Differential hepatic gene expression of caged FHMs from Nebraska watersheds. *Environmental Science and Technology* 46:1877-1885.

Sellin Jeffries MK, Abbott KI*, Cowman T, Kolok AS. 2011. Occurrence and endocrine effects of agrichemicals in a small Nebraska watershed. *Environmental Toxicology and Chemistry* 30:2253-2260.

Sellin Jeffries MK, Conoan N*, Cox M, Sangster J, Balsiger HA*, Bridges AA*, Cowman T, Knight LA*, Bartelt-Hunt SL, Kolok AS. 2011. The anti-estrogenic activity of sediments from agriculturally-intense watersheds: Assessment using *in vivo* and *in vitro* assays. *Aquatic Toxicology* 105:189-198.

Presentations at Meetings and Invited Seminars:

Sellin Jeffries MK, Stultz AE, Rawlings J, Belanger S, Oris JT**.** 2013. Webinar: Update on the development of alternative testing strategies and endpoints for determining whole effluent toxicity in fishes. Health and Environmental Science Institute Technical Committee Effluent Project Webinar.

Sellin Jeffries MK, Stultz AE, Rawlings J, Belanger S, Oris JT. 2013. The development of alternative strategies and additional endpoints for whole effluent toxicity testing in fishes. Society of Toxicology and Environmental Chemistry North America 34rd Annual Meeting, Nashville, TN.

Oris JT, **Sellin Jeffries MK**, Stultz AE, Zhang J, Bailer AJ. 2013. A Path Toward Effluent Toxicity Test Alternatives With Fish. Society of Toxicology and Environmental Chemistry North America 34rd Annual Meeting, Nashville, TN.

Thornton LM**, Oris JT, **Sellin Jeffries MK**. 2013. Development of the sheepshead minnow, *Cyprinodon variegatus*, as a model organism for immunotoxicity. Society of Toxicology and Environmental Chemistry North America 34rd Annual Meeting, Nashville, TN.

Sellin Jeffries MK. 2012. Endocrine disruption in ecotoxicology: Minnows, manure, municipalities and immunity. University of North Carolina – Greensboro Department of Biology Seminar Series. Greensboro, NC.

Sellin Jeffries MK. 2012. Fish on steroids: Defeminized females and immunocompromised males. University of the Pacific Department of Biological Sciences Seminar Series. Stockton, CA.

Sellin Jeffries MK, Arivett BA, Fiester SE, Coffey DD*, Thornton LM*, Smith AW*, Actis LA, Oris JT. 2012. Development of two small fish species, *Pimephales promelas* and *Cyprinodon variegatus*, as model organisms for immunotoxicity. Society of Toxicology and Environmental Chemistry North America 33rd Annual Meeting, Long Beach, CA.

Kolok AS, **Sellin Jeffries MK**, Bartelt-Hunt S. 2012. Agrichemicals and sediments: The hourglass. Society of Toxicology and Environmental Chemistry North America 33rd Annual Meeting, Long Beach, CA.

Sellin Jeffries MK, Stultz AE, Rawlings J, Belanger S, Oris JT. 2012. Alternative strategies for assessing effluent toxicity in fish: A comparison of the fish embryo test and the larval growth and survival test. Society of Toxicology and Environmental Chemistry North America 33rd Annual Meeting, Long Beach, CA.

Rawlings J, Böhler S, **Sellin Jeffries MK**, Stultz AE, Oris JT, Braunbeck T, Norberg-King TJ, Belanger S. 2012. Progress towards the development of a FHM embryo test and comparison to the zebrafish embryo test for assessing acute fish toxicity. Society of Toxicology and Environmental Chemistry North America 33rd Annual Meeting, Long Beach, CA.

Sellin Jeffries MK. 2012. Fish on steroids: Defeminized females and immunocompromised males. Texas Christian University Department of Biology Seminar Series. Fort Worth, TX.

Thornton LM*, Oris JT**, Sellin Jeffries MK**. 2012. Development of the sheepshead minnow (*Cyprinodon variegatus*) as model organisms for immunotoxicity. Ohio Valley Society of Toxicology & Environmental Chemistry Regional Meeting, Oxford, OH.

Stultz AE**, Sellin Jeffries MK**, Oris JT. 2012. Alternative strategies for assessing effluent toxicity in fish: A comparison of the fish embryo test and the larval growth and survival test. Ohio Valley Society of Toxicology and Environmental Chemistry Meeting, Oxford, OH.

Kolok AS, **Sellin Jeffries MK**. 2012. Agrichemicals and sediments: The hourglass. American Water Resources Association Summer Specialty Conference, Denver, Colorado.

Oris JT, **Sellin Jeffries MK**, Stultz AE. 2012. Exploring animal alternatives: Seeking a replacement for whole effluent toxicity testing in fish. The 6th Society for Environmental Toxicology and Chemistry World Congress, Berlin, Germany.

Sellin Jeffries MK, Mehinto AC, Carter BJ, Denslow ND, Kolok AS. 2011. Microarrays in the field: Are differential gene expression patterns consistent with differences in contaminant loads between sites? Society of Environmental Toxicology and Chemistry North America 32nd Annual Meeting, Boston, MA.

Sellin Jeffries MK, Claytor C, Stubblefield W, Pearson W, Oris JT. 2011. Development and application of a quantitative model to predict the risk of PAH phototoxicity in herring following the *Exxon Valdez* oil spill. National Society of Environmental Toxicology and Chemistry North America 32nd Annual Meeting, Boston, MA.

Kolok AS, **Sellin MK**. 2011. Sediments from agriculturally intensive watersheds defeminize female fish via anti-estrogenic activity. ASLO Aquatic Sciences Meeting, San Juan, Puerto Rico.

Extramural Funding:

National Science Foundation – Catalyzing New International Collaborations (CNIC) Program. 2014-2015. Catalyzing New International Collaborations: US-Kazakhstan workshop and pilot study- Pesticide occurrence and ecological effects in the Syr Darya River Basin. $49,751. Dan Snow, Alan Kolok, Shannon Bartelt-Hunt and Marlo Jeffries. (Pending)

American Association of Laboratory Animal Science – Grants for Laboratory Animal Science. 2014-2015. Towards the 3Rs in fish toxicity testing. $47,495. Marlo Jeffries. (Pending)