EFFECTS OF DICLOFENAC EXPOSURE ON THE GASTROINTESTINAL TRACT OF *XENOPUS LAEVIS* METAMORPHS

A Report of a Senior Study

by

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ABSTRACT

Pharmaceutical drugs are commonly found in the environment due to global reliance and improper disposal. Diclofenac is one of these compounds that is often found in waterways and in wildlife. Due to its nature as a non-steroidal anti-inflammatory drug, research suggests it can have harmful effects on organ systems among vertebrates. The purpose of this experiment was to determine the impact of an environmentally relevant dose of diclofenac on the gastrointestinal tract of *Xenopus laevis* metamorphs. Thirty tadpoles were exposed to 0.125µg/l of diclofenac for 21 days. After the exposure period, histology was performed on the small intestine. The experimental group showed a significant reduction in intestinal dimensions compared to controls. The mean epithelial cell height decreased from 42.57 ± 5.3 µm (control) to 36.55 ± 6.51µm ($p = 0.00066$). The mean intestinal width decreased from 1.31 ± 2.12 mm to 1.14 ± 2.72 mm ($p = 0.02$). These results indicate that diclofenac exposure caused measurable atrophy of the intestinal tissue in *Xenopus laevis* metamorphs. The experimental groups would have smaller intestines was supported. Further research is recommended to examine cellular mechanisms responsible for diclofenac-induced damage on the small intestine and other organ systems.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Pharmaceutical drugs are a pillar of modern medicine. Thousands of these compounds exist to help people manage anything from relieving allergies to easing chronic pain. Drugs come in many forms of administration, including pills, injections, topical creams, and liquids, which are all absorbed into the body and eventually passed. As global reliance on pharmaceuticals increases, so does the concern about their presence in water sources. Six-hundred thirty-one pharmaceutical substances have been detected in aquatic environments across 71 countries (Aus et al., 2016). Topical creams leaching directly into water bodies, compounds passing through wastewater management systems without being fully removed, and improper disposal by consumers are factors in this growing concern. Triclosan and diclofenac are two pharmaceutical drugs that are present in water systems and in aquatic organisms. Research indicates many harmful effects these substances can have on wildlife, including hormone disruption, organ damage, and interference with reproduction and behavior. These effects can contribute to the decline of populations, threatening biodiversity. The broader implications of pharmaceutical bioaccumulation highlight the urgency of how

these compounds entering the environment could also pose a risk to human health through the food chain and drinking water.

Triclosan and Diclofenac

Triclosan is a synthetic compound utilized for its antimicrobial properties. The chemical was introduced to the healthcare world as a surgical scrub in the 1970s. Now, it is used as a disinfectant, and its presence is found in antibacterial hand soap, hand sanitizers, cosmetics, mouthwash, and textiles (Weatherly & Gosse 2017). Triclosan's antifungal and antibacterial properties include reducing *Toxoplasma gondii*, which creates harmful birth defects, and *Plasmodium falciparum*, which is the deadliest strain of malaria (Freundlich et al., 2007; Neville et al., 2015). Although triclosan has been added to products like toothpaste for decades, debate of the necessity of using triclosan for its antimicrobial power arises when discussing the potential environmental and human health-related risks. In September of 2016, the U.S.A Food and Drug Administration (FDA) banned triclosan in soap products, considering evidence of soap containing the chemical worked better than soap lacking it could not be supported (Weatherly & Gosse 2017). It remains in other personal care products and surgical grade soap.

Vast usage of triclosan creates opportunities for the drug to enter the human body. Dermal exposure of the drug through creams or cleaning products leads to percutaneous absorption (Queckenberg et al, 2010). 2,517 participants representing the United States general population provided urine samples, in which triclosan was present in 74.6% of the samples (Calafat et al., 2008). Triclosan was found in human breast milk samples, likely due to exposure from personal care products (Aldoffson-Erici et al., 2002). The consistent use of triclosan-containing products leads to an increased concentration of the compound in

humans, allowing it to enter water systems through urination and being washed down sinks. Water treatment facilities are not able to completely remove the chemical. Research suggests that triclosan is present in water in low concentrations, with the amount dependent on wastewater treatment systems; triclosan is readily biodegradable under aerobic conditions and not in anaerobic conditions (McAvoy et al., 2002). Triclosan's presence in water spans across the globe. In China, triclosan concentrations in surface water range from 0.06 to 612 ng/L (Zhenyao et al., 2024).

Previous mammalian studies support the idea that exposure to triclosan can have harmful effects on wildlife. Male mice were shown to have accelerated hepatocellular carcinoma development after eating food 0.08% triclosan (Yueh et al., 2014). After exposing the female wistar rat to triclosan for 21 days, an increased uterine weight was recorded and serum thyroid hormone concentrations were suppressed (Stoker et al., 2010). Various studies show the adverse effects triclosan has on the frog species, *Xenopus laevis* (Table 1). These results suggest triclosan as a potential carcinogen and endocrine disruptor.

Table 1. Dosage levels and corresponding effects of triclosan used on *Xenopus laevis* across 6 independent studies.

Dosage	Results	Study
0.6, 1.5, 7.2, or 32.3 µg TCS/l.	Environmentally relevant TCS concentrations did not alter the normal course of thyroid	Fort, Douglas J., et al. 2017
1.5 µg/l	Reduction of larval growth occurred at exposure day 21	
10uM	Decreased TMRM fluorescence in mitochondria	Thomson, Alexander H., and Christopher K. Thompson. 2024
30uM, 10uM, 1uM	Increased oxygen consumption rates dose-dependently	
20, 100, and 200 µg/l	Plasma Vg in all TCS treatment groups were lower than that of control group	Matsumura, Naomi, et al. 2005
benzo[a]pyrene and triclosan (50 ng.L-1 35 each)	Female tadpoles developed liver steatosis	Usal, Marie, et al. 2019
25 µg l–1	Increased growth during the metamorphic stages relative to the control, but did not influence growth during the post-metamorphic phase	Fort, Douglas J., et al. 2010
0.15 ± 0.03 µg/L	Increased hindlimb development and a decrease in total body weight	Veldhoen, Nik, et al. 2006
0.03 µg/L	Alteration of thyroid hormone receptor mRNA expression	
2 mg/L	Death of frogs was observed on the 4th day	Tenkov, Kirill S., et al. 2022
0.5 mg/L,	Frogs remained viable for 11 days; triclosan caused damage to the liver tissue; no effect on the number of frog red blood cells, but reduced osmotic resistance	

Diclofenac is a nonsteroidal anti-inflammatory drug. Synthesized in 1973, it is prescribed to treat mild to moderate pain and reduce swelling. Diclofenac is comparable in efficacy and tolerability as other pain relievers such as ibuprofen, sulindac, and diflunisal (Brogden et al., 1980). The global usage of the drug has allowed it to enter waterways. Its presence has been measured in 50 countries, with concentrations exceeding predicted no-effect levels (Aus et al., 2016). Because of potential toxicity on aquatic life, it is considered an emerging environmental contaminant (Lonappan et al., 2016). Diclofenac concentrations in marine environments range from 0.000001 μg/L to 0.843 μg/L, with the highest levels detected in estuaries (Bonnefille et al., 2018).

Although a ban has not been implemented, diclofenac is increasingly regulated. For instance, in 2015, the drug was reclassified as a prescription only medication as a result of the increased risk of cardiological events in patients with pre-existing conditions at a higher rate than other nonsteroidal anti-inflammatory medications (Spitz 2013). Also, diclofenac was banned for veterinary use in South Asia in 2006, subsequently improving vulture conservation efforts (Dama 2014). As vultures would feed on carcasses of animals that were exposed, they would suffer from kidney failure and visceral gout (Mahapatro & Arunkumar 2014). Replacing diclofenac with other anti-inflammatory drugs, less vultures have succumbed to the effects.

In addition to vultures, diclofenac shows potential risk against freshwater organisms (Acuña et al., 2015). Diclofenac showed accumulation in crucian carp (*Carassius auratus*), with the maximum bioconcentration factors being 121 L kg^{-1} in the liver, and 52.3 L kg^{-1} in the gills after 14 days of exposure (Lu et al., 2017). Diclofenac exposure to *Xenopus* embryos resulted in promotion of teratogenicity and morphological anomalies (Chae et al., 2015). Other research suggests behavioral and physiological changes in *Xenopus laevis* after diclofenac exposure (Table 2). These adverse effects further suggest that diclofenac is an emerging environmental concern, specifically in regard to presence in water ways and that impact on wildlife.

Table 2. Dosage levels and corresponding effects of diclofenac used on *Xenopus laevis* across 4 independent studies.

Dosage	**Results**	**Study**
125 μg L^{-1}, 250 μg L^{-1}	swimming distance, velocity and global activity decreased	Peltzer et al. 2019
1000 μg L^{-1}, 2000 μg L^{-1}	heart frequency and ventricular systole interval reduced	
22.3 and 11.1, 25.7 and 18.7, 47.8 mg active substance/L and 45.3 mg/L	teratogenic and growth inhibitory	Emre et al. 2024
9.56 mg L^{-1}	malformations and death	Cardoso-Vera et al. 2017
10 mg mL^{-1}	malformations and embryo mortality	Chae et al. 2015

NSAIDS, including diclofenac, are suggested to cause gastrointestinal (GI) injury. A meta-analysis of 280 randomized trials involving NSAIDs demonstrated a significant increase in upper GI complications across all treatment regimens, with diclofenac showing a statistically significant association with a p-value of 0.0106 (Coxib et al., 2013). These injuries arise from a combination localized toxic metabolite effects leading to cellular stress, induction of apoptosis, disruption of the epithelial barrier, and subsequent inflammatory responses (Boelsterli et al., 2012). Side effects are present across vertebrate classes, including mammals, birds, and amphibians. When exposed to 7.5 mg/kg of diclofenac, rats experienced damage to the mucosal membrane of the small intestine, along with a significant shortening of the villi (Zhang et al., 2009).

Xenopus laevis as a model organism

Xenopus laevis, commonly known as the African clawed frog, is a commonly used model organism for scientific research. They are tetrapod vertebrates and have a close evolutionary relationship with higher vertebrates. *Xenopus* is useful for research as they have a high fecundity rate as well as large embryos (Tandon et al., 2017). They have been observed in multiple studies where they are exposed to pharmaceutical drugs (Table 3). Given the widespread presence of diclofenac in water, and the evidence supporting its harmful impact on wildlife, exposing this aquatic species to relevant concentrations of diclofenac is necessary to assess any potential adverse effects to organ systems.

Table 3. Effects of various drug exposure to *Xenopus laevis*

Drug	**Dosage**	**Results**	**Study**
Streptomycin	1 μg/mL, 10 μg/mL	dysbiosis in the microbiome	Ortega 2019
Benzodiazepines	1 μg/L, 5 μg/L, 10 μg/L	decreased heart rate and motility, induced marked cephalic and abdominal edema, intestinal and retinal defects	Fogliano, et al. 2022
Fluoxetine	10 μL	reduced growth at metamorphosis	Conners, et al. 2009
Sertraline	10 μL	reduced growth at metamorphosis	
Sertraline	0.1 and 1 μL	reduced growth at metamorphosis, acceleration of development	
Ethynylestradiol (EE2)	6 pM, 60 pM, 600 pM	sex-reversal was implied, reduced fertilization rates, among frogs with ovaries there was a significantly higher percentage that lacked oviducts	Gyllenhammar, et al. 2009

The organs of interest for this experiment include the gonads of female and male *Xenopus laevis* and the thyroid, both of which are endocrine glands; the small intestine will also be observed. The location of these organs on the chosen model organism can be seen on Figure 1. The thyroid gland secretes thyroxine (T4), which is converted into the biologically active triiodothyronine (T3) at other organs. These hormones are responsible for a wide variety of processes, such as brain development across all vertebrates (Fini et al., 2012).

In amphibians and fishes, T3 triggers metamorphosis. For instance, in *Xenopus laevis*, metamorphosis includes absorbing the tail, developing limbs, and anatomical changes of organs and the skull. In mammals, the hormone maintains essential homeostatic processes including basal metabolism, thermogenesis, and heart rate (Zwahlen et al., 2024). The gonads of vertebrates are responsible for reproduction and hormone production. The gonadotropins, luteinizing hormone, and follicle stimulating hormone, are required for proper reproductive development in vertebrates (Urbatzka et al., 2010). They are necessary to produce gametes and the synthesis of sex steroids – estrogen, progestins, and androgens.

The small intestine serves the essential function of digesting food and absorbing nutrients, playing a critical role in the overall digestive process of *Xenopus laevis*. The small intestine of *Xenopus laevis* consists of columnar epithelial cells, arranged along longitudinal folds that functionally resemble mammalian villi (McAvoy & Dixon 1978). These villi increase the internal surface area, enhancing nutrient absorption and interaction with substances such as diclofenac. The measured diameter of the small intestine among *Xenopus laevis* ranges between 1 and 2 mm in post-metamorphic individuals (Tamaoki et al., 2016), which aligns with commonly reported anatomical values for this genus.

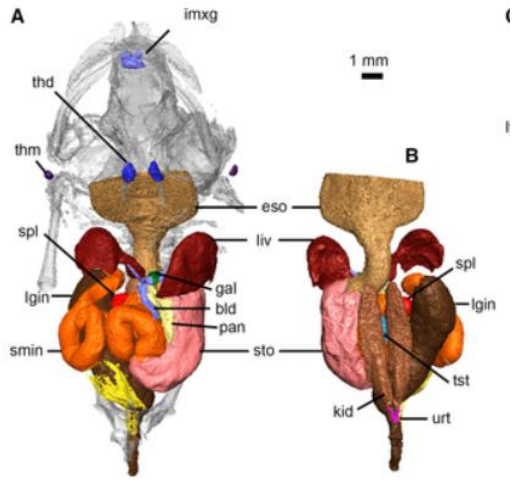

Figure 1. Digital dissection of *Xenopus laevis*. The organs of interest are labeled "thd" for thyroid and "tst" for testes. The small intestine is colored orange and labeled "smin" (Porro & Richards 2017, pg 188).

Purpose of Study

The purpose of this study is to determine the effects of diclofenac exposure on the gastrointestinal tract of *Xenopus laevis* metamorphs. Due to previous research suggesting potential impact on the gastrointestinal system, the small intestine will be analyzed. Because diclofenac disrupts the epithelial cells that line the small intestine via mechanisms such as apoptosis and triggering inflammatory responses, it is hypothesized that the small intestine of the experimental tadpoles will be smaller than the control group (Boelsterli et al., 2012). Observing tadpoles after exposure and analyzing tissues will give insight to the impact of diclofenac on essential organ systems.

CHAPTER II

METHODOLOGY

Sixty albino *Xenopus laevis* tadpoles were purchased from Xenopus Express (www.xenopus.com) and organized into tanks based on developmental stage. All procedures were approved by the Maryville College IACUC committee (see Appendix). There were 20 tadpoles in stage 63, 64, and 65. The 60 tadpoles were divided into 6 tanks, with 1 experimental and 1 control tank holding 10 tadpoles per stage. Water was conditioned by adding 5ml of Aqueon water conditioner to 5 gallons of tap water. Four liters of conditioned water were added to each tank. A solution of 0.005g diclofenac dissolved in 100 mL dimethyl sulfoxide (DMSO) was added into the experimental tanks at 10 microliters. Ten microliters of DMSO was added to the control tanks. Tadpoles were fed ad lib and water was changed once a day; for each liter of water changed 2.5 microliters of the chemical (DMSO and diclofenac solution at 0.125micrograms/l concentration or pure DMSO) was added to the tank. The tadpoles were exposed to the chemicals for 21 days. After the exposure period, the tadpoles were weighed and anesthetized with tricaine mesylate (MS222). Once unconscious, a dissection microscope and dissection tools were used to remove the liver, gastrointestinal tract, kidney, gonads, and head from the organisms (see Figure 2). The organs were fixed in 7.5 ml of Bouin's solution for 7 days. Bouin's was removed from tube, and organs were

rinsed with 70% ethanol until liquid was clear. The small intestine was separated from the gastrointestinal tract using dissection scissors. Histology was performed on 59 small intestines as (Nieuwkoop et al., 1967) described. The small intestines were oriented to obtain transverse cuts for the slides. Slides were stained and a cover was added. Slides were examined under 4x and 40x magnification on a light microscope. Per organ, 20 cells were measured. The diameter of the cell and microvilli height (1 per cell) were measured using an ocular ruler. Measurements were multiplied by respective conversion factors to obtain micrometer size. Statistical analysis of an unpaired t-test was performed using Microsoft Excel.

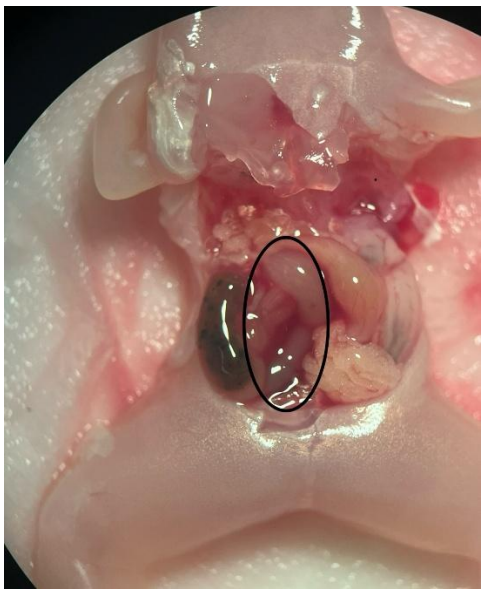

Figure 2. Dissection of *Xenopus laevis* photographed through dissection scope lens. The black circle indicates the small intestine of the frog. Image obtained by author.

CHAPTER III

RESULTS

Quantitative Results

The average width of the small intestine of the among the control group was significantly (p=0.02) larger (1.31 μm ±2.12 mm) than the experimental group (1.14 mm ±2.72 mm; see Figures 3 & 5). The average height of the columnar epithelial cell among the control group was 42.57μm (±5.3μm). The experimental group had an average height of 36.55 μm (±6.51 μm) (see Figures 4 & 6). The p-value between the two groups is 0.00066.

Qualitative Observations

On day 11 of the experiment, a frog from the stage 65 control group died. Throughout the experiment, the tanks of the experimental groups were noticeably dirtier than the control tanks (see Figure 7). There was no observable difference in behaviors such as activity level or swimming speed between the experimental and control groups. Independent of stage, all control and experimental groups ate the same amount of food at similar speeds

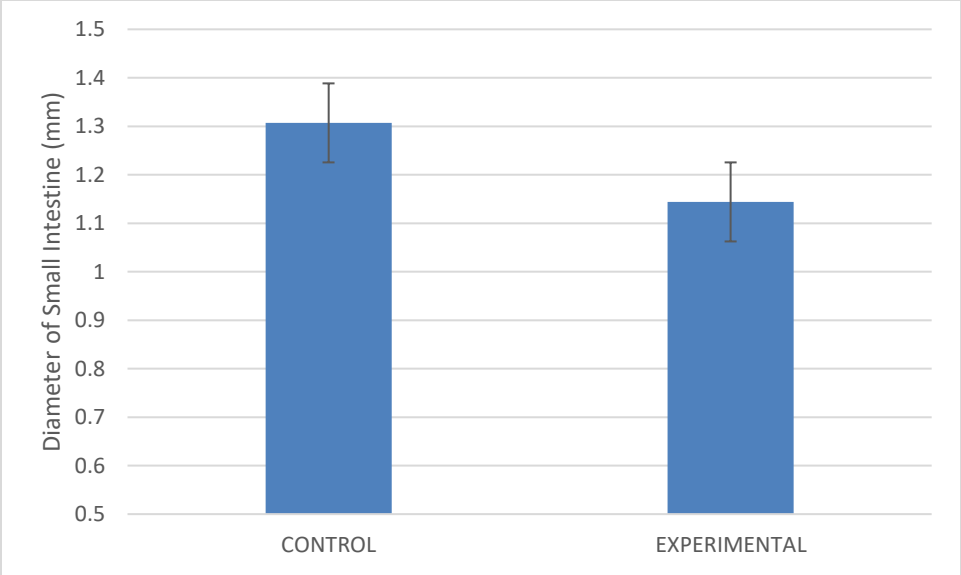

Figure 3. Comparison of small intestine diameter of *Xenopus laevis* metamorphs after exposure to diclofenac. The mean width of the small intestine of the control group is 1.31mm (±2.12 mm). The mean diameter width of the experimental group is 1.14 mm (±2.72 mm). The p-value between the two groups is 0.02.

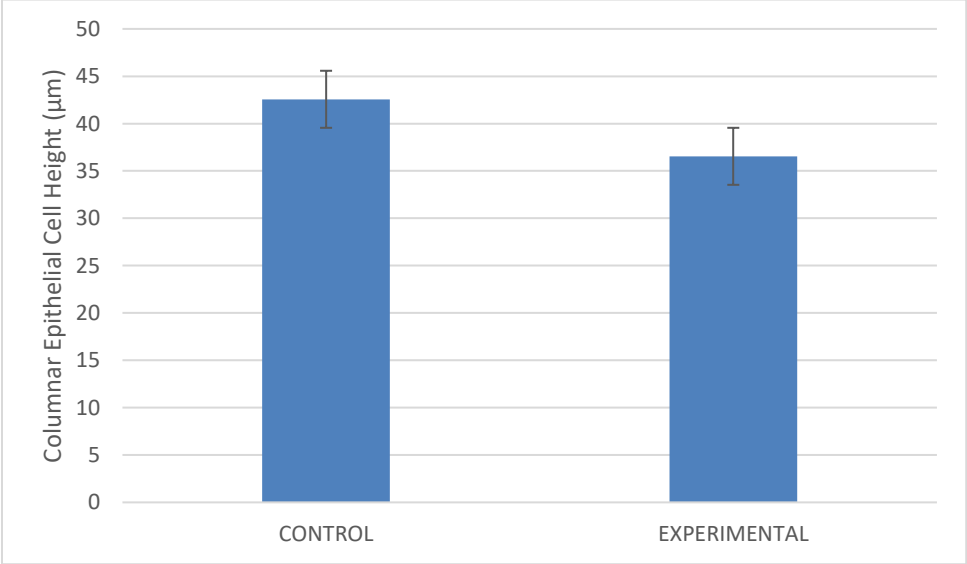

Figure 4. Comparison of columnar epithelial cell height within the small intestine of *Xenopus laevis* metamorphs after exposure to diclofenac. The mean height of the columnar epithelial cell among the control group is 42.57µm (±5.3µm). The mean height of the experimental group is 36.55 µm (±6.51 µm). The p-value is 0.00066.

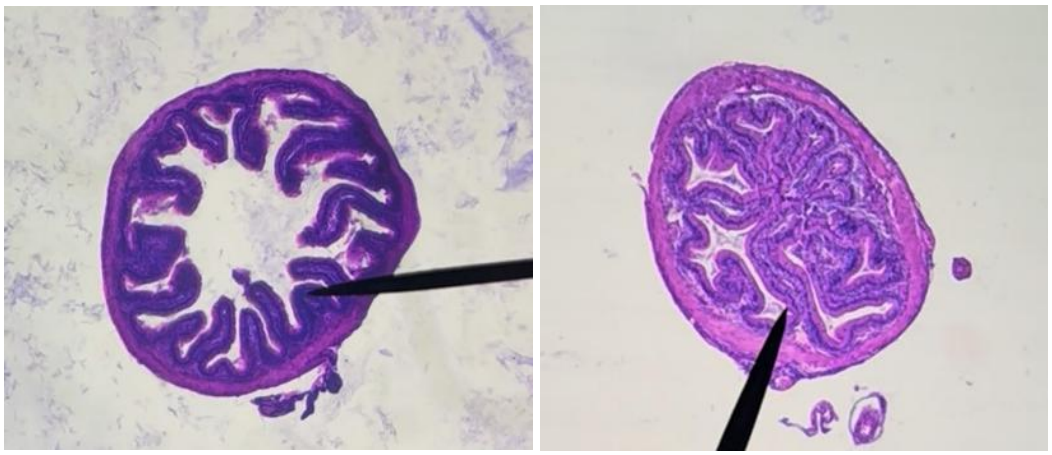

Figure 5. Comparison of small intestine diameter of *Xenopus laevis* after exposure to diclofenac. The control group (left) is seen to have a wider diameter, as well as more open space within the cell compared to the experimental group (right) Photos are viewed under 4x magnification. Images photographed by author.

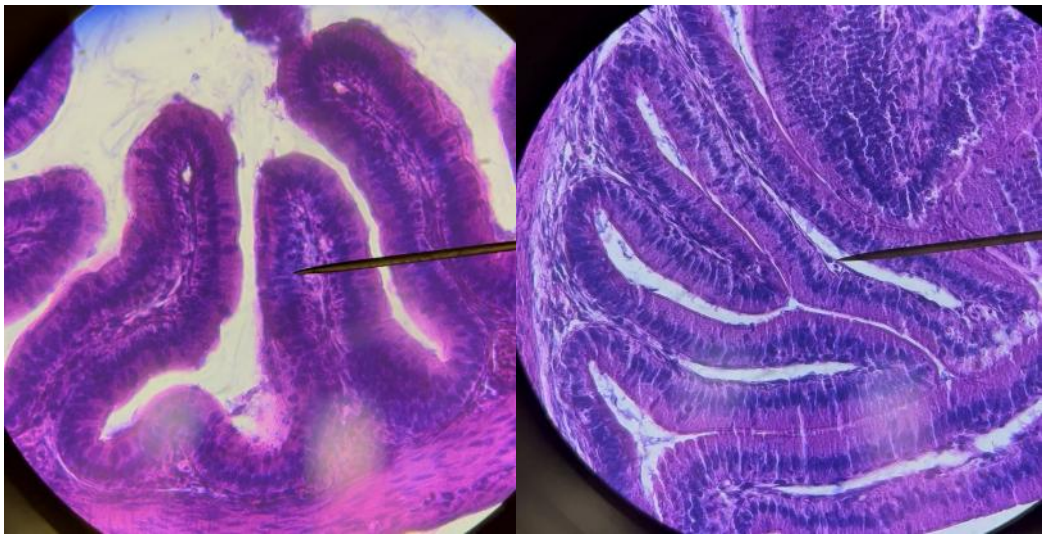

Figure 6. Comparison of columnar epithelial cell height found in small intestine of *Xenopus laevis* after exposure to diclofenac. The control group (left) is seen to have larger cells compared to the experimental (right). The control group also is seen to have more space between plicae circularis where each cell is found. Photos are viewed under 40x magnification. Images photographed by author.

Figure 7. Comparison of water cleanliness of *Xenopus laevis* control and experimental stages during exposure period. Experimental tanks (right) have a higher concentration of fecal matter compared to control tanks (left).

CHAPTER IV

DISCUSSION

The variation in columnar epithelial cell height and small intestine diameter of observed *Xenopus laevis* metamorphs was expected due to physiological effects of diclofenac. The hypothesis that the experimental group would have smaller measurements compared to the control group was supported. Considering the tadpoles were exposed to the drug at stages from 63-66, it can be concluded that the drug caused atrophy or degradation of the structure, rather than inhibiting growth or maturation. This aligns with previous findings that NSAIDs disrupt the epithelial cells that line the small intestine, although total mechanisms are not completely understood.

Mechanistic studies have suggested that NSAID-induced intestinal damage is multifactorial, following a "multiple-hit" pathogenesis; the multiple hits begin with a pharmacokinetic process in which glucuronidated NSAID or oxidative metabolite conjugates are excreted via the hepatobiliary route into the distal small intestine, where bacterial β-glucuronidase releases aglycones that are subsequently absorbed by enterocytes and metabolized by intestinal cytochrome P450 enzymes into potentially reactive intermediates (Boelsterli et al., 2012). This inducts stress within the endoplasmic reticulum and mitochondria, eventually leading to apoptosis and necrosis of epithelial cells (Tsutsumi et al.,

2004). The second hit to the intestines involves toll-like receptor 4 triggering tumor necrosis factor-mediated cell injury (Nadatani et al., 2018). This second hit triggers an inflammatory response, creating tissue injury through oxidative and immune-mediated pathways. Supporting this, the experimental tanks were noticeably dirtier than the control (see Figure 4). This observation supports histological findings and previous research, suggesting that the intestines were damaged from the diclofenac, leading to more waste output due to impaired nutrient reabsorption.

The thyroid and gonads were not analyzed due to time constraints, however, their inclusion in future research would provide insight into the broader endocrine impacts of diclofenac exposure. The amphibian digestive tract is correlated with thyroid activity, specifically the influence of thyroid hormone on intestinal remodeling (Ishizuya & Shi 2005). Diclofenac and other nonsteroidal anti-inflammatory drugs have been identified as potential endocrine disruptors, including those involving thyroid hormones (Reis et al., 2024). Considering the findings of intestinal epithelial degradation in this experiment, it is hypothesized that diclofenac may exert its toxicity through both direct tissue effects and endocrine-mediated mechanisms. Future histological and biochemical examination of the thyroid and gonads in experiments could determine whether the intestinal changes observed are influenced by endocrine disruption as well, providing more information about the mechanisms of diclofenac's developmental and physiological toxicity in *Xenopus laevis*.

Beyond amphibians, these findings carry broader implications for vertebrate physiology. In humans, NSAIDs such as diclofenac, indomethacin, and naproxen are well-documented to cause gastrointestinal injury, often manifesting as mucosal ulceration, bleeding, and even perforation of the small intestine (Wolfe, Lichtenstein, & Singh, 1999). In

the United States alone, NSAID-related gastrointestinal complications account for over 100,000 hospitalizations each year, with approximately 16,000 of these cases resulting in fatalities (Peura, 2002). The mechanisms underlying NSAID-induced toxicity may be expressed across vertebrates, as the epithelial damage in *Xenopus laevis* is similar to injury found within mammalian intestines. These findings bridge ecological toxicology and clinical pharmacology, offering a more comprehensive understanding of how a widely used pharmaceutical can exert parallel effects across diverse vertebrate lineages.

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APPENDIX 1: IACUC APPROVAL

MARYVILLE COLLEGE INSTITUTIONAL ANIMAL CARE & USE COMMITTEE
Application for Use of Vertebrate Animals in Student Research

Provide information after each bold item
Student Name:
Diya Patel and Emily Miller
Student Email Address:
diya.pate1@my.maryvillecollege.edu; emily.g.miller@my.maryvillecollege.edu
Date:
April 4, 2025
Senior Study Advisor:
Crain
Species to be used:
Xenopus laevis
Age of animals:
Stage 63-66
Number of animals in study:
30
Duration of study:
August 20-September 20
Location of animals during the study (building and room):
Sutton 114

List personnel to call if problems with animals develop:

Name	Daytime Phone	Nighttime Phone	Emergency No.
Drew Crain	8652928737	86520287 37	

What will happen to the animals at the end of the study? If euthanasia is required, state the specific methods.
Euthanasia via MS222

(Do not write below line: For MC IACUC Use)

Maryville College IACUC Approval Number: 202502
Date Approved: Apr. 15, 2025
Signed:

WORKS CITED

Acuña V, Ginebreda A, Mor JR, Petrovic M, Sabater S, Sumpter J, Barceló D. 2015. Balancing the health benefits and environmental risks of pharmaceuticals: Diclofenac as an example. Environ Int. 85:327-333.

Adolfsson-Erici M, Pettersson M, Parkkonen J, Sturve J. 2002. Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. Chemosphere. 46(9-10):1485-1489.

Aus der Beek T, Weber FA, Bergmann A, Hickmann S, Ebert I, Hein A, Küster A. 2016. Pharmaceuticals in the environment—global occurrences and perspectives. Environ Toxicol Chem. 35(4):823-835.

Boelsterli UA, Redinbo MR, Saitta KS. 2013. Multiple NSAID-induced hits injure the small intestine: underlying mechanisms and novel strategies. Toxicol Sci. 131(2):654-667.

Bonnefille B, Gomez E, Alali M, Rosain D, Fenet H, Courant F. 2018. Diclofenac in the marine environment: a review of its occurrence and effects. Mar Pollut Bull. 131:496-506.

Brogden RN, Heel RC, Pakes GE, Speight TM, Avery GS. 1980. Diclofenac sodium: a review of its pharmacological properties and therapeutic use in rheumatic diseases and pain of varying origin. Drugs. 20:24-48.

Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. 2008. Urinary concentrations of triclosan in the U.S. population: 2003-2004. Environ Health Perspect. 116(3):303-307.

Cardoso-Vera JD, Islas-Flores H, Gómez-Oliván LM, Sánchez-Ocampo EM, Osten JR-V, Hernández-Navarro MD. 2017. Comparative study of diclofenac-induced embryotoxicity and teratogenesis in Xenopus laevis and Lithobates catesbeianus, using the frog embryo teratogenesis assay: Xenopus (FETAX). Sci Total Environ. 574:467-475.

Chae JP, Cho DH, Won DH, Gye MC. 2015. Evaluation of developmental toxicity and teratogenicity of diclofenac using Xenopus embryos. Chemosphere. 120:52-58.

Conners DE, Rogers ED, Armbrust KL, Kwon JW, Black MC. 2009. Growth and development of tadpoles (Xenopus laevis) exposed to selective serotonin reuptake inhibitors, fluoxetine and sertraline, throughout metamorphosis. Environ Toxicol Chem. 28(12):2671-2676.

Coxib and traditional NSAID Trialists' (CNT) Collaboration, Bhala N, Emberson J, Merhi A, Abramson S, Arber N, Baron JA, Bombardier C, Cannon C, Farkouh ME, et al. 2013. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. Lancet. 382(9894):769-779.

Dama M. 2014. The diclofenac ban is helping vulture conservation; what further pharmaceutical threats loom ahead? Curr Sci. 107(4):564-565.

Emre FB, Turhan DÖ, Güngördü A. 2024. Toxicity of commercial and pure forms of three nonsteroidal anti-inflammatory drugs in Xenopus laevis embryos before and after ozonation. Drug Chem Toxicol. 47(6):1004-1017.

Fini JB, Le Mével S, Palmier K, Darras VM, Punzon I, Richardson SJ, Clerget-Froidevaux MS, Demeneix BA. 2012. Thyroid hormone signaling in the Xenopus laevis embryo is functional and susceptible to endocrine disruption. Endocrinology. 153(10):5068-5081.

Fogliano C, Raucci F, Cimini AM, Pastore A, Motta CM, Bernabò N, Guerriero V, Varricchio E. 2022. Environmental concentrations of a delorazepam-based drug impact on embryonic development of non-target Xenopus laevis. Aquat Toxicol. 250:106244.

Fort DJ, Rogers RL, Gorsuch JW, Navarro LT, Peter R, Plautz JR. 2017. Effect of triclosan on anuran development and growth in a larval amphibian growth and development assay. J Appl Toxicol. 37(10):1182-1194.

Fort DJ, Mathis MB, Hanson W, Fort CE, Navarro LT, Peter R, Buche C, Walker M, Tocqueville JF, Grizzle TB. 2010. Triclosan and anuran metamorphosis: no effect on thyroid-mediated metamorphosis in Xenopus laevis. Toxicol Sci. 113(2):392-400.

Freundlich JS, Wang F, Tsai HC, Kuo M, Shieh HM, Anderson JW, Nkrumah LJ, Valderramos JC, Yu M, Kumar TRS, et al. 2007. X-ray structural analysis of Plasmodium falciparum enoyl acyl carrier protein reductase as a pathway toward the optimization of triclosan antimalarial efficacy. J Biol Chem. 282(35):25436-25444.

Gyllenhammar I, Holm L, Eklund R, Berg C. 2009. Reproductive toxicity in Xenopus tropicalis after developmental exposure to environmental concentrations of ethynylestradiol. Aquat Toxicol. 91(2):171-178.

Ishizuya-Oka A, Shi YB. 2005. Molecular mechanisms for thyroid hormone-induced remodeling in the amphibian digestive tract: a model for studying organ regeneration. Dev Growth Differ. 47(9):601-607.

Lu G, Xie Z, Zhang Z. 2018. Effects of dissolved organic matter, feeding, and water flow on the bioconcentration of diclofenac in crucian carp (Carassius auratus). Environ Sci Pollut Res Int. 25(8):7776-7784.

Lonappan L, Brar SK, Das RK, Verma M, Surampalli RY. 2016. Diclofenac and its transformation products: environmental occurrence and toxicity - a review. Environ Int. 96:127-138.

Mahapatro GK, Arunkumar K. 2014. The case for banning diclofenac and the vanishing vultures. Biodiversity. 15(4):265-268.

Matsumura N, Ishibashi H, Hirano M, Nagao Y, Watanabe N, Shiratsuchi H, Kai T, Nishimura T, Kashiwagi A, Arizono K. 2005. Effects of nonylphenol and triclosan on production of plasma vitellogenin and testosterone in male South African clawed frogs (Xenopus laevis). Biol Pharm Bull. 28(9):1748-1751.

McAvoy DC, Schatowitz B, Jacob M, Hauk A, Eckhoff WS. 2002. Measurement of triclosan in wastewater treatment systems. Environ Toxicol Chem. 21(7):1323-1329.

McAvoy JW, Dixon KE. 1978. Cell specialization in the small intestinal epithelium of adult Xenopus laevis: structural aspects. J Anat. 125(Pt 1):155-169.

Nadatani Y, Watanabe T, Shimada S, Otani K, Tanigawa T, Fujiwara Y. 2018. Microbiome and intestinal ischemia/reperfusion injury. J Clin Biochem Nutr. 63(1):26-32. doi:10.3164/jcbn.17-137.

Neville AJ, Zach SJ, Wang X, Larson JJ, Judge AK, Davis LA, Vennerstrom JL, Davis PH. 2015. Clinically available medicines demonstrating anti-Toxoplasma activity. Antimicrob Agents Chemother. 59(12):7161-7169.

Nieuwkoop PD, Faber J. 1967. The normal table of Xenopus laevis (Daudin): a systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis. Amsterdam: North-Holland Publishing Company.

Ortega FJ. 2019. The effect of streptomycin on the microbiome of Xenopus laevis [master's thesis]. [Place unknown]: [publisher unknown].

Peltzer PM, Lajmanovich RC, Martinuzzi CS, Attademo AM, Colussi CL, Curi LM, Culzoni MJ, Goicoechea HC. 2019. Biotoxicity of diclofenac on two larval amphibians: assessment of development, growth, cardiac function and rhythm, behavior and antioxidant system. Sci Total Environ. 683:624-637.

Porro, Laura B., and Christopher T. Richards. "Digital dissection of the model organism Xenopus laevis using contrast-enhanced computed tomography." *Journal of Anatomy* 231.2 (2017): 169-191.

Queckenberg C, Meins J, Wachall B, Doroshyenko O, Tomalik-Scharte D, Bastian B, Abdel-Tawab M, Fuhr U. 2010. Absorption, pharmacokinetics, and safety of triclosan after dermal administration. Antimicrob Agents Chemother. 54(1):570-572.

Reis R, Dhawle R, Girard R, Frontistis Z, Mantzavinos D, de Witte P, Cabooter D, Du Pasquier D. 2024. Electrochemical degradation of diclofenac generates unexpected thyroidogenic transformation products: implications for environmental risk assessment. J Hazard Mater. 472:134458.

Spitz S. 2013. Physicians should be cautious when prescribing diclofenac. CMAJ. 185(6):470.

Stoker TE, Gibson EK, Zorrilla LM. 2010. Triclosan exposure modulates estrogen-dependent responses in the female Wistar rat. Toxicol Sci. 117(1):45-53.

Tamaoki K, Okada R, Ishihara A, Shiojiri N, Mochizuki K, Goda T, Yamauchi K. 2016. Morphological, biochemical, transcriptional and epigenetic responses to fasting and refeeding in intestine of Xenopus laevis. Cell Biosci. 6:2.

Tenkov KS, Grebenshchikova AV, Safronov VV, Selivanova MA, Kladchenko ES, Soldatov AA. 2022. An in vivo study of the toxic effects of triclosan on Xenopus laevis (Daudin, 1802) frog: assessment of viability, tissue damage and mitochondrial dysfunction. Comp Biochem Physiol C Toxicol Pharmacol. 259:109401.

Thomson AH, Thompson CK. 2024. Acute triclosan exposure alters mitochondrial form and function in Xenopus laevis tadpoles, including the developing brain. bioRxiv. doi:10.1101/2024.11.

Tsutsumi S, Gotoh T, Tomisato W, Mima S, Hoshino T, Hwang HJ, Takenaka H, Tsuchiya T, Mori M, Mizushima T. 2004. Endoplasmic reticulum stress response is involved in nonsteroidal anti-inflammatory drug-induced apoptosis. Cell Death Differ. 11:1009-1016.

Urbatzka R, Bottero S, Mandich A, Lutz I, Kloas W. 2010. Expression profiles of LHβ, FSHβ and their gonadal receptor mRNAs during sexual differentiation of Xenopus laevis tadpoles. Gen Comp Endocrinol. 168(2):239-244.

Usal M, Veyrenc S, Darracq-Ghitelman-Rey L, Quartier M, Perrin L, Dis SS, Boulahtouf A, Bonnet N, Leon C, Vasseur P, et al. 2019. Concomitant exposure to benzo[a]pyrene and triclosan at environmentally relevant concentrations induces metabolic syndrome with multigenerational consequences in Silurana (Xenopus) tropicalis. Sci Total Environ. 689:149-159.

Veldhoen N, Skirrow RC, Osachoff H, Wigmore H, Clapson DJ, Gunderson MP, Van Aggelen G, Helbing CC. 2006. The bactericidal agent triclosan modulates thyroid hormone-associated gene expression and disrupts postembryonic anuran development. Aquat Toxicol. 80(3):217-227.

Wang Z, Li X, Li Y, Liu H, Lin CSK, Sun J, Wang Q. 2024. Unveiling the occurrence and ecological risks of triclosan in surface water through meta-analysis. Environ Pollut. 361:124901.

Weatherly LM, Gosse JA. 2017. Triclosan exposure, transformation, and human health effects. J Toxicol Environ Health B Crit Rev. 20(8):447-469.

Wolfe MM, Lichtenstein DR, Singh G. 1999. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med. 340:1888-1899.

Yueh MF, Taniguchi K, Chen S, Evans RM, Hammock BD, Karin M, Tukey RH. 2014. The commonly used antimicrobial additive triclosan is a liver tumor promoter. Proc Natl Acad Sci U S A. 111(48):17200-17205.

Zhang S, Lü B, Si JM, Chen FM, Meng LN, Wu WF, Yu LM. 2009. [Effect of non-steroidal anti-inflammatory drugs on small intestinal barrier function in rats]. Zhonghua Nei Ke Za Zhi. 48(1):44-47. Chinese.

Zwahlen J, Dornburg A, Boekhoff S, Denver RJ, Dugas MB, Hammond S, Heimeier R, Hu F, Kulkarni SS, Norris D, et al. 2024. The ecological function of thyroid hormones. Philos Trans R Soc Lond B Biol Sci. 379(1898):20220511.