Histological Analysis of the Integument of the Zigzag Salamander, *Plethodon dorsalis*

By

Breanna C. Davis

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Faculty Advisor: Kevin M. Gribbins, Ph.D.

Executive Director of Honors: James B. Williams, Ph.D.

Abstract

Studies conducted on the integument of amphibians within Plethodontidae revealed these salamanders have mucous, granular, and modified composite multicellular glands in their skins. Unfortunately, few species have been studied to date. Thus, the purpose of this study was to investigate the histological diversity of the integumentary glands found on the ventral and dorsal surfaces of the Zigzag Salamander, *Plethodon dorsalis*, and compare these features to those found on the Northern Two-Lined Salamander, *Eurycea bislineata*, and the Cave Salamander, *Eurycea lucifuga.* These three species are found in the same family, Plethodontidae, which is comprised of more than 250 species. The data was then used to form a preliminary comparative study that investigated the histological diversity of integumentary glands found on the ventral and dorsal surfaces of terrestrial adult lungless salamanders. Results showed that *P. dorsalis* had polarization to its two skin surfaces, with mucous glands dominating ventrally and poison glands dominating dorsally. Additionally, *P. dorsalis* had similar morphology to their glands as that observed in other lungless salamanders with the distinction of the stratum corneum lining the ducts of both poison and mucous glands in a valve like manner.

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Statement of Purpose

Chytrid disease, or chytridiomycosis, has been one of the leading causes of amphibian deaths in recent years, with studies mostly focusing on the decline in frog populations (Weinstein et al. 2009; Retallick et al. 2004). Uniquely, the few studies that focus on the effects of chytridiomycosis in salamander populations have shown that salamander populations with the disease in the wild have not declined (Weinstein et al. 2009). Multiple species of salamanders have yet to be studied and these could provide insights into what distinguishing features may allow salamanders to survive chytridiomycosis while other amphibians die off. Chytridiomycosis is caused by the pathogenic fungus, *Batrachochytrium dendrobatidis*, which often causes epithelial infections and skin lesions, making integumentary features the most likely source of the survival features salamanders possess to fight this disease (Nichols et al. 2001).

Plethodon dorsalis is a terrestrial lungless salamander, whose integument histology has never before been studied. This study provides a preliminary histological investigation into the integumentary features observed in *P. dorsalis* as well as clear visual drawings of the key distinguishing features of this salamander's skin. It also gives a preliminary comparative analysis of the integumentary features found in the Plethodontidae family based on *Plethodon dorsalis*, *Eurycea bislineata*, and *Eurycea lucifuga* salamanders. It was hypothesized that the dorsal and ventral surfaces of *P. dorsalis* would differ in features such as gland number, type, and pigment, and that plethodontid salamanders would have similar skin gland morphologies to that of other previously studied lungless salamanders with varying distribution of gland types between species.

Introduction

Chytridiomycosis

Chytridiomycosis is caused by the fungus *Batrachochytrium dedrobatidis* (Weinstein et al. 2009). Thought to have originated in Africa, this disease has been found in amphibians all over the world and continues to play a significant role in their decline (Weldon et al. 2004). Most studies have focused on the effects of chytridiomycosis on frogs and found that environmental factors may play a part in how the disease affects frog populations (Weinstein et al. 2009; Retallick et al. 2004). Nichols et al. (2001) studied the effect of chytridiomycosis in poison dart frogs, *Dendrobates tincttorius* and *D. auratus*, and found that all the frogs exposed to the fungus developed a fatal skin disease and skin lesions. Weinstein et al. (2009) studied the effect of chytridiomycosis in salamanders and found that those collected with chytridiomycosis infection always died in captivity, but populations with the disease studied in the wild did not show signs of decline. A study conducted on the effect of chytridiomycosis in Tiger Salamanders, *Ambystoma tigrinum*, indicated that several harbored extensive infections in their sloughed skin but were not morbidly affected (Davidson et al. 2003). This led the scientists to conclude that the rapid rate of sloughing in salamanders may be the distinguishing feature that allows them to survive the disease, but further experimentation would need to be conducted to support their hypothesis (Davidson et al. 2003).

The specific features that allow salamanders to survive with chytridiomycosis are still under investigation as there are still many species of salamander that have not been studied to date. Even fewer histological studies have been conducted to investigate the skin, or integument, features in these salamanders that might offer more insight into the survival mechanisms they possess.

Plethodon dorsalis

Plethodon dorsalis, more commonly referred to as the Zigzag salamander, is part of the Plethodontidae family (Dodd et al. 2004). This family comprises over 250 species of lungless salamanders and can be found along the west coast of North America and throughout the eastern United States (Heying et al. 2003). This family is characterized by a loss of several cranial bones, are all lungless, and possesses four legs and four digits on each foot (Heying et al. 2003). The word *plethore* in Greek means fullness or full of, and *odon* means teeth so the name *Plethodon* refers to the many teeth found on both the jaw and the roof of the mouth of this salamander taxon (Dodd et al. 2004). The term *dorsal* means back in Latin, so *dorsalis* refers to the pattern found along the back of *P. dorsalis.*

While amphibians have evolved to be able to successfully live in both aquatic and terrestrial ecosystems by undergoing metamorphosis (Bonnett and Chippindale 2006), *P. dorsalis* is a completely terrestrial salamander. *Plethodon dorsalis* practices direct development, meaning that all developmental stages occur within the egg and neonates hatch from their terrestrial eggs as miniature adults, thus skipping the larval stage (Heying et al. 2003). As no histological studies have ever been conducted on *P. dorsalis,* it is unknown what developmental changes occur in the skin glands as it develops within the egg or whether their skin glands differ from other larval or adult salamanders that are not fully terrestrial.

Integumentary System

The integumentary system is comprised of the skin and its derivatives, such as hair and exocrine glands (Barclay 1999). Studies conducted on the integument of the plethodontid salamander family have shown mucous, granular, and modified composite multicellular glands in their skin (Fontana et al. 2006). Histological studies of the skin, such as those done within the

Gastrophryne carolinesis and *Proteus anguinus* (Siegel et al. 2008; Mali and Bulog 2004), can be utilized to understand the specific structure and location of the multitude of different multicellular glands and cellular structures unique to amphibian skin. Data on the morphology of Caudata skin glands is limited to a handful of studies between aquatic and terrestrial salamanders or their larvae (Bonnet and Chippindale 2006; Fontana et al. 2006; Mali and Bulog 2004; Sever et al. 2016). The authors of one comprehensive study on the skin in the salamander *Karsenia koreana* found and described mucous, granular, and modified granular glands (Sever et al. 2016). Evidence from several other studies has shown that there are two types of dermal glands found in all adult amphibians: the granular and mucous glands (see Fontana et al. 2006 for review). This study will provide histological and ultrastructural data on the integumentary glands, compare their distribution on the ventral and dorsal surfaces of *P. dorsalis*, and then compare our data with the other salamanders in the Plethodontidae family to analyze potential similarities and differences of integumentary features among terrestrial adult salamanders.

Methodology

Role of Advisor

As my advisor, Dr. Gribbins, provided guidance and training as the project was completed. We met in person at least once every two weeks to discuss my progress, sometimes more often if questions arose while executing the project. He provided me with lab safety information and helped me refine the procedures necessary for the use of dangerous chemicals during the embedding process. Because of the dangerous nature of these chemicals, as shown in appendix B, I adorned gloves, goggles, and a lab coat for most of the embedding process. A fume hood was also utilized while completing the embedding process for the transmission electron microscope (TEM) samples, which can contain toxic fumes. Once the samples were prepared for analysis, Dr. Gribbins and I photographed the samples using light and electron microscopes. These images were then referenced when drawing the distinguishing features found in the poison and mucous glands of the integument. He also then provided resources for labeling and determining the identity of the structures found in the skin. Lastly, Dr. Gribbins assisted me in preparing to present my results by conducting the final edits and critiques of both my poster and formal manuscript.

Timeline

My project began in September 2018 as I began learning microscopy and staining techniques by practicing on already embedded samples utilized just for practice. In January of 2019, the dissection and embedding of *P. dorsalis* for light microscopy was completed. Once this was accomplished, I began sectioning and staining samples to use as preliminary data for the Undergraduate Research Conference (URC) and the Indiana Academy of Science (IAS) conference. Dr. Gribbins then took the samples I sectioned and used the more advanced Leica

Ultracut S microtome, that he does not allow undergraduate students to use, to get clear-cut sections of integumental tissues. These sections provided good images that were easily analyzed to identify structures. My proposal was submitted to the honors counsel on March 18, 2019 and accepted with minor revisions. After having my proposal accepted, I presented a poster at the IAS conference on March 30, 2019 and the URC at Butler University on April 12, 2019. Then in August 2019, I began embedding samples for TEM analysis. By early October, I had finished sectioning and Dr. Gribbins had produced images from his TEM. I then added the TEM results to my poster and presented at the National Collegiate Honors Council conference in November 2019. The present manuscript on *P. dorsalis* was completed in early 2020.

Tissue Preparation for Light and Transmission Electron Microscopy

P. dorsalis were overdosed using an anesthetic known as MS222. The salamander body cavity was opened and exposed and then the entire salamander was placed in a solution of Trumps fixatives in order to prevent autolysis, bacterial attack, and to aid in tissue fixation. Forty-eight hours after fixation, dorsal and ventral skin pieces were dissected from the body using a dissecting scope. The skin was then cut into multiple squares approximately 3x3 millimeters in size.

For light microscopy, gloves, goggles, and a lab coat were adorned while rinsing the samples three times for ten minutes each in cacodylate buffer. Once the rinses were complete, the samples were then stored in 70% ethanol until ready to complete the dehydration and embedding process. To use epon plastic to embed the skin samples, a modified method was used based off of the process described by Seigel et al. (2008). Before starting the dehydration process, a solution of plastic was made by mixing the plastics ERL-4221, DER-736, NSA, and DMAE for about two and a half hours and then covered with parafilm until ready for use.

Then the dehydration process was performed on skin samples, using an ascending series of ethanol washes to remove water and fixative from the skin samples. Once the ethanol washes reached 100% the samples were infiltrated with the plastic made earlier in a solution of two-parts ethanol and one-part plastic. The skin samples were then incubated in a 1:1 ethanol/plastic solution, and then finally in 100% plastic overnight. The skin tissues were then incubated in a new batch of 100% plastic for two hours. Lastly the samples were placed in disposable molds and put in a vacuum oven set at 70°C. After 24 hours, the skin samples were taken out of the oven and removed from the disposable molds.

For transmission electron microscopy, the 3x3 millimeters samples were rinsed in cacodylate buffer three times for five minutes. The samples were then post-fixed in osmium tetroxide for an hour and a half before being rinsed again in cacodylate buffer three times for two minutes. The samples were then stored in 70% ethanol until ready to complete the dehydration and embedding process. From here the steps are the same as those for the dehydration and embedding of the light microscopy samples detailed above.

Sectioning and Staining

Once the plastic hardened, it provided enough support for the tissue samples to be cut into very thin sections via microtomy. These sections were then placed on microscope slides and stained with LAD dye in order to clearly see the integumentary structures. The slides were then analyzed under light and transmission electron microscopes to identify the structures and architecture of the *P. dorsalis* skin.

Drawing the Structures

Once all the sectioning was complete, Dr. Gribbins verified that the poison and mucous glands comprised the most distinguishing features of the integument of *P. dorsalis.* With this

verification, illustrations of both gland types were drawn to clearly depict the unique structure and features of each gland and provide a clear comparison. A sketchpad and graphite pencils were used to sketch out the structures seen in the photographs and under the light microscope. By utilizing multiple section images, an idealistic illustration of each gland was drawn emphasizing all the features unique to each gland. This allowed all the distinguishing features to be observed at once in the drawings, which were not always represented in each of the histological sections at the same time. The graphite illustrations and the photographs of the microscopic images used as references were then shown to Professor Viewegh for critiques and edits. Once the accuracy of the sketches was approved by Professor Viewegh and Dr. Gribbins, the sketches were retraced using micron pens and digitally scanned to incorporate into the final manuscript.

Familial Comparison

After the preliminary findings were completed for *P. dorsalis,* a comparison was done including the preliminary histological findings of the integument of *E. bislineata* and *E. lucifuga* completed by other students of Dr. Gribbins. The preliminary results of *E. bislineata* and *E. lucifuga* are shown in Figures 4 and 5 in the results section. As all three salamanders were of the Plethodontidae family, this comparison was considered a preliminary familial comparison. Specific key features such as gland types present ventrally and dorsally, abundance of chromatophores, and the presence of stratum corneum lining the excretory ducts were analyzed across all three species to find similarities and differences within their integuments.

Results

Plethodon dorsalis

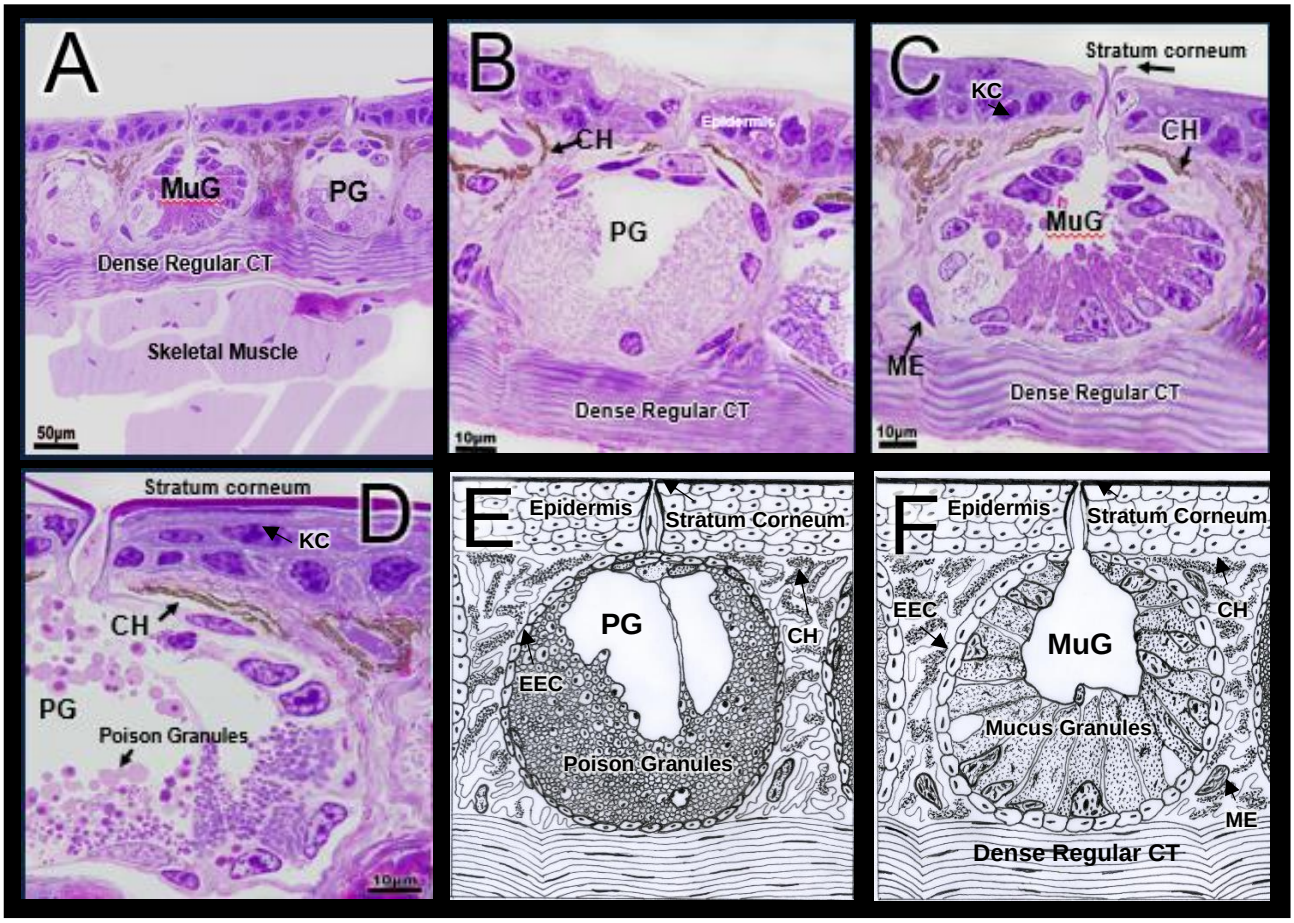

Figure 1 A-D: Histological sections of the dorsal skin from *P. dorsalis* taken using a light microscope at varying powers depicts both poison (A,B,D) and mucous (A,C) glands with E and F as idealized illustrations for comparison. Mucous gland, MuG; Poison gland, PG; Chromatophores, CH; Myoepithelial cells, ME; Connective tissue, CT; Epidermal Epithelial Cells, EEC; Keratinocyte, KC. Bars = 50 and 10µm.

The dorsal integument of *P. dorsalis* shows both mucous and poison glands present in the dermis (Fig. 1A; MuG, PG). The stratum corneum, which serves as the outermost layer of the epidermis, is present all the way around the body superficially and lines the ducts of both gland types (Fig. 1C,D; MuG, PG). The stratum corneum lining the ducts appears valve-like with the thickness and angle of the lining towards the opening at the apical surface (Fig 1C,D). Keratinocytes, the main cell type of epidermis, are seen clearly in the epidermis of the

integument (Fig. 1C,D; KC). The main gland found on the dorsal side is the poison gland and, as shown, are relatively the same size as the mucous glands (Fig. 1A; MuG, PG). Chromatophores are seen throughout the dermis surrounding the glands and produce clusters of small granules (melanin) around the glands that give the skin its pigment (Fig. 1B,C,D; CH, MuG, PG). Myoepithelial cells are observed in and around the mucous and poison glands as they help expel mucous and poison from the glands, through the ducts and onto the skin surface (Fig 1C; ME, MuG). Dense irregular connective tissue makes up the basal layer of the dermis with skeletal muscle found deep to the dermis (Fig 1A).

The illustrations of the poison and mucous glands provide an idealized depiction of the structures present in the integument of *P. dorsalis* (Fig. 1E,F; PG, MuG). The epidermis clearly shows the stratum corneum as the most superficial layer that also lines the inside of the gland ducts forming a valve like structure (Fig. 1E,F). The poison and mucous glands are shown as relatively the same size in the dermis layer with epidermal epithelial cells forming the barrier between the dermis and the interior of the glands (Fig. 1E,F; EEC). Chromatophores are found throughout the dermis surrounding both gland types and myoepithelial cells are most easily observed around the mucous glands (Fig. 1E,F; CH,ME, MuG). Poison granules, differing in size and density, are depicted within the poison gland and mucus granules are depicted within the mucous gland (Fig. 1E,F; PG, MuG). Dense regular connective tissue is shown as the basal layer of the dermis (Fig. 1E,F). The illustrations emphasize the distinguishing features between the poison and mucous glands to make them more easily discernible (Fig. 1E,F).

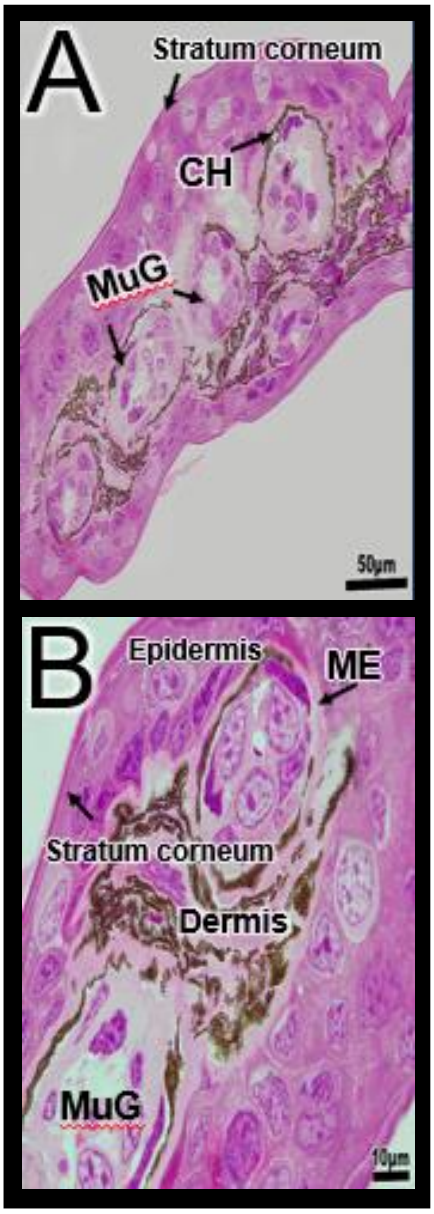

Figure 2 A,B: Sections of ventral skin of the *P. dorsalis* taken with a light microscope at different magnifications shows multiple mucous glands. Mucous gland, MuG; Chromatophores, CH. Bars = 50 and 10µm.

The ventral integument of *P. dorsalis* only has mucous glands present in the dermis (Fig. 2A,B; MuG). Mucous glands are more abundant in the ventral integument in comparison to the dorsal integument (Fig.2A and Fig. 1A; MuG). The mucous glands seen ventrally are developing mucus glands and have not produced or concentrated mucin granules like those shown in Fig.1C (Fig. 2B; MuG). Myoepithelial cells are present in the dermis and around the

mucous glands, similar to those found in the dorsal integument seen in Fig. 1C (Fig. 2B; ME, MuG). The stratum corneum is superficially lining the epidermis of the ventral skin as seen in the dorsal skin in Fig. 1A,C,D (Fig. 2 A,B). Chromatophores are grouped around the mucous glands throughout the dermis, similar to those seen around the poison and mucous glands on the dorsal surface (Fig. 1A,B,C,D; CH, PG, MuG and Fig.2A,B; CH, MuG).

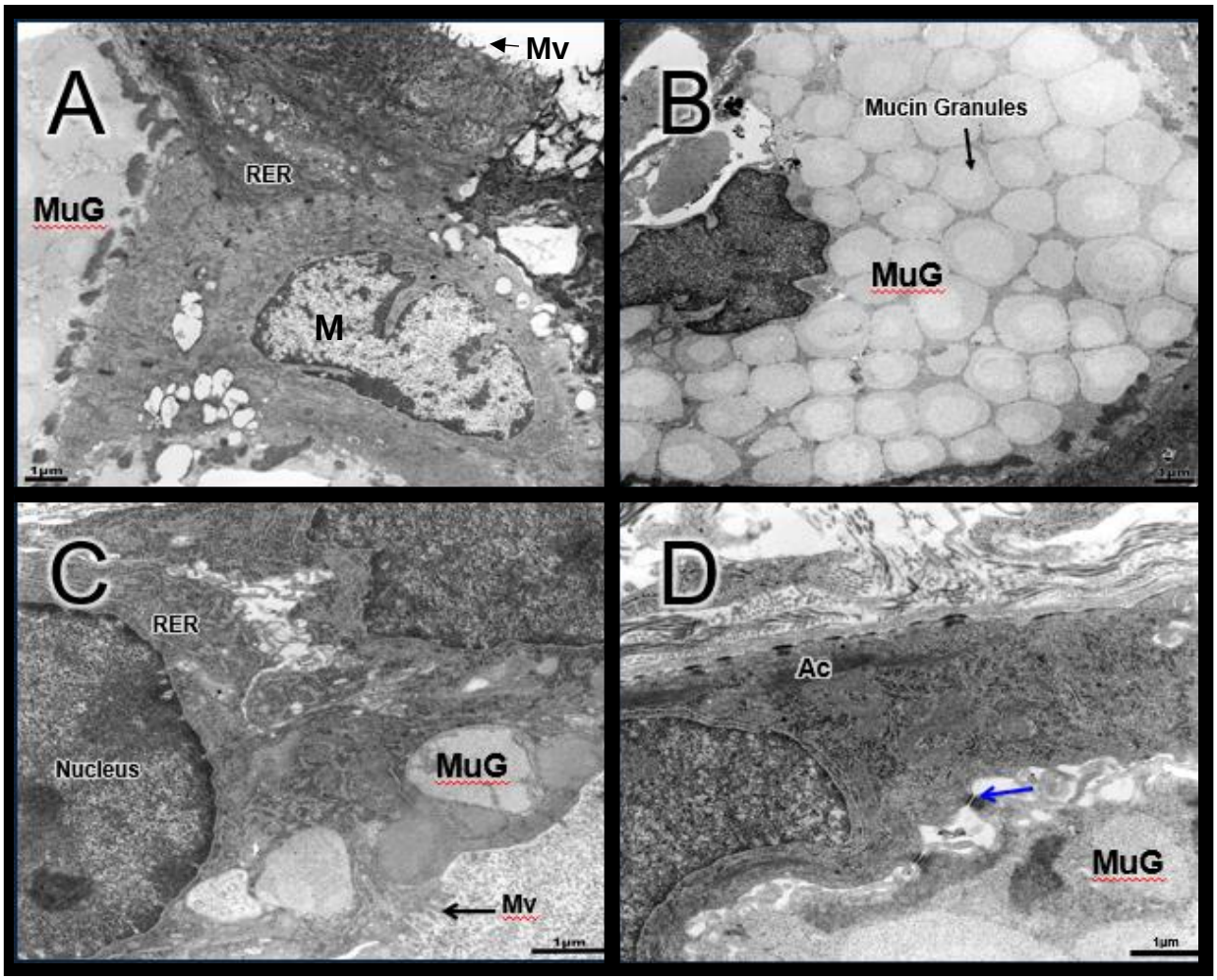

Figure 3 A-D: Transmission electron microscope (TEM) micrographs of a *P. dorsalis* myoepithelial cell (D) and mucous gland, focusing on specific features within the gland (A,B,C) at various magnifications. Mucous gland, MuG; Microvilli, Mv; Rough endoplasmic reticulum, RER; Actin, Ac; Mitochondria, M; Blue arrow = desmosome junction. Bars = 1µm.

Mucous glands are found on both the dorsal and ventral surfaces of *P. dorsalis*. Transmission electron microscope images are used to analyze these glands subcellularly.

Mucin producing cells within mucous glands have mitochondria and rough endoplasmic reticulum dominating the organelle composition within these cells (Fig.3A; M, MuG, RER). The nuclei of mucous gland cells clearly show light staining euchromatin and large conspicuous nucleoli, which is a clear indication of a cell active in transcription and the cytoplasm is filled with enlarged and active rough endoplasmic reticulum (Fig. 3C; RER). As these nuclei are active, these cells are starting production of a new wave of mucin protein granules (Fig. 3C). Prominent mucin granules can be seen within the mucous gland that would have been developed and secreted to form mucus from actively secreting glands (Fig. 3B; MuG). The surfaces of the mucous cells show microvilli that increase surface area (Fig. 3A; Mv). The nucleus and cytoplasm of a myoepithelial cell can be seen in Fig. 3D. Actin appears to be in great abundance within the cytoplasm of these myoepithelial cells with multiple desmosome junctions (blue arrow) located between them and the mucus producing cells of the gland (Fig. 3D; Ac).

Eurycea bislineata

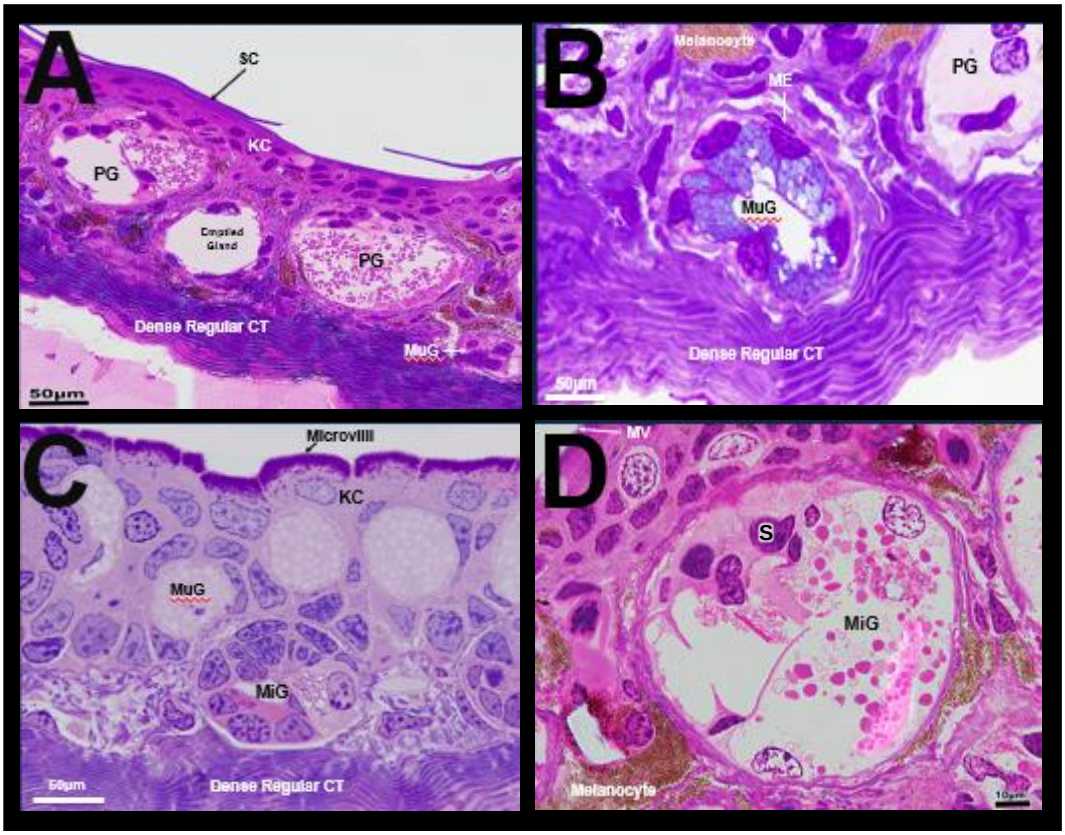

Figure 4 A-D: Integument from the *E. bislineata* at varying degrees of power via light microscopy, shows both poison and mucous glands on the dorsal surface (A,B) and mucous and mixed glands on the ventral surface (C,D). Poison gland, PG; Stratum corneum, SC; Mucous gland, MuG; Keratinocyte, KC; Mixed gland, MiG; Myoepithelial cell, ME; Serous cell, S; Microvilli, MV; Connective tissue, CT. Bars = 50 and 10µm.

Part of the Plethodontidae family comparison includes the study of the integument of *Eurycea bislineata*. Multiple poison glands as well as some mucous glands are seen on the dorsal integument of *E. bislineata* (Fig. 4A; PG, MuG). Some of the glands shown are empty of poison granules while others are partially or completely full of poison granules (Fig. 4A; PG). The stratum corneum thinly lines the apical surface of the integument with dense regular connective tissue forming the basal layer of the dermis (Fig. 4A; SC). Melanocytes, also known

as chromatophores, are scattered throughout the dermis and myoepithelial cells are seen near the mucous and poison glands (Fig. 4B; ME).

The ventral integument of *E. bislineata* shows multiple mucous glands as well as some mixed glands used to perform both serous and mucous secretions (Fig. 4C; MuG, MiG). Poison glands are not found on the ventral surface (Fig.4C). Microvilli are seen on the superficial layer of the epidermis (Fig. 4C; Mv). Dense regular connective tissue is also seen making up the basal dermis layer and keratinocytes are seen throughout the epidermis of the integument (Fig. 4C; KC). One of the key features within the ventral integument of *E. bislineata* is the mixed glands that are present (Fig. 4D, MiG). Mucous granules can be clearly seen within the mixed gland as well as a few serous cells within the apical region of the gland (Fig. 4D; MiG, S, MG). Melanocytes are seen grouped around the glands and microvilli are found on the superficial layer of the epidermis (Fig. 4D; Mv).

Eurycea lucifuga

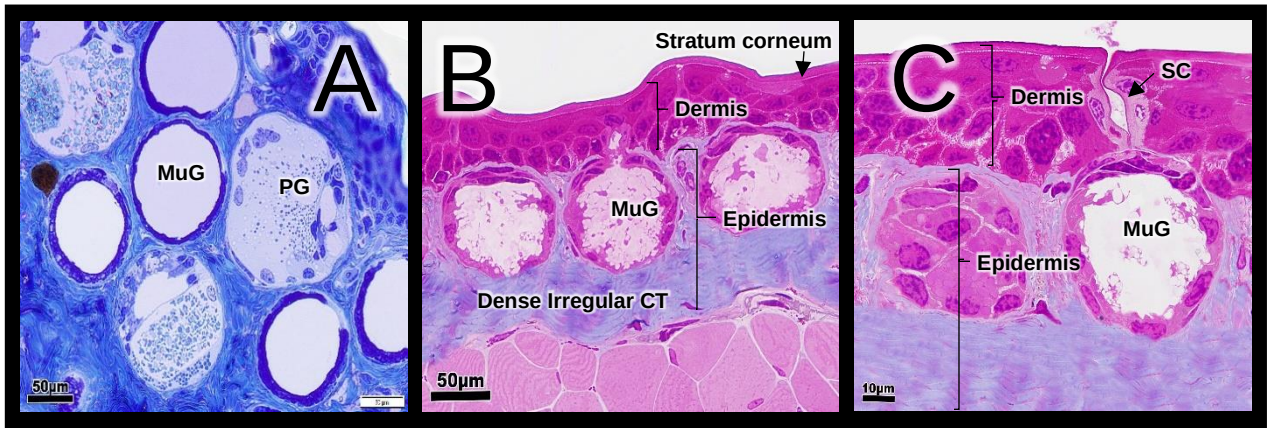

Figure 5 A-C: *E. lucifuga* integument at different magnifications shows both poison and mucous glands dorsally (A) and mucous glands ventrally (B,C). Mucous gland, MuG; Poison gland, PG; Stratum corneum, SC; Connective Tissue, CT. Bars = 50 and 10µm.

Eurycea lucifuga integument is also studied for the Plethodontidae family comparison. The dorsal surface of *E. lucifuga* integument has both poison and mucous glands (Fig. 5A; PG, MuG). Most of the mucous glands seen dorsally are empty with only a thin lining of epithelium cells making up the outer edges of the gland to form a barrier between the gland and the rest of the dermis. (Fig. 5A; MuG). Poison granules seen within the poison glands show that the glands were ready for secretion (Fig. 5A; PG).

The ventral surface shows an abundance of mucous glands (Fig. 5B,C; MuG). The stratum corneum forms a thin apical layer at the top of the epidermis similar to that seen in both *P. dorsalis* and *E. bislineata* (Fig. 2B; Fig. 4A; Fig. 5B). The basal layer of the dermis is dense irregular connective tissue that provides a sturdy connection point for the skeletal muscle seen deep to the integument (Fig.5B). An empty mucous gland and a full mucous gland of *E. lucifuga* are shown in Fig. 5C. Within the epidermis the mucous gland duct provides an opening from the mucous gland to the apical surface of the epidermis (Fig. 5C; MuG). The stratum corneum is also observed lining the duct similar to that found in *P. dorsalis* but the

lining of *P. dorsalis* appears significantly thicker and more like a valve (Fig. 1C,D; compare to Fig. 5C). Very few chromatophores, or melanocytes, are seen in Fig. 5C and they appear in less abundance in the epidermis of *E. lucifuga* compared to that of *P. dorsalis* and *E. bislineata* (Fig. 1C; Fig. 2A; Fig. 4A; Fig. 5C).

Analysis/Conclusion

Plethodon dorsalis

The integumentary system includes the skin and any of its derivatives such as nails or glands (Barclay 1999). The few studies conducted on the integument of plethodontid salamanders have shown mucous, granular, and modified composite multicellular glands in their skin (Fontana et al. 2006). *Plethodon dorsalis* has mucous glands as well as granular glands, more commonly known as poison glands, present in the dermis. The dorsal integument of *P. dorsalis* has both gland types, while the ventral integument only has mucous glands. This is most likely due to poison glands being used to secrete a deterrent as protection from predators that typically strike from above (Arrivillaga and Brown 2018). The mucous and poison glands found in *P. dorsalis* are relatively similar in size, but in histological studies of other amphibians, such as *G. carolinesis*, the poison glands are significantly larger in size in comparison to the mucous glands (Siegel et al. 2006).

The overall structure of the *P. dorsalis* integument is similar to that of other amphibians in that the epidermal and dermal layers have many of the same features, including: stratum corneum, keratinocytes, chromatophores, myoepithelial cells, and dense irregular connective tissue (Garton and Mushinsky 1979). The stratum corneum makes up the most superficial layer of the epidermis providing the first line of defense against pathogens attempting to enter the body. Keratinocytes are found in the epidermis and produce keratin proteins that act as intermediate filaments for anchoring structures and forming junctions. When keratin proteins build up, they make the epidermis hard (Eckert and Rorke 1989). Chromatophores found throughout the dermis provide the integument with the pigment that makes distinct patterns and colors used for breeding and protection. Myoepithelium cells are found around glands and help

secrete the granules from these glands (Balachander et al. 2015). Dense irregular connective tissue is found at the basal layer of the dermis and provides the structural support of the integument as well as an anchorage point for the skeletal muscle found deep to the dermis. These commonly found integument structures make up much of the *P. dorsalis* integument.

The most distinguishing feature of the *P. dorsalis* integument is the stratum corneum lining the ducts of both gland types. Studies of other amphibians did not show the stratum corneum contributing to the lining of any of the gland ducts (Garton and Mushinsky 1979; Siegel et al. 2006). The stratum corneum of *P. dorsalis*, however, lines the excretory ducts of the poison and mucous glands of the dorsal integument as well as the mucous gland ducts of the ventral integument. This morphological feature is also found in the Plethodontid salamander, *Karsenia korenea* (Sever et al. 2016). The way the stratum corneum is angled and positioned in the ducts of *P. dorsalis* suggests that it acts as a plug, or more likely a valve, that may aid in the building of pressure before the gland releases its contents, helping the gland's secretion efficiency.

The ventral integument of *P. dorsalis* is found to be structurally the same as the dorsal integument with the most notable difference being the lack of poison glands. In comparison to the dorsal integument, the ventral integument has a larger abundance of mucous glands. Transmission electron microscope analysis of the mucous glands shows the mucoid producing cells that make up the lining of the mucous glands and produce the mucin granules that are secreted. The mucoid producing cells show enlarged nucleoli in the nucleus indicating active transcription as well as an abundance of rough endoplasmic reticulum seen in the cytoplasm, a clear indicator of protein production destined for exocytosis. These subcellular structures are important for the protein production function of these cells. Desmosome junctions are found between the mucus producing cells and myoepithelial cells; these junctions are like spot welds

and hold these cell types together. Since myoepithelial cells have high actin content, the contractions of these cells most likely pulls on the desmosome junctions forcing the mucus producing cells together. Thus, the lumen of each gland would be constricted, squeezing the contents of the glands into the duct and out of the body. These structures make up the mucous glands and allow them to produce and secrete the mucin granules (Balachander et al. 2015).

In conclusion, based on the histological analysis of the *P. dorsalis* integument, the dorsal and ventral surfaces have the same structural profile with the significant difference being that the ventral surface lacks poison glands and has a larger abundance of mucous glands. The *P. dorsalis* integument also has a similar morphology to that of other amphibians with the same structural components found in the epidermis and dermis. The most significant distinction between other amphibian integuments and that of *P. dorsalis* is the stratum corneum found lining the ducts of both poison and mucous glands potentially acting as a valve. As this is the first study done examining the integument of *P. dorsalis*, more studies will need to be conducted to build upon these findings as well as verify them. A TEM analysis of the poison glands and a closer examination of the stratum corneum could provide support for its possible valve-like function. Future studies can be conducted on embryonic salamander integument and then compared to the adult salamander integument from this study to gain a better understanding of the morphology that occurs between the embryonic and adult stages of salamanders.

Familial Comparison

The histological integument studies of the Northern Two-Lined salamander, *E. bislineata,* Cave salamander, *E. lucifuga,* and Zigzag salamander, *P. dorsalis,* conducted by students of Dr. Gribbins, were used to form a preliminary familial comparison of the integument within the Plethodontidae. All three of these salamander species have similar

morphological features to those found in the epidermis and dermis of other amphibians, such as poison glands, mucous glands, keratinocytes, stratum corneum, and chromatophores (Garton and Mushinsky 1979). The most distinct differences found between the integument of *P. dorsalis* and that of *E. lucifuga* and *E. bislineata* is the mixed glands found in the dermis of *E. lucifuga* and *E. bislineata* but not in *P. dorsalis* and the valve-like lining of the ducts in *P. dorsalis*.

All three species of lungless salamanders show the stratum corneum lining the ducts of their excretory glands such as the poison, mucous, and mixed glands. This structural characteristic is not found in other amphibians and appears to be unique and possibly synapomorphic to the Plethodontidae family (Garton and Mushinsky 1979; Siegel et al. 2006). *Plethodon dorsalis* appears to have a thick lining of stratum corneum within the ducts that angles to form what appears to be a valve, while the stratum corneum lining the ducts of *E. bislineata* and *E. lucifuga* is rather thin in comparison. The valve-like lining of stratum corneum appears to be unique to *P. dorsalis*.

The three species also all show poison glands only in the dorsal integument most likely used to secrete a deterrent for protection. One characteristic of the *P. dorsalis* integument is that the poison and mucous glands are similar in size. The poison, mucous, and mixed glands of *E. bislineata* and *E. lucifuga* are not all relatively the same size. Instead, the mucous glands are found to be smaller in comparison to the poison and mixed glands in *E. bislineata* and *E. lucifuga.* A distinguishing feature of the *E. lucifuga* integument is the significantly smaller number of chromatophores seen in the dermis compared to the amount found in the dermis of *E. bislineata* and *P. dorsalis* both dorsally and ventrally.

In conclusion, the integument of Plethodontidae salamanders is found to be morphologically similar to that of other amphibians with the unique characteristic of having

the stratum corneum lining the ducts of excretory glands. The distinct characteristics of the *P. dorsalis* skin is the lack of mixed glands and the valve-like stratum corneum lining the excretory ducts. The most notable characteristic of *E. lucifuga* is the less abundant grouping of chromatophores in the dorsal and ventral integument. All three species of lungless salamanders have poison glands only in the dorsal integument. Future studies should build upon this study to include a TEM analysis of the poison and mixed glands for more detailed comparisons. This familial comparison can be used in future studies to determine any different integument features found in salamanders that are not found in other amphibians, such as frogs. The data from this study could be used as a control to compare to the integument of Plethodontid salamanders proven to be infected with chytridiomycosis. A study such as this could give insight as to why salamanders have been able to survive chytridiomycosis (Weinstein et al. 2009).

Reflection

This project has been extremely informative, both academically and professionally. I began this project my sophomore year and worked hard to learn the technical skills needed to complete this project. This included learning to obtain clean sections of integument for staining and the intense protocols needed to embed the integument in plastic. Throughout this project, I also learned to overcome my setbacks and relish my successes.

My first setback occurred while learning to use the microtome to cut thin sections of tissue. It took me many hours to learn what adjustments needed to be made to the microtome based on the sections I was producing. Often the angle of the blade or the embedded plastic needed to be adjusted to achieve a clean section that provided intact tissue structures that could be clearly observed. I had not anticipated it to take as long as it did for me to become proficient at using the microtome, but I learned it is a machine best mastered by practice. Another setback I encountered occurred the first time I embedded. The embedding process itself is rather intense as it takes almost two days to complete and requires someone to be available most of the first day to move the tissues between different concentrations of solutions at exact time intervals. When we finally added the tissues to the wells of plastic, I learned how hard it is to get the tissue to orient the way you want in the plastic as the tissue liked to rotate within the liquid plastic. I lost a few samples due to bad orientation or having the label float up and cover the tissue, but I learned from that experience so the second time I embedded I did not lose any samples.

Another major problem I had while completing this project was procrastination. As my course load progressively increased, this project became less of a priority as it did not need to be completed for several years. Completing this project seemed like a dauntless task that could wait until my more pressing schoolwork was done, especially when it came to the final

manuscript writeup. While taking this approach allowed me to be successful in my classes, it did cause me more stress later as the project deadline loomed ever closer. Looking back, if I had been better about my time management I would not have been as stressed about completing the project.

The corona virus pandemic also posed a problem as it prohibited me from performing any further TEM analysis on the integument. Originally, TEM analysis of both the mucous and poison glands of *P. dorsalis* was going to be included in my final manuscript. The poison gland analysis was going to be completed in early March and added to the already completed manuscript, but due to the restrictions caused by the outbreak it was not possible to complete this analysis in time to add to my manuscript. Further TEM analysis of the *P. dorsalis* integument will need to be conducted in future studies.

Personally, this project taught me stress management. I have never undertaken a project of this magnitude, and to do it while taking some of my most rigorous college classes caused a great deal of stress. As a biology major hoping to be accepted into graduate school to become a veterinarian, I knew that completing this project would teach me how to better manage my time as well as stress. Veterinarians have a relatively high suicide rate due to the stress of their job, so I decided to persevere and complete my project so I could begin learning to manage my stress before entering graduate school. I learned that taking some time for myself every day, even if it was just ten minutes, really reduced my anxiety. Painting was another way I relieved stress while completing this project. Taking art classes to obtain my art minor, I realized that getting lost in the beauty of making an image come to life on paper or canvas was very cathartic for me and rejuvenated me when I felt stressed.

Academically, this project forced me to learn embedding and sectioning techniques I never would have learned otherwise. It also introduced me to histology and integumentary features with the aid of my mentor before ever taking classes that taught these subjects. This project also gave me the opportunity to present my research multiple times and even travel across the country for one of these presentations. Finally, the independent research done for this project presented me with unique experiences that aided me in promoting myself to graduate schools.

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Appendices

Appendix A- CITI Training

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI PROGRAM)

COMPLETION REPORT - PART 1 OF 2
COURSEWORK REQUIREMENTS*

* NOTE: Scores on this Requirements Report reflect quiz completions at the time all requirements for the course were met. See list below for details. See separate Transcript Report for more recent quiz scores, including those on optional (supplemental) course elements.

- **Name:** Breanna Davis (ID: 6907866)
- **Institution Affiliation:** University of Indianapolis (ID: 473)
- **Institution Email:** davisbc@uindy.edu
- **Institution Unit:** Math
- **Phone:** 788-3537

- **Curriculum Group:** Responsible Conduct of Research (RCR)
- **Course Learner Group:** Group 8: Responsible Conduct of Research
- **Stage:** Stage 1 - Basic Course

- **Record ID:** 29396742
- **Completion Date:** 08-Nov-2018
- **Expiration Date:** 07-Nov-2023
- **Minimum Passing:** 80
- **Reported Score*:** 89

REQUIRED AND ELECTIVE MODULES ONLY	DATE COMPLETED	SCORE
Authorship (RCR-Basic) (ID: 16597)	08-Nov-2018	5/5 (100%)
Collaborative Research (RCR-Basic) (ID: 16598)	08-Nov-2018	4/5 (80%)
Conflicts of Interest (RCR-Basic) (ID: 16599)	08-Nov-2018	5/5 (100%)
Data Management (RCR-Basic) (ID: 16600)	08-Nov-2018	4/5 (80%)
Mentoring (RCR-Basic) (ID: 16602)	08-Nov-2018	5/5 (100%)
Peer Review (RCR-Basic) (ID: 16603)	08-Nov-2018	4/5 (80%)
Research Misconduct (RCR-Basic) (ID: 16604)	08-Nov-2018	5/5 (100%)
Introduction to RCR (RCR-Basic) (ID: 17009)	08-Nov-2018	3/3 (100%)
Plagiarism (RCR-Basic) (ID: 15156)	08-Nov-2018	4/5 (80%)
Research, Ethics, and Society (RCR) (ID: 15198)	08-Nov-2018	3/5 (60%)
Research Involving Human Subjects (RCR-Basic) (ID: 13566)	08-Nov-2018	5/5 (100%)

For this Report to be valid, the learner identified above must have had a valid affiliation with the CITI Program subscribing institution identified above or have been a paid Independent Learner.

Verify at: www.citiprogram.org/verify/?k63591311-7e31-4f0e-9f40-8713b29e4e1f-29396742

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COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI PROGRAM)

COMPLETION REPORT - PART 2 OF 2
COURSEWORK TRANSCRIPT**

** NOTE: Scores on this Transcript Report reflect the most current quiz completions, including quizzes on optional (supplemental) elements of the course. See list below for details. See separate Requirements Report for the reported scores at the time all requirements for the course were met.

- **Name:** Breanna Davis (ID: 6907866)
- **Institution Affiliation:** University of Indianapolis (ID: 473)
- **Institution Email:** davisbc@uindy.edu
- **Institution Unit:** Math
- **Phone:** 788-3537

- **Curriculum Group:** Responsible Conduct of Research (RCR)
- **Course Learner Group:** Group 8: Responsible Conduct of Research
- **Stage:** Stage 1 - Basic Course

- **Record ID:** 29396742
- **Report Date:** 24-Feb-2019
- **Current Score**:** 89

REQUIRED, ELECTIVE, AND SUPPLEMENTAL MODULES	MOST RECENT	SCORE
Research Involving Human Subjects (RCR-Basic) (ID: 13566)	08-Nov-2018	5/5 (100%)
Plagiarism (RCR-Basic) (ID: 15156)	08-Nov-2018	4/5 (80%)
Research, Ethics, and Society (RCR) (ID: 15158)	08-Nov-2018	3/5 (60%)
Authorship (RCR-Basic) (ID: 16597)	08-Nov-2018	5/5 (100%)
Collaborative Research (RCR-Basic) (ID: 16598)	08-Nov-2018	4/5 (80%)
Conflicts of Interest (RCR-Basic) (ID: 16599)	08-Nov-2018	5/5 (100%)
Data Management (RCR-Basic) (ID: 16600)	08-Nov-2018	4/5 (80%)
Mentoring (RCR-Basic) (ID: 16602)	08-Nov-2018	5/5 (100%)
Peer Review (RCR-Basic) (ID: 16603)	08-Nov-2018	4/5 (80%)
Research Misconduct (RCR-Basic) (ID: 16604)	08-Nov-2018	5/5 (100%)
Introduction to RCR (RCR-Basic) (ID: 17009)	08-Nov-2018	3/3 (100%)

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COMPLETION REPORT - PART 1 OF 2
COURSEWORK REQUIREMENTS*

* NOTE: Scores on this Requirements Report reflect quiz completions at the time all requirements for the course were met. See list below for details. See separate Transcript Report for more recent quiz scores, including those on optional (supplemental) course elements.

- Name: Breanna Davis (ID: 6907566)
- Institution Affiliation: University of Indianapolis (ID: 472)
- Institution Email: davisbc@uindy.edu
- Institution Unit: Math
- Phone: 765-3537

- Curriculum Group: Human Subjects Research (HSR)
- Course Learner Group: Group 3: Non-Health Related Research
- Stage: Stage 1 - Basic Course

- Record ID: 30753604
- Completion Date: 02-Mar-2019
- Expiration Date: 01-Mar-2021
- Minimum Passing: 80
- Reported Score*: 93

REQUIRED AND ELECTIVE MODULES ONLY	DATE COMPLETED	SCORE
Belmont Report and Its Principles (ID: 1127)	02-Mar-2019	3/3 (100%)
History and Ethical Principles - SBE (ID: 490)	08-Feb-2015	5/5 (100%)
Defining Research with Human Subjects - SBE (ID: 491)	08-Feb-2015	5/5 (100%)
The Federal Regulations - SBE (ID: 502)	08-Feb-2015	5/5 (100%)
Assessing Risk - SBE (ID: 503)	08-Feb-2015	5/5 (100%)
Informed Consent - SBE (ID: 504)	08-Feb-2015	5/5 (100%)
Privacy and Confidentiality - SBE (ID: 505)	08-Feb-2015	5/5 (100%)
Populations in Research Requiring Additional Considerations and/or Protections (ID: 16650)	09-Feb-2015	4/5 (80%)
Students in Research (ID: 1321)	09-Feb-2015	5/5 (100%)
Internet-Based Research - SBE (ID: 510)	09-Feb-2015	5/5 (100%)
Conflicts of Interest in Human Subjects Research (ID: 17464)	10-Feb-2015	4/5 (80%)
Unanticipated Problems and Reporting Requirements in Social and Behavioral Research (ID: 14925)	10-Feb-2015	4/5 (80%)
Cultural Competence in Research (ID: 15166)	10-Feb-2015	5/5 (100%)
Records-Based Research (ID: 5)	10-Feb-2015	3/3 (100%)
Research with Prisoners - SBE (ID: 506)	10-Feb-2015	4/5 (80%)
Research with Decisionally Impaired Subjects (ID: 16510)	02-Mar-2019	4/5 (80%)
Research with Older Adults (ID: 16502)	10-Feb-2015	4/5 (80%)
Research with Children - SBE (ID: 507)	10-Feb-2015	5/5 (100%)

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COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI PROGRAM)

COMPLETION REPORT - PART 2 OF 2
COURSEWORK TRANSCRIPT**

** NOTE: Scores on this Transcript Report reflect the most current quiz completions, including quizzes on optional (supplemental) elements of the course. See list below for details. See separate Requirements Report for the reported scores at the time all requirements for the course were met.

- Name: Breanna Davis (ID: 6907566)
- Institution Affiliation: University of Indianapolis (ID: 473)
- Institution Email: davisbc@uindy.edu
- Institution Unit: Math
- Phone: 765-3537

- Curriculum Group: Human Subjects Research (HSR)
- Course Learner Group: Group 3: Non-Health Related Research
- Stage: Stage 1 - Basic Course

- Record ID: 30753604
- Report Date: 02-Mar-2019
- Current Score**: 93

REQUIRED, ELECTIVE, AND SUPPLEMENTAL MODULES	MOST RECENT	SCORE
Belmont Report and Its Principles (ID: 1127)	02-Mar-2019	3/3 (100%)
Records-Based Research (ID: 5)	10-Feb-2015	3/3 (100%)
Research and HIPAA Privacy Protections (ID: 14)	10-Feb-2015	4/5 (80%)
Research with Older Adults (ID: 16502)	10-Feb-2015	4/5 (80%)
Populations in Research Requiring Additional Considerations and/or Protections (ID: 16650)	09-Feb-2015	4/5 (80%)
Students in Research (ID: 1321)	09-Feb-2015	5/5 (100%)
Defining Research with Human Subjects - SBE (ID: 491)	05-Feb-2015	5/5 (100%)
The Federal Regulations - SBE (ID: 502)	05-Feb-2015	5/5 (100%)
Assessing Risk - SBE (ID: 503)	05-Feb-2015	5/5 (100%)
Research with Decisionally Impaired Subjects (ID: 16810)	02-Mar-2019	4/5 (80%)
Informed Consent - SBE (ID: 504)	05-Feb-2015	5/5 (100%)
Privacy and Confidentiality - SBE (ID: 505)	05-Feb-2015	5/5 (100%)
Research with Subjects with Physical Disabilities & Impairments (ID: 16657)	10-Feb-2015	5/5 (100%)
Research with Prisoners - SBE (ID: 506)	10-Feb-2015	4/5 (80%)
Research with Children - SBE (ID: 507)	10-Feb-2015	5/5 (100%)
Internet-Based Research - SBE (ID: 510)	09-Feb-2015	5/5 (100%)
Cultural Competence in Research (ID: 15166)	10-Feb-2015	5/5 (100%)
Unanticipated Problems and Reporting Requirements in Social and Behavioral Research (ID: 14925)	10-Feb-2015	4/5 (80%)
History and Ethical Principles - SBE (ID: 490)	05-Feb-2015	5/5 (100%)
Conflicts of Interest in Human Subjects Research (ID: 17464)	10-Feb-2015	4/5 (80%)

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Appendix B- Chemical Safety Sheets

bioWORLD
MOLECULAR TOOLS FOR LIFE SCIENCES

SAFETY DATA SHEET

Create Date: 2017-07-21 00:00:00/ Revision Date: 2017-08-01 00:00:00/ Print Date: 2017-08-18

SECTION 1: COMPANY AND PRODUCT INFORMATION

1.1 Product identifiers

Product name	:	Sodium Cacodylate Buffer 0.2 M, pH 7.4
Product code	:	40120084
CAS number	:	Not Available
Synonyms	:	Not Available

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	For laboratory and research use only.

1.3 Details of the supplier of the safety data sheet

Address	:	4150 Tuller Rd. Suite 228 Dublin, OH 43017
Email	:	tech@bio-world.com
Phone	:	614-792-8680, Toll free: 1-888-bio-PLUS
Fax	:	614-792-8685

1.4 Emergency telephone number

Emergency phone	:	1-888-bio-PLUS

SECTION 2: HAZARDS IDENTIFICATION

2.1 Classification of substance or mixture

Carcinogenicity (Category 2), H351
Reproductive Toxicity (Category 2), H361
Acute Toxicity (Category 4), H302
Acute Toxicity (Category 4), H312
Acute Toxicity (Category 4), H332
Eye Irritation (Category 2B), H320
Chronic Aquatic Toxicity (Category 1), H410

2.2 GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Carcinogenicity (Category 2), H351
Reproductive Toxicity (Category 2), H361
Acute Toxicity (Category 4), H302
Acute Toxicity (Category 4), H312
Acute Toxicity (Category 4), H332
Eye Irritation (Category 2B), H320
Chronic Aquatic Toxicity (Category 1), H410

2.3 Label elements and precautionary statements

Pictogram : 

Signal word	:	Danger
Hazard statement(s)	:	H302: Harmful if swallowed. H312: Harmful in contact with skin. H320: Causes eye irritation. H332: Harmful if inhaled. H351: Suspected of causing cancer. H361: Suspected of damaging fertility or the unborn child. H410: Very toxic to aquatic life with long-lasting effects.
Precautionary statement(s)	:	P201: Obtain special instructions before use. P202: Do not handle until all safety precautions have been read and understood. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P271: Use only outdoors or in a well-ventilated area. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection. P301 + P310 + P330: IF SWALLOWED: Immediately call a POISON CENTER/doctor. Rinse mouth. P304 + P340 + P311: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor. P308 + P313: IF exposed or concerned: Get medical advice/ attention. P391: Collect spillage. P403 + P233: Store in a well-ventilated place. Keep container tightly closed. P405: Store locked up. P501: Dispose of contents/ container to an approved waste disposal plant.

2.4 Hazards not otherwise classified (HNOC) or not covered by GHS

No unclassified hazards known.

2.5 NFPA Rating

Health hazard	:	2
Fire hazard	:	0
Reactivity hazard	:	0

2.6 HMIS Rating

Health hazard	:	2
Chronic health hazard	:	*
Reactivity hazard	:	0
Flammability	:	0
Physical hazard	:	0

SECTION 3: COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

TED PELLA, INC.
Microscopy Products for Science and Industry

Safety Data Sheet
Product No. 18306-4221 ERL 4221 Epoxide Resin
Issue Date (04-09-15)
Review Date (08-31-17)

Section 1: Product and Company Identification
Product Name: ERL 4221 Epoxide Resin
Synonym: none
Company Name
Ted Pella, Inc., P.O. Box 492477, Redding, CA 96049-2477
Inside USA and Canada 1-800-237-3526 (Mon-Thu. 6:00AM to 4:30PM PST; Fri 6:00AM to 4:00PM PST)
Outside USA and Canada 1-530-243-2200 (Mon-Thu. 6:00AM to 4:30PM PST; Fri 6:00AM to 4:00PM PST)
CHEMTREC USA and Canada Emergency Contact Number 1-800-424-9300 24 hours a day
CHEMTREC Outside USA and Canada Emergency Contact Number +1-703-741-5970 24 hours a day

Section 2: Hazard Identification
2.1 Classification of the substance or mixture
GHS Pictograms

GHS07

GHS Categories
GHS07: Irritant
Skin Sens. 1 H317: May cause an allergic skin reaction.

2.2 Label elements
Hazard Pictograms

GHS07

Signal Word: Warning

Hazard-determining components of labeling: 7-Oxabicyclo[4.1.0]heptane-3-caboxylic acid, 7-oxabicycl[4.1.0]hept-3-ylmethly ester

Hazard Statements
H317 May cause an allergic skin reaction.

Precautionary Statements
P261 Avoid breathing dust/fumes/mist/vapors/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P321 Specific treatment (see on this label).
P363 Wash contaminated clothing before reuse.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.

P501 Dispose of contents/container in accordance with local/regional/national/ international regulations.

2.3 Other hazards

Classification according to Directive 67/548/EEC or Directive 1999/45/EC
Irritant: May cause sensitisation by skin contact.
Information concerning particular hazards for human and environment: The product has to be labeled due to the calculation procedure of international guidelines.
Classification system: The classification was made according to the latest editions of international substances lists, and expanded upon from company and literature data.
Label elements
Labeling according to EU guidelines: The product has been classified and marked in accordance with directives on hazardous materials.
Code letter and hazard designation of product: Irritant.
Hazard-determining components of labeling:
7-Oxabicyclo[4.1.0]heptane-3-caboxylic acid, 7-oxabicycl[4.1.0]hept-3-ylmethly ester.
Risk phrases:
May cause sensitisation by skin contact.
Safety phrases:
Do not breathe gas/fumes/vapor/spray (appropriate wording to be specified by the manufacturer).
Avoid contact with skin.
Wear suitable gloves.
This material and its container must be disposed of as hazardous waste.

Health Effects:
NFPA Hazard Rating: Health: 2; Fire: 1; Reactivity: 1
HMIS® Hazard Rating: Health: 2; Fire: 1; Reactivity: 1
(0=least, 1=Slight, 2=Moderate, 3=High, 4=Extreme)

Results of PBT and vPvB assessment: A Chemical Safety Assessment has not been carried out.
PBT: NA
vPvB: NA

Emergency overview
Appearance: Colorless liquid.
Immediate effects: ND
Potential health effects
Primary Routes of entry: Ingestion, inhalation, eye and skin contact.
Signs and Symptoms of Overexposure: ND
Eyes: ND
Skin: May cause sensitisation by skin contact.
Ingestion: ND
Inhalation: ND
Chronic Exposure: ND
Chemical Listed As Carcinogen Or Potential Carcinogen: None listed
See Toxicological Information (Section11)
Potential environmental effects
See Ecological Information (Section 12)

Section 3: Composition / Information on Ingredients

TED PELLA, INC.
Microscopy Products for Science and Industry

Safety Data Sheet

Product No.18310 D.E.R.™ 736 Epoxy Resin
Issue Date (04-09-15)
Review Date (08-31-17)

Section 1: Product and Company Identification
Product Name: D.E.R.™ 736 Epoxy Resin
Synonym: Diglycidyl Ether of Poly (propylene glycol), Polymer of epichlorohydrinpolyglycol
Company Name
Ted Pella, Inc., P.O. Box 492477, Redding, CA 96049-2477
Inside USA and Canada 1-800-237-3526 (Mon-Thu. 6:00AM to 4:30PM PST; Fri 6:00AM to 4:00PM PST)
Outside USA and Canada 1-530-243-2200 (Mon-Thu. 6:00AM to 4:30PM PST; Fri 6:00AM to 4:00PM PST)
CHEMTREC USA and Canada Emergency Contact Number 1-800-424-9300 24 hours a day
CHEMTREC Outside USA and Canada Emergency Contact Number +1-703-741-5970 24 hours a day

Section 2: Hazard Identification
2.1 Classification of the substance or mixture

GHS Pictograms

GHS07

GHS Categories
GHS07: Irritant

Acute Tox. 4	H302: Harmful if swallowed.
Acute Tox. 4	H332: Harmful if inhaled.
Skin Irrit. 2	H315: Causes skin irritation.
Skin Sens. 1	H317: May cause an allergic skin reaction.

2.2 Label elements

Hazard Pictograms

GHS07

Signal Word: Warning

Hazard Statements

H302	Harmful if swallowed.
H315	Causes skin irritation.
H317	May cause an allergic reaction.
H332	Harmful if inhaled.

Precautionary Statements

P261	Avoid breathing dust/fume/gas/mist/vapors/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P321	Specific treatment (see on this label).
P362	Take off contaminated clothing and wash before reuse.
P301+P312	IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

2.3 Other hazards

Classification according to Directive 67/548/EEC or Directive 1999/45/EC
Label elements
Labeling according to EU guidelines: The product has been classified and marked in accordance with directives on hazardous materials.
Harmful - Harmful by inhalation and if swallowed.
Irritant - Irritating to eyes and skin. May cause sensitization by skin contact.
Information concerning particular hazards for human and environment: NA
Code letter and hazard designation of product:
Label: Harmful
Risk phrases:
Harmful by inhalation and if swallowed.
Irritating to eyes and skin.
May cause sensitization by skin contact.
Safety phrases:
Keep container in a well-ventilated place.
Avoid contact with skin.
Wear suitable gloves.
This material and its container must be disposed of as hazardous waste.

Health Effects:
NPFA Hazard Rating: Health: 1; Fire: 0; Reactivity: 0
HMIS® Hazard Rating: Health: 1; Fire: 0; Reactivity: 0
(0=least, 1=Slight, 2=Moderate, 3=High, 4=Extreme)

Results of PBT and vPvB assessment:
PBT: NA
vPvB: NA

TED PELLA, INC.
Microscopy Products for Science and Industry

Safety Data Sheet
Product No. 18301 Nonenyl Succinic Anhydride, (NSA)
Issue Date (04-09-15)
Review Date (08-31-17)

Section 1: Product and Company Identification
Product Name: Nonenyl Succinic Anhydryde Modified (NSA)
Synonym: NSA (Nonenyl Succinic Anhydride Modified)
Company Name
Ted Pella, Inc., P.O. Box 492477, Redding, CA 96049-2477
Inside USA and Canada 1-800-237-3526 (Mon-Thu. 6:00AM to 4:30PM PST; Fri 6:00AM to 4:00PM PST)
Outside USA and Canada 1-530-243-2200 (Mon-Thu. 6:00AM to 4:30PM PST; Fri 6:00AM to 4:00PM PST)
CHEMTREC USA and Canada Emergency Contact Number 1-800-424-9300 24 hours a day
CHEMTREC Outside USA and Canada Emergency Contact Number +1-703-741-5970 24 hours a day

Section 2: Hazard Identification
2.1 Classification of the substance or mixture
Classification according to Regulation (EC) No 1272/2008

GHS Pictograms

GHS07

GHS Categories

Acute Tox. 4	H302: Harmful if swallowed.
Acute Tox. 4	H312: Harmful in contact with skin.
Acute Tox. 4	H332: Harmful if inhaled.
Skin Irrit. 2	H315: Causes skin irritation.
Eye Irrit. 2	H319: Causes serious eye irritation.

2.2 Label Elements

GHS07

Signal Word: WARNING

Hazard statements

H302+H312+H332	Harmful if swallowed, in contact with skin or if inhaled.
H315	Causes skin irritation.
H319	Causes serious eye irritation.

Precautionary statements

P284	Wear respiratory protection.

P280	Wear protective gloves/protective clothing/eye protection/face protection.
P301+P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

2.3 Other Hazards
Results of PBT and vPvB assessment:
PBT: Not applicable.
vPvB: Not applicable.

Classification according to Directive 67/548/EEC or Directive 1999/45/EC
R20/21/22: Harmful by inhalation, in contact with skin and if swallowed.
R36/38: Irritating to eyes and skin.
Classification system:
The classification was made according to the latest editions of international substances lists, and expanded upon from company and literature data.

Health effects:
NFPA Hazard Rating: Health: 1; Fire: 1; Reactivity: 0
HMIS® Hazard Rating: Health: 1; Fire: 1; Reactivity: 0
(0=least, 1=Slight, 2=Moderate, 3=High, 4=Extreme)

Emergency overview
Appearance: Yellow liquid
Immediate effects: Harmful by inhalation, in contact with skin and if swallowed. Irritating to eyes and skin.
Potential health effects
Primary Routes of entry: Ingestion, inhalation, and skin and eye contact.
Signs and Symptoms of Overexposure: ND
Eyes: Rinse opened eye for several minutes under running water. If symptoms persist, consult a doctor.
Skin: Immediately wash with water and soap and rinse thoroughly
Ingestion: Call for a doctor immediately.
Inhalation: Supply fresh air. If required, provide artificial respiration. Keep patient warm. Consult doctor if symptoms persist. In case of unconsciousness; place patient stably in side position for transportation.
Chronic Exposure: ND
Chemical Listed As Carcinogen Or Potential Carcinogen: No
See Toxicological Information (Section11)
Potential environmental effects
See Ecological Information (Section 12)

Section 3: Composition / Information on Ingredients

Principle Component(s) (chemical and common name(s)) (Cas. No)	%	OSHA PEL mg/m3	ACGIH TLV mg/m3	NTP	IARC	OSHA regulated
Nonenyl Succinic Anhydride	ND	NE	NE	No	No	No

TED PELLA, INC.
Microscopy Products for Science and Industry

Safety Data Sheet
Product No. 18315 Dimethylaminoethanol (DMAE)
Issue Date (04-09-15)
Review Date (08-31-17)

Section 1: Product and Company Identification
Product Name: Dimethylaminoethanol (DMAE)
Synonym: 2- Dimethylaminoethanol
Company Name
Ted Pella, Inc., P.O. Box 492477, Redding, CA 96049-2477
Inside USA and Canada 1-800-237-3526 (Mon-Thu. 6:00AM to 4:30PM PST; Fri 6:00AM to 4:00PM PST)
Outside USA and Canada 1-530-243-2200 (Mon-Thu. 6:00AM to 4:30PM PST; Fri 6:00AM to 4:00PM PST)
CHEMTREC USA and Canada Emergency Contact Number 1-800-424-9300 24 hours a day
CHEMTREC Outside USA and Canada Emergency Contact Number +1-703-741-5970 24 hours a day

Section 2: Hazard Identification
2.1 Classification of the substance or mixture

GHS Pictograms

GHS02 GHS05 GHS07

GHS Categories

GHS02	Flammable	
	Flamm. Liq. 3	H226: Flammable liquid and vapor
GHS05	Corrosive	
	Skin Corr. 1B	H314: Causes severe skin burns and eye damage
GHS07	Irritant	
	Acute Tox. 4	H302: Harmful if swallowed
	Acute Tox. 4	H312: Harmful in contact with skin
	Acute Tox. 4	H332: Harmful if inhaled

Signal Word: DANGER

2.2 Label elements
Labeling according to EU guidelines
This product has been classified and marked in accordance with GHS guidelines.

Hazard-determining components of labeling
2-dimethylaminoethanol

Hazard statements

H226	Flammable liquid and vapor.
H302+H312+H332	Harmful if swallowed, in contact with skin or if inhaled.
H314	Causes severe skin burns and eye damage.

Precautionary statements

P210	Keep away from heat/sparks/open flames/hot surfaces. - No smoking.
P303+P361+P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER/doctor.
P322	Specific measures (see on this label).
P405	Store locked up.
P501	Dispose of contents/container in accordance with local/regional/ national/international regulations.

2.3 Other Hazards
Results of PBT and vPvB assessment:
PBT: Not applicable.
vPvB: Not applicable.

Classification according to Directive 67/548/EEC or Directive 1999/45/EC
Corrosive: Causes burns.
Harmful: Harmful by inhalation, in contact with skin and if swallowed.
Flammable
Information concerning particular hazards for human and environment: Not applicable.
Label elements:
Labeling according to EU guidelines:
The product has been classified and marked in accordance with directives on hazardous materials.
Code letter and hazard designation of product:
Label: Corrosive
Risk phrases
Flammable.
Harmful by inhalation, in contact with skin and if swallowed.
Causes burns.
Safety phrases
Keep locked up and out of the reach of children.
Avoid contact with eyes.
In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
Wear suitable protective clothing, gloves and eye/face protection.

Health Effects
NFPA Hazard Rating: Health: 3; Fire: 3; Reactivity: 0
HMIS® Hazard Rating: Health: 4; Fire: 3; Reactivity: 0
(0=least, 1=Slight, 2=Moderate, 3=High, 4=Extreme)

Emergency overview
Appearance: Colorless liquid
Immediate effects: ND
Potential health effects
Primary Routes of entry: Inhalation, ingestion, eye and skin contact.
Signs and Symptoms of Overexposure: ND
Eyes: Strong caustic effect
Skin: Harmful by contact with skin.
Ingestion: Harmful if swallowed.
Inhalation: Harmful by inhalation.

SAFETY DATA SHEET

Airgas

Ethanol

Section 1. Identification

GHS product identifier	:	Ethanol
Chemical name	:	ethanol
Other means of identification	:	ethyl alcohol; Denatured Alcohol; ALCOHOL; Ethyl alcohol (Ethanol)
Product use	:	Synthetic/Analytical chemistry.
Synonym	:	ethyl alcohol; Denatured Alcohol; ALCOHOL; Ethyl alcohol (Ethanol)
SDS #	:	001114
Supplier's details	:	Airgas USA, LLC and its affiliates 259 North Radnor-Chester Road Suite 100 Radnor, PA 19087-5283 1-610-687-5253
Emergency telephone number (with hours of operation)	:	1-866-734-3438

Section 2. Hazards identification

OSHA/HCS status	:	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
Classification of the substance or mixture	:	FLAMMABLE LIQUIDS - Category 2

GHS label elements

Hazard pictograms :

Signal word	:	Danger
Hazard statements	:	Highly flammable liquid and vapor. May form explosive mixtures with air.

Precautionary statements

General	:	Read label before use. Keep out of reach of children. If medical advice is needed, have product container or label at hand.
Prevention	:	Wear protective gloves. Wear eye or face protection. Keep away from heat, sparks, open flames and hot surfaces. - No smoking. Use explosion-proof electrical, ventilating, lighting and all material-handling equipment. Use only non-sparking tools. Take precautionary measures against static discharge. Keep container tightly closed. Use and store only outdoors or in a well ventilated place.
Response	:	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
Storage	:	Store in a well-ventilated place. Keep cool.
Disposal	:	Dispose of contents and container in accordance with all local, regional, national and international regulations.
Hazards not otherwise classified	:	None known.

Section 3. Composition/information on ingredients

Substance/mixture : Substance

Chemical name : ethanol

Other means of identification : ethyl alcohol; Denatured Alcohol; ALCOHOL; Ethyl alcohol (Ethanol)

CAS number/other identifiers

CAS number : 64-17-5

Product code : 001114

Ingredient name	%	CAS number
ethanol	100	64-17-5

There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

Occupational exposure limits, if available, are listed in Section 8.

Section 4. First aid measures

Description of necessary first aid measures

Eye contact : Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Continue to rinse for at least 10 minutes. Get medical attention if irritation occurs.

Inhalation : Remove victim to fresh air and keep at rest in a position comfortable for breathing. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. Get medical attention if adverse health effects persist or are severe. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.

Skin contact : Flush contaminated skin with plenty of water. Remove contaminated clothing and shoes. Get medical attention if symptoms occur. Wash clothing before reuse. Clean shoes thoroughly before reuse.

Ingestion : Wash out mouth with water. Remove dentures if any. Remove victim to fresh air and keep at rest in a position comfortable for breathing. If material has been swallowed and the exposed person is conscious, give small quantities of water to drink. Stop if the exposed person feels sick as vomiting may be dangerous. Do not induce vomiting unless directed to do so by medical personnel. If vomiting occurs, the head should be kept low so that vomit does not enter the lungs. Get medical attention if adverse health effects persist or are severe. Never give anything by mouth to an unconscious person. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.

Most important symptoms/effects, acute and delayed

Potential acute health effects

Eye contact : No known significant effects or critical hazards.

Inhalation : No known significant effects or critical hazards.

Skin contact : No known significant effects or critical hazards.

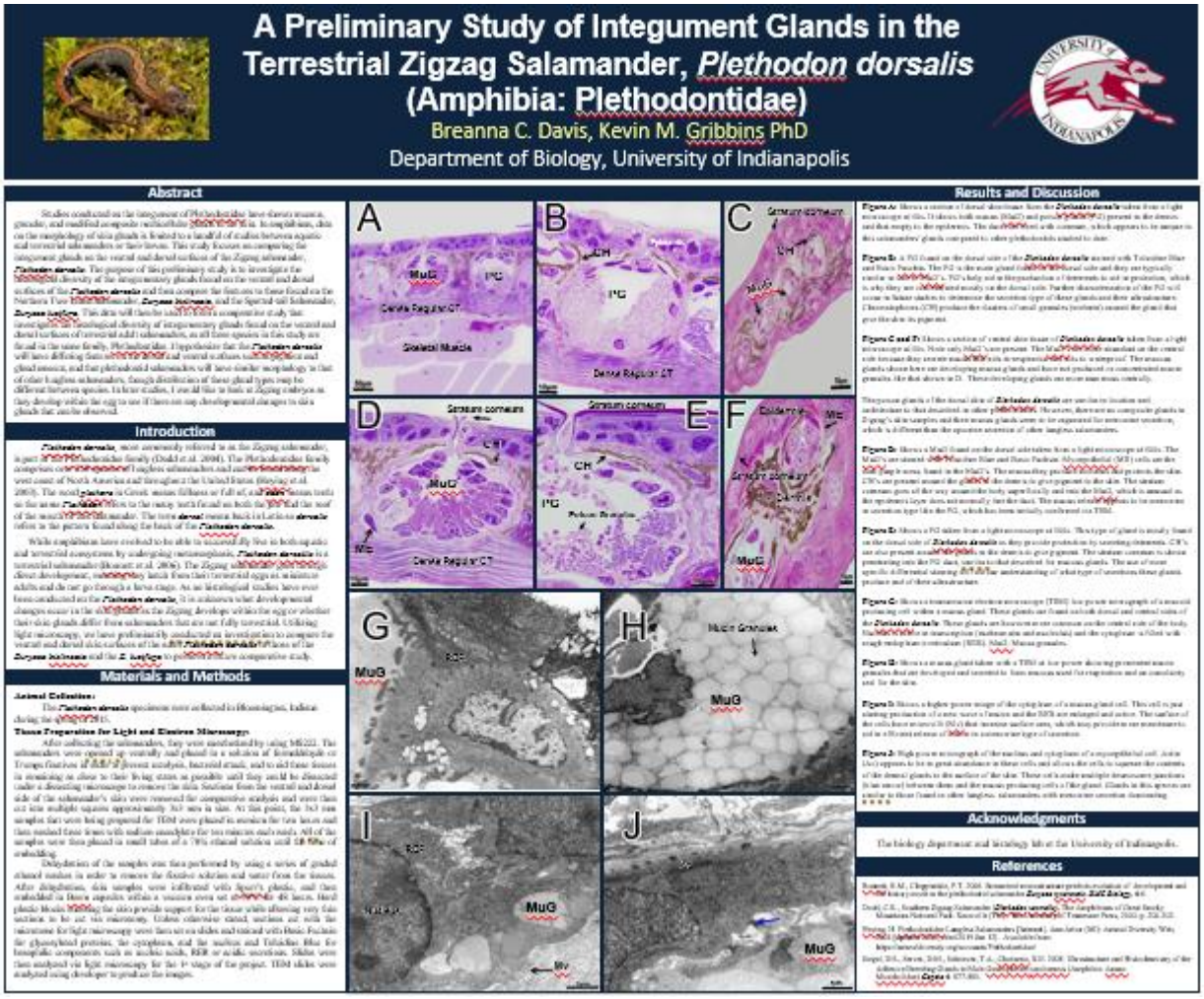
Frostbite : Try to warm up the frozen tissues and seek medical attention.

Ingestion : No known significant effects or critical hazards.

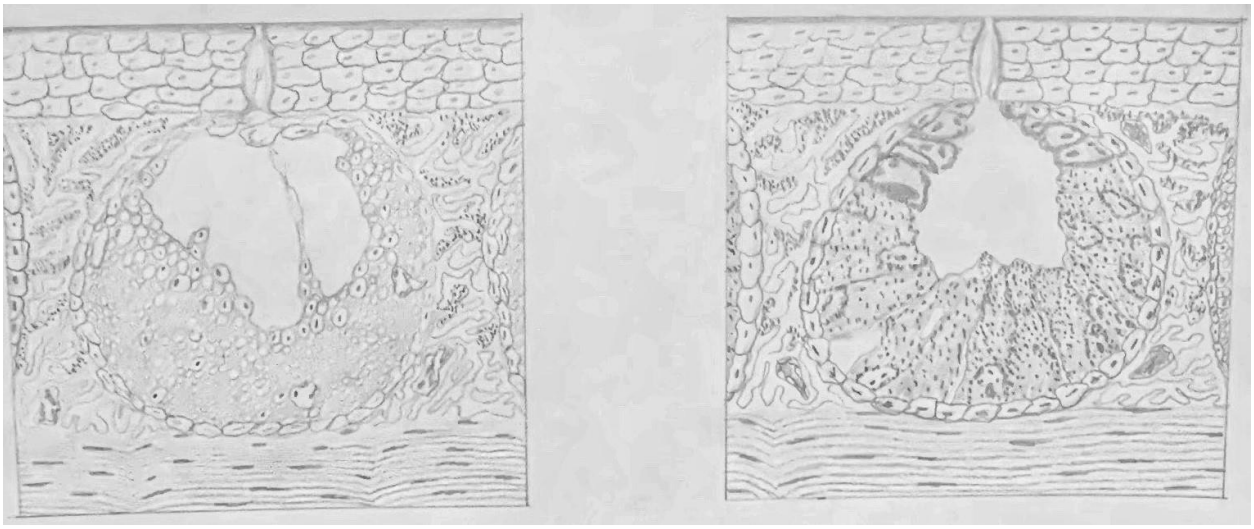
Over-exposure signs/symptoms

Eye contact : No specific data.

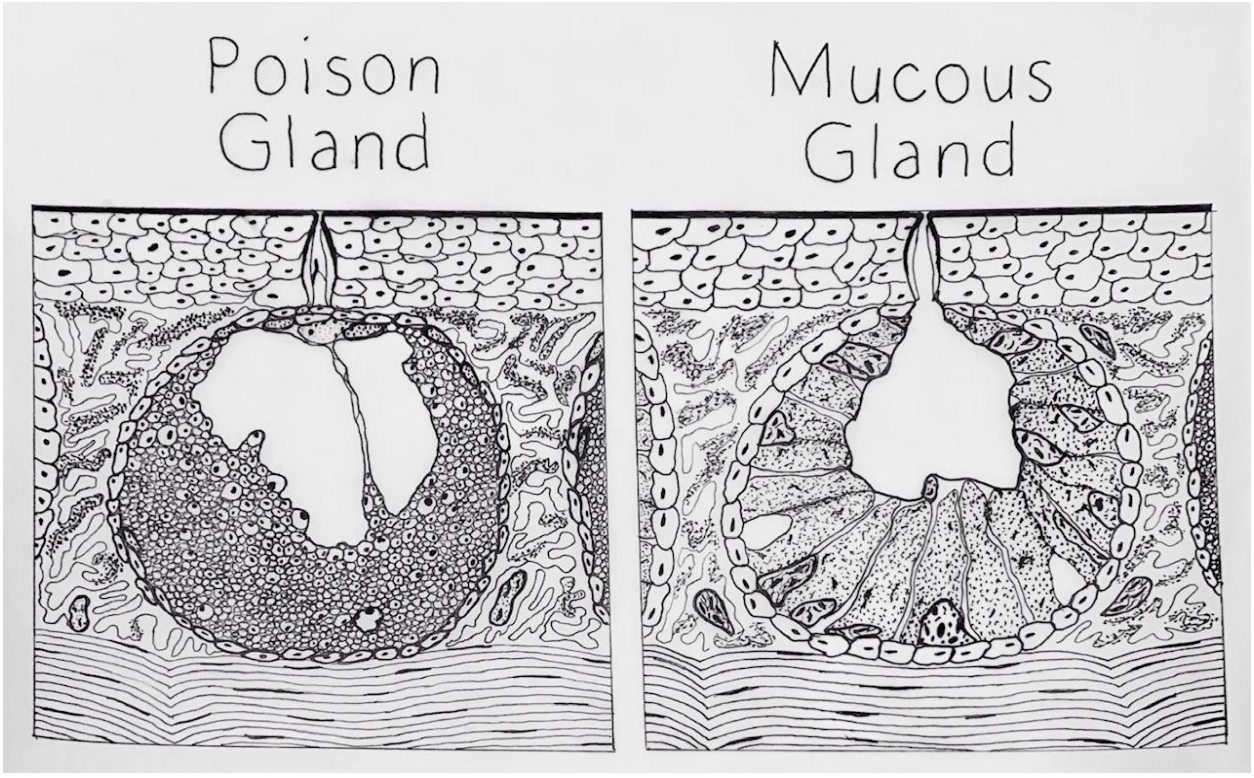
Appendix C- Scientific Poster



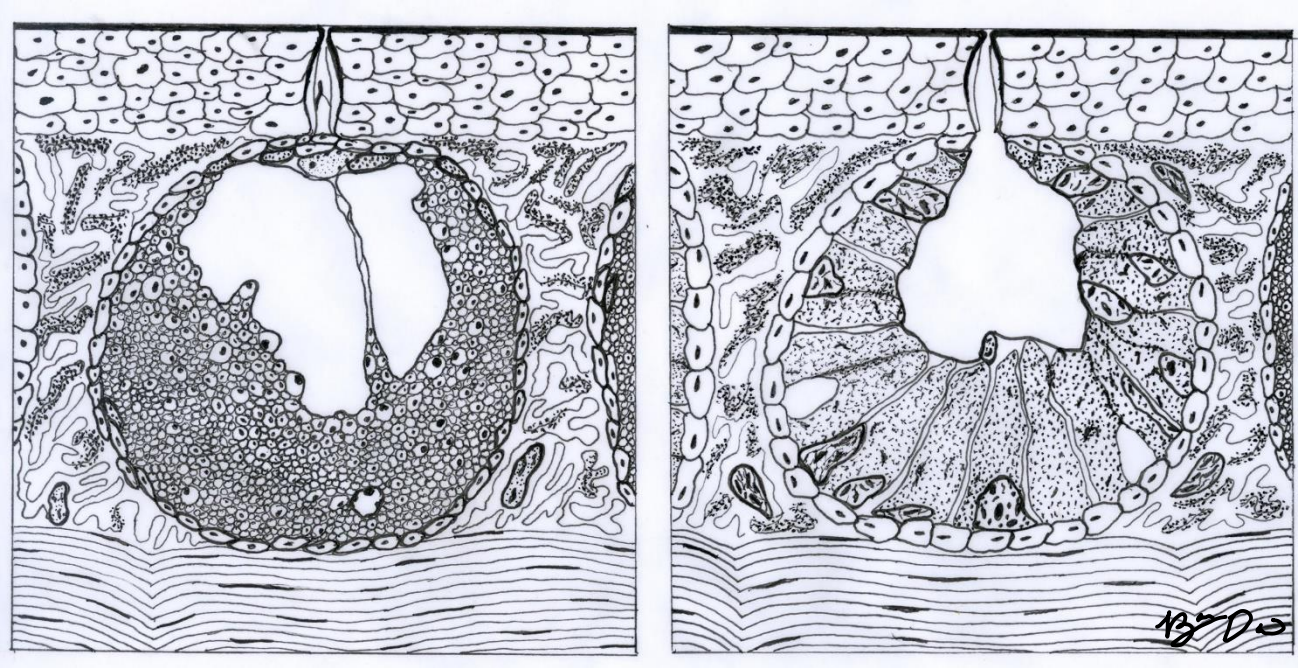
Appendix D- Illustration Development



Graphite Illustration of Poison and Mucous Gland (above)



Traced Micron Pen Illustration of Poison and Mucous Gland (above)



Scanned Micron Pen Illustration of Poison and Mucous Gland (above)