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Pseudoephedrine HCl Exposure-Induced Developmental Toxicity in Regenerating D. tigrina Planaria

ABSTRACT

As a precursor for the illicit drug methamphetamine, there is a compelling need to investigate the potential adverse effects of PSE. While existing research has addressed the effects of chronic drug overdose using the model organism, Dugesia tigrina planaria, pseudoephedrine (PSE) has yet to be tested. This study aims to investigate the direct impact of PSE on planarian regeneration, assessing how varying doses influence regeneration, morphology, and motility as a model for wound healing in humans. A regeneration assay was conducted over a 10 day period where each planarian was decapitated to separate the bi-lobed brain from its ventral nerve cord prior to being exposed to PSE solutions in dosages of 30 mg, 120 mg, and 240 mg. A negative correlation between PSE concentration and rate of regeneration was observed. Notably, planaria exposed to the 30 mg solution exhibited a 56.66% increase in mortality rates compared to those exposed to the 240 mg solution. This study concluded that concentrations of PSE pose extensive harm in D. tigrina planaria although lower concentrations possess a greater rate of lethality in terms of faster absorption, therefore constituting significant risk towards humans when abused in excessive fast-absorbing quantities. These findings have practical implications for pharmacologists, pharmacy personnel, and patients alike by urging for a greater level of caution towards PSE drug abuse.

Introduction

In the past few decades, the ubiquity of pseudoephedrine (PSE) as a precursor for the illegal manufacture of crystal methamphetamine, a highly addictive stimulant drug, has posed a consistent threat (U.S. Government Accountability Office, 2013). PSE, a non-prescription nasal decongestant, is available at most retail pharmacies, therefore making it an easily accessible source for people who operate illegal meth labs to obtain (Centers for Disease Control and Prevention, 2013). Although the Food and Drug Administration (FDA) claims that PSE is safe when taken as directed, there have been multiple reports of adverse side effects throughout the years (Harvard Health, 2023). Currently, PSE is found as a hydrochloride (HCl) or sulfate in doses ranging from 30 to 120 mg (Głowacka, 2021), but for the purpose of this study, only PSE HCl was used. The extent to which PSE can potentially cause significant harm to the developing human brain and impede the healing process after sustaining an injury remains uncertain.

To investigate the dangers of PSE at a subcellular level, this study utilizes the flatworm, *D. tigrina* planaria, as a model organism. Planaria has emerged as one of the best-characterized animal models for neuropharmacology and developmental biology due to its remarkable regenerative capabilities (Newmark and Sánchez Alvarado, 2002). Planaria's cell morphology and physiology exhibit a high degree of resemblance to vertebrate nervous systems in mammals such as humans (Sarnat and Netsky, 1985, 2002). In toxicology studies, planaria primarily absorb chemicals in the water by epithelial diffusion (Hagstrom, 2016). Planarian amputation is followed by the activation of cells called neoblasts, or adult stem cells that facilitate its regenerative process (Baguna, 2012). These neoblasts are essential to study in planarian regenerative research due its pluripotency, allowing it to give rise to various cell types that were previously damaged (Rink, 2012). Neoblast progeny then generates a blastema, or regenerative outgrowths at wounds, to replenish the missing tissues (Reddien, 2013).

In this study, various concentrations of PSE (30 mg, 120 mg, 240 mg) were introduced to cultures of planaria flatworms (n = 300) upon decapitation. For a period of 10 days, each amputated planaria was observed to track their mortality rates, regeneration, and morphological characteristics in response to PSE exposure. Using a quantitative and qualitative correlational approach, the following question was investigated: To what extent does pseudoephedrine (Sudafed®) affect the regeneration of *Dugesia tigrina* planaria?

Literature Review

1.1 Precursor for Methamphetamine

In 2005, Congress passed the Combat Methamphetamine Epidemic Act as an effort to ban over-the-counter sales of medicines containing pseudoephedrine (PSE), an ingredient involved in the illicit manufacture of methamphetamine (Drug Enforcement Administration, 2005). Despite this, methamphetamine, a powerful and highly addictive stimulant, has continued to purge across the United States, attributing to over 106,000 drug overdose deaths in 2021 alone (National Center for Health Statistics, 2021). Furthermore, a 2019 research report written by the National Institute on Drug Abuse describes the origin of most methamphetamine in the United States being produced by "transnational criminal organizations (TCOs) in Mexico" that can easily be made in illegal laboratories "with relatively inexpensive over-the-counter ingredient such as pseudoephedrine, a common ingredient in cold medications" (NIDA, 2019). Therefore, the prevalence of PSE as a rising precursor for methamphetamine corroborates a need for further investigation to study the extent of its negative influence on the human body.

1.2 The Nature of *Dugesia Tigrina* Planaria

For over forty years, planarians have gained the attention of both regenerative biologists and pharmacologists alike. Having emerged as model organisms in behavioral pharmacology and regenerative medicine studies, planaria have been commonly used to investigate the pharmacology of drug abuse. Danielle Hagstrom, a biology professor at the University of California in San Diego, wrote an article titled "Freshwater Planarians as an Alternative Animal Model for Neurotoxicology" (2015) where she discusses the suitability of planaria as a model because of its unique regenerative abilities. As the author mentions, "neoblasts" are undifferentiated stem cells that make up approximately 30% of planaria and form a "blastema" which are an aggregate of cells. This allows for planaria's regenerative features since damage towards the flatworm allows for the proliferation, or growth, of neoblasts that form to help replace the impaired cells. To support this idea, Dr. Michael Levin from Tufts University claims planaria to be the center of regenerative medicine in his article titled "Planarian regeneration as a model of anatomical homeostasis: Recent progress in biophysical and computational approaches" (2018). As the author references, in most "metazoans" or multicellular organisms, stem cells are used to replace aged or damaged cells, so planarians provide an insight into mechanistic investigation of "in-vivo stem cell regulation" or natural regeneration in organisms, which are likely to inform the functioning of stem cells in all animals including humans.

Therefore, through planarians as an effective model for regenerative medicine, development and regeneration can be observed through similar processes allowing us to induce neurodevelopment "at will" through amputation. Although scholarly works have addressed the effects of chronic drug overdose, spanning from opioids to cannabinoids, benzodiazepines, cocaine, and other substances using the model organism, *D. tigrina* planaria, PSE has yet to be tested.

1.3 Planarians in Behavioral Pharmacology and Regenerative Studies

Current research that investigates the effects of pharmaceuticals using planarian behavior, regeneration, or morphology as endpoints conclude that planaria display withdrawal-like behavior to a number of addictive drugs. In "Adult and Regenerating Planarians Respond Differentially to Chronic Drug Exposure" (2022), Kevin Bayingana and his colleagues from the Department of Biology at Swarthmore College examined the effects of chronic exposure to common drugs and stimulants on the developing nervous system of adult and regenerating planarians. Their findings concluded that common drugs and stimulants, including caffeine, histamine, fluoxetine, and sertraline, brought about sublethal morphological and behavioral effects on both samples of planarian species (Bayingana et. al, 2022). In this peer-reviewed journal, the authors use terminology such as "significant lethality" to indicate the negative influence high concentrations of the drugs had on planaria and "developmental neurotoxicity" to describe the nature of planarian nervous system growth under drug exposure (Bayingana et. al, 2022).

Despite having addressed the extent to antidepressant lethality over a prolonged period of time, their results substantiate the need for further safety evaluation of other common drugs. Furthermore, the authors did not examine the sublethal effects of the medication PSE, nor did it address how varying doses of each drug would alter the results in a concentration-dependent manner thereby posing a gap in the research.

Aside from utilizing morphological changes as an endpoint to investigate significant lethality, other research aims to examine locomotion and motility to reach similar conclusions. Oné R. Pagán and his colleagues

from the Department of Biology at West Chester University determined that acute exposure to the cholinergic compounds, nicotine and carbamylcholine, decreased planarian motility in a concentration-dependent manner (Pagán et. al, 2009). In their journal titled, "A Cembranoid from Tobacco Prevents the Expression of Nicotine-Induced Withdrawal Behavior in Planarian Worms" (2009), terminology such as "stimulants" referring to drugs that reverse effects of fatigue on mental and physical tasks and "benzodiazepines" to indicate depressants that produce sedation and hypnosis, relieving anxiety, muscles spasms, and seizures, was used.

Despite having tested *D. dorotocephala* planarians through observing its motility decrease, Pagán and his colleagues did not address planarian regeneration post-decapitation to model wound healing in humans (Pagán et. al, 2009). Furthermore, similar to Bayingana's study mentioned previously, the authors do not mention the use of PSE in their research.

While Bayingana studied morphological changes, Pagán centered his research on planarian locomotion and motility. However, it is notable that both articles have yet to address PSE and use regeneration as an endpoint, therefore posing a gap and substantiating the need for further research.

Materials and Methods

1.1 Planaria Specimens

The specimens of *Dugesia tigrina* planaria used in all experiments were purchased from Carolina Biological Supply Co. (Burlington, NC), maintained and acclimated to temperature-controlled (18-22°C) room conditions in the jars in which they were shipped, and tested within 3 days of receipt. 60 planaria was added to each petri dish (30 heads, 30 tails), accounting for a total of 300 experimental subjects. Petri dishes were stored in an Igloo Laguna 9 cooler to maintain a controlled temperature with minimal exposure to light. During the observational period, specimens were fed 1 gram of egg yolk 3 times a week and residual particles were cleaned and removed to prevent contamination. Prior to the start of the assay, planarians were starved ≥ 5 days to reduce metabolic inconsistencies during absorption. To study the regenerative process, on Day 3, intact worms were amputated with a sterilized razor blade and immediately submerged in their respective solutions no more than 3 hours prior to the start of the assay.

1.2 Test Compounds

Pseudoephedrine HCl (30 mg, 120 mg, 240 mg), caffeine tablets (200 mg), and ethyl alcohol (70%) were purchased from CVS Pharmacy (Simi Valley, CA). Each chemical was mixed in separate petri dishes. Pseudoephedrine HCl and caffeine tablets were crushed and then diluted with a solution of planarian water to obtain the desired concentrations. Arrowhead 100% Mountain Spring Water (San Bernardino, CA) used as a base solution for the planaria was purchased from Target (Simi Valley, CA) and fell within a reasonable pH range (neutral pH of 7.2). To mitigate diminishing effects due to weekly water changes, all solutions were replaced daily. Table 1 summarizes tested chemicals and concentrations.

TABLE 1: Chemicals and Concentration Ranges Tested

Compound	Concentration	Group
Pseudoephedrine HCl	30 mg	Low Treatment (PSE 30)
Pseudoephedrine HCl	120 mg	Medium Treatment (PSE 120)
Pseudoephedrine HCl	240 mg	High Treatment (PSE 240)
Caffeine	200 mg	Positive Control (PC)
Spring Water	24 mL	Negative Control (NC)

1.3 Preparation of Materials

All equipment, tools, and containers were sanitized with a 70% ethanol solution and then thoroughly rinsed with distilled water. 5 petri dishes were marked and labeled as follows: positive control (PC) caffeine, negative control (NC) spring water, PSE 30 mg (low), PSE 120 mg (med), PSE 240 mg (high). Figure 1 summarizes labeled petri dishes. An OMAX 40X-2000X Compound Lab Microscope and digital microscope was used to assess behavioral and morphological changes for all planarians involved. 1 mercury-free thermometer was placed in each of the 5 petri dishes to ensure the maintenance of a temperature-controlled environment at 21°C.

1.4 Horizontal Bisection Amputation

To slow down and immobilize planaria, the worms were placed on wet lens paper wrapped around a glass slide. Using the compound microscope for accuracy, the sterilized scalpel was used to make horizontal bisections and amputate each planarian head and tail (Fig. 2). Remnants, including both the planarian head and tail, were placed back into their respective petri dishes for the regeneration assay. A baseline photo was taken for each of the 6 conditions.

1.5 Regeneration Assay

Planarians were observed every 24 hours for a 10 day period (Fig. 1). Each day, the length was measured (rate of regeneration) and photographed under the compound microscope to determine any morphological changes. Pipettes were used to transfer the planaria to a blank microscope slide and assessed under the compound microscope until they were then returned back to their initial solution. Planarian morphological changes were observed and compared to the standard anatomical characteristics of the flatworm. Planarian motility was analyzed using a technique where a grid of 5 mm by 5 mm squares was placed underneath the petri dish. Then, a timer was set for 30 seconds and the number of squares one planarian traveled within that period of time was recorded. To measure the length in centimeters, a standard ruler was used after transferring each individual planarian to a wet lens paper slide to slow down movement and maximize accuracy.

During visual inspection, the following regeneration stages were observed: (1) Pre-wound closure: no visible regrowth to fill gap from wound closure, (2) Wound closure: cut has flattened and started to regrow, (3) Pattern formation: wound closed and round, formation visible, (4) Head translucent but visible, (5) Head still mostly translucent, shape starting to peak, early photoreceptors now visible, (6) Head and photoreceptors visible, but more translucent than rest of body, (7) Head fully peaked, photoreceptors visible, head color same as the remainder of body.

1.6 Statistical Analysis

Over the course of 10 days, planarian mortality rates within each of the 5 cultures were recorded. A planarian's regenerative process was considered to be inhibited once it ceased to exhibit signs of life. At Day 3 when mortality rates among the 5 cultures could best be compared, a chi-square (x^2_c) test was used with $p < 0.01$ as the comparative value to assess the significance of the results (Fig. 5). The null hypothesis declared there to be no statistically significant relationship between PSE concentrations and mortality rates. Chi-squared was used to determine whether observed differences represented a relationship in the population rather than merely random fluctuations in the sample. Chi-square tests were generated using FourmiLab. The mean ($\bar{x}$), population standard deviation (σ), and population variance (σ^2) for mortality incidence and life expectancy was generated using 365 Data Science. Normal distributions were computed using Stapplet and graphed as shown (Fig. 10).

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The above equation signifies a chi-squared test. Expected values (E_i) are indicated by the value 33.40 (active planaria at Day 3) and 26.60 (deceased planaria at Day 3) for the given population. Observed values (O_i) are denoted by the observed number of active and deceased planaria for each concentration. A chi-square test was used to determine whether a statistically significant association exists between PSE concentration and mortality rates.

1.7 Disposal

To ethically dispose of the planaria, they were submerged in bleach, placed in a sealed container, and left in the freezer for 48 hours. Planaria and their containers were carefully wrapped in a biohazard bag, and without containing any potentially sharp objects that might puncture the bag, they were tied closed. Planarians were then disposed of accordingly.

Results

1.1 Drug Toxicology Profile for PSE in *D. tigrina* Planaria

To identify an appropriate exposure profile for pseudoephedrine HCl (PSE) in the *D. tigrina* species of planaria, the mortality incidence over the course of the period post-amputation was examined. This data revealed a complex correlation between PSE concentration and mortality rates.

At the end of the 10 day regeneration assay, all 60 planaria in each of the PSE solutions (30 mg, 120 mg, 240 mg) were recorded as deceased. This resulted in a 100% overall mortality rate after exposure to PSE in the span of 3-10 days. Furthermore, a statistically significant correlation between PSE concentration and mortality incidence was verified through the chi-square test, generating a value of 181.6127 for p-value of < 0.00001 (Fig. 5). The result is significant at $p < 0.01$. Therefore, it is evident that exposure to PSE HCl causes an adversely negative effect in *D. tigrina* planaria.

1.2 Assessment of Mortality Incidence

To assess mortality incidence, each planarian was tracked throughout the 10 day period to count how many active planaria remained after each day. In this study, active planaria was defined as (1) responsive to light stimuli and (2) exhibition of motility to a certain degree. On Day 1, 6 planaria were reported as deceased in the 30 mg solution, 3 planaria for the 120 mg solution, and 4 for the 240 mg solution (Fig. 6). This trend continues in Day 2 where a rapid increase of 56 planaria were observed as deceased in the 30 mg solution, 23 for the 120 mg, and 21 for the 240 mg (Fig. 6). Most notably, all 60 planaria were reported dead by Day 4 in the 30 mg solution, while it took until Day 5 for the 120 mg solution, and until Day 10 for the 240 mg solution (Fig. 6). Comparatively, the negative control resulted in an overall mortality of 4 planaria while the positive control resulted in a mortality of 2 planaria by Day 10 (Fig. 6).

Upon exposure of PSE, all solutions exhibited an immediate decline in regeneration rates and blastema formation while beginning a rapid incline in mortality. This trend was best compared on Day 3 when mortality incidence was assessed. For the negative control, there was a 3.33% mortality rate and the positive control displayed a rate of 1.67% (Fig. 7). However, all concentrations of PSE revealed a mortality incidence $\leq 41.67\%$ (Fig. 7). The highest mortality was depicted in PSE 30 (98.33%) which was 56.66% above the greatest solution of PSE 240 (41.67%) (Fig. 7). Furthermore, PSE 120 exhibited a mortality rate of 76.67%, placing it between the highest and lowest mortality rates of the 3 PSE solutions (Fig. 7).

To compare the amount of planaria that died each day on average in response to PSE, the mean mortality rate was recorded over the 10 day period (Fig. 8). The negative control displayed an average mortality of 0.4 planarians per day with a standard deviation of $\pm$ 0.66 (Fig. 8). For the positive control, 0.2 planarians were reported as deceased per day with a standard deviation of $\pm$ 0.4 (Fig. 8). PSE 30 exhibited the highest average mortality rate per day with a mean of 15 planaria and a standard deviation of $\pm$ 29.29 (Fig. 8). PSE 120 resulted in a mortality of 12

planaria and a standard deviation of ± 8.34 while PSE 240 had an mortality average of 6 planaria and a standard deviation of ± 4.38 (Fig. 8).

1.3 Assessment of Average Lifespan

The life expectancy of planaria was averaged over the 10 day period under exposure of each PSE concentration (Fig. 9). The lowest concentration, PSE 30, exhibited an average lifespan of 1.983 days with a standard deviation of ± 0.465 and a variance of 0.216 (Fig. 9). PSE 120 averaged a lifespan of 2.983 days with a standard deviation of ± 1.147 and a variability of 1.316 (Fig. 9). PSE 240 showcased an average lifespan of 4.367 days, a standard deviation of ± 2.51, and a variability of 6.299 (Fig. 9). Therefore, an incline in life expectancy was observed with increasing concentrations of PSE.

The likelihood of planarian life expectancy being ≤ 3 days was also assessed (Fig. 10). Under exposure to PSE 30, there was a 98.56% chance the planaria would possess a lifespan of ≤ 3 days (Fig. 10a), while PSE 120 exhibited a 50.13% probability (Fig. 10b), and PSE 240 showcased a 25.21% chance (Fig. 10c). Therefore, PSE 30 and 120 demonstrated a ≤ 50% average in mortality within 3 days of exposure, whereas planaria under PSE 240 were 74.79% more likely to survive within the first 3 days (Fig. 10). Therefore, an increase in life expectancy above 3 days was shown with increasing concentrations of PSE.

1.4 PSE Exposure Alters Regeneration Morphology

When observed under a compound microscope, distinct features of the planaria indicated high stress in all concentrations of PSE. In comparison to baseline conditions (Fig. 3a), planaria responded adversely to PSE exposure with distinct loss of viability, structural deterioration, and malformation (Fig. 3b).

As observed on Day 1, planaria in negative and positive control environments exhibited physiological standards of the regenerative process (Fig. 4). However, planaria in PSE 30 and PSE 120 displayed immediate morphological responses including body scrunching, wherein planarians curl up to avoid the harmful stimulus (Fig. 4). In PSE 30, reaction to the stimulus within ≤ 5 hours took the form of right/left bisections demonstrating scrunching behaviors and curling up to protect their internal organs (Fig. 4). In PSE 120, this behavior occurred similarly to PSE 30 except within ≤ 12 hours of exposure (Fig. 4). In PSE 240, planarians began to curl within ≤ 24 hours, but did not respond as adversely compared to lower concentrations of PSE 30 and 120.

After the 10 day assay, planaria in all PSE concentrations displayed high levels of turgor collapse, contortion, and were unresponsive to stimuli. The positive control solution showcased successful levels of regeneration, similar to the negative control to a lesser extent (Fig. 4). However, in PSE 30, 120, and 240 all planarian viability was lost (Fig. 4). Each concentration displayed a cessation of biological activity and metabolic processes indicative of cellular dysfunction and altered pigmentation (Fig. 4). All PSE concentrations revealed a disintegration of tissues and cellular integrity, likely due to the effects of toxicity (Fig. 4). Therefore, a correlation between PSE exposure over a 10 day period and declining planaria metabolic activity was established.

FIGURE 1: Experimental Design

FIGURE 2: Horizontal Bisection of Planaria

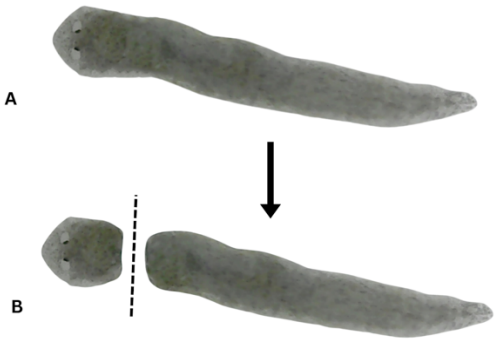
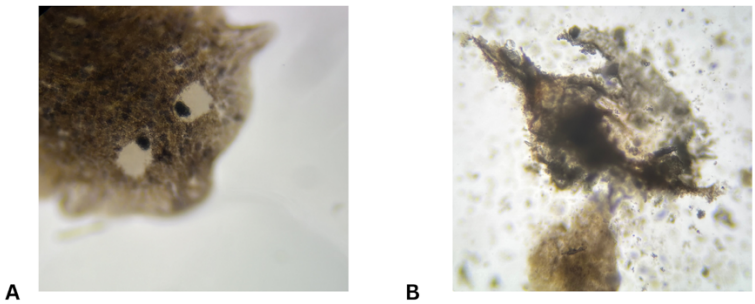
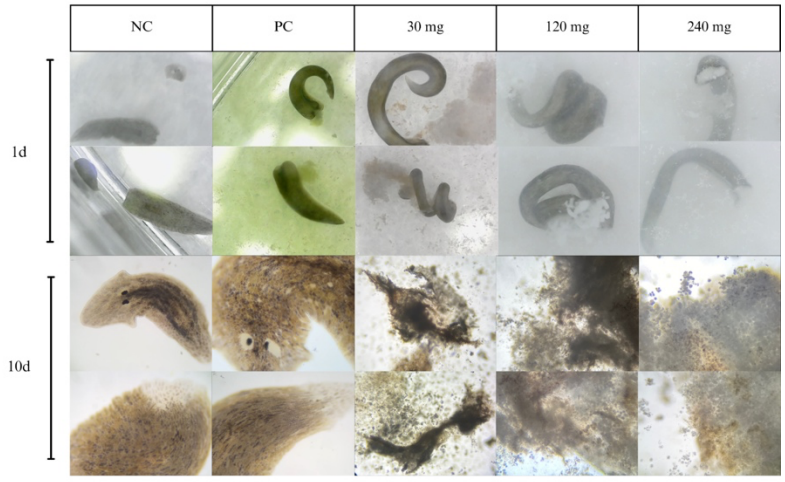


FIGURE 3: Exposure of Pseudoephedrine (PSE) Induces Mortality



(Fig. 3A): Baseline conditions of planaria. Eyes are visible and auricles, or chemoreceptors, are present on both sides of its head. (Fig. 3B): After a 10 day exposure to PSE 30 mg, the planarian head and tail is no longer visible. Epithelial tissue is torn and scattered throughout the sample, indicative of its death.

FIGURE 4: Regeneration Assay Under PSE Exposure



"1d" indicates 1 day under exposure to the substances and the baseline conditions of the experiment. "10d" indicates 10 days post-exposure. "NC" represents negative control (spring water) and "PC" is the positive control group (diluted caffeine solution)

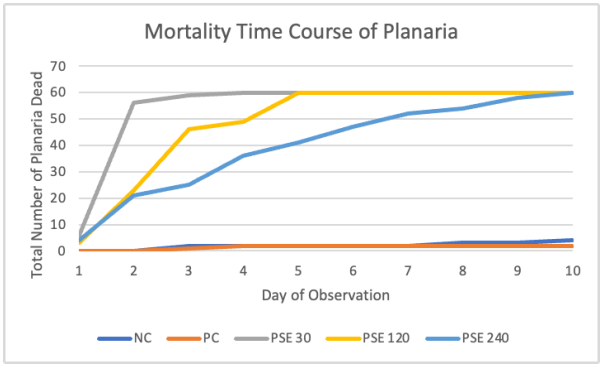
FIGURE 5: Chi-Square Test for Planarian Mortality on Day 3

	Active	Deceased
NC	58 (33.40) [18.12]	2 (26.60) [22.75]
PC	59 (33.40) [19.62]	1 (26.60) [24.64]
PSE 30	1 (33.40) [31.43]	59 (26.60) [39.46]
PSE 120	14 (33.40) [11.27]	46 (26.60) [14.15]
PSE 240	35 (33.40) [0.08]	25 (26.60) [0.10]
Column Totals	167	133

The chi-square statistic is 181.6127. The p-value is < 0.00001. The result is significant at p < .01. There is a statistically significant correlation between PSE and mortality rates in planaria on Day 3.

FIGURE 6: Mortality Time Course of Planaria

A



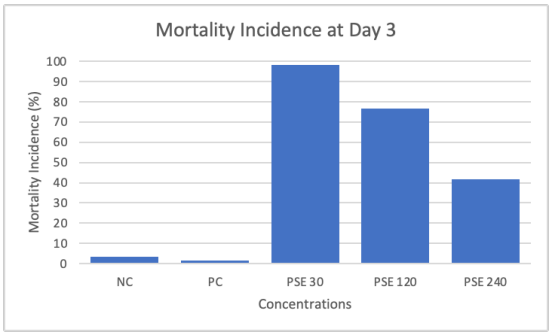
B

Day	NC	PC	PSE 30	PSE 120	PSE 240
1	0	0	6	3	4
2	0	0	56	23	21
3	2	1	59	46	25
4	2	2	60	49	36
5	2	2	60	60	41
6	2	2	60	60	47
7	2	2	60	60	52
8	3	2	60	60	54
9	3	2	60	60	58
10	4	2	60	60	60

(Fig. 6A): Mortality rates were recorded throughout the 10-day post-amputation period. Results were graphed.
(Fig. 6B): Values for each dead planarian were recorded each day, summing to a total of 60 planaria per petri dish.

FIGURE 7: Mortality Incidence at Day 3

A



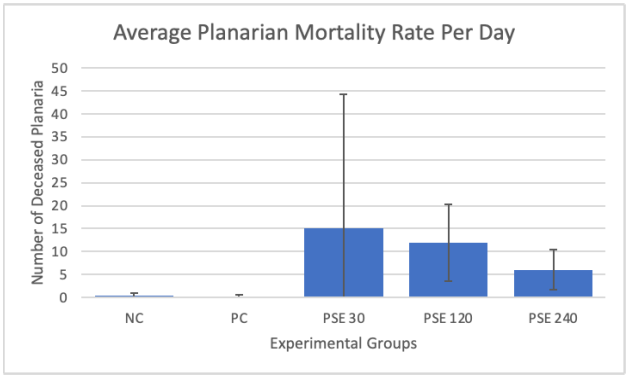
B

	NC	PC	PSE 30	PSE 120	PSE 240
Mortality %	3.33%	1.67%	98.33%	76.67%	41.67%

(Fig. 7A): Mortality incidence was recorded throughout the 10-day post-amputation period. Results were graphed.
(Fig. 7B): Values were expressed in a percent for each dead planarian, summing to a starting total of 100% planaria per petri dish.

FIGURE 8: Average Planarian Mortality Rate Per Day

A



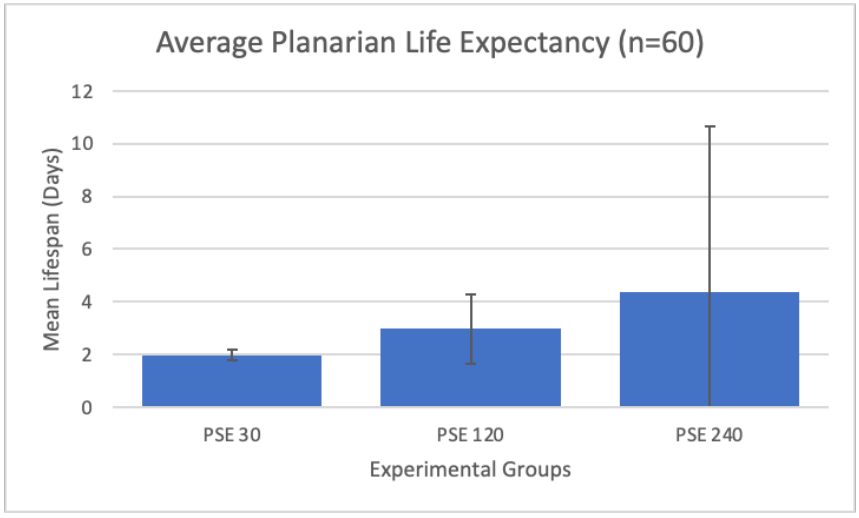
B

	Mean	SD
NC	0.4	0.66
PC	0.2	0.4
PSE 30	15	29.29
PSE 120	12	8.34
PSE 240	6	4.38

(Fig. 8A): Planarian mortality per day was averaged and recorded on a graph. (Fig. 8B): Data graphed above is expressed in a table, in terms of number of planaria.

FIGURE 9: Average Planarian Life Expectancy

A

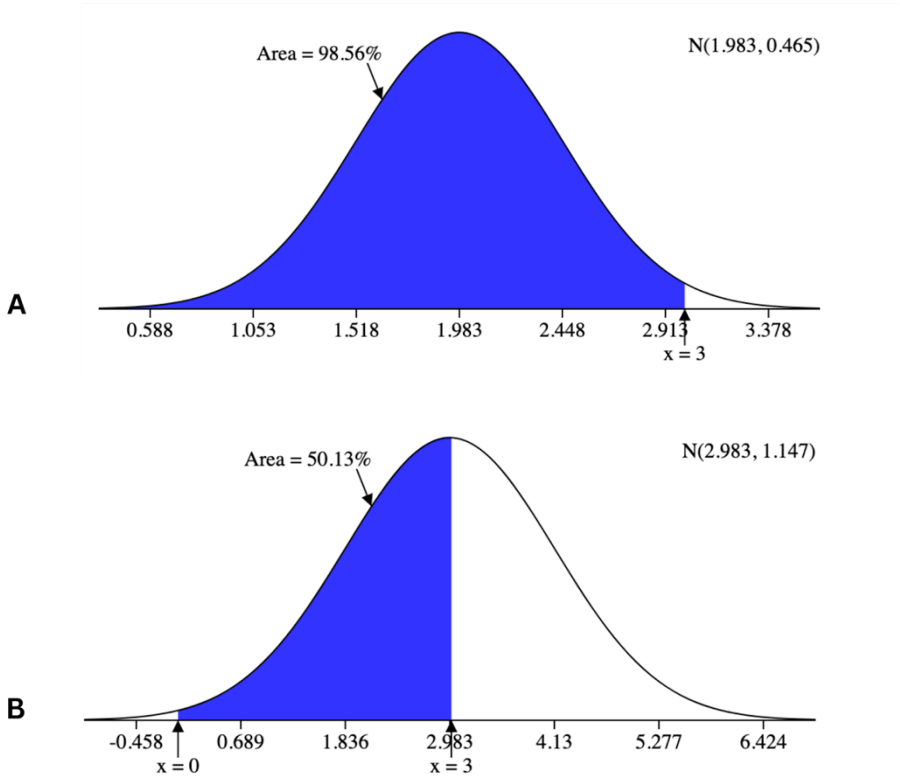


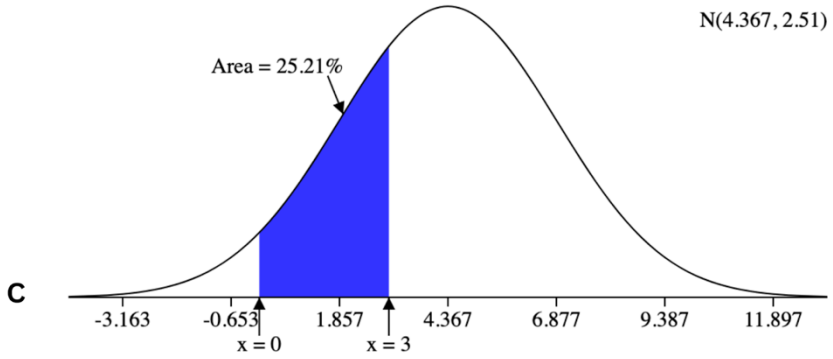
B

	Mean	SD	Variance
PSE 30	1.983	0.465	0.216
PSE 120	2.983	1.147	1.316
PSE 240	4.367	2.51	6.299

(Fig. 9A): Planarian life expectancy per day was averaged and recorded on a graph. (Fig. 9B): Data graphed above is expressed in a table, in terms of days.

FIGURE 10: Likelihood of Mortality Within 3 Days





(Fig. 10A): 98.56% chance that the planaria will have a lifespan of ≤ 3 days. (Fig. 10B): 50.13% chance that the planaria will have a lifespan of ≤ 3 days. (Fig. 10C): 25.21% chance that the planaria will have a lifespan of ≤ 3 days.

Discussion

1.1 Assessing PSE Absorption

Due to PSE's inherently stimulative nature, it was hypothesized that low concentrations (30 mg) would lead to enhanced regeneration and accelerated formation of a blastema. It was also hypothesized that high concentrations of PSE (120 to 240 mg) would lead to heightened mortality rates and delayed regeneration, considering its linkage with adverse side effects at high dosages in human-related drug abuse.
In this study, results provided contradictory to the initial hypothesis. Although there was a consensus that PSE was linked to planarian mortality and inhibited regeneration, the planaria underwent faster absorption of PSE 30 in comparison to higher concentrations of PSE, attributing to inclining mortality rates within lower concentrations. Planaria primarily absorb chemicals in the water via epithelial diffusion, and since PSE 30 is commonly used as a 4-6 hour treatment in humans, planaria absorb it quicker (Fig. 4). PSE 120 represents a 12 hour treatment while PSE 240 is a 24 hour treatment, which is why the planaria took longer to absorb higher concentrations of PSE and display adverse effects in response to exposure.

1.2 Causes of Mortality: Alpha-Adrenergic Stimulation

To understand why the planaria reacted negatively to PSE exposure, it is crucial to understand its stimulative properties. PSE's primary mode of action involves the activation of alpha-adrenergic receptors, leading to its vasoconstrictive properties. In planaria, which lack a true circulatory system with blood vessels, excessive vasoconstriction-like effects disrupt the distribution of nutrients and oxygen to its cells. Therefore, this explains the adverse effects of PSE on planaria physiological function.

Furthermore, this investigation demonstrated declining lifespan associated with faster absorption of PSE concentrations (Fig. 9). The reason for this stems from planaria possessing a simple nervous system, and since PSE works to overstimulate neurotransmission, the planaria displayed disruptions in neuronal signaling which led to abnormal behavior, impaired movement, and sudden death.

1.3 Effect on Regeneration

With increasing mortality rates associated with faster absorption, PSE was found to negatively impact regeneration as well (Fig. 8). It is suspected that vertical bisection was likely to have disrupted a pathway that affects planarian motility, causing their distinct curling-up response. For the planaria, this was a way for them to protect their exposed internal structures rather than actively move away from the irritant, as seen in PSE 30 and PSE 120 within ≤ 12 hours of exposure (Fig. 4).

Since planaria form a blastema, or an aggregation of stem cells, to recover from injury in the form of an amputation, exposure to PSE caused a lack of blastema formation, particularly in PSE 30 (Fig. 3). The reason for this is likely due to the toxicity of PSE which exerted toxic effects on planarian neoblasts, or stem cells, that interfered with its metabolic processes and cellular homeostasis ultimately leading to cell death.

D. tigrina planaria lack specific receptors and regulatory mechanisms crucial for modulating the effects of PSE. With the absence of a system in place to metabolize and eliminate the substance efficiently, this led to an accumulation of PSE and increased susceptibility to its adverse effects as seen with PSE 30's lifespan of ≤ 2 days (Fig. 9).

Therefore, PSE's vasoconstrictive properties were likely responsible for planaria's delayed regeneration, although the existence of additional targets of PSE in this species cannot be ruled out by the data.

1.4 Limitations

Various limitations in this study include temperature fluctuations, light exposure, a single-species focus, lack of a detailed molecular analysis, and sample size constraints.

Although efforts were made to reduce significant temperature fluctuations that could have skewed experimental results, environmental parameters cannot be ruled out entirely. Thermometers were placed in each petri dish to regulate conditions and an insulated housing container provided temperature consistency throughout the duration of the experiment. However, potential variability due to factors beyond strict laboratory control could have taken place.

This species of planaria are known to display adverse effects to prolonged exposure under light. So to mediate these effects, a controlled environment was maintained using a housing container that provided little to no access to light. However, it is worth considering that the only accessible method for planarian regeneration to be tracked under a microscope involved exposure to a light source. Therefore, it is possible that planaria were observed to exhibit exaggerated effects to PSE since upon microscopic observation, light played a significant factor.

Another limitation to consider is the use of a single species in this investigation. The results proved in this experiment are only applicable towards the *Dugesia tigrina* species of planaria, and cannot be verified for *Schmidtea mediterranea*, *Girardia dorotocephala*, or other unmentioned species of flatworms without further research. Furthermore, since planaria was used in this study as a model organism for humans, there still remains notable differences between the two species. These results cannot be conclusive towards humans, and only serve as supportive material.

An OMAX 40X-2000X Compound Lab Microscope and digital microscope was used in this experiment. In order to verify molecular or subcellular effects of PSE exposure, a higher powered microscope would be preferred. This study utilized a sample size of 300 planaria, including 60 planaria (30 heads, 30 tails) in each of 5 petri dishes. To verify existing results, a larger sample size involving n ≥ 300 planaria should be used.

1.5 Implications and Future Directions

There remains a wide variety of paths for further research to take place in PSE and planarian regeneration studies. Implications include ecotoxicological relevance, fundamental biological mechanisms in regenerating tissues, and public awareness of PSE drug overdose.

In terms of ecotoxicological relevance, the toxicity of PSE on planaria underscores the potential consequences of reckless PSE disposal in aquatic environments. There remains a high level of environmental risk for aquatic species such as *D. tigrina* planaria with the use and disposal of pharmaceutical compounds into bodies of water.

One of the most important insights drawn from this study includes the enhancement of our understanding in regenerative patterns and developmental processes. Due to increasing mortality rates and declining rates of

regeneration displayed under PSE exposure, this provides insight into the potential interference of pharmaceutical contaminants with fundamental biological mechanisms in regenerating tissues.

With the use of PSE as a precursor for methamphetamine in the past 2 decades, concern for PSE drug abuse is likely to remain high. This study verified the negative effects of PSE exposure, urging the public to avoid excessive quantities of PSE 30 mg due to its faster absorption rate into the body. As proven in planaria, this is likely to cause adverse effects in terms of morphology, wound healing, and cellular metabolism, so it is advisable that the public draws a great amount of caution towards PSE-containing medications.

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