

The Effect of Ultra-high Dilutions of Thyroxine on the Morphogenesis of *Xenopus laevis* Tadpoles

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Abstract

Background: The morphogenesis of *Xenopus laevis* is dependent on the thyroid system and the production of thyroxine. Numerous studies using the amphibian model have shown tadpoles to be responsive to ultra-high dilutions of Thyroxine. Ultra-high dilutions used in Homeopathy are not suitable to pharmacokinetic investigation due to their lack of detectable active ingredient and the lack of analytical methods with sufficient sensitivity; however, laboratory and clinical studies are providing experimental evidence contributing to the pharmacodynamics of high dilution remedies.

Method: The experiment consisted of four groups, labelled according to the dilution each group was administered, Control (no treatment), Thyroxine 6C, Thyroxine 30C and Thyroxine 200C. Each group consisted of 90 tadpoles divided into three tanks of 30 tadpoles each. The respective dilutions were administered to the water the tadpoles were housed in every eight hours from Day 32 until Day 58 of the experiment. Tail length was measured every three days from Day 32 to Day 48 and every day from Day 48 to Day 58.

Results: Thyroxine 6C was shown to have had a stimulatory effect while Thyroxine 30C had an inhibitory effect on the growth phase of the *Xenopus laevis* tadpole tail. Thyroxine 6C, 30C and 200C were shown to have had an inhibitory effect on the reduction phase of the *Xenopus laevis* tadpole tail.

Conclusion: Where the results were shown to have had a stimulatory effect on the growth phase of the *Xenopus laevis* tadpole tail, it was likely due to a physiological effect, mimicking the action of the naturally circulating thyroxine. The results that were shown to have had an inhibitory effect are in line with the "Law of Similars" and the fact that a homeopathic preparation of Thyroxine would have an opposing effect to that of naturally circulating thyroxine.

Keywords: Homeopathy; Ultra-high dilution; Thyroxine; Morphogenesis; *Xenopus laevis*; Tadpole; Amphibian

Introduction

The morphogenesis of *Xenopus laevis* is entirely dependent on the thyroid system and the production of thyroxine; they require the initiation and maintenance of high levels of circulating thyroid hormones to make the transition from tadpole to juvenile frog. Exposure of tadpoles to exogenous thyroid hormones can induce precocious morphogenesis, whereas withholding thyroid hormones by surgical ablation of the thyroid gland will prevent any further development until such time that the hormone is replaced [1].

Endler et al. presented a model involving highly dilute Thyroxine and the metamorphosis of highland amphibians inspired by studies at the time of experimental intoxication and subsequent detoxification of organisms using high dilutions of the same substance. The amphibian model being analogous to the intoxication model by the fact that physiological thyroxine levels during metamorphosis are high compared to any other developmental stage [2].

This led to the hypothesis that highly dilute Thyroxine, prepared according to homeopathic principles of succussion and dilution would have an inverse effect to that of molecular thyroxine and any effect observed would lean towards information transfer from the crude substance to the diluent [3].

According to a study conducted by Harrer, 48 hourly applications of Thyroxine 30D have an inhibitory effect on the morphogenesis of highland amphibians [4]. The purpose of Harrer's study was to reproduce an experiment conducted by Endler et al. using diluted Thyroxine on amphibians [5]. The studies focused on two parameters; entry into the 4-legged stage and tail reduction.

The Thyroxine 30D and the Water 30D test solutions were prepared in a similar manner by successive succussions and dilutions [4]. The results of Harrer's study (2013) were in line with that of Endler's study; there was a clear inhibitory trend on both parameters. For those that entered the 4-legged stage, pooled Thyroxine 30D values for tail length were 10.1% smaller than the Water 30D control group for the initial study ($p < 0.01$ and a large effect size, $d > 0.08$), while five independent researchers that replicated the study showed to be 12.4% smaller when pooled together ($p < 0.01$ and a large effect size, $d > 0.08$).

An analysis of 107 studies on highly dilute substances compared the results of repeated studies from an internal-laboratory team (the initial study team with at least two publications and a follow-up trial of the initial publication), a multicentre team (independent experiments in a different location with one study coordinator) and an independent team (an independent researcher, independent laboratory with an independent publication).

The studies comprised of 30 initial studies and 77 replication studies. Of the effects found in the initial studies, 83% were re-observed in internal-laboratory replication, 67% in multicentre replication and 44% in independent replication studies. The authors proposed the idea that the higher number of positive outcomes in the initial studies, apart from publication bias or random success, may have been due to expertise, knowledge, superior handling and general know-how [6].

The ability to repeat experiments is considered an integral part of modern science, for this reason, a similar model to Endler et al. and various studies that followed involving amphibians is adopted for the purpose of this study. The specific species *Xenopus laevis* was chosen as it is considered of “least concern” on the IUCN Red List of Threatened Species with an increasing population trend [7]. To date, most of the research available involving the topic of amphibians, ultra-high dilutions of thyroxine and homeopathy have been conducted using 30D potencies and although the exact results have varied, they have all shown an inhibitory trend on metamorphosis [2-5].

The aim of this study is to use a model that has been tried and tested by various independent researchers to investigate the effect of ultra-high dilutions of Thyroxine on the morphogenesis of *Xenopus laevis* tadpoles using potencies of 6C, 30C and 200C, to determine if the effect is stimulatory or inhibitory to morphogenesis and whether the various potencies had similar or dissimilar effects on morphogenesis. It is hypothesised that ultra-high dilutions of Thyroxine in centesimal potencies may inhibit or stimulate morphogenesis of *Xenopus laevis* tadpoles when considering the stage and duration of tail reduction.

Methods

The principles of the 3Rs according to The National Centre for the replacement, refinement and reduction of animals in research were scrutinised during the development, approval and experimental phases of the study [8].

Replacement

The process of morphogenesis is well suited to demonstrate the subtle effects of homeopathy without causing any permanent or adverse effects to the tadpole. Morphogenesis of *Xenopus laevis* tadpoles is dependent on thyroxine; they require the initiation and maintenance of high levels of circulating thyroid hormones to make the transition from tadpole to juvenile frog. Exposure to exogenous thyroid hormones can induce precocious morphogenesis, whereas surgical ablation of the thyroid gland will prevent any further development until the hormone is reintroduced [1].

In order to demonstrate the potential effects of a homeopathic dilution in a physiological environment and to substantiate the “Law of Similars”, the *Xenopus laevis* tadpole was chosen as a suitable sample group. No significant short-term harm or any long-term harm was expected to be endured by the tadpoles throughout the experiment. Since thyroxine is a naturally occurring hormone, treatment with

homeopathic dilutions of the same substance can only speed up or slow down, if it has any effect at all, on what will naturally occur without external influence.

Refinement

A quantitative, experimental, factorial study was conducted under the supervision of qualified laboratory technicians at the University of Johannesburg, Zoology Department and Faculty of Science on the Auckland Park campus. The research proposal was submitted to the Health Research Ethics Committee (HREC) accredited by the National Health Research Ethics Council of South Africa (NHREC Registration no: REC-241 112-035) and reviewed by 2 members of the HREC not affiliated with the Department of Homeopathy. Ethical clearance was granted by the HREC (Ethical Clearance: REC-01.159.2015).

Special care regarding the housing and upkeep of the animals is taken as set out by the South African National Standard (SANS) for the care and use of animals for scientific purposes, the Royal Society for the Prevention of Cruelty to Animals (RSPCA), as well as the EU Directive 2010/63/EU on the protection of animals used for scientific purposes [9-11].

Possible risks to the *Xenopus laevis* tadpoles may include handling and husbandry errors, however special care is taken in this regard (see Husbandry and Data Collection). The potential benefits of the study may demonstrate the effects of highly dilute thyroxine and how the various effects differ in relation to the potency.

This may lead to a more detailed understanding of homeopathic dilutions and their potential for therapeutic application. There are no long-term adverse effects anticipated and the study is considered low risk with considerable scientific benefit. The findings of this study may add to the fundamental research available on the “Law of Similars” and lend to theories of physiochemical properties and pharmacodynamic mechanisms of information transfer where there is non-molecular information imprinted on a solvent during the process of succussion and dilution [12].

Reduction

The sample group consisted of 360 newly hatched tadpoles. According to the IUCN red list of threatened species *Xenopus laevis* is considered of “least concern” with an increasing population trend [7]. Three pairs of sexually mature *Xenopus laevis* frogs were primed for mating with a series of human chorionic gonadotropin (hCG) hormone injections to induce ovulation, egg laying and fertilisation [9]. The hCG used was supplied in 1 mL ampoules of 5000 international units (IU). The males were given a primer injection of 50 IU (0.01 mL), while the females were injected with 100 IU (0.02 mL) of hCG into the dorsal lymph sac.

A final injection was given 48 hours after the primer injection; this time the males were given 100 IU, while the females were given 300 IU of hCG. Over the course of the 48 hours following the primer injection, the cloacal labia became swollen and red, while the males developed darker nuptial pads on the underside of the forearms and fingers, indicating that they were ready to mate.

Amplexus was not observed but is assumed to have occurred roughly 36 hours post final injection at some point during the night. The eggs observed the following day marked “Day 1” of the experiment. It was estimated that roughly 1000 tadpoles developed and of these, 360 tadpoles were used for the experiment, the remaining

640 tadpoles were donated back to the institution for practical laboratory purposes.

Husbandry

The newly hatched tadpoles were housed in an environmental control room where the photoperiodism is set to 14 hours light and 10 hours dark, mimicking their natural habitat, according to the RSPCA. The temperature was checked daily and maintained within a range of 23°C-25°C. Quantity and depth of water provisions are poorly outlined, as long as the animal can swim around freely, lie fully submerged underwater and avoid contact with other animals or tank walls if desired. The quality of water is more important, with a pH maintained between 6.5 and 8.5.

The researcher followed standard operating procedures when handling the animals whilst priming them for mating; they were closely monitored for key signs of ill-health and procedures of egg-harvesting were adhered to as set out by the RSPCA. Qualified laboratory assistants supervised to ensure that all procedures were conducted in an acceptable, responsible and humane way, keeping in line with the ethical requirements as set out by Guidelines on Ethics for Medical Research: Use of Animals in Research and Training, SANS and the UE Directive 2010/63/EU on the protection of animals used for scientific purpose [9-11,13].

To minimise variability, the researcher decided to house all the tadpoles together, but due to the number of tadpoles and an inadequate tank size, they were spread across three tanks until they reached 32 days old, at which point they were randomly divided into the experimental tanks.

The feed consisted of a mixture of spirulina, commercial fish food and a maize based cereal mixed in a ratio of 1:1:1. One part spirulina (15 g), one part commercial fish food (15 g) and one part maize cereal (15 g) in a 250 mL beaker. The components were blended in a coffee grinder to a fine powder. For the first 10 days, the mixture was added to 200 mL of purified water and allowed to settle in the refrigerator overnight.

The liquid was then siphoned off the top of the remaining pulp and 10 mL added to each of the three tanks daily. A new mixture was prepared every two days. For the remainder of the experiment, dry mixture was sprinkled over the top, as much as they would consume within five minutes to prevent fouling of the water, roughly 5 g of powder in each tank once daily.

Laboratory technicians fitted aquarium filters to each tank to prevent the water from becoming murky; they cleaned and replaced the filter floss once a week. It was anticipated that the filters would be sufficient to keep the water clean and free from any debris. By Day 35, the filters were no longer able to keep the water clean and clear. A complete water change was done on Day 40 and again on Day 45.

Experimental groups

The experimental tanks were made of glass with an opaque bottom and two sides, the front and back side being transparent for easy observation. The tanks measured 50 cm × 50 cm × 50 cm and were filled with 50L of distilled water. The experiment consisted of four groups, the Control, Thyroxine 6C, Thyroxine 30C and Thyroxine 200C. Each group consisted of three replicates (tanks) housing 30 tadpoles each, totaling 90 tadpoles per group and a total of 360 tadpoles for the experiment. It was agreed to house 30 tadpoles per

tank due to the size of the tanks available as well as to minimize the number of tadpoles affected per group should any adverse events occur throughout the study.

Preparation and administration of the ultra-high dilutions of thyroxine

Levothyroxine Sodium Salt Pentahydrate was received in powder form and triturated to 3C potency according to the German Homoeopathic Pharmacopoeia HAB Method 6, for the trituration of solid raw materials. The 3C potency was then converted to a liquid potency according to the German Homoeopathic Pharmacopoeia HAB method 8A, for liquid preparations made from triturations. Potentisation was done according to the Hahnemannian multi-vial method. To make the 5C potency 43% v/v ethanol was used. Further potencies, 6C to 15C were made with 90% v/v ethanol. The potencies 16C to 196C were prepared in 20% v/v ethanol. The potencies from 197C to 200C were prepared in 90% v/v ethanol.

Bulk containers were then prepared for the experiment. Stock potencies prepared were 5C, 29C and 199C, each in volumes of 1300 mL in 20% v/v ethanol [14,15]. The researcher then diluted and succussed the administration dose to the desired potency as and when needed. The administration dose was then calculated as one percent of the volume of the tank. Having used 50L of water per tank, a single administration dose was calculated as 500 mL. The researcher diluted the stock potency according to the Hahnemannian method in a ratio of 1:99, calculated as 5 mL of stock solution added to 495 mL of purified water.

The 500 mL solution was then succussed 100 times against a resilient surface. The appropriate potency per tank was administered every eight hours from Day 32. Each tank in Thyroxine 6C Group was given 500 mL of 6C Thyroxine, Thyroxine 30C Group was given 500 mL of 30C Thyroxine and Thyroxine 200C Group was given 500 mL of 200C Thyroxine. With every dose, 500 mL of water was removed from the tank to maintain the water volume at 50L. The Control Group remained untreated.

Data Collection

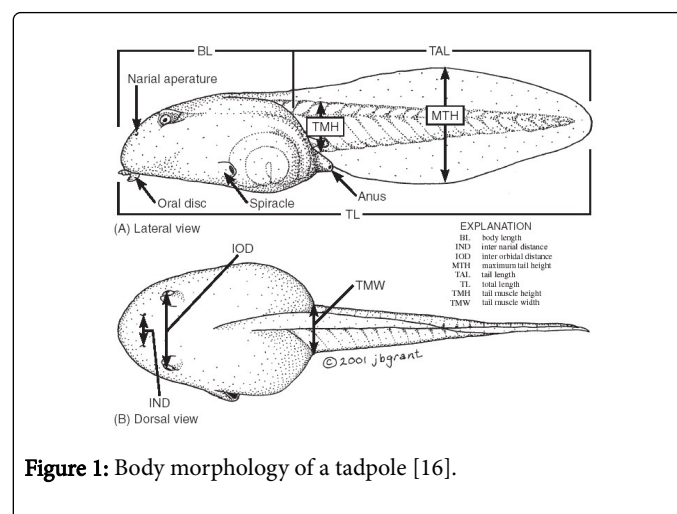


Figure 1: Body morphology of a tadpole [16].

Tail length was measured every three days from Day 32 (Stage 55), the day administration of the remedy started, until Day 47 (Stage 60). From Day 48 (Stage 61) to Day 58 (Stage 66), tail length was measured

daily. Starting with Thyroxine 6C Group, one tank at a time, all the tadpoles were removed from the tank using a fine nylon aquarium net.

They were gently placed in a 500 mL plastic container whilst they awaited measurement. To measure tail length, each tadpole was placed on a transparent petri dish and placed over a ruler marked in 0.5 mm increments.

As can be seen in Figure 1, the tails were measured from the top of the anus to the tip of the tail (TAL) [16]. Once measurement was completed, they were placed back in the tank to avoid duplicate measurements. The process was repeated for each of the 12 tanks.

Data analysis

A Univariate Analysis of Variance (ANOVA) was conducted to explore the impact of various ultra-high dilutions on the tail growth and reduction of *Xenopus laevis* tadpoles at specific stages. Post Hoc tests were performed to determine where these differences lie and are only performed if ANOVA established that there was a significant difference.

Multiple Comparison Tests were used to establish which group means differ from others once the overall F-test demonstrated that there was a statistically significant difference.

The Tukey method is preferred if the number of groups is large. Tukey HSD is the most conservative of the post-hoc tests as it is the most likely to accept the null hypothesis of no group variances [17].

Upon conclusion of the study, all animals were donated back to the University of Johannesburg, Zoology Department for practical laboratory purposes.

Even though development of the tadpoles may have been affected during the treatment phase, no developmental abnormalities were incurred once the treatment phase of the study was concluded. The tadpoles continued to develop normally and matured into juvenile frogs.

Results

Tail length was measured every three days from Day 32 (Stage 55), the day administration of the ultra-high dilutions of Thyroxine started, until Day 47 (Stage 60). From Day 48 (Stage 61), when tail reduction is theoretically said to begin, until Day 58 (Stage 66), tail length was measured daily [1].

Day 32 intergroup analysis-homogenous subsets

The Univariate AVOVA revealed a statistically significant main effect for treatment, $F=21.574$, with a p-value of <0.001 and a large effect size (partial eta squared=0.154).

A Tukey HSD multiple comparison test using harmonic mean sample size and an alpha value of 0.05 found that the thyroxine 6C group and the thyroxine 30C group were homogeneous and therefore comparable with no significant difference between them, while the thyroxine 200C group and the control group were homogeneous and comparable with no significant difference between them, as can be seen in Table 1.

Therefore, for the remainder of the study, only groups within each of the Homogenous Subsets are compared to each other.

Group	No. of Tadpoles	Homogeneous Subset Mean Tail Length (mm)	
		1	2
Control-No treatment	90	24.53	-
Thyroxine 6C	90	-	26.87
Thyroxine 30C	90	-	26.91
Thyroxine 200C	90	23.89	-
p-Value	-	0.532	1

Table 1: Means for groups in homogeneous subsets-day 32.

Growth phase day 32 to day 48

On the evening of day 39 of the experiment, an electrical power failure occurred for roughly 4 hours which prevented access to the laboratory resulting in a missed treatment dose and may have caused temperatures in the aquarium to drop slightly.

Although it is possible to assume that this may have resulted in thermal shock and possibly contributing to the mortalities that occurred thereafter, it is likely not the reason for the deaths as the temperature could not have dropped so significantly considering they were housed in an environmental control room sealed off within the surrounding aquarium. It is worth noting that all tadpoles in the study were equally affected and the temperature was back up to the regulated 24°C by the time access to the laboratory was restored.

It is believed that *Xenopus laevis* become stressed by prolonged periods of exposure to temperatures below 14°C and above 26°C with optimal temperatures above 21°C [10]. It is not anticipated that the temperature would have dropped more than 3°C. Table 2 summarises the change in mean tail length and sample size from Day 32 to Day 48.

The mean tail growth is illustrated in Figure 2 for each group from Day 32 to Day 48.

Group	Day	Mean Tail Length (mm)	Change in Tail Length (mm)	Number of Tadpoles
Control	Day 32	24.53	3.59	90
	Day 48	28.12		52
Thyroxine 6C	Day 32	26.87	10.89	90
	Day 48	37.76		59
Thyroxine 30C	Day 32	26.91	6.7	90
	Day 48	33.61		49
Thyroxine 200C	Day 32	23.89	8.44	90
	Day 48	32.33		76

Table 2: Descriptive statistics-mean tail growth from day 32 to day 48.

	Day 58	25.41		69
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Table 4: Descriptive statistics-mean tail reduction from day 48 to day 58.

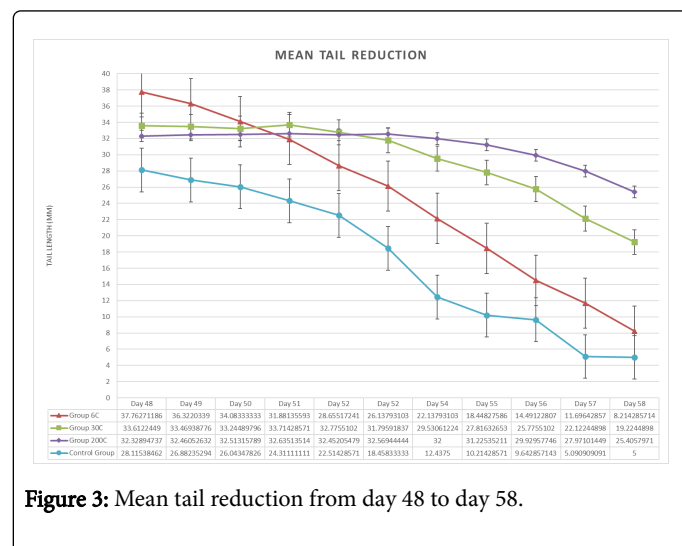


Figure 3: Mean tail reduction from day 48 to day 58.

Group	Group	Mean Difference in Reduction (mm)	Sig.
Control	Thyroxine 6C	0.71	p=0.971
Control	Thyroxine 30C	2.34	p=0.480
Control	Thyroxine 200C	4.96	p=0.007
Thyroxine 6C	Thyroxine 30C	3.04	p=0.128
Thyroxine 6C	Thyroxine 200C	5.66	p<0.001
Thyroxine 30C	Thyroxine 200C	2.62	p=0.198

Table 5: Tukey HSD multiple comparison test - mean difference in tail reduction.

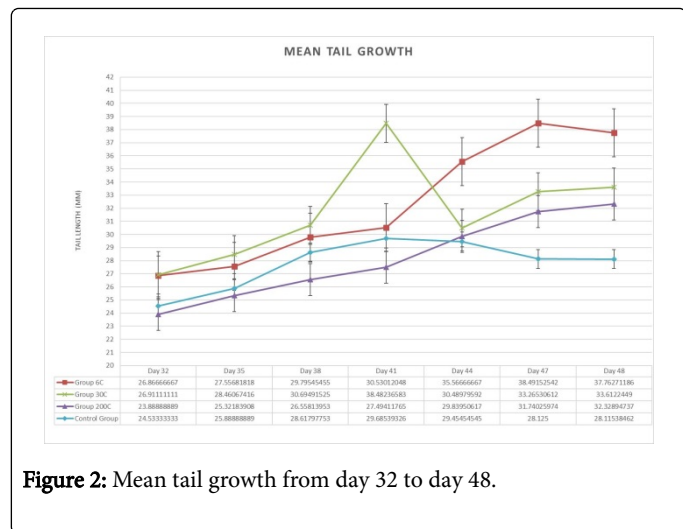


Figure 2: Mean tail growth from day 32 to day 48.

An intergroup analysis was conducted to compare the overall mean change in tail growth from Day 32 to Day 48 and to see if there was any statistically significant difference between the groups. The Univariate ANOVA revealed a statistically significant main effect for treatment, $F=44.747$, with a p-value of <0.001 and a large effect size (partial eta squared=0.186). Post Hoc comparisons using the Tukey HSD multiple comparison tests was used to determine where the significance lies. Table 3 summarises the findings.

Group	Group	Mean Difference in Growth (mm)	Sig.
Control	Thyroxine 6C	5.34	p<0.001
Control	Thyroxine 30C	3.43	p<0.001
Control	Thyroxine 200C	1.91	p=0.002
Thyroxine 6C	Thyroxine 30C	1.91	p=0.002
Thyroxine 6C	Thyroxine 200C	3.43	p<0.001
Thyroxine 30C	Thyroxine 200C	1.52	p=0.021

Table 3: Tukey HSD multiple comparison test-mean difference in tail growth.

Reduction phase-day 48 to day 58

Group	Day	Mean Length (mm)	Tail	Change in Tail Length (mm)	Number of Tadpoles
Control	Day 48	28.12		23.12	52
	Day 58	5.00			
Thyroxine 6C	Day 48	37.76		29.55	59
	Day 58	8.21			
Thyroxine 30C	Day 48	33.61		14.39	49
	Day 58	19.22			
Thyroxine 200C	Day 48	32.33		6.92	76

The Univariate ANOVA revealed a statistically significant main effect for treatment, $F=19.746$, with a p-value of <0.001 and a large effect size (partial eta squared=0.247). Table 4 represents mean change in tail length from Day 48 to Day 58. Figure 3 shows the mean tail reduction for each group from Day 48 to Day 58. An intergroup analysis was conducted to compare the overall mean change in tail reduction from Day 48 to Day 58 and to see if there was any statistically significant difference between the groups. The Tukey HSD Multiple Comparison Test was used to determine where the significance lies. Table 5 summarises the findings.

Discussion

Naturally circulating thyroxine in the *Xenopus laevis* tadpole will stimulate tail reduction by activating lytic enzymes and inducing programmed cell death [18]. According to the “Law of Similars”, a homeopathic preparation of Thyroxine would have an opposing effect to that of naturally circulating thyroxine [19]. On Day 32 (NF Stage 55) naturally circulating thyroxine levels of the *Xenopus laevis* tadpole

are believed to be at its highest.²⁰ It was at this stage that the administration of the ultra-high dilutions of Thyroxine began.

Growth phase-day 32 to day 48 intergroup analysis

On day 32, the Thyroxine 200C Group and the Control Group (Homogeneous Subset 1), were found to have had a statistically significant shorter mean tail length than that of the Thyroxine 6C Group and the Thyroxine 30C Group (Homogeneous Subset 2). Homogenous Subset 1 and Homogenous Subset 2 are therefore not comparable from the start. The Control Group shows the least amount of growth with 3.59 mm while its comparable Thyroxine 200C Group grew on average 8.44 mm.

Thyroxine 6C Group showed the most amount of growth with an average of 10.89 mm while its comparable Thyroxine 30C Group grew an average 6.7 mm. The Tukey HSD Multiple Comparison Test revealed a statistically significant mean difference in tail growth from Day 32 to Day 48 for Homogeneous Subset 1, with a mean difference in tail growth of 1.91 mm and a p-value of 0.002 and Homogeneous Subset 2, with a mean difference in tail growth of 1.91 mm and a p-value of 0.002.

Homogenous subset 1-control group versus thyroxine 200C group

The Control Group as well as the Thyroxine 200C Group did not grow as much as what was naturally expected to occur, therefore it cannot be deduced that the lack of expected growth of the Thyroxine 200C group was due to the effect of the highly dilute Thyroxine 200C as the Control Group failed to show adequate growth.

However, assuming that without application of the highly dilute Thyroxine 200C, the Thyroxine 200C Group might have grown as much as what was naturally expected to occur and that perhaps the application of the highly dilute Thyroxine 200C may have inhibited normal growth and development, given that there was a statistically significant difference between the two groups by Day 48.

This finding would be in line with the “Law of Similars” and the fact that a homeopathic preparation of Thyroxine should have an opposing effect to that of naturally circulating thyroxine, which is responsible for normal growth and development at this stage [11,19,20].

Assuming that both the Control Group and the Thyroxine 200C Group were never going to grow as much as what was naturally expected to occur due to various husbandry reasons such as sudden temperature changes, water pH variances, poor hygiene, handling errors, feeding errors or microbial infection, it may then be interpreted that the Thyroxine 200C might have stimulated growth given that there was a statistically significant difference between the two groups at this stage.

Thyroxine 200C Group being 1.91 mm longer than that of the control group. Again, this is not in line with the homeopathic “Law of Similars” and the homeopathic dilution of 200C goes beyond that of Avogadro’s constant, implying there’s no active compound in solution for a physiological effect, leaving no reasonable explanation for the finding. Perhaps it was purely husbandry factors, as previously mentioned, that led to the difference between the two groups [9,11,19].

Homogenous subset 2-thyroxine 6c group versus thyroxine 30C group

The Thyroxine 6C Group grew as much as what was naturally expected to occur, while the Thyroxine 30C Group did not. Therefore, one of two assumptions can be made; it can be deduced that the lack of growth of the Thyroxine 30C Group was due to the inhibitory effect of the highly dilute Thyroxine 30C, or the adequate growth of the Thyroxine 6C Group was due to the stimulatory effect of the highly dilute Thyroxine 6C.

If the application of the highly dilute Thyroxine 30C inhibited normal growth and development, this finding would be in line with the “Law of Similars” and the fact that a homeopathic preparation of Thyroxine would have an opposing effect to that of naturally circulating thyroxine, which is responsible for normal growth and development at this stage [11,19,20].

Similar findings were reported by Bonamin & Endler in which a combination of Santoninim and Cina 30C and a combination of Eupatorium perfoliatum and Arsenicum album 30C were shown to have inhibited parasite multiplication of *Trichinella spiralis* and *Plasmodium berghei* respectively [21].

If the application of the Thyroxine 6C stimulated growth and development, this finding would be in line with having had a possible physiological effect. Given that a homeopathic dilution of 6C is well below that of Avogadro’s constant, which is equivalent to a 12C dilution, implying that there is still a detectable amount of active compound in solution; this would allow Thyroxine 6C to stimulate growth and mimic the tadpoles own natural thyroxine [12,19]. Similar findings were reported by le Roux in which growth and limb bud development of the *Xenopus laevis* tadpole was stimulated by eight hourly applications of Thyroxine 4C and Thyroxine 7C [22].

Reduction phase-day 48 to day 58 intergroup analysis

During the reduction phase, the Control Group reduced by 23.12 mm, Thyroxine 6C Group reduced the most by 29.55 mm, Thyroxine 30C Group reduced by 14.39 mm and Thyroxine 200C Group reduced the least by 6.92 mm. The Tukey HSD Multiple Comparison Test revealed a statistically significant difference in mean tail reduction for Homogenous Subset 1 with a mean difference in tail reduction of 4.96 mm and a p-value of 0.007, while the same test revealed no statistically significant difference for Homogenous Subset 2 with a mean difference in tail reduction of 3.04 mm and a p-value of 0.128.

Homogenous subset 1-control group versus thyroxine 200C group

Given that these two groups fall in Homogenous Subset 1, being comparable from the start and even though they were significantly different on Day 48, the Control Group measuring 28.12 mm and the Thyroxine 200C Group measuring 32.33 mm, they reduced at significantly different rates by Day 58, indicating that the Thyroxine 200C had an inhibitory effect of the reduction phase of the tadpole tail, resulting in a mean tail length of 25.41 mm, compared to that of 5 mm for the Control Group on Day 58.

In this instance, the degree of inhibition of the reduction phase was large enough to affect the “rate” at which the tails reduced. However, as much as it appears that the rate of the Thyroxine 200C Group was reduced compared to that of the Control Group, it’s more likely a

matter of how much the Thyroxine 200C inhibited tail reduction, in accordance with the “Law of Similars”.

In this case, tail reduction was inhibited such that it appears to slow down the rate of reduction, when in fact reduction is not dependent on time, but rather on influences affecting natural thyroxine levels [1]. Similar findings were reported by Konar & Sukul in which Nux Vomica 200C was shown to increase the level of free water in the brain of the teleost fish exposed to ethanol counteracting the effect of the ethanol which is responsible for dehydrating or decreasing free water in the brain [23].

Homogenous subset 2-thyroxine 6C group versus thyroxine 30C group

Even though these two groups were significantly different on Day 48, the Thyroxine 6C Group measuring 37.76 mm and the Thyroxine 30C Group measuring 33.61mm, they reduced at significantly different rates by Day 58. The Thyroxine 30C Group measured 19.22 mm compared to 8.21 mm for the Thyroxine 6C Group by Day 58, both indicating a possible inhibitory effect on the reduction phase of the tadpole tail as neither of them reduced as much as what was naturally expected, however, the Thyroxine 30C showed a greater inhibitory effect on the reduction phase than that of the Thyroxine 6C.

As per Homogenous Subset 1, the degree of inhibition of the reduction phase between the two groups was large enough to affect the “rate” at which the tails reduced. In this case, two different potencies showed the same inhibiting effect, but to different degrees.

Tail reduction is not dependent on time, but rather on influences affecting natural thyroxine levels, in this case the highly dilute Thyroxine 30C showed a greater inhibiting effect than the highly dilute Thyroxine 6C [1]. Preparing homeopathic dilutions involve a two-step process known and “succussion and dilution”. With each “succussion and dilution” step, the potency is said to increase as well as the therapeutic strength [19]. This may account for the fact that the highly dilute Thyroxine 30C had more of an inhibitory effect than the highly dilute Thyroxine 6C.

The pharmacodynamic effects of high dilution remedies have been investigated using laboratory models to show the mechanisms of action in cells, tissues and animals [24]. A study performed by Khuda-Bukhsh et al. in 2011 supports the fact that high dilutions are able to modulate cell receptors and protein synthesis. Significant remodulation was observed on treatment of a chemically induced murine papilloma with Secale cornutum 30C by downregulation of aryl hydrocarbon receptors assessed by western blot analysis [25].

The ability of highly dilute substances to modulate gene expression has been proven in numerous studies [26-30]. Datta et al. concluded that Arsenicum album 30C and 200C reduced genotoxic effects of arsenic trioxide in mice, but failed to express their protective effect in the presence of transcription blocker Actinomycin D, suggesting that the highly dilute Arsenicum album operates through active gene transcription [31].

It is believed that the effect of homeopathic dilutions is due to non-molecular information transfer to the solvent during the process of succussion and dilution. Hormesis is a biological phenomenon with a biphasic dose response characterised by beneficial low dose stimulation and toxic high dose inhibition.

Hormesis can be initiated by homeopathic remedies and nanoparticles, which are perceived as a threat within a biological environment and act as a trigger to a systemic response prompting metabolic pathways to address the change induced by the nanoparticles of that specific remedy. It is at this point where the organism itself is responsible for the change and no longer the remedy [32].

Several research models using homeopathic remedies demonstrate hormesis through pre- and post-conditioning in which low dose stimuli, when administered before or after a toxic or detrimental event, may enhance the adaptive ability of the biological environment [33,34]. A wide variety of nanoparticles have been shown to induce biphasic dose responses *in vivo* and *in vitro* models, the quantitative features of which are in line with those of hormetic dose responses induced by pharmaceutical chemicals [35].

Conclusion

When analysing the growth phase of the *Xenopus laevis* tadpole tail from Day 32-Day 48, the Thyroxine 6C had a stimulatory effect on tail growth while the Thyroxine 30C had an inhibitory effect on tail growth. When analysing the reduction phase of the *Xenopus laevis* tadpole tail from Day 48-Day 58, Thyroxine 6C, Thyroxine 30C and Thyroxine 200C had an inhibitory effect on tail reduction. The hypothesis that ultra-high dilutions of Thyroxine would either inhibit or stimulate morphogenesis of *Xenopus laevis* tadpole when considering the stage and duration of tail reduction can be accepted. It is also noted that the Thyroxine 6C and Thyroxine 30C had different effects during the growth phase, while Thyroxine 6C, Thyroxine 30C and Thyroxine 200C all had a similar effect on the reduction phase of the *Xenopus laevis* tadpole tail. These findings also lend to theories of physiochemical properties and pharmacodynamic mechanisms of information transfer where there is non-molecular information imprinted on a solvent during the process of succussion and dilution [12].

Limitations and Recommendations

Possible contributing factors to the number of mortalities encountered may have been due to trauma experienced during handling [9]. The deaths encountered may have influenced the statistical significance of the study. For future studies, the following recommendations can be made:

- Reduce measurements to the reduction phase of development only to keep handling to a minimum.
- Reduce daily measurements to every second or third day to keep handling to a minimum.
- Measure the pH of the water daily to avoid deviations from tolerable ranges.
- Investigate a single dilution of Thyroxine compared to a control group.
- Consider blinding the researcher to the control and treatment groups to substantiate any findings.
- Prepare water in a similar manner to the Thyroxine for the treatment groups, by succussing and diluting and then administering to the Control Group for consistency across groups.
- Allocate two tanks to one group, allowing water in the second tank to acclimatise to the environment and the researcher to check the

- pH of the water before the tadpoles are moved into fresh water, making the change less traumatic for them.
- Consider measuring the total length of the tadpole, from head to tail end, for a more accurate measurement, as opposed to just measuring the tail.
 - Consider other parameters such as limb bud development and weight for measurement.
 - Consider automating applications or single applications of the ultra-high dilutions of Thyroxine.
 - Avoid a maize based cereal as it seems to cloud the water rapidly requiring more frequent cleaning and water changes.
 - Avoid spirulina as an addition to the feed as this may alkalise the water to levels that are intolerable for the *Xenopus laevis* tadpoles.

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