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Amphibians and ultra high diluted thyroxine – further experiments and re-analysis of data

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Background: **A model of thyroxine and metamorphosis of highland amphibians is frequently mentioned as an example of experiments on extremely diluted substances in discussions around 'homeopathy'.**
Methods: **The model was scrutinized by reanalysing the results of the initial researcher *A* and a second researcher *B* as well as of 5 external researchers *C* between 1990 and 2013. *Rana temporaria* larvae were taken from an alpine highland biotope. The test solution was thyroxine 10^{-30} (T30x), tetra-iodo-thyronine sodium pentahydrate diluted with pure water in 26 steps of 1:10, being agitated after each step. Analogously prepared water (W30x) was used for control. Tadpoles were observed from the 2-legged to the 4-legged stage. Experiments were performed in different years, at different times of season, and their duration could vary. Frequencies of 4-legged animals, effect sizes and areas under the curves (AUCs) were calculated and regression analyses were performed to investigate possible correlations between year, season, duration etc. Experiments were in line with animal protection guidelines.**
Results: **The total set of data *A* + *B* + *C* as well as subsets *A* (initial researcher, N = 286 + 293), *B* (second centre, 965 + 965) and *C* (5 external researchers, 690 + 690) showed an effect of extremely diluted agitated thyroxine reverse to that known of molecular thyroxin, i.e. test values were below control by 11.4% for A, 9.5% for B and 7.0% for C ($p < 0.001$ for each of the subsets). The effect size (Cohen's d) was >0.8 (large) for both A and B and 0.74 (medium) for C.**
Conclusion: **Although a perfect reproducibility was not obtained, this paradoxical phenomenon was generally consistent in different observations. Correlations were found between details of laboratory handling, as well as environment temperature, and the size of the results.** *Homeopathy* (2015) **104**, 250–256.

Keywords: Homeopathy; High dilution; Thyroxine; Amphibians

Introduction

In 'Ultra High Dilution' (UHD) 1994, a model involving highly diluted thyroxine and the metamorphosis of highland amphibians was presented.[1] Since then, in discussions around 'homeopathy', there is frequent mention of that model as an example of experiments on extremely diluted substances (e.g.[2,3]). At the time of its interception in the 1990s this model was inspired by studies on experimental intoxication of organisms and subsequent detoxification using a highly diluted, agitated solution of the same substance.[4] Using this protocol, an enhanced detoxification has been described in comparison with control by some,[5] but not all researchers.[6] The (vague) analogy of the amphibian experiment to an intoxication model is that physiological thyroxine levels during metamorphosis are high compared to other stages of development,[7] or compared to other vertebrata. This led to the speculation that highly[8] or even extremely diluted thyroxine,[9] prepared according to instructions derived from homeopathy in a

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process of stepwise dilution and agitation, may have an effect inverse to that of molecular thyroxine. Since any dilution beyond 6.02×10^{-23} is theoretically 0-molar, any effects observed would point towards information transfer from the diluted substance to the diluent.[10,11]

Freedom of research, but also repeatability of experiments are important criteria of modern science. A bibliographic analysis of international basic research studies on probes stepwise succussed and diluted beyond theoretical 0-molarity compared the results of laboratory-internal, multicenter and independent repetition trials.[12] A total of 107 studies comprising 30 initial and 77 replication studies revolving around this topic were identified. Of the total number of effects found in the initial studies (100%), 83% were re-observed in laboratory-internal replication studies, 67% in multicenter replication and 44% in independent replication studies. The authors discussed the possibility that, apart from random success or publication bias, the higher number of positive outcomes in the initial studies may have come about through superior handling know-how.[12]

The experiment with extremely diluted agitated thyroxine on amphibians from highland biotopes above the tree line was one of the models covered by the above bibliographic study. The purpose of the study presented here was to analyse, rather than further add to, a body of data that has accumulated over the years on that model. The authors compared the results of the initial researcher[12] and a second researcher[12] as well as of external researchers.[13–17] They scrutinized reproducibility, and tried to determine the influence of external factors on the experimental outcome. Their hypothesis was that results would prove similar across the categories defined and that factors determining the outcome could be identified. Other types of experiments performed by the authors, e.g. with *Rana temporaria* from lowland biotopes,[18] were not considered. A detailed account of the difficulties and pitfalls of research on animals from lowland biotopes has been published.[18,19]

Methods

All data on experiments from 1990 through 2013 with highland *R. temporaria* that underwent treatment with thyroxine 30x applied at 48-h intervals on entering the standardized 2-legged stage were analysed. The experiments were split into those performed by the initial researcher PC Endler, Zoological Institute Graz University, in 1990 (experiments A)[11]; the initial second centre researcher W Scherer-Pongratz, Boltzmann Institute for Homeopathy, Graz, between 1990 and 2000 (B)[13]; and independent researchers (C) from the Department for Molecular Cell Biology of Utrecht University (C_1) in 1990,[13] the Federal Institute of Veterinary Medical Investigation, Graz (C_2) in 2000,[14] the Zoological Institute of Vienna University (C_3) in 2000,[14] a researcher suggested by KIKOM, Bern University (C_4) in 2010,[15] and from the Patienteninformation für Naturheilverfahren, Berlin (C_5) in 2011[16] and 2013.[17]

In these experiments *R. temporaria* larvae were taken from an alpine highland biotope above tree line. For preparation of the test solution thyroxine 10^{-30} (T30x), a stock solution was prepared from tetra-iodo-thyronine sodium pentahydrate (T_4, Sigma) diluted/suspended at 10^{-4} parts by weight in pure double distilled water (10 ml). Different to the Homeopathic Pharmacopoeia water was used for solvent in order to avoid any possible effects of alcohol. This was further diluted with pure water in 26 steps of 1:10 being agitated (succussed) after each step by banging the half-filled bottle 30 times against a rubber impediment at intervals of approximately 0.5 s. Each dilution step was done with a new pipette and a new hard glass bottle (nominal volume 20 ml). The water control solution (W30x) was prepared analogously. Three microlitres of probe dilutions (T30x or W30x) were added per animal and 300 ml of basin water (chlorine-free tap water) at intervals of 48 h[13] For reasons of laboratory convenience (danger of cross-contamination due to intricate handling) only one bottle was used per substance in each of the experiments.

The starting stage of treatment was defined as the point at which the hindlegs of the 2-legged tadpoles are straddled such that one can only just see through the triangle formed by thigh, shank, and tail. This occurs during Gosner's stage 31.[20] White plastic basins were assigned to each group (T30x and W30x) in a random procedure. This gave a rectangle of basins with identical treatment groups arranged diagonally adjacent to one another. A total of 60–100 animals per group were randomly allotted to the basins in each of 24 sub-experiments. The number of animals per basin was the same for the T30x and the W30x groups, in most cases 20. Indirect natural light was used. The tadpoles were fed with blanched lettuce *ad libitum*. Temperature was 18–27°C. Tadpole development was monitored at intervals of 8 h. The experiments were performed blind. Tadpoles were observed until their forelegs broke through (forelegs are preformed under the skin within days and break through within minutes, thus this is a clear cut parameter), and the animals had entered the 4-legged stage.

Results were compared on the basis of a relative time scale as standardized in[13] and described in the following. Experiments on amphibian metamorphosis can vary considerably in duration. The problem of artificial differences in variability when comparing and pooling data from several experiments was approached by normalization with respect to time based on the development of both the test and the control animals. The range from 0% to 90% over which the fraction of 4-legged animals out of the total number of animals progresses in the course of an experiment is divided into 10%-intervals, defining a relative time scale comprising 10 levels. Each measurement is then assigned to the level on the relative time scale to which it is closest. Subsequent analysis in each of the experiments was then based on the individual values observed in the test and the control group at each of the 11 time levels (also referred to in the following as measuring points).