Transdisciplinarity and Translationality in High Dilution Research

Transdisciplinarity and Translationality in High Dilution Research:

Signals and Images GIRI Series

Edited by

Leoni Bonamin and Silvia Waisse

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PREFACE

LEONI V. BONAMIN, SILVIA WAISSE
& STEPHAN BAUMGARTNER

Evidence for biological effects of high dilutions (HD, dilutions above Avogadro's number) and homeopathic preparations has been documented in scientific publications included in databases such as PubMed since the 1980s, being almost 6,000 listed up to 2018. However, this evidence is still hardly visible to part of the global scientific community. As a result, the state of the art in HD research needs to be unveiled.

A considerable part of the research on this subject is developed by members of GIRI—*Groupe International de Recherche sur l'Infinitésimal*—namely, the first international scientific society organised for this purpose. GIRI was founded by Prof Madeleine Bastide in 1986, after gathering researchers interested in the subject, and who used to meet regularly at *Les Entretiens Internationaux de Monaco*, organised by *S.A.S* Princess Antoinette. Since its foundation, GIRI holds annual conferences in which researchers in different areas can meet and discuss their recent findings. To consolidate the knowledge on HD research, besides the individual (or collaborative) scientific papers published by members every year, a book with selected research presented in the GIRI meetings is published every ten years. The present is the third volume of the series entitled *Signals and Images*. The first one was edited by Prof Madeleine Bastide in 1997, and the second by Prof Leoni V Bonamin in 2008.

The aim of this volume is to provide the scientific community direct access to the main results of HD research presented at GIRI meetings from 2009 to 2019. The book includes comprehensive and critically analysed information on current explanatory models for HDs, which are built under different points of view; physical properties of HDs; effects of HDs on cell biology, plant, animal and microbiological models; and the aspect of translationality in homeopathy research in infectious and epidemic diseases.

In short, this book describes the state of the art in HD/homeopathy research with emphasis on fundamental research.

The contributions answer with a sound, evidence-based "no" to the question "Is homeopathy really that implausible?" As such, this book represents an essential contribution to the current debate on this particular aspect of complementary and alternative medicine, much needed by practitioners, patients and governments for the formulation of healthcare policies.

PART ONE:

AN OVERVIEW OF HOMEOPATHIC RESEARCH

CHAPTER ONE

EXPLANATORY MODELS FOR HOMEOPATHY PART 2: STATE OF THE ART

SILVIA WAISSE & LEONI V. BONAMIN

A model for homeopathy: state of the art

In a previous article (Waisse & Bonamin, 2016) we analysed the theoretical model put forward by Samuel Hahnemann, the founder of homeopathy, to account for the mechanism of homeopathic treatment. As was shown, Hahnemann's model was based on the assumption of a vital force exclusive to living beings, which was a common 18th-century notion. However, the existence of such *sui generis* force of nature was refuted together with the formulation of the first law of thermodynamics by the mid-1800s. From that time onward, several models were suggested to explain the basic homeopathic tenets (therapeutic similitude, small doses, etc.). In this chapter we describe the results of a survey of the models currently applied to account for the effects of homeopathic medicines on living beings.

We reviewed in February 2015 articles published in the previous five years and included in database PubMed. Unfortunately, the number of publications on homeopathy/high dilutions (HD) was not too large. In addition, as was emphasised once and again, researchers in HD do not use a controlled vocabulary, which makes the use of search terms impossible. Therefore, not to miss any article, we first selected word "homeopathy" alone and searched for articles published from 2010 to February 2015 which titles suggested they dealt with fundamental research in homeopathy. Then we read all the corresponding abstracts to select the studies for full-text analysis. We also searched relevant articles in *International Journal of High Dilution Research* manually. Since the purpose of the present study is to discuss models for homeopathy, we also included review and theoretical studies for analysis. Finally, as some articles reported the latest steps of larger research projects, in some cases we also reviewed articles published

before 2010.

A large number of articles on fundamental research fell into the category Zacharias (personal communication) qualifies as "philatelic": an effect is described, without any explanatory context, but like stamps on an album. For instance, effects of homeopathic medicines on some isolated *in vitro* cellular phenomena, like apoptosis (Arora et al., 2013; Preethi et al., 2012), cytotoxicity (Toliopoulos et al., 2013; Ive et al., 2012; Frenkel et al., 2010), genotoxicity and clastogenicity (Preethi et al., 2008), oxidative stress (de Camargo et al., 2013), cell proliferation (Coelho Moreira et al., 2012), and macrophage function (de Oliveira et al., 2011). The experimental design described in these articles suggests that the researchers used conventional tools and rationales, and "tested" homeopathic HDs just to see whether some effect appeared. All these studies are doubtlessly highly relevant, as they demonstrate effects of HDs at the cellular level. However, they do not contribute to the understanding of their mode of action, and thus are tangentially relevant to a scientific model for homeopathy.

Other authors made attempts at giving explanations for the observed effects. The articles that specifically focused on putative explanations for the action of HDs might be categorised as follows:

1) **Weak quantum theory**: based on an original paper by Atmanspacher, Römer and Walach (2002), several studies (Walach, 2000; Milgrom, 2010, 2012, 2014; Beauvais, 2013; Almirantis, 2013; Weingärtner, 2007) suggest that the effects of HDs do not involve local (causal) interactions, but a kind of connectedness modelled on the entanglement exhibited by subatomic particles with a common origin, or on Carl G. Jung's notion of synchronicity. In this case, HDs operate as signs (see below), however, their sign characteristic is not fixed by any information content in the remedy (Walach, 2000). As, however, this model has not yet been tested (Fisher, 2013) its basic properties cannot be assessed, and thus it is no further discussed here.

2) The effects of HDs depend on the **interaction between starting substance and solvent**. This is to say information is somehow conveyed from source substance to biological target. It is worth observing that this line of studies agrees with the current paradigm in mainstream biology (Waisse & Bonamin, 2016).

According to some researchers, information transmission from the original substance to the biological target is strongly associated with the physical or physical-chemical properties of HDs. Countless studies were thus conducted to demonstrate measurable changes in HDs, including the famous "memory of water" (Thomas, 2007; Chaplin, 2007; Elia et al., 2007), thermoluminescence (Rey, 2003), delayed luminescence (Lenger et al.,

2008), dielectric dispersion (Mahata, 2013; Maity et al., 2010), fluorescence (Sharma & Purkait, 2012), ultraviolet light transmission (Marschollek et al., 2010; Wolf et al., 2011), magnetic properties (Botha & Ross, 2008), impedance and other electrical properties (Assumpção, 2008; Smith, 2008; Holandino et al., 2008), analogy to spin supercurrents in superfluids (Boldyreva, 2011), and formation of aqueous nanodomains (Czerlinski & Ypma, 2010). In particular, it is worth calling the attention to the studies on proton nuclear magnetic resonance relaxation starting 1985 (Demangeat, 2013) and the more than 20 years of research on electromagnetism (Weingärtner, 2007). A more recent study gathered experimental evidence of stable water nanostructures in homeopathic HDs by means of Fourier transform infrared spectroscopy (FTIR), ultraviolet-visible spectroscopy, fluorescent microscopy and atomic force microscopy (Elia et al., 2014).

Once again, despite the tremendous contribution of these studies to the understanding of some properties of HDs, none such properties have yet been correlated to any biological effect, not even from the theoretical point of view, despite some interesting results obtained by Betti et al. (2011).

3) **Transmission of information involving biological systems** was demonstrated in an ongoing series of studies by a group of researchers from India chaired by Sukul using the anti-alcoholic effect of homeopathic *Nux vomica* on live toads as model. These authors first showed that such effect could be transferred from one batch of toads subjected to treatment to a control batch through a cotton thread soaked with water (Sukul et al., 2012). Next, they interposed a live toad in the system, with one hind limb immersed in *Nux-v* 200c solution and the other in a beaker with distilled water. The latter was connected through wet cotton treads to five beakers containing control toads (Chakraborty et al., 2014a). In parallel, they used the same model to verify relationships (complementarity, antagonism) among various homeopathic medicines (Konar et al., 2014). The ability of water to convey information between living systems was also tested in plants, using the heat shock model and homeopathic medicine *Cantharis vesicatoria* 200c (Mondal et al., 2012). According to the authors, the information of the starting substance is carried by H-bonded water structures preserved by ethanol. They subjected this hypothesis to test by investigating possible variation in free and bound water molecules by means of FTIR – Fourier Transformed Infra Red Spectroscopy (Chakraborty et al., 2014b). The results showed that the homeopathic medicines (*Natrum muriaticum* 8cH, 32cH, *Canth* 8cH, 32cH, *Nux-v* 8cH, 32cH) exhibited greater number of free water molecules compared to the control (0.03 M ethanol), that this number differed between them, and that the alcohol O-H bond strength varied between the drugs. They explained these findings on the grounds of the

differences in the H-bonded network between the various drugs, as well as in the bond strength induced by serial dilution and agitation.

Transfer of biologically relevant information was also suggested to occur by means of high-voltage electric fields, which allegedly would imprint this information into water or a water solution (Ruzic et al., 2008). This hypothesis was tested using cress seedlings exposed to heat stress. Although the assessed biological effects (plant growth stimulation/inhibition) were, indeed, imprinted into a water solution, the authors admit they were unable to assert whether this phenomenon had any connection with the effects of the starting substances (diluted herbicide glyphosate and plant growth regulator [cytokinin] BAP).

Next, a considerable number of studies might be considered as attempts to answer two questions: **What** HDs plausibly do? And **how**?

What HDs plausibly do?

HDs regulate gene / protein expression

This is the hypothesis which oriented two remarkable lines of research, as well as several related studies. Twenty years ago, Khuda-Bukhsh (1997) suggested that homeopathic drugs act through regulation of gene expression. He put this hypothesis to test in tens of experiments involving a multiplicity of models. In 2013 he was able to demonstrate, by means of gene expression profiling using the global microarray technique, that the effect of *Condurango* 30cH and *Hydrastis canadensis* 30cH on the gene expression profile of HeLa cells was significantly different from placebo (alcohol 30cH) relative to more than 100 genes (Saha et al., 2013).

In this line, the Italian group chaired by Bellavite designed a quite refined series of studies. The model, in this case, was the anxiolytic-like effect of homeopathic drug *Gelsemium sempervirens* (*Gels*). First, these authors sought to establish this effect on experimental grounds in an animal model (Magnani et al., 2010; Bellavite et al., 2011). Then they investigated the mechanisms of this action in nervous cells lines using *in vitro* models. For this purpose, they analysed the gene expression profile of human SH-SY5Y neuroblastoma cells exposed to various dilutions of *Gels* by means of microarray assay (Marzotto et al., 2014). The results showed changes in the expression of 56 genes, being 49 down- and 7 up-regulated. Among the former, several corresponded to G protein-coupled receptor signalling pathways, calcium homeostasis, inflammatory response and neuropeptide receptors. More significantly, genes TAC4 and GALR2 were downregulated, being that the former encodes neuropeptide haemokinin-1, and the latter

galanin receptor 2, both of which are involved in the psycho-neuro-immune-endocrine axis function which is associated with emotional responses. The effects were comparable between all the tested dilutions, from 2cH up to 30cH (above Avogadro's number).

Similarly, Bigagli et al. (2014) demonstrated that homeopathic remedy *Apis mellifica*, in mother tincture and dilutions 3c, 5c and 7c, modulated hundreds of genes. Cluster analysis revealed groups of up- or down-regulated genes specifically involved in cytokine expression, inflammation, antioxidative responses and proteasome degradation. These results were confirmed by means of reverse transcription polymerase chain reaction (RT-PCR) of five candidate genes. These findings allowed the authors conclude that *Apis* up to dilution 7c modulated gene expression, resulting in inhibition of processes involved in the regulation of inflammation, what suggests the mechanism of hormesis.

This type of model was also applied to plants. Marotti et al. (2014) used microarray assay to establish whether 7-day old wheat seedlings grown from seeds poisoned or not with a sublethal dose of arsenic trioxide showed different gene expression profiles after application of ultra–high diluted As_2O_3. The results clearly demonstrated that the expression profile of several classes of genes in the plants subjected to treatment significantly differed from the ones of plants treated with placebo (water). The authors observed that along with genes associated with various metabolic functions, also changes in the expression profile of many genes known to be involved in or affected by general abiotic stresses were detected. As a result, they concluded that the evidence thus gathered supported the aforementioned hypothesis by Khuda-Bukhsh.

Gene regulation was also investigated in apoptosis induced by homeopathic dilutions of *Ruta graveolens* in human colon cancer cells (Arora & Tandon, 2015) and in the anti-inflammatory effect of *Rhus toxicodendron* in homeopathic dilutions on chondrocytes (Huh et al., 2013). Hofbauer et al. (2010) showed that homeopathic *Nux-v* and *Calendula officinalis* were able to reduce the *Helicobacter pylori*-induced gene expression of heparin-binding epidermal growth factor (HB-EBF) in a cell line model.

HDs regulate endogenous system networks

This kind of observation is only possible when *in vivo* studies are performed, with special emphasis on the effect of high-diluted endogenous substances. One example is provided by thymulin (Sato et al., 2012). When thymulin 5cH was added to a water container offered to broiler chickens, protection against development of viral arthritis and lack of mobility due to

overpopulation stress could be clearly seen one week before slaughter, i.e. the period associated with the highest levels of mortality in commercial broiler breeding. This protection was related to improved immune response, as manifested by changes in the histological organisation of different immune organs. In more refined models (Bonamin et al., 2013; Rodrigues de Santana et al., 2014a) thymulin 5cH was able to improve B1 mouse peritoneal stem cells, leading them to the phagocyte phenotype, with differentiation and migration to the local inflammation site induced by both, BCG (Bacillus Calmette-Guerin) or *Leishmania (L.) amazonensis.* The most important point is that this effect was not isolated. Contrariwise, changes in the kinetics of B2 and T lymphocytes, as well as in macrophage activity and maturation in the infection site, also occurred as a function of time, involving a coordinate balance of several components of the immune and inflammation response. The final outcome of all these changes was remission of infection after 21 to 60 days.

Vertical (mother-foetus) transmission of homeopathic adaptive information could also be detected in a dexamethasone model. In the study by Bonamin et al. (2012) treatment of pregnant rats with ultra-high diluted homeopathic dexamethasone 15cH (10^{-33} M)—thus, above Avogadro's number—did not induce any major change in the offspring development when adult. Instead, the F1 generation developed a particular cell and molecular pattern of inflammation regulation when challenged with a standard irritant agent (carrageenan). In these animals, several parameters related to acute inflammation predominated compared to controls, such as increase of the mast cell ability to release histamine granules, increase of the vascular activity, polymorphonuclear cells migration, etc. All these changes involve different molecular mechanisms and pathways, which are the opposite to steroid regulation of inflammation.

Also exogenous substances prepared as homeopathic medicines exhibit modulation ability on several biological parameters in a non-linear manner. In a similar *Leishmania (L.) amazonensis* infection model (Rodrigues de Santana et al., 2014b), treatment of mice with *Antimonium crudum* 30cH increased the peritoneal B1 cells in the acute phase and the peritoneal and spleen B2 cells in the chronic phase of infection. The result was decrease of the local inflammation response and increase of the number of non-phagocytised parasites in the subcutaneous tissue. Since *Leishmania* is an intracellular mandatory parasite, this outcome implies interruption of the parasite cycle.

In short, the skin, spleen, peritoneum, lymphoid organs, mast cells, leukocytes and even parasites, all of them participate in the response to the homeopathic stimuli in a coordinated and organised regulation of the host–

parasite relationship. Similar studies on changes in the host-parasite relationship were published by the Brazilian group chaired by Marques (Sandri et al., 2015; Aleixo et al., 2012; Ferraz et al., 2011).

How do HDs act?

Early experimental models

Here we allude to the basophil degranulation model developed starting at the end of the 1980s (Poitevin et al., 1988; Davenas et al., 1988), and which became famous as a function of the controversy it elicited. A detailed account of the work of the two groups involved in these studies, one chaired by Poitevin and Benveniste, and the other by Sainte-Laudy and Belon, is given in the 2006 review by Bellavite et al. (2006) to which we refer the readers. The two latest chapters of this almost 30-year long story are a retrospective account made by Sainte-Laudy and Belon (2009) of their endeavours and a more sceptical review by Ennis (2010).

In their later publication Sainte-Laudy and Belon (2009) explained that the aim of their 28-year work, since 1981, was to set up an *in vitro* model to test the similia principle with the sensitivity needed to measure the intended effects, be simple enough to be easily reproducible, and be based on the use of a purified commercially available compound with fully described and accepted pharmacological characteristics. According to them, the negative feedback induced by histamine on basophil and mast cell activation via the H_2 receptor met their research goals.

However, Ennis (2010) review of this model is highly critical. In particular, she pointed to variability not only among different laboratories, but also within one and the same group of investigators. While she conceded that there is some evidence of effect when HDs are used, the suggestions she made for future studies (performance of multicentre trials with previous clarification of a number of issues including: use of one or many cell donors, methods used to prepare histamine solutions, type of water used as diluent, methods used to detect activation, use of a number of anti-IgE dilutions, whether anti-IgE is the best agonist to use, and systematic performance of negative control experiments to assess the stability of the experimental system) makes one infer that, as a fact, this model is nowhere close to the ideal requirements listed by Sainte-Laudy and Belon.

Plausibility/operation of the similia principle in experimental models

Wiegant and Van Wijk (2010) sought to develop a model to test the similia principle in cells. Within that context, they defined "disease" as the inability of the body (cells) to heal itself, following the action of a stressor (here, heat shock). Such "disease" is characterised by "symptoms," like in a homeopathic pathogenetic trial (HPT), in this case, the phenomenon of proteotoxicity. The latter can be measured based on the type and amount of heat shock proteins (HSPs) that are expressed. Their initial hypothesis was that a high-dose stressor not only deranges the body (cells), but also sensitises it to the action of the same stressor in lower dose, which in turn results in tolerance (reduced sensitivity) to the stimulus. Their first series of experiments tested the "isopathic" condition on Reuber H35 rat hepatoma cells: a lower dose stressor (hyperthermia) was administered after the higher dose one. The results showed that the level of HSPs was initially suboptimal, to become then enhanced. In turn, cells subjected to the lower dose stressor alone did not exhibit any change in HSP expression compared to the controls. On these grounds, the authors concluded that the phenomenon of sensitisation (by disease) followed by development of tolerance (induced by the same stressor in lower dose) was demonstrated. The second step focused on the "heteropathic" condition: the lower dose, curative stimulus was no longer the same stressor (heat shock), but different, heavy metals (arsenite, cadmium, lead and copper) and oxidative agents (menadione and diethyldithiocarbamate–DDTC). First the "pathogenetic" effects of all the stressors were investigated, to wit, the qualitative and quantitative patterns of HSPs and glucose-regulated protein expression. The relative percentage of similarity to the effects of heat shock (used as standard) was calculated. The cells were then subjected to high-dose heat shock, followed by low-dose treatment with all eight tested stressors. The survival of cells subjected to high-dose heat shock was used as standard to calculate the "survival stimulation factor" (SSF) corresponding to each treatment. While mild hyperthermia exhibited the highest SSF, followed by arsenite, lead, cadmium and DDTC exhibited the lowest values, the ones of mercury, copper and menadione being intermediate. Finally, they plotted the percentages of relative similarity versus the SSF values and found highly significant correlation. Thus, they concluded that the evidence gathered demonstrated the validity of the similia principle at the cellular level by molecular mechanisms.

Nanoscience

Recent studies detected nanoparticles (NPs) of starting materials in homeopathic remedies diluted above Avogadro's number (Chikramane et al., 2010; Upadyhay & Nayak, 2010). In addition, being traditionally prepared in glass vials, homeopathic HDs also contain silica NPs (Demangeat, 2013; Chikramane et al., 2010; Upadyhay & Nayak, 2010) which detach during agitation (Witt et al., 2006; Holandino et al., 2007). The presence of silica NPs might represent a fundamental component of the mechanism of action of homeopathic remedies (Demangeat, 2013; Upadyhay & Nayak, 2010; Witt et al., 2006; Ives et al., 2010). Nanosilica might form stable 3-dimensional structures using DNA, proteins or living cells as moulds (epitaxial growth) (Bell & Koithan, 2012). Epitaxy is a common occurrence in material science and technology, the manufacture of superconductors in particular. It consists in the transfer of non-material information from the surface of one material, usually solid, to another, usually liquid (Rao et al., 2007). If silica is, indeed, present in HDs, it might serve as vehicle for structural or electromagnetic specific information. In other words, silica NPs might act as carriers of the starting substance information, which might then be identified and interpreted by biological systems. This is the core of the epitaxy hypothesis.

Bell and colleagues (2013) formulated a broad-encompassing model for the action of homeopathic medicines on living beings which takes practically all the hypotheses mentioned in the present review into consideration, called nanoparticle-allostatic cross-adaptation-sensitisation (NPCAS) model. According to it, homeopathic medicines are considered sources of NPs and/or silica NPs modified by the starting substance. These NPs initiate a complex adaptive response in the allostatic network (which includes neural, endocrine, immune and metabolic pathways) with cascading effects that emerge across the entire organism over time. Disease is understood as the result of progressive allostatic overload of the organism by high-intensity stressors, consisting in a unique, complex, nonlinear and dynamic pattern of maladaptive functions determined by genetic and epigenetic factors. This phenomenon involves metaplastic priming, i.e. the past history of synaptic activity determines the current plasticity, including the direction and magnitude of the response to subsequent stimuli. That is which in the past was known as "predisposition." Within this context, the remedy NPs act on the disease-primed system as new physiological stressors which reflect its dysfunction, eliciting amplified, cross-adapted changes in the opposite direction to those caused by disease. Bell and Koithan stress that the remedy effects are mediated by physiological, not by

pharmacological processes, including metaplasticity, hormesis, cross-adaptation and time-dependent sensitisation (TDS).

On these grounds, they developed a research program which seeks to demonstrate four basic hypotheses: 1) variability in NP size, morphology and aggregation influence the remedy properties and effects, and the reproducibility of findings; 2) homeopathic remedies modulate patterns of allostatic responses, with measurable effects based on systemic priming by previous higher-intensity stressors; 3) active remedies initiate and/or elicit TDS-based reversal of stressor reactivity, whereby the effects of the simillimum remedy NPs on the organism are cross-adapted and cross-sensitised to the previously established maladaptive processes which led to the emergence of disease; and 4) the simillimum remedy administered to a sick person produces persistent improvement in systemic resilience, including global wellbeing and resolution of local symptoms.

Theoretical models

In a very interesting paper Bellavite et al. (2013) had resource to Boolean networks to show how self-organisation and adaptation are relevant to the understanding of the principle of therapeutic similarity. Their point of departure is the idea that homeopathy ought to be understood as a network regulatory approach, and that the phenomena of state-dependency, i.e. the response of a biological system to a stimulus, is not only determined by the quality or quantity of such stimulus, but also by the state of activation of the target system. This might account for the fact that a homeopathic drug elicits a series of symptoms in a healthy individual and heals them in a sick one (therapeutic similarity).

According to Bellavite et al., in a Boolean network, external perturbations might have pathological effects resulting in permanent self-sustaining alterations. Contrariwise, changes that enable the system to find its way back to its original state might induce therapeutic effects by causing specific changes in attractors. In other words, if pathology is considered an alteration in the self-organisation of a complex network, then it is theoretically possible to restore its normal (healthy) behaviour through targeted perturbations. This approach, state the authors, is essentially different from that which merely seeks to suppress the symptoms of disease and might even transiently exacerbate them (“homeopathic aggravation”). The reason is that the changes induced by the perturbation lead the system far from its equilibrium, which requires a plus of energy. Contrariwise, Bellavite et al.’s approach is consistent with that of homeopathy, the similia principle in particular. The reason is that it presupposes that the intrinsic

tendency to self-recovery might be actively assisted through the application of adequate stimuli to a sensitive system.

Shortly, this hypothesis states that the homeopathic remedy pushes the system out of its pathological adaptation and settles it in a new self-organising state of equilibrium closer to the healthy state (positive adaptation). The homeopathic remedies thus behave as low-level danger signals to the biological stress response networks. Finally, this model predicts that the more sensitive a system is to a particular regulation, the lower should be the intensity of the stimulus required to regulate it an effective manner, i.e. the homeopathic low doses. Thus being, the model suggested by Bellavite et al. accounts for the basic features of the homeopathic approach to medicine and might be particularly relevant to research lines like the ones pursued by the groups chaired by Bonamin and Marques, for instance, which investigate global homeostatic phenomena, or homeodynamics, in Bellavite et al.'s words.

The informational/semiotic model

From a different perspective, starting in the 1990s, HDs and their interactions with biological systems began to be understood as semiotic phenomena, i.e. sign processes. Attuned to the paradigm shift in biology, Lagache and Bastide analysed the mechanisms for biological transmission of information, and concluded that, in addition to molecules, living systems also exchange non-molecular information with their environment. The result was the so-called "theory of the corporeal signifiers," (Bonamin et al., 2008) which fully agrees with the theoretical views formulated about 100 years earlier by American polymath Charles Sanders Peirce (1839-1914), the acknowledged founder of modern semiotics (Bonamin & Waisse, 2015). Walach (1991) explicitly applied Peirce's notions of sign and semiosis to the phenomena involving HD in general, and their relationship to the therapeutic similarity, in particular.

Walach described homeopathic practice as "pure semiotics," on the grounds that Hahnemann had defined disease as unknowable as such (*an sich*, like a Kantian *noumenon*) (Rosenbaum & Waisse-Priven, 2004), but knowable only through perceptible manifestations (signs, symptoms). Thus, the latter operate as tokens (***stand for*** disease) or according to Peirce's fundamental definition of sign:

> A sign, or representamen, is something which *stands to somebody for something* in some respect or capacity. It addresses somebody, that is, creates in the mind of that person an equivalent sign or perhaps a more developed sign. The sign which it creates I call the *interpretant* of the first

> sign. The sign stands for something, its *object*. It stands for that object, not in all respects, but in reference to a sort of idea, which I have sometimes called the *ground* of representation (Peirce, 1931, our emphasis).

Assuming that homeopathic remedies "carry" the message of the original substance, or in other words, that the latter somehow "imprints" its structure into the solvent, and based on the triadic structure of Peirce's sign (object/referent, representamen/sign and interpretant/meaning), Walach constructed two triads, one for HPTs and the other for therapeutic application of homeopathic remedies, which are virtually mirror-like images:

	Triad 1	Triad 2
Object	Starting substance	Disease
Representamen	Remedy	Disease symptoms
Interpretant	Set of symptoms	Remedy

In triad 1 the object referred by the sign is the starting substance, represented by the corresponding homeopathic remedy given to a healthy volunteer in a HPT. The volunteer's body interprets the information received and produces its meaning, i.e. the set of symptoms of the remedy. In this case, the remedy as sign shares in something of the substance, for which reason it might be classified as an *icon*, according to Peirce's classification of signs.

In triad 2 the object referred by the sign is disease, which is represented by the set of symptoms exhibited by the patient, and signifies the means of its cure, i.e. the remedy. In this case, the sign points as the index finger to the remedy needed to achieve the cure of disease, for which reason is classified as an *index*. More important than that, says Walach, is the link connecting the two triads, namely, the law of similarity mediating between the symptom picture of the remedy in HPTs and the symptom picture exhibited by the ill individual.

About 20 years later, the semiotic perspective was carried on by Jurj (2010). Through an independent line of analysis, he reached some same conclusions as Walach. For instance, the iconic nature of HDs as signs, and the indexical nature of symptoms. However, Jurj chose to emphasise the essential nature of signs, which is *mediation*—as also Lagache and Bastide had remarked. Peirce was the first thinker since Aristotle to formulate an integrated ontology-logic-epistemology, and he did so by replacing the category of substance by that of relationship (mediation) as the core (Bonamin & Waisse, 2015). Therefore, Jurj agrees with Lagache and Bastide upon asserting that HDs do not involve matter transmission, but semiotic transmission. As a result, it does not matter how much a substance

is diluted, since the image (icon) of the biological effects of the starting substance is conveyed in a semiotic, iconic manner: "At each step of dilution, the [supporting] medium changes, but the icon is kept, always referring to the same referent, able to be interpreted by a sensitive system."

Examples of semiotic models range from experimental designs to clinical findings, including the NP-silica hypothesis described above, since silica NPs have a true sign function per definition.

In the 1990s, Youbicier-Simo et al. (1993, 1996) demonstrated that *in ovo* administration of HDs of bursin—a hormone derived from the bursa of Fabricius which induces the development of the B-lymphocyte system—stimulated the development of the chicken immune system in bursectomised embryos, as manifested by normal antibody production to a given antigen. This, per definition, is the typical mediation-based operation of a sign: something that *stands for* something *to* someone. Or in simpler words: bursin in HD "tricked" the chicken embryo system and made it "believe" that the hormone was there.

Metamorphosing frog larvae are extremely sensitive to thyroxine. Along more than 20 years, starting in 1991, Endler et al. performed countless multicentre tests with many variations of the core model parameters to test the hypothesis that non-molecular information is transferred in living systems (Endler et al., 1991; Oberbaum, 2013). In addition to demonstrating that high-diluted thyroxin (30x) slows down the thyroxin-regulated metamorphosis of *Rana temporaria*, they succeeded in designing a highly reproducible model (Endler et al., 2010; Harrer, 2013). Guedes et al. (2004, 2010, 2011) confirmed Endler's findings in *Rana catesbeiana* and further demonstrated that diluted triiodothyronine (T3) modifies the effect of T_3 in pharmacological dose on apoptosis. The dramatic resorption of the tadpole tail is a target of much attention as an experimental system of cell death (Yaoita & Nakajima, 1997).

De Paula Coelho et al. (2006) showed that heat-induced itching behaviour in rats could be reversed by prolonged administration of increasing dilutions of *Dolichos pruriens*. Interestingly, the molecular and cellular mechanisms involved in both forms of itch induction are completely different. Heat induces itch in rats through vasodilatation secondary to a neural reflex, while *Dolichos pruriens* pod powder induces typical type I hypersensitivity. Thus being, no receptor antagonistic binding may be hypothesised in this case.

At the clinical level, Jurj et al. (2010) reported a case series of epidermolysis bullosa (EB) with dramatic improvement following homeopathic treatment. EB is a group of rare genetic diseases characterised by recurrent blistering resulting from even slight traction of epithelial lined

surfaces, most remarkably the skin. Also here the homeopathic medicines somehow "tricked" the patient's body to believe that the missing proteins were there, i.e. they *stood for* the proteins *to* the children's body. Once again, this summarises the operation of signs, and in addition to the abovementioned examples, it might also account for the phenomena known as "sensations as if" which arise in HPTs and have much value for prescription purposes.

Similarly, in another study (Banerjee et al., 2010) 38 patients with ß-thalassemia were given homeopathy as adjuvant to treatment with hydroxyurea (HU) and were compared to 38 patients treated with HU alone. ß-thalassemia is a group of hereditary disorders characterised by a genetic deficiency in the synthesis of ß-globin chains. Patients do not develop anaemia until the foetal (γ) globin genes are silenced; the ones with persistent high levels of foetal haemoglobin (HbF) have less severe anaemia, milder clinical syndromes, and are often transfusion independent. On these grounds, several agents were investigated to increase the synthesis of HbF, including HU. The preliminary results of the study indicated significant decrease in the serum ferritin and increase in the HbF levels in the group treated with HU plus homeopathy. In addition, the size of the spleen exhibited significant decrease in most patients with splenomegaly, and improvement of their general health condition, with longer intervals between transfusions for most of patients in this group.

Final remarks

In its two parts, the present and (Waisse & Bonamin, 2016), our study shows that scientific modelling of the action of homeopathic medicines on biological systems was attempted since the very inception of homeopathy at the turn of the 19th century. Remarkably, all such models meet contemporary epistemological standards. Naturally, as scientific notions and methods evolve over time, also assumptions, experimental designs and hypotheses change. Nevertheless, in no instance one can detect any attempt at deviating from the mainstream scientific ethos. Therefore, the assertion that research in homeopathy is "alternative" does not hold from any point of view.

Critics assert the implausibility of homeopathy as an absolute *a priori*, on the grounds that since HDs contain nothing but water, they cannot have any effect by principle (Shang et al., 2005). However, also by principle, this would be a non-scientific attitude, since science does not accept *a priori* dogmas. One might concede that trials conducted in human beings are not the best means to establish the plausibility or not of the homeopathic HDs.

In addition to the influence of placebo (Walach, 2011), highly complex neuro-immunoendocrine networks are involved, which are poorly susceptible to deterministic assays. The case of research with animals, and more particularly with plants and cells (Waisse, 2017; Teixeira & Carneiro, 2017) is much different, and thus should be the target of the analysis of partisans and critics alike.

Then, there is the view of some traditional homeopathic practitioners, who grounded on doctrinary notions, assert that homeopathy is by principle the polar opposite to "Western reason," and as such absolutely irreconcilable with mainstream medicine and science. Nevertheless, this is no new phenomenon, but one was denounced as early as in the 1850s by the respected physician Robert E. Dudgeon (1854). Some of the historical reasons were explained by Jütte (1999).

Our main intention was to provide a "scientific biography" of homeopathy to the community of researchers on HDs, and more particularly to homeopathic practitioners, to promote a broader understanding of the scientific basis of homeopathy. As was shown, biology, and consequently also medicine, is undergoing a dramatic paradigm shift from the matter-force theory to the informational/biosemiotic view. Within this context, the explanatory models for homeopathy far from being "alternative" to those of mainstream science were, and are, in full continuity with it. Naturally, beyond the boundaries circumscribed by Avogadro's limit.

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