

ORIGINAL PAPER

Animal models for studying homeopathy and high dilutions: Conceptual critical review

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Introduction: This is a systematic review of the animal models used in studies of high dilutions. The objectives are to analyze methodological quality of papers and reported results, and to highlight key conceptual aspects of high dilution to suggest clues concerning putative mechanisms of action.

Methods: Papers for inclusion were identified systematically, from the Pubmed-Medline database, using 'Homeopathy' and 'Animal' as keywords. Only original full papers in English published between January 1999 and June 2009 were included, reviews, scientific reports, thesis, older papers, papers extracted from Medline using similar keywords, papers about mixed commercial formulas and books were also considered for discussion only. 31 papers describing 33 experiments were identified for the main analysis and a total of 89 items cited.

Results: Systematic analysis of the selected papers yielded evidence of some important intrinsic features of high dilution studies performed in animal models: a) methodological quality was generally adequate, some aspects could be improved; b) convergence between results and *materia medica* is seen in some studies, pointing toward to the possibility of systematic study of the *Similia* principle c) both isopathic and *Similia* models seem useful to understand some complex biological phenomena, such as parasite–host interactions; d) the effects of high dilutions seem to stimulate restoration of a 'stable state', as seen in several experimental models from both descriptive and mathematical points of view. *Homeopathy* (2010) 99, 37–50.

Keywords: Systematic review; animal; concepts; methodology; *Similia* principle; isopathy; steady state

Introduction

Several systematic reviews of high dilution research have been published classifying the methodological quality and positive or negative results.^{1–4} Other authors have pointed out methodological or conceptual problems in high dilution research, such as the standardization of experimental models to demonstrate, understand and characterize the main features of *Similia* principle. Less problematic are the variations *isopathy* and *iso-endopathy*, meaning the

use of highly diluted substances used to treat intoxication by the same substance (*isopathy*) or highly diluted endogenous substance used to treat physiological disturbances related to it (eg highly diluted thymulin to treat immunosuppression). Since these approaches are closer to the traditional scientific approach, many studies in fundamental research have focused on such models, using *in vivo* or *in vitro* protocols.^{5,6}

In fact, studies based strictly on the *Similia* principle are not numerous in the literature. Most experiments concentrate on the simple demonstration (or not) of some effect of high dilutions, without clarifying their physiopathological aspects or biological meaning. In this sense, the development of animal models to investigate the *Similia* principle is still preliminary and deserves more attention by the scientific community.

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Received 24 June 2009; revised 26 October 2009; accepted 3 November 2009

In this systematic review some of these conceptual aspects are evaluated, based on studies published in Medline indexed journals between 1999 and 2009.

Methods

Papers to be included for evaluation were selected by the following criteria: a) Indexed in Pubmed-Medline with 'Homeopathy' and 'Animal' as keywords; b) English language original on-line available full papers about experimental studies; c) published between January 1999 and June 2009. Clinical Veterinary trials were excluded.

The selection was restricted to the last 10 years because there appears to have been improvement in methodology during this period. 31 original full papers, describing 33 experiments, were selected, identified and analyzed from two points of view:

- a) Assessment of methodological quality
- b) Assessment of critical conceptual aspects.

Reviews, scientific reports, thesis, older papers, papers about mixed commercial formulas and book (chapters), papers listed in Medline with slightly different keywords, in English or other languages, were considered for discussion only; 89 such items were identified.

Assessment of methodological quality was based on the following criteria:

- a) Adequacy of reporting
- b) Blinding of measurement of outcomes
- c) Nature of control
- d) Randomization
- e) Statistical analysis
- f) Result

Assessment of conceptual aspects was based on:

- a) Convergence of data toward a consistent conclusion
- b) Adoption of a theoretical baseline to be challenged by the study
- c) Construction of innovative protocols and model designs.

The possible convergences and coherences among the studies are discussed.

Results

Methodological quality

The general evaluation of quality is presented in Table 1. Of the 33 experiments analyzed, 10 used isopathic models and 23 *Similia* models. All papers used randomized samples, except,⁷ in which sensitive rats were selected to be treated according to the *Similia* principle.

No correlation between blinding or type of control substance and positive or negative results was detected (Table 2). The statistical methods reported varied. Student *t*-test and ANOVA were the most used. Few reports included a justification for the chosen test.

Table 1 summarizes the main characteristics of selected papers classified according to the experimental model used (see details in discussion). Rats, mice, frogs and

chickens were the species used; the majority of experiments were on rodents. Most experiments were randomized. However, the use of selected sample according by prospectively defined criteria can be a valuable tool to study the *Similia* principle, since inclusion of non-responsive animals could generate false-negative results.⁷

Conceptual aspects

An overview of conceptual aspects is presented in Table 3.

Convergence of results between different experiments or coherence between results and *materia medica* was often observed, although there were some exceptions.⁸⁻¹⁰ In these cases, differences between protocols resulted in different conclusions, although the reasons for these discrepancies are unknown.

One young but interesting field revealed in this review is the parasite-host interactions model, but the results of single studies still require replication.¹¹⁻¹⁴

Discussion

Methodological quality

Blindness and statistics: The statistical analysis shown in Table 2 revealed no bias in terms of positive or negative results related either to, type of control substance or blinding. Of course, blindness is a very important criterion of quality in all kinds of experimental studies – including classical pharmacology. But the variations among the different studies regarding these items seemed not to alter the conclusion. The use of blind protocols seems critical in histopathological analysis because of its obvious subjective character (Table 1). The use of blind protocols associated to objective digital methods of histomorphometry and quantification can improve such analysis.

Another problem identified was the choice of statistical method (Table 1). Some results were analyzed by *t*-test even when comparing more than two groups, when the best choice for these cases is the use of parametric or non-parametric ANOVA. A priori tests to verify the Gaussian distribution of data, such as Bartlett test, were used only in few studies. Few authors justified their choice of statistical method. However, authors who used an inappropriate test in their first studies often changed to more appropriate tests in subsequent studies.^{15,16} In all cases, there was convergence of results between old and new experiments, despite the discrepancy of statistical methods. Thus, this problem was not sufficient to change the general conclusion.

Control: What kind of negative control is most appropriate? Succussed or unsuccussed vehicle? Many authors consider succussed vehicle the more appropriate, because some studies have demonstrated the presence of traces of silicon, from glass vials, in simple succussed water, which could cause biological effects.^{1,4} The mean concentration of silicates in homeopathic solutions beyond Avogadro's number is approximately 20–30 mM, however, much higher quantities of silicates are required to elicit biological responses *in vitro* – 500 μ M to 1 mM.³

Table 1 Summary of experiments classified in different experimental model categories, and type with methodological quality criteria. See Table 3 for identifying the theoretical basis of each study (Similia principle or isopathy)

Reference	Model	Species	Treatment	Variables measured	Blinding	Control	Randomization	Statistics adequate?	Results	Comments
Behavior										
Ruiz Vega <i>et al.</i> ⁵²	EEG	Rat	<i>Coffea cruda</i> mother tincture	Delta band spectrum	Yes	Unsuccussed vehicle and water	Yes	Yes	+	Definition of EEG pattern of pathogenetic/pharmacological effect of <i>Coffea cruda</i>
Sukul <i>et al.</i> ⁷	Ethanol intake	Rat	<i>Nux vomica</i> 30c ultra-sonic homogenizer	Alcohol/water preference	Yes	Untreated and strychnine (alcohol control tested previously)	No, instead, susceptible rats were selected by the Porsoit test	No (t-test) #	+	ANOVA would be more appropriate for a general comparison among all groups followed by a post-hoc test. Insertion of two new methods: selection of susceptible rats and ultra-sonic succussion
Ruiz Vega <i>et al.</i> ⁵³	EEG	Rat	<i>Coffea cruda</i> 30c and 200c	Delta band spectrum	Yes	Succussed vehicle	Yes	Yes	+ (30c) - (200c)	Proposal of a biological pattern marker for high diluted <i>Coffea cruda</i>
Ruiz Vega <i>et al.</i> ²⁵	EEG	Rat	<i>Coffea cruda</i> 30c	Delta band spectrum	Yes	Succussed vehicle	Yes	Yes	+	Proposal of mathematical model after data plotting: recovery of basal pattern
Ruiz Vega <i>et al.</i> ²⁷	EEG*	Rat	<i>Histaminum</i> 30cH	Delta band spectrum	Yes	Unsuccussed vehicle	Yes	Yes	+	Proposal of mathematical model after data plotting: recovery of basal pattern
Coelho <i>et al.</i> ²³	Itching behavior	Rat	<i>Dolichos pruriens</i> 9, 12 and 30cH	General activity; skin itching-induced lesions	Yes	Unsuccussed vehicle and no treated rats	Yes	yes	+	Recovery of basal behavioral pattern after treatment with progressive repeated potencies
Pinto <i>et al.</i> ²⁰	Stress and depression	Mouse	<i>Chamomilla</i> 6cH	General activity; forced swim; blood cells	Yes	Unsuccussed vehicle; diazepam; amytriptiline	Yes	Yes	+	Recovery of basal behavioral pattern after treatment; use of allopathic positive control for comparison; trouble of using alcohol as control
Intoxication										
Kundu <i>et al.</i> ³¹	Arsenic toxicity	Mouse	<i>Arsenicum album</i> 30c (isopathy)	Enzymatic changes; visual histopathology	No	Succussed alcohol	Yes	Yes	+	Blinded quantitative histomorphometry would make histological observations more objective
Mallik <i>et al.</i> ³²	Arsenic toxicity	Mouse	<i>Arsenicum album</i> 30c and 200c (isopathy)	Enzyme levels	Yes	Succussed alcohol	Yes	No (t-test)	+	ANOVA would be more appropriate for a general comparison among all groups followed by a post-hoc test
Banerjee <i>et al.</i> ^{15,79}	Geno-toxicity	Mouse	<i>Arsenicum album</i> 200	Cytogenetical; biochemical parameters	Yes	Succussed vehicle; no treated mice	Yes	No (t-test)	+	ANOVA would be more appropriate for a general comparison among all groups followed by a post-hoc test

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Table 1 (continued)

Reference	Model	Species	Treatment	Variables measured	Blinding	Control	Randomization	Statistics adequate?	Results	Comments
Banerjee <i>et al.</i> ¹⁶	Arsenic intoxication	Mouse	<i>Arsenicum album</i> 200c (isopathy)	Enzymatic changes; electron microscopy; endocrine framework; metalloproteinase zymography	Yes	Successful vehicle	Yes	Yes	+	Proper statistical test and coherence among several parameters and previous results toward the protective isopathic effect
Inflammation										
Bertani <i>et al.</i> ⁸	Acute inflammation	Rat	Mineral homeopathy complex	Paw edema	Yes	Saline with the same osmolarity	yes	Yes	+	Results varying according to the time of treatment
Bonamin <i>et al.</i> ³³	Acute inflammation	Mouse	Dexamethasone 7cH; 15cH (isopathy)	Paw edema; leukocyte migration	No	Unsuccussed vehicle	yes	Yes	+	Observation of immediate effect on leukocytes
Araujo Prato-Neto <i>et al.</i> ⁹	Acute; chronic inflammation	Rat	<i>Causiticum</i> 30cH	Paw edema; foreign body induced granuloma	No	Successful vehicle	Yes	Yes	+	(*) positive result only for acute inflammation
Macedo <i>et al.</i> ¹⁹	Acute inflammation	Rat	<i>Arnica montana</i> 6cH	Nystatin; carrageenan-induced paw edema	No	Successful; unsuccussed vehicle	Yes	Yes	+	(*) positive only for carrageenan induced acute inflammation
Almeida <i>et al.</i> ⁵¹	Caries induction	Rat	<i>Kreosotum</i> 6cH	Qualitative dental lesions; skin and behavioral symptoms	Yes	Unsuccussed vehicle; sodium fluoride	Yes	n/a	-	No caries were verified but other symptoms related to <i>Kreosotum</i> pathogenesis
Pedalino <i>et al.</i> ³⁶	LPS induced peritonitis	Mouse	<i>Atropa belladonna</i> ; <i>Equinacea angustifolia</i> 'Potency chord'	Leukocyte counting; phagocytosis	No	Buffer; dexamethasone	Yes	Yes	+	Modulation of peritonitis with cytoprotective effects on local leukocytes introduction of accord of potencies model
Conforti <i>et al.</i> ¹⁰	Acute inflammation	Rat	Several	Paw edema	Yes	Unsuccussed saline; indometha-cine	Yes	Yes	+	Results with different conclusions according to group/housing organization
Santos <i>et al.</i> ³⁷	Acute inflammation	Rat	<i>Rhus tox</i> 6, 12, 30, 200 cH	Paw edema and others	Yes	Unsuccussed vehicle; indometha-cine	Yes	Yes	+	Convergence of results toward the anti-inflammatory effect
Carcinogenesis										
Biswas; Khuda-Buksh ⁷⁵	Hepato-carcinogenesis	Mouse	<i>Chelidonium</i> 30cH; 200cH	Enzymatic activity; clastogenic effect	Yes	Untreated mice	Yes	no (t-test)	+	ANOVA would be more appropriate for a general comparison among all groups followed by a post-hoc test; observation of potency and time related effects

Biswas <i>et al.</i> ⁷⁷	Hepato-carcinogenesis	Mouse	<i>Carcinosinum</i> 200c; <i>Chelidonium</i> 200c	Biochemical parameters and histopathology	Yes	Successed vehicle	Yes	Yes	+	Improvement of therapeutic effect after association of both medicines; convergence of results toward the protective effect
Tissue growth and differentiation										
Endler <i>et al.</i> ¹⁵	Meta-morphosis	Frog	T4 1:10 ⁸ (D8) (iso-endopathy)	Metamorphosis rate	Yes	Successed vehicle	Yes	Yes	+	Multi-centric study with good but not 100% of convergence of results
Guedes <i>et al.</i> ⁴⁴	Meta-morphosis	Frog	Thyroid 1:10 ²⁶ (iso-endopathy)	Metamorphosis rate	Yes	Unsuccessed control	Yes	Yes	+	Convergence of results with those from Endler <i>et al.</i> 2003
Thangapazham <i>et al.</i> ⁸⁰	Tumor cell inoculation	Rat	<i>Thuja occidentalis</i> 1000c; <i>Sabal serulata</i> 200c	Tumor cell growth; apoptosis identification	Yes	Successed vehicle; tap water	Yes	Yes	+	Identification of pro-apoptotic effect
Mc Laughlin <i>et al.</i> ⁸¹	Tumor cell inoculation	Mouse	<i>Sabal serulata</i> 200c	Cell growth	Yes	Successed water	Yes	Yes	+	Anti-tumor effect in mice converges to <i>in vitro</i> results
Werkman <i>et al.</i> ²⁵	Bone repair	Rats	<i>Calcarea phosphorica</i> 6cH	Bone growth and organization; qualitative histopathology	No	Water and sham operated rats	Yes	Yes	+	Blinded quantitative histomorphometry would make histological observations more objective, although the presented microphotographs show obvious differences between groups
Weber <i>et al.</i> ⁴³	Meta-morphosis	Frog	T4 1:10 ³⁰ (iso-endopathy)	Metamorphosis rate after several physical interferences on remedies	Yes	Successed water	Yes	Yes	+	Microwave and cell phone radiation impaired the biological effect of medicines (pilot study)
Experimental infection – host–parasite interaction										
Velkers <i>et al.</i> ¹¹	<i>E coli</i> infection	Chicken	Homeopathy complex and isopathy	Mortality; body weigh	No	No treatment	Yes	Yes	–	No correlation between blindness and positive or negative result; no vehicle control
Berchieri <i>et al.</i> ¹²	<i>Salmonella</i> infection	Chicken	Isopathy in drinking water (D30)	Number of CFU from cloacae smear	No	Water	Yes	Yes	+	No correlation between blindness and positive or negative result; no vehicle control
Lira-Salazar <i>et al.</i> ¹³	<i>Plasmodium berghei</i> infection	Mouse	<i>Eupatorium perfoliatum</i> , <i>Arsenicum album</i> 30cH	Parasite proliferation and differentiation	No	Successed vehicle	Yes	Yes	+	Parasite cycle arrest
Almeida <i>et al.</i> ¹⁴	<i>Trypanosoma cruzi</i> infection	Mouse	Isopathy 12D; <i>Phosphorus</i> 12D	Parasitemia; leukocyte response; mortality	No	Unsuccessed vehicle	Yes	Yes	+	No mortality but increase of parasitemia in phosphorus treated mice; improvement of immune response in isopathy

Glossary: * EEG = electroencephalogram; n/a = not applicable (qualitative observation); LPS = lipopolysaccharide from *E. coli*; CFU = colony forming units; + significant effect compared to control; – non significant effect compared to control.

Table 2 Contingency tables of result (positive or negative) with experimental design: blindness and type of control (succussed or unsuccessful). No significant difference was observed. For blinding $p=0.6456$; for type of control $p=1.0000$; Fisher Exact test

Result	Blind	Not blind
A		
Positive	19	7
Negative	4	3
Result	Unsuccussed	Succussed
B		
Positive	14	14
Negative	2	3

Some *in vitro* studies have demonstrated effects of succussed vehicle sufficient to mask statistical significance and induce false-negative or false-positive results, compared to unsuccessful vehicle. The latter gives more stable results.^{17,18} In this case, considering all the experiments performed in a study is crucial. The convergence of results is an useful criterion to discriminate non-specific contaminant-related effects of succussed solvent from specific test-substance effects of homeopathic preparation.¹⁸ But this problem seems irrelevant in studies performed *in vivo*, in which succussed, unsuccessful vehicle and untreated animals usually present the same biological pattern.^{16,19} Also the nature of vehicle is sometimes more important than succussion itself, particularly in behavioral models, as pointed in.²⁰ Two or more negative control groups – *i.e.* succussed, unsuccessful vehicle and untreated animals – could be an important quality criterion for future *in vivo* experiments. Besides statistical significance, the convergence of results may give important pointers to understand the biological action of high dilutions.¹⁶

Another point that requires careful analysis is the possibility of type II errors (false-negative) because of communication between groups, as suggested by Bengston and Moga²¹ and Conforti and colleagues.¹⁰ In this case, authors used the rat paw edema method to study the effects of high diluted substances in inflammation, showing that cage organization and steps of blindness can be crucial: putting animals receiving different treatments in the same cage, instead of separating animals receiving the same treatment in the same cage, as is done traditionally, can drastically change the results in terms of statistical significance.¹⁰

This kind of error (type II) has previously been observed in non-homeopathic research. Fernandes²² demonstrated a type II error in a study when mice submitted to immunization and the control was kept in the same room: no statistically significant differences regarding behavior and physiological parameters were found. But when the control animals were kept in a separate room, under the same environmental conditions, there were clear and significant differences compared to immunized mice. This suggests that some kind of non-visual communication between mice (for instance pheromones) could be involved in neuro-immune control, generating bias. Repetitions of such studies are needed to clarify these hypotheses, but the careful planning of control group conditions is a critical element

in experimental studies about high dilutions, particularly those on behavioral models.

Conceptual aspects

In previous reviews, 727 studies on animal models from a database of basic research were classified according to whether the theoretical basis was proving, prophylactic or therapeutic. There was no time limit. One of the main remarks of the authors is that there are a huge variety of studied biological systems, and this dispersion of data makes it impossible to determine “which model organisms have been utilized most effectively in the study of the *Similia* Principle”.^{5,6}

But our general overview reveals an important common feature of the reviewed models: the effect of high dilutions is often to restore living systems to a certain ‘stable state’. This is seen in studies of behavior, tissue organization and infection^{13,14,20,23–25} and in some cases demonstrated by mathematical models.^{26,27} By the concept of ‘stable state’ we mean the recovery of a biological system to control or normal parameters, after some kind of physiological perturbation. Each model, however, presents specific details that deserve to be discussed.

The isopathy model: Between 1998 and 2009, Khuda-Bukhsh's group published a series of well-conducted studies on the protective role of *Arsenicum album* at two different high potencies (30c and 200c) in the experimental intoxication of mice with arsenic trioxide.^{15,16,28–32} Considering their work as a whole, the protective action of *Arsenicum album* 30c was verified in blind experiments, using succussed and unsuccessful controls. Several complementary parameters were evaluated, including histopathological changes provoked by arsenic trioxide, as well as reduction of oxidative stress measured by enzyme activity.^{31,32} Experiments using *Arsenicum album* 200c were performed measuring genotoxicity, liver and spleen arsenic levels and oxidative stress in these organs and ultra-structural evaluation.^{15,16,32}

These studies show methodological evolution over time: statistical methods became more appropriate, standard methods of measurement were adopted, such as atomic absorption spectrometry and electronic microscopy. The cumulative data during these 10 years show a narrowing coherence and high convergence with previous studies, allowing the authors to hypothesize an action of high dilutions on gene expression and ‘molecular switch’ toward a more adapted state in face of the noxious stimulus.¹⁶

Other isolated studies about isotherapy were also published during this period. Bonamin and colleagues showed that simultaneous injections of high potencies of dexamethasone (10^{-33} M or 15cH) inhibit the action of pharmacological doses of dexamethasone *in vivo*, both on inflammation process and tumor leukocyte response.³³ These effects were observed immediately and only in host related parameters, no effect was seen on inoculated tumor cells. The design of this study included detailed description of drug preparation, individual measurements made in duplicate, positive and negative controls, and standardization for season and time of day.³⁴

Table 3 Overview of key conceptual aspects extracted from the selected papers. The classification according to experimental model is maintained, as in Table 1

Reference	Convergence of data	Theoretical basis	Innovation
Behavior			
Ruiz Vega <i>et al.</i> ²⁶	Yes With: Ruiz Vega <i>et al.</i> 2000 Ruiz Vega <i>et al.</i> 2002	<i>Similia</i> principle	Yes Identification of markers; Proposal of mathematical model
Sukul <i>et al.</i> ⁷	Yes With: Sukul <i>et al.</i> 1999	<i>Similia</i> principle	Yes Selection of susceptible rats; ultra-sonic succussion
Ruiz Vega <i>et al.</i> ²⁷	Yes With: Ruiz Vega; Salgado, 2008	<i>Similia</i> principle	Yes Proposal of mathematical model
Coelho <i>et al.</i> ²³	Yes With: <i>Dolichos pruriens materia medica</i>	<i>Similia</i> principle	Yes Model to test itching behavior
Pinto <i>et al.</i> ²⁰	Yes With: <i>Chamomilla materia medica</i>	<i>Similia</i> principle	Yes Proposal of a model for investigation about sick mate stress
Intoxication			
Mallick <i>et al.</i> ³²	Yes With: Kundu <i>et al.</i>	Isopathy	No Classical protocol used
Banerjee <i>et al.</i> ¹⁶	Yes With: Banerjee <i>et al.</i> 2007	Isopathy	Yes Insertion of new and accurate techniques
Inflammation			
Bertani <i>et al.</i> ⁸	No Results varying according to the time of treatment	Complex – <i>Similia</i> principle	No Classical protocol used
Bonamin <i>et al.</i> ³³	Yes Comparable results between 7cH and 15cH	Isopathy	Yes Simultaneous injections revealed the immediate effect
Araújo Prado Neto <i>et al.</i> ⁹	No Effects seen in acute but not in chronic inflammation	<i>Similia</i> principle	Yes Comparison of acute and chronic models reveals the <i>Similia</i> principle
Macedo <i>et al.</i> ¹⁹	Yes With: <i>Arnica montana materia medica</i>	<i>Similia</i> principle	No Classical models used
Almeida <i>et al.</i> ⁵¹	Yes With: <i>Kreosotum materia medica</i>	<i>Similia</i> principle	Yes Original model used
Pedalino <i>et al.</i> ³⁶	n/a No previous study of potency chord to compare	<i>Similia</i> principle	Yes First systematic study about accord of potencies
Conforti <i>et al.</i> ¹⁰	No Results with different conclusions according to group/housing organization	<i>Similia</i> principle	Yes Changing of housing systems of animals from different groups leads to changing conclusion
Santos <i>et al.</i> ³⁷	Yes Different models were applied in the same study with similar results	<i>Similia</i> principle	No Classical models were used
Carcinogenesis			
Biswas <i>et al.</i> ⁷⁷	Yes With: Biswas; Khuda-Buhksh 2002	<i>Similia</i> principle	Yes Demonstration of additive effects between two medicines
Tissue growth and differentiation			
Guedes <i>et al.</i> ⁴⁴	Yes With: Endler <i>et al.</i> 2003 (and previous results of same group)	Endo-isopathy	No Replication of a classical protocol developed by Endler
Thangapazham <i>et al.</i> ⁸⁰	Yes With: McLaughlin <i>et al.</i> 2006	<i>Similia</i> principle	Yes Identification of apoptosis related to the treatment
Werkman <i>et al.</i> ²⁵	n/a No previous study about histological bone organization found	<i>Similia</i> principle	Yes No previous study about histological bone organization was found

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Table 3 (continued)

Reference	Convergence of data	Theoretical basis	Innovation
Weber <i>et al.</i> ¹⁸	Yes With: Guedes <i>et al.</i> 2004; Endler <i>et al.</i> 2003	Endo-isopathy	Yes Original results about physical interferences on high diluted substances
Experimental infection – host–parasite interaction			
Velkers <i>et al.</i> ¹¹	n/a No previous study about <i>E. coli</i> infection in poultry found	Isopathy and complex	Yes No previous results about <i>E. coli</i> infection in poultry was found
Berchieri <i>et al.</i> ¹²	n/a No previous study about <i>Salmonella</i> sp infection in poultry found	Isopathy	Yes No previous study about <i>Salmonella</i> sp infection in poultry was found
Lira-Salazar <i>et al.</i> ¹³	n/a No previous study about <i>Plasmodium</i> sp infection found	Similia principle	Yes No previous study about <i>Plasmodium</i> sp infection was found
Almeida <i>et al.</i> ¹⁴	n/a No previous study about <i>T. cruzi</i> infection found	Similia principle and isopathy	Yes No previous study about <i>T. cruzi</i> infection was found

n/a = not applicable.

The observation of comparable results in different models – inflammation and cancer host response – leads to the hypothesis that genetic disturbances might interfere on the action of dilutions, since tumor cells seems not to be themselves responsible. This hypothesis is in accordance to the *in vitro* studies performed by Walchli and co-workers³⁵ and Siqueira.¹⁸

The examples discussed above point toward the importance of convergence of results from different models and variables, to create a background on which a theoretical model can be built. This kind of approach is not aimed at proving that the biological action of high dilution is not an artifact – which would be better done by multi-centric repetitions of the same protocol – but to create a template to propose hypotheses concerning the mode of action to be tested in the future. Studies using inflammation models applied to high dilution research are particularly illustrative in this sense.^{8,9,36–38}

There are also divergent studies in the literature.²⁴ For instance in¹¹ and,¹² commercial broiler chickens were experimentally inoculated with bacteria and treated with isopathic preparations. Opposite results were obtained in these studies, indicating that the question of the practical usefulness of isotherapies and homeopathic complexes in epidemic conditions remains open.

A study by Kuzeff and colleagues³⁹ shows that the isopathic preparation of (+)-trans-(1S,2S)-U50488 hydrochloride enantiomer was able to inhibit the toxicity of (–)-U50488 HCl *in vivo*. The same conclusion was obtained by the authors in a similar model, using (+)-propranolol hydrochloride.⁴⁰ This was the first time that isomer specificity has been considered as a determinant factor in the activity of isopathic preparations.

Iso-endopathy: One of the most studied models of the effect of high dilutions of endogenous substances is the modulation of metamorphosis rate of frogs by homeopathically prepared thyroxin [T4], diluted beyond Avogadro's number. The effect of potentised T4 on Alpine frogs (*Rana temporaria*) metamorphosis has been a stable and

well reproducible result since early 1990's, including repetitions in five different laboratories with a total $N > 3,000$ tadpoles.^{41–43} Opposite to the effect of T4 in measurable doses, a high dilution of T4 slowed down metamorphosis. Similar results were obtained by Guedes and co-workers in Brazil, it means, in different climatic conditions and using a different species of frog (*Rana catesbeiana*).⁴⁴

The stability of results obtained in this series lead to another series of studies discussing factors that influence the action of high dilutions, such as succussion, initial T4 blood levels and temperature, in obtaining coherent results. Non-alpine tadpoles treated with pharmacological doses of T4 before treatment with potentised T4 responded better than untreated tadpoles⁴⁵; in alpine tadpoles, pre-treatment with molecular T4 did not help to enhance the effect of potentised T4⁴² – which suggests that alpine tadpoles have a higher physiological T4 blood level or are more susceptible to T4. In general, however, the effect of potentised T4 on both populations was opposite to that of molecular T4. Herein, a similar "tendency to normality" effect, as also observed by^{23,46} in completely different *in vivo* experimental models, is verified.

Still regarding to external influences, a recent pilot study suggests the blocking effect of certain magnetic fields, such as microwave oven and cell phone derived fields on homeopathic preparations of T4.⁴³ The specificity of T4 has not yet been verified, by using as control, instead of T4, another similar molecule without physiological action.

Other studies on iso-endopathic phenomena have also been reported, including the effect of high dilutions of glutamate on neuroprotection of rats from glutamate toxicity⁴⁷ and the effect of high diluted thymulin in host response to tumor cells, BCG inoculation and immune system histopathology.^{48–50}

'Animal proving': Three reports of animal pathogeneses were found. Almeida and colleagues, fed rats a cariogenic diet and treated them with Kreosotum 6cH.⁵¹ The animals showed signs compatible with the *materia medica* of Kreosotum: deposits on dental surface associated with fur loss. However, this was an incidental observation in a therapeutic

model study. Another experimental model designed specifically to verify the pathogenetic effects of *Dolichos pruriens* given to rats in rising potencies (9, 12, 18, 30cH) failed to demonstrate the pathogenetic symptoms.²³

A more detailed model was described by⁵² using a mathematical model to demonstrate objectively the pathogenetic effects of *Coffea cruda* 30c on the sleep pattern of rats, monitored by electroencephalogram (EEG). Waves corresponding to the NREM sleep (delta band) were recorded, and subjected to a Fast Fourier Transformation (FFT) the power in the 0.5–2.5 Hz band was computed to assess sleep intensity. The variation of EEG pattern after *Coffea cruda* treatment was statistically significant compared to baseline. Further studies of different potencies of *Coffea cruda* compared variability within-group and between groups. The main conclusion of this study was the definition of delta band and slow/delta power ratio as markers of *Coffea cruda* activity.⁵³ This approach corroborates those of,⁵⁴ in which rhythmical electrical events could be identified as indicators of biological actions of homeopathic remedies.

The systematic observation and experimentation of pathogenesis in animals is still a new field in homeopathy research; most of positive results observed were from incidental findings and very few systematic studies are seen in current scientific literature.

Inflammation models: The development of experimental inflammation models has been useful in the comparison about the action of low and high dilutions of active substances. Five papers included in this review focus on this question.

Bertani and colleagues explored the anti-inflammatory effect of a mineral complex, containing 34 different salts in potencies varying between D2 and D7, using a blind protocol designed based on the carrageenan-induced paw edema model in rats.⁸ The results varied according to time of administration: no anti-inflammatory effect was seen when the complex was administered, as a previous treatment, directly into the inflammatory site before the injection of irritant stimulus, but a significant anti-edematous effect was seen when the complex was given in a curative manner, it means, after carrageenan inoculation.

A study performed by Araújo Prado Neto and colleagues, showed positive effects of Causticum on carrageenan-induced rat paw edema, comparable to those of Indometacin.⁹ These positive results were reproducible in all variations of the protocol: pre- or post-treatment, using hydro-alcohol solution or water as vehicle, in low (6cH and 12cH) or high (30cH and 200cH) dilutions. Negative results were obtained in a foreign body induced granuloma model, an inflammatory pattern that does not manifest edema – erythema symptoms, a pattern that is symptomatically analogous with the pathogenesis of Causticum. This study also used a “*similia control*”, subcutaneous injection of concentrated Causticum, the same source used for preparing the dilutions. As expected, this caused acute paw edema with all cardinal signals of inflammation.

The same group³⁷ tested the putative anti-inflammatory effect of *Rhus toxicodendron* using various experimental models in rats and mice. This illustrates of how using various models can hint at the physiopathological mechanisms

of high dilution effects. 6, 12, 30 and 200cH potencies were able to reduce paw edema induced by carrageenan in a significant manner. Since 6cH potency exhibited the most evident effect, this potency was tested in other models, such as dextran-induced edema, croton oil induced erythema, vascular permeability induced by histamine injection, writhing test in mice, and stress induced gastric lesions. Taken as a whole the data indicate that when prostaglandin production is the most important chemical event inducing acute inflammatory symptoms, results were positive, independent of the protocol – pre- or post-treatment. However, the models in which inflammation was dependent on histamine action gave contradictory results. Analyzing this inconsistency more closely, in the dextran-induced edema model, only the treatment given at the same time of as irritant inoculation produced an anti-inflammatory output. For vascular permeability, induced by histamine injection, neither pre- nor post-treatment was effective. If one supposes that some active principle from *Rhus toxicodendron* acts on histamine release or on a specific receptor, the negative result obtained in the last model could not be explained. On the other hand, if one considers the *Similia* principle to understand these results, only the paw edema model shows gross symptomatic mimesis – or similar template – to the *materia medica* of *Rhus tox*. Thinking ‘homeopathically’, it makes sense, although it is not sufficient to ‘prove’ the existence of the *Similia* principle.

Other experimental models that include prostaglandin-mediated acute inflammation signals also have high level of reproducibility, but the modulator effects of high diluted preparations cannot be interpreted as a simple blocking or anti-inflammatory activity comparable to non-steroidal anti-inflammatory drugs. They fit more complex models, which cannot be completely understood in the present level of knowledge.^{8,19,33,38,55}

Another original study was.³⁶ This was one of the rare experimental works made to observe the behavior of ‘potency chords’, meaning the mixture of different preparations in different dilutions from the same matrix. These preliminary results strongly suggest that the mixture of low and high potencies of *Atropa Belladonna* and *Echinacea angustifolia* generates a synergistic effect, considering the effects on modulation of inflammatory processes.

The level of dilution in an experimental study is crucial for a correct interpretation. The studies described above concern to dilutions above Avogadro’s number and the results point toward subtle interferences in biological states of stability, considering the general organization of the living system, as is often seen in biosemiotic studies.^{56–58} On the other hand, experiments using low potencies often show linear or log relation between concentration and effect, in other words, a classic molecular pattern.⁵⁹ The superposition of both frameworks reveals two different biological phenomena. These differences must be considered *a priori*, before planning an experimental design.

Host–parasite interaction: There is also in the recent literature, some works designed to explore the behavior of the body after to be stimulated by parasitic stressor and treated with homeopathic medicines. Three interesting

experimental models are described, using protozoa or worm infection.

Cina 30c and *Santoninum* 30c were effective in reduction of *Trichinella spiralis* larvae infection in muscle tissue.⁶⁰ Lira-Salazar and co-workers, demonstrated the effect of *Eupatorium perfoliatum* 30cH and *Arsenicum album* 30cH on *Plasmodium berghei* infected mice.¹³ In both cases parasite multiplication was inhibited, but the most interesting observation was the increase in the number of schizonts (the *Plasmodium* parasite form found inside the red blood cells), suggesting an interruption of *Plasmodium* life cycle in this intracellular phase. Although the mechanism involved cannot be explained by these studies, it is plausible that this change of infection dynamics could be related to immune-mediated interactions between host and parasite.

Other example is the infection of mice with *Trypanosoma cruzi* – the parasite that causes the Chagas disease in humans – and treatment with an isopathic preparation of the same parasite (*T. cruzi* 12dH) or with *Phosphorus* 12dH, chosen by repertorization of *T. cruzi* infection symptoms.¹⁴ In this study the differences between biological reactions triggered by the two treatments are clear, suggesting that each involves a different mechanism. Isopathic pre-treatment decreased the patent period of parasitaemia, increased blood lymphocytes count, reduced parasitaemia and mortality rate. But post-treatment with *Phosphorus* was associated with a longer patent period of parasitaemia, higher maximum parasitaemia, significant reduction of lymphocyte numbers but no mortality. The infected control had the highest mortality rate. Although isopathy pre-treatment could be related to a vaccine-like specific stimulation of immune system, the *similia* based treatment (*Phosphorus*) produced changes in parasite pathogenicity. Since the pathogenicity of *T. cruzi* is quite dependent on host-parasite interactions, one can hypothesize that *Phosphorus* treatment interferes in modulatory immune-mediated mechanisms.

Other studies showing parasite-related immune-modulation effects of commercial formulations that include homeopathic medicines (Canova[®]) are also described.^{61,62}

Behavior models: Studies related to behavioral experimental models demonstrate a particular feature of the action of high dilutions: the tendency to gradually restore normal levels of the measured parameters. The curve shape obtained in this kind of effect is quite different from the classical dose-response curve of modern pharmacology. To observe it, it is necessary to select an animal sample able to manifest spontaneous and peculiar behavior.

Nuxvomica 30c and *Nuxvomica* MT were able to reduce alcohol effects and voluntary ethanol intake in predisposed (susceptible) rats selected by the classical method described by Porsolt, in the decade of 60.^{7,63} This data confirm the pilot results obtained by the same group.⁶⁴ The selection of sensitive rats as part of experimental design for studying high dilutions was first described by Cristea.⁶⁵ This principle was also used by⁶⁶ in a model of hyperactive/hypoactive rats selected by the open-field method and treated with *Rhus tox* 200cH. Only hyperactive rats showed significantly different behavior after treatment. A similar result was obtained by,⁶⁷ using *Bryonia alba* 200cH and hypoactive

rats. Coelho and co-workers observed gradual and significant reduction of itching behavior in rats submitted to heat after prolonged treatment with rising potencies of *Dolichos pruriens* (9, 12 and 30cH),²³ finally resulting in values equal to control group. Similarly in a pilot study, the recovery of normal basal behavior after *Chamomilla* 6cH treatment was observed by²⁰ in two different models of rat depression.

A solid study about the effects of *Gelsemium sempervirens* in different potencies was recently published, showing the influence of this medicine in behavior of mice submitted to novel environments.⁶⁸ These findings are in accordance with previous studies performed *in vivo* and *in vitro*.^{69,70}

All the results observed in behavior experimental models lead to the same conclusion proposed by Ruiz Vega and Salgado⁴⁶: homeopathic stimuli can drive the living system to restore a state of equilibrium.

Tissue organization: Werkman and colleagues compared *Calcarea phosphorica* 6cH and Sodium risedronate (1 mg/kg) in bone regeneration in a rat osteoporosis model.²⁵ Serial digital histomorphometric analysis of bones showed quantitative but, mainly qualitative differences between treatments and between treated and control rats. In osteoporotic bones, the osseous turnover is inadequate because, bone formation is diminished and resorption increased. The *Calcarea phosphorica* treated rats had less bone callus than control, displaying thin lamellar compact bone, instead of immature trabecular pattern. The sodium risedronate treated rats had greater quantity of bone formation, but it maintained its immature appearance up to 28 days of treatment. This suggests that *Calcarea phosphorica* 6cH had a modulating effect on bone organization but not on rate of bone formation. Another recent study based on morphometry of liver tissue regeneration showed analogous results. While high dilutions of dexamethasone (7cH or 10⁻¹⁷ M) did not change the mass of liver regeneration it did change the reticulin deposition pattern during the process, leading to a more delicate stroma organization of the newly formed liver tissue.^{71,72}

Recently, similar results and conclusions were found by Almeida and co-workers using *Plumbum* in a bone regeneration model.⁷³

Cancer: Several studies were published during the last 10 years about the effect of homeopathic medicines used according to the *Similia* principle, on biology of tumor cells *in vivo*.

The first experiments to evaluate the action of *Arnica* 30c as a protective agent against cytogenetic damage-induced by ultrasonication in mice were performed in 2001.⁷⁴ Mice with hepatic tumors induced by p-DAB had reduced tumor incidence and improved genotoxic parameters after treatment with *Chelidonium* 30c and 200c; the results of treatment with 200c were better than 30c. Although the control group was not ideal (no vehicle, no succussion), the study was blind and different parameters were coherent.⁷⁵ Continuation of this work with better control groups and more refined measurements – including enzyme activity and electronic microscopy yielded congruent results and a clear positive interference of *Chelidonium* 200c on

hepatic tumor progression.^{76,77} Positive results were also observed using *Lycopodium* 30c in the same model.^{78,79}

Two interesting papers published in 2006 comparing *in vitro* and *in vivo* models of human prostate cancer have shown the protective effect induced by *Sabal serrulata*, *Thuja occidentalis* and *Conium maculatum* 200cH.^{80,81} Different parameters were analyzed, such as apoptosis gene expression *in vitro* and tumor growth, PCNA expression ('proliferating cell nuclear antigen', a cell proliferation marker) and frequency of apoptosis *in vivo*. Similarly to the observations in isopathic experiments, no direct action of these medicines was seen toward tumor cells *in vitro*, however, reduction of tumor volume and PCNA positive cells, as well as, increase in apoptotic cell number were seen in intact animals.

More recently, the anti-tumor effect of *Ruta graveolens* 200c and *Hydrastis Canadensis* 200c in transplanted tumors in mice⁸²; as well as the anti-tumor effects of *Ruta graveolens* 200c in 3-methylcolanthrene-induced sarcoma in rats and hepatic carcinoma were described.^{83,84}

Although all these findings suggest the participation of immune response, no immunological parameter was analyzed in these studies. The hypothesis that some kind of genetic control was involved in the process is plausible, but it is important to consider that epigenetic mechanisms could also participate in the phenotype changes of initiated treated cells. Thus, further works are needed in this field.

Regarding immune anti-tumor response, positive effects of the commercial homeopathic complex Canova[®] on circulating NK ('natural killer') and B cell in sarcoma-180 bearing mice were verified.⁸⁵

Mathematical modeling: Building on their previous work on the effect of *Coffea cruda* 30c and 200c on the sleep pattern of rats,^{52,53} the group of Ruiz Vega *et al.* conducted a blinded study to evaluate, using a mathematical approach, the kinetics of the effect of *Coffea cruda* 30c before and after the treatment of rats with pharmacological doses of caffeine.²⁶ In this study, two experimental sets were used: one in which rats were treated with caffeine then with *Coffea cruda* 30c; and another in which rats were treated with *Coffea cruda* 30c before caffeine. The spectral density of delta and slow band patterns of EEG as a function of time were fitted to an analytical function (log-log fitting). Animals pre-treated with caffeine and then treated with *Coffea cruda* 30c showed a significantly different curve from control, tending to restoration of the homeostatic state, similar to untreated rats. However, rats treated with caffeine after the exposure to *Coffea cruda* 30c had increased caffeine effect; thus, two different populations of rats were found to respond to caffeine stimulus with a different pattern. In such circumstances, homeopathic medicine could act as 'homologous priming' of biological events and susceptibility could define the pattern of this priming.

According to the authors, the homeopathic stimulus could drive the living system spontaneously between critical states, named 'attractors'. Homeostasis could be viewed as a process for restoring stable states.⁴⁶

The same group came to similar conclusions with high dilution of histamine to evaluate modification of sleep

patterns in rats: treatment with histamine 30c increased wakefulness.^{27,46} The comparison of different potencies, however, resulted in an oscillatory pattern. Other *in vitro* and isolated organ studies reveal the same oscillatory pattern of potency/effect curve.^{17,86-89} The possibility of considering this kind of behavior as a possible marker of high dilution phenomenon is discussed.⁴⁶ In short, mathematical modeling can be useful to establish precise conceptual parameters, maybe, a *sine qua non* condition to build solid hypothesis about mechanisms.

Conclusion

The systematic analysis of the selected papers allowed to put in evidence some important intrinsic features of high dilutions studies performed in animal models: a) methodological rigor was generally adequate, even if some particular aspects could be still improved. b) an expressive convergence between results and classical *materia medica* is seen among studies, pointing toward to the plausibility of considering the *Similia* principle as a potential object of systematic study. c) both isopathic and *Similia* principle models seem useful to understand complex biological phenomena, such as parasite-host interactions. d) the effects of high dilutions seem to tend to restore living systems to a "stable state", recovering normal parameters similar to control, as seen in several experimental models from both, descriptive and mathematical modelling points of view.

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