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# *The 4th Generation Folate*

*Innovative compounds*



## INTRODUCTION & APPLICATIONS

Quatrefolic® represents **an innovative dietary ingredient** designed to act as a nutrient in all areas where folic acid and folate supplementation has been recommended and allowed.

**Pregnancy  
and lactation**



**Infants &  
children**



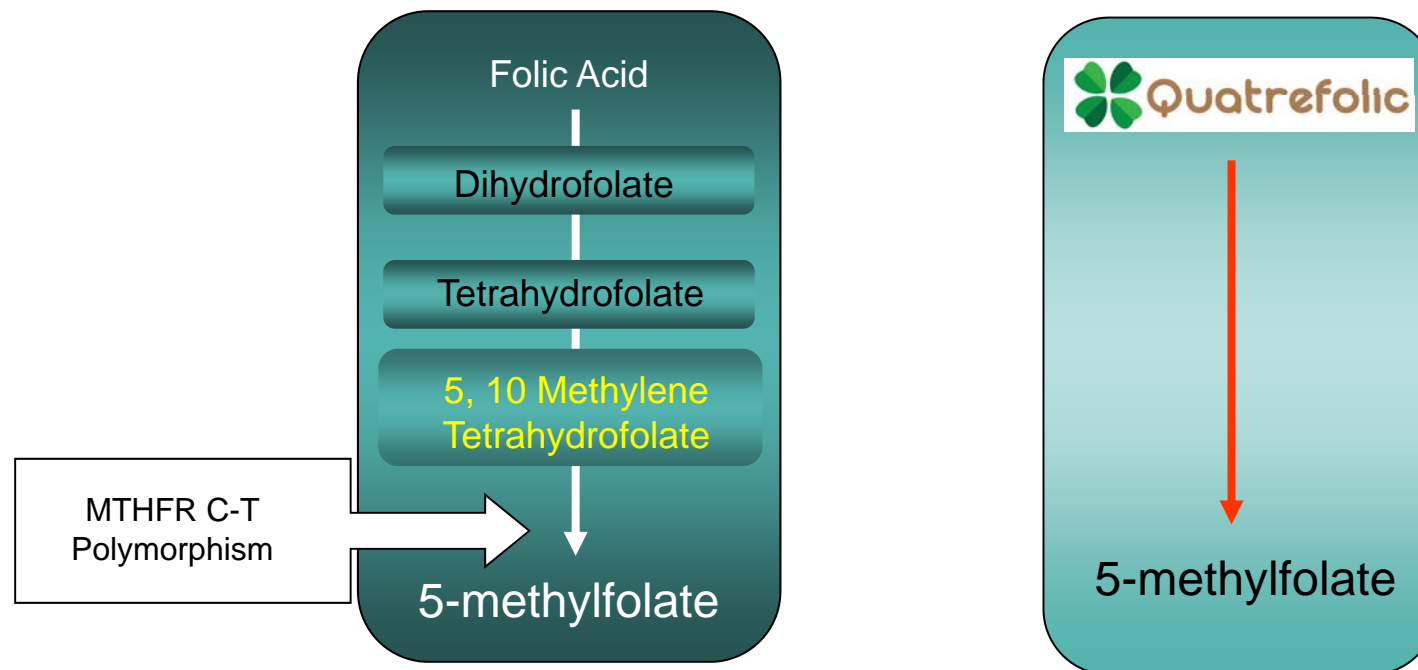
**Adults  
& Older people**



**Other  
conditions**



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**Conversion steps in our body**

Folate and folic acid must be converted to 5-methyltetrahydrofolate before they can participate in the two key metabolic pathways: **methylation processes** and **DNA synthesis**.

All the natural folates ingested are turned into 5-methyltetrahydrofolate as unique transportable form.

It does not interfere on B12 vitamin detection.

It is the essential form in which folates occur and are stored in the human body.

## 5-METHYLTETRAHYDROFOLATE

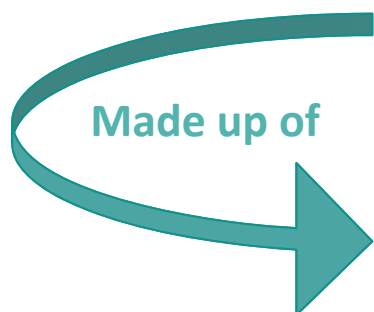
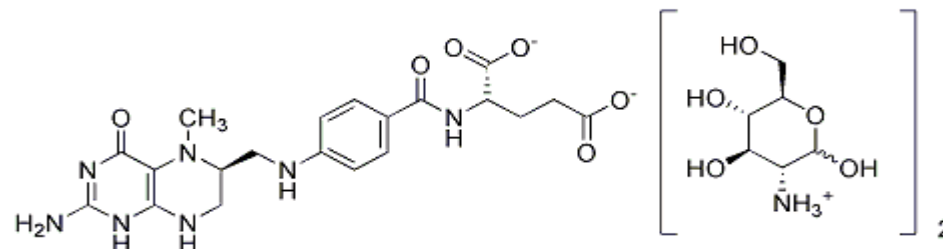
It is the only folate crossing the blood-brain barrier.

It is immediately and completely bioavailable.



## THE MISLEADING CONCEPT

[D-glucosamine-  
(6S)methyltetrahydro  
folate]



Made up of



5-methyltetrahydrofolate  
&  
D-Glucosamine

The innovative salt form **overcomes the 5-methyltetrahydrofolate calcium salt limits** (the third generation) with significant advantages:



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The innovative salt form **overcomes the 5-methyltetrahydrofolate calcium salt limits** (the third generation) with significant advantages:

- § High water solubility
- § Improved bioavailability
- § Long lasting stability
- § Established safety





## HIGH WATER SOLUBILITY

Quatrefolic® is 100 times more soluble in water than calcium salt.



Quatrefolic®



5-methyltetrahydrofolate calcium salt

Quatrefolic® demonstrates a surprisingly high solubility in water, compared with the slight solubility of the reference compound, (6S)-5-methyltetrahydrofolate calcium salt.

High water solubility means the product may be better absorbed by mucosal cells which may facilitate access to the blood and circulation with the potential for improved bioavailability.

Possible  
Applications



**Food and beverage industries**



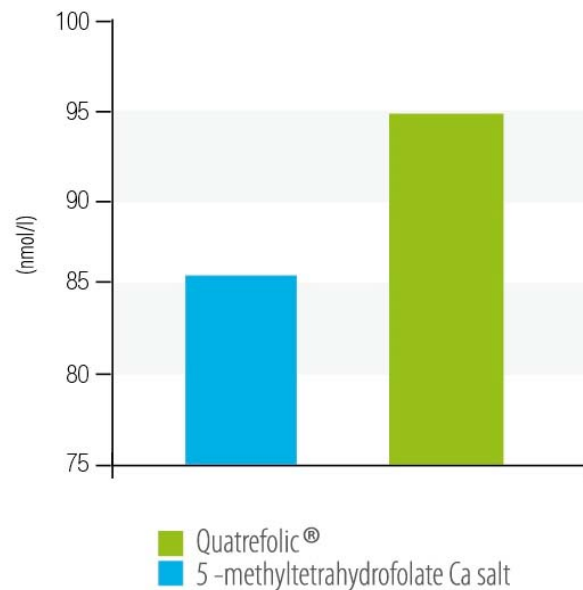
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## IMPROVED BIOAVAILABILITY

### Quatrefolic® and 5-methyltetrahydrofolate: pharmacokinetic comparison

PK parameters (C<sub>max</sub>) – (6S)-5-methyltetrahydrofolate Ca salt vs. Quatrefolic®  
(400µg dose) (PAS – Gnosis 2009)



5-methyltetrahydrofolate bioavailability studies were performed in rats after p.o administration.

The studies were performed also in healthy volunteers according to GLP.

The human clinical study confirmed the experimental findings in the rat: Quatrefolic® has better bioavailability **(+10%)** than 5-methyltetrahydrofolate.



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## Stability of Quatrefolic® at room temperature (25° C)

	Time 0		6 months		12 months		19 months	
	Purity	Assay	Purity	Assay	Purity	Assay	Purity	Assay
Quatrefolic®	99.3%	55.7%	99.0%	55.4%	98.5%	55.1%	98.3%	55.0%

Besides the high chemical stability, Quatrefolic® showed a very high solubility in water - **even higher than 1 g/ml** compared with the slight water solubility of 5-methyltetrahydrofolate calcium salt (1.1 g/100 ml)

Gnosis patent claims the innovative product, its use and the combination with several ingredients including SAM-e.



## ESTABLISHED SAFETY

### Toxicological studies

#### IN VITRO

##### Bacterial mutation in *S. typhimurium* and *E. coli*

The bacterial mutation assay was performed in order to assess the compound's ability to induce gene mutations in *S. typhimurium* and *E. coli*. The reverse mutation assay was run in bacterial strains already mutant at a locus whose phenotypic effects are easily detected, and, since many chemicals can demonstrate mutagenic activity only after metabolism to some reactive forms, the test was performed in presence and in absence of a rat liver metabolic system (S9 microsomal fraction).

The test concluded that Quatrefolic™ does not induce reverse mutation in *S. typhimurium* and *E. coli* at doses up to 5,000 µg/plate.

##### Mutation in LS178YTK<sup>-/-</sup> mouse lymphoma cells.

The assay was done in order to confirm the inability of Quatrefolic™ to induce mutations in LS178YTK<sup>-/-</sup> mouse lymphoma cells cultured after *in vitro* treatment, and in absence or presence of a rat liver microsomal system. The test concluded that Quatrefolic™ does not induce mutations at concentrations up to 5,000 µg/ml.

##### Chromosome aberrations in Chinese hamster ovary cells (CHO) *in vitro*.

The assay was made in order to demonstrate the inability of Quatrefolic™ to induce any chromosomal aberration in presence or absence of a S9 liver microsomal fraction.

No chromosomal aberrations were observed in CHO after *in vitro* treatment with concentrations of Quatrefolic™ up to 5,000 µg/ml.

#### IN VIVO

##### Single dose oral toxicity

The acute toxicity of Quatrefolic™ was assessed in rats of both sexes, dosing the product by gavage at 500 mg/kg level.

After dosing, animals were observed for a 7 day period and finally sacrificed.

EU	When
Toxicological studies	Complete
GRAS report	July 2010
NDIN filing (USA)	November 2010
EFSA filing (EU)	Q3 2010

In 2010 Gnosis makes commercially available Quatrefolic® [D-glucosamine-(6S)-methyltetrahydrofolate], the fourth generation folate endowed with long lasting stability, very high water solubility and better bioavailability than the commercially available methylated reduced folates.



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## DOSAGES

The intended uses of **Quatrefolic®** and use levels will be same as that of folic acid, expressed on the bases of the "Recommended Dietary Allowances for Folate for Children and Adults".

AGE (years)	Males and Females (µg/day)	Pregnancy (µg/day)	Lactation (µg/day)
-	Folate	-	-
1-3	150	-	-
4-8	200	-	-
9-13	300	-	-
14-18	400	600	500
19+	400	600	500



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Gnosis is the only company that has three NDIN's filled and GRAS recognition about folate products with FDA.

**Two patents on 5-methyltetrahydrofolate have been filed in 2008:**

1. *Process for the diastereoisomeric resolution of L-5-Methyltetrahydrofolic acid ( U.S. Patent No. 7,947,662 - PCT/EP2008/052037)*
2. *New revolutionary organic salt of 5-methyltetrahydrofolate showing an unexpected long last stability as well as a peculiarly high-water solubility instead of the reported slight water solubility of calcium salt of 5-methyltetrahydrofolate . (PCT/EP2008/052034).*



### Most Effective Strategic Product Development

The independent and impartial expert judging panel assembled exclusively from leading healthcare organizations and top business school academics recognized Gnosis and its new compound **Quatrefolic®**, for excellence in:

- ✓ innovative patent design
- ✓ project planning and execution and overcoming research and development challenges.
- ✓ high quality product development
- ✓ flexibility and choice for the consumer
- ✓ recognizing market and consumer demands and trends
- ✓ strategic market orientation



## Finalist NutrAward 2012

### Best New Ingredient

The NutrAward program was created over 10 years ago, to reward and recognize companies that are investing in rigorous and measurable scientific studies to prove the efficacy of their proprietary ingredients, products or technologies.

Independent panel of judges evaluated:

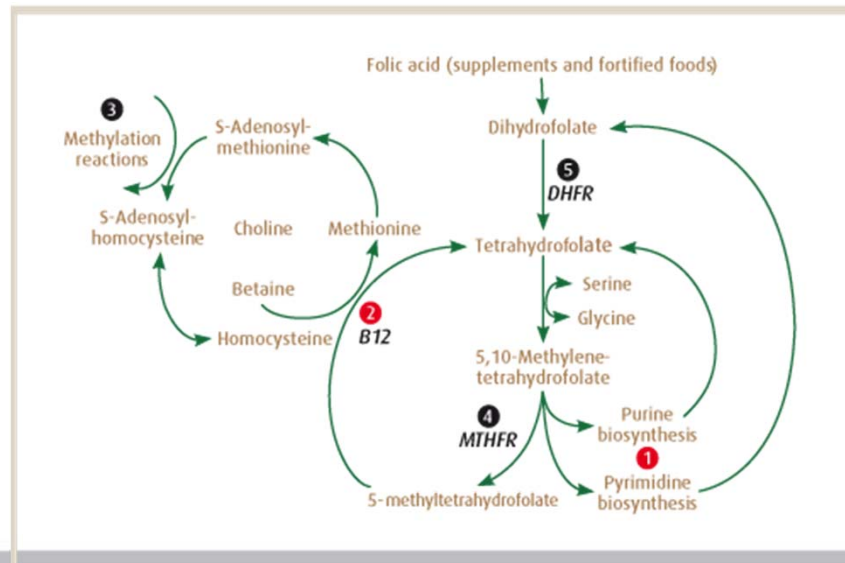
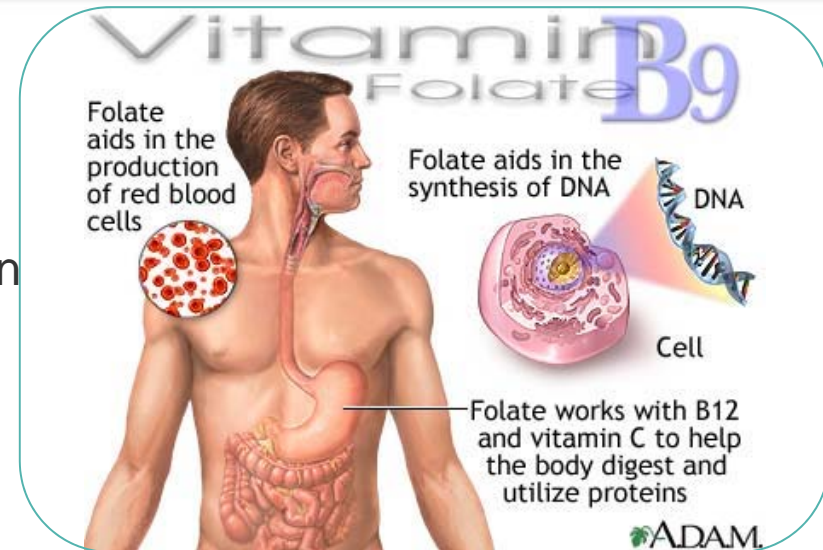
- ✓ Scientific merit
- ✓ efficacy
- ✓ health-related benefits
- ✓ safety and innovation
- ✓ market potential
- ✓ credibility recognition in the marketplace





Folate is an important vitamin for everyone required for all dividing cells, including the production of red blood cells.

Folate is a substrate for an important reaction that involves a series of enzymatic reactions and cofactors, as vitamin B12, and it is necessary for the synthesis of DNA.

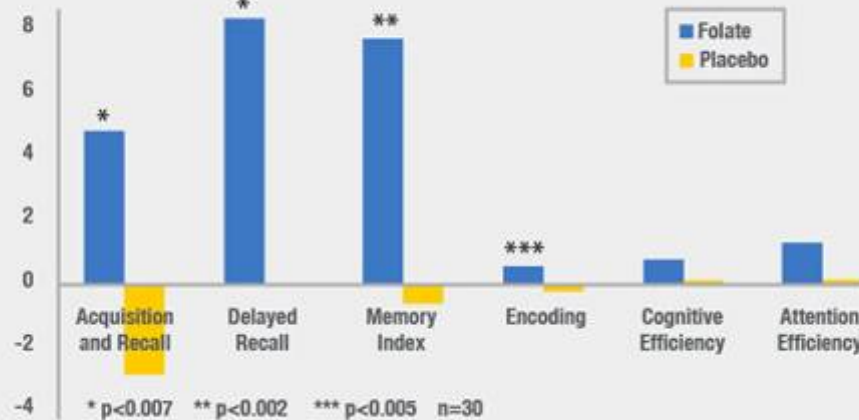


Absorbed folates are metabolized in intestinal mucosal cells through a reduction and methylation to 5-methyltetrahydrofolate.



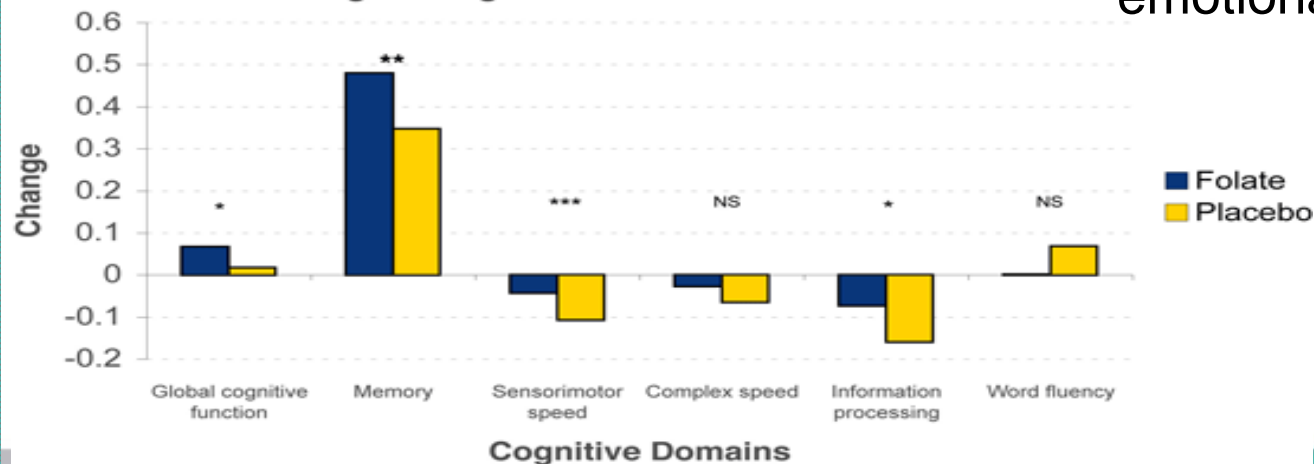
## FOLATE: COGNITIVE DECLINE

### FOLATE IMPROVES MEMORY IN PATIENTS WITH COGNITIVE DEFICITS



No adverse events reported greater than placebo.

### Change in Cognitive Function Attributed to Folate



\* p=0.03 \*\*p=0.01 \*\*\*p=0.05

*The Lancet. 2007;369:208-215.*

In the recent years several studies have been published describing the biological pathways in which the reduced folate is involved.

Folate is crucial for proper brain function and plays an important role in mental and emotional health.



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