G-PUR/Purified Clinoptilolite Whitepaper

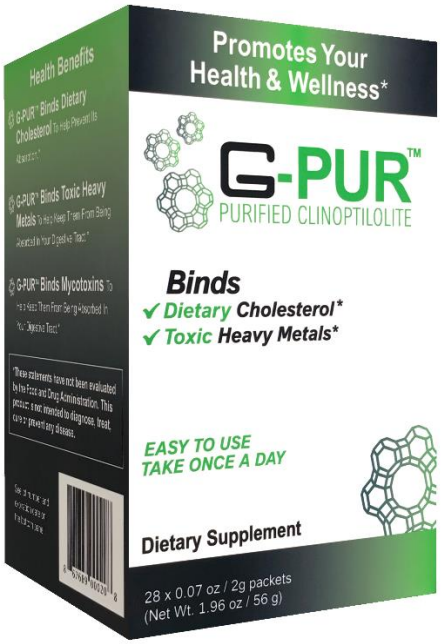

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Table of Contents

Page

List of Figures and Tables

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1 Introductory information

1.1 Disclaimer

This document is intended to serve as a summary of scientific evidence related to G-PUR and purified Clinoptilolite for scientific professionals use only. The information presented herein is sourced from published and unpublished research, articles, and books, as cited. The document is for informational purposes only. This document does not constitute medical advice, is not intended to serve as the basis for any kind of medical advice, and should not be considered or used to replace the care of a licensed health professional. As with all dietary supplements, consumers should carefully review all warnings and consult a healthcare professional prior to use. See G-PUR label for directions for use and warnings.

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2 G-PUR – Product identity

2.1 G-PUR Composition

G-PUR consists of Clinoptilolite (CLN). Clinoptilolite is a natural zeolite that occurs mainly in alteration products of sedimentary and/or volcanic rocks or altered basalts or clay deposits. The color of Clinoptilolite varies from pale green, blue-green, grey-green, to beige. The solid rock breaks irregularly, into sharp-edged, splinter-shaped or board-shaped fragments, with conchoidal smooth to uneven rough fracture surfaces.

2.2 Names and Other Identities

The major raw material of G-PUR is the mineral Clinoptilolite. Clinoptilolite is a crystalline, hydrated Calcium Potassium Sodium Magnesium Aluminosilicate belonging to the group of

natural zeolites. In contrast to natural zeolites that are in great quantities found in zeolite-tuff deposits, synthetic zeolites are man-made. The Chemical Abstracts Service (CAS) registry number for Clinoptilolite is CAS 12173-10-3.

G-PUR consists of a Clinoptilolite that has been purified by a patented process to minimize the content of contamination by unwanted substances that naturally occur in Clinoptilolite.

2.3 Formation of Clinoptilolite

In the lower to middle Badenian (approx. 15 million years ago), intensive volcanism led to the emission of large amounts of acidic volcanic ash which was deposited in the nearby border areas of the Paratethys and subsequently compacted under the growing compressive load of younger volcanic and also clastic sediments. The highly alkaline sea water of the Paratethys was thereby partly trapped in the pore space of the rock. The growing compressive load and tectonically induced subsidence of the original marine sedimentation space led to pressure and temperature increases in the sediment layers. This further triggered the chemical reactions of the alkaline pore water with the acidic rock mass already taking place. Over the course of the following millions of years, the rock went through a weak metamorphosis. During this time the acidic ash material - but mainly the contained volcanic glass components - was partially dissolved under the influence of alkaline pore waters, pressure and temperature. From the resulting (with different elements) highly saturated solution, over time, secondary minerals precipitated. The main representative of these metamorphosis products is the mineral Clinoptilolite. [Coombs 1997, Ames 1960, Sheppard 1971, Mumpton and Fishman 1977]

2.4 Clinoptilolite Source/ G-PUR Raw Material Source

There are many Clinoptilolite deposits of differing qualities found around the world, including the USA, Cuba, Mexico, Turkey, Greece, Ukraine, and China. [Margeta 2013]

The presence of impurities and coexisting phases may significantly affect the range of use of Clinoptilolites; even within a single mineral deposit, raw material may have varying composition and chemistry. This is the reason why any application of Clinoptilolite must be subjected to standardization concerning quality level and processing techniques. [Colella 2011]

G-PUR is exclusively sourced from a quarry in eastern Slovakia. This deposit provides Clinoptilolite of ultimate quality and, unlike many other deposits, Clinoptilolite which has an extremely low heavy metal content.

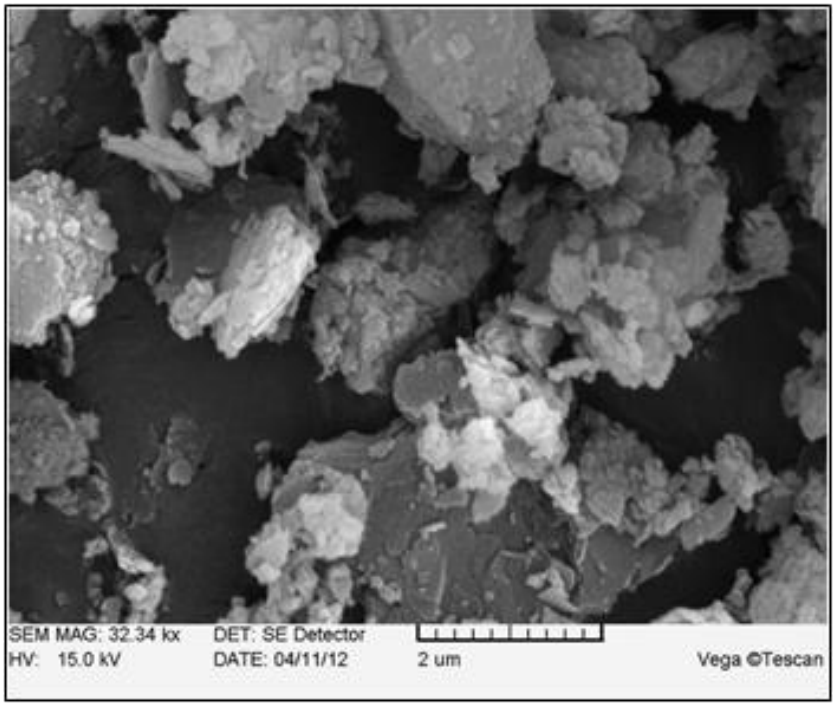

Figure 1 Pictures of G-PUR taken at high resolution (SE image, magnification 32340, Tescan Vega)

The fine-grained to aphanitic rock (individual mineral grains are not visible with the naked eye) consists essentially of an extremely fine matrix, in which crystal clasts are embedded. A typical appearance of idiomorphic Clinoptilolite crystals can been seen in Figure 2. What is extremely unique about this raw material from the Slovakian quarry is its very high homogeneity and the fact that it consists of two different Clinoptilolite modifications.

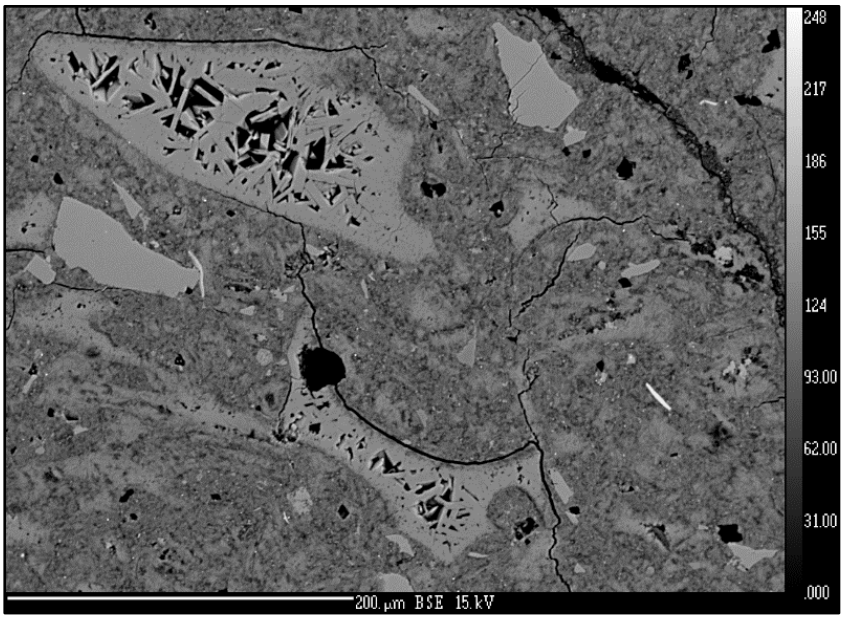

Figure 2 Electron Probe Micro-Analysis with back-scattered electron imaging of G-PUR raw material

One of these modifications shows the coffin-shaped Clinoptilolite crystals (Figure 3 middle) and the fine Clinoptilolite matrix crystals (Figure 3 left, not resolvable even with high resolution imaging techniques).

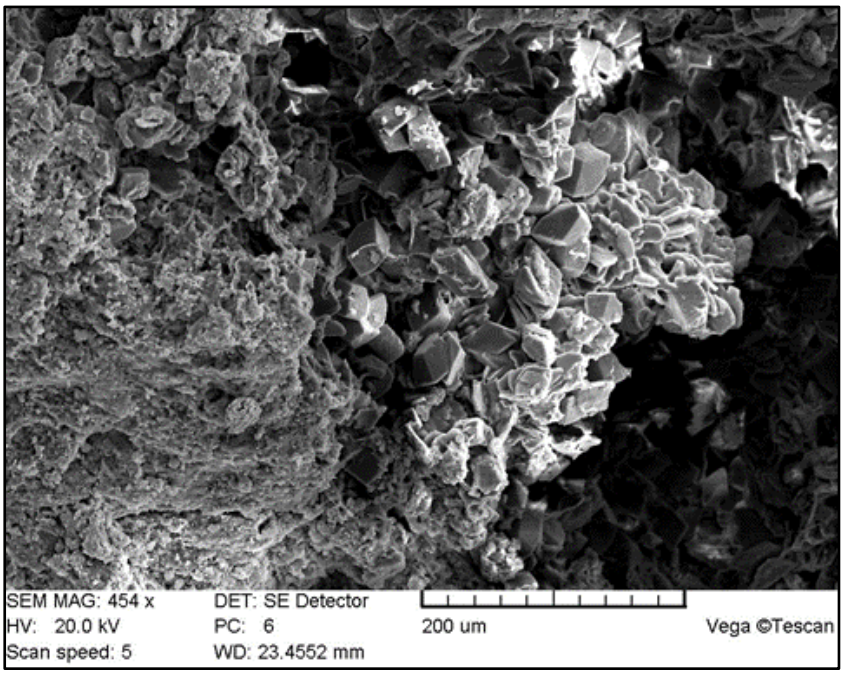

Figure 3 A section of G-PUR raw material at low resolution (SE image, magnification 454, Tescan Vega)

None of the samples from the Slovakian quarry, analyzed by electron microscopy, revealed fibrous or needle-shaped structures. The several hundred quality control samples, that have been analyzed by X-ray diffraction for their mineralogical composition, never showed traces of such fibrous zeolites (like Mordenite or Erionite which are both mineralogical similar, but unhealthy zeolites). [Stephenson 1999, Baris 2006]).

2.5 Basic Structure and Characterization

Clinoptilolite is a natural zeolite comprising a microporous arrangement of silica and alumina tetrahedra. The name is derived from the Greek words klino (oblique), ptylon (feather) and lithos (stone). The primary building unit of the Clinoptilolite framework is the tetrahedron with 1 silicium or aluminium atom in the centre and 4 oxygen atoms at the verticals. Some of the Si^{4+} is substituted by Al^{3+}. The typical Si/Al ratio for Clinoptilolite is > 4. The combination of several primary building units form a three-dimensional structure, the reason why Clinoptilolite is part of the tectosilicate group. Channels within the three-dimensional Clinoptilolite framework consist of different types: one type consists of the 10-member ring

(4.4 – 7.2 Å) and the second type consists of an 8-member ring with a size of 4.1 – 4.7 Å. Besides these two kinds of pores, Clinoptilolite has also some meso-/macro-pores with diameters from 10nm-10µm. Macropores also allow small molecules to reach and adsorb onto the “inner” surface.

Substitution of Si^{4+} by Al^{3+} defines the overall negative net charge and makes Clinoptilolite a so called ion-exchanger for positively charged substances (=cations). The negative charge of the framework is compensated by monovalent or divalent cations (called exchange or extra-framework cations) located together with water molecules in structural channels. Cations, which are small enough for exchange mechanism (= smaller than the Clinoptilolite pores) can enter or leave the channels. For each ion that is removed from a solution, an equivalent amount of ions from the Clinoptilolite channels are exchanged into the solution. The overall structure of Clinoptilolite remains geometrically unchanged during this fully reversible process. The silicium and aluminium atoms, as being part of the tectosilicate structure (tetrahedral or framework cations), are not exchanged under ordinary conditions.

The Si/Al substitution leads to possible external surface interactions (sorption effects) either with polar molecules, or with other cations, provided that they are large enough to prevent entry into the Clinoptilolite channels/pores. These adsorptive effects allow formation of aggregates, characterized in some cases by very high stability (e.g. for cholesterol or microbial toxins like aflatoxins and endotoxins or even some pesticides). The surface area of G-PUR for such adsorption effects exceeds several hundred square feet per gram. [Breck 1974, Bailey 1999, Baerlocher 2007, Colella 2011, Spyrnsky 2008, Petrakakis 2007, Bočarov-Stančić 2011, Armbruster 2001]

2.6 Human Application – Summary of G-PUR Characteristics

During digestion, G-PUR stays inside the digestive tract and is eliminated via the stool. As G-PUR is stable even in an acid pH environment, there is no major degradation of the structure. The main effects are ion exchange and surface adsorption. When G-PUR is in contact with an electrolyte solution, specific extra- framework cations may be removed from their sites within the Clinoptilolite and replaced by other cations from the solution, depending on concentration, size and affinity. Due to size, larger cations (like most organic molecules) cannot enter pores (pore size 4Å, e.g. sugar: > 8Å). Despite its large internal surface, G-PUR has a medium-sized (a few hundred square feet/gram) negatively charged, external surface. This results in possible external interactions either with polar molecules, or with other cations, provided that they both are large enough to prevent entry into the G-PUR channels/pores. Binding capacity to aflatoxins (fungal toxins which can be highly toxic &

carcinogenic when consumed in large quantities) is the main reason why Clinoptilolite has been used in animal breeding for more than a decade. In Europe, Clinoptilolite is mixed with feed and reduces bioavailability of fungal toxins.* [Colella 2011, Ceyhan 2007, Rivera 1998, Pond 1989, EFSA 2013, Bočarov-Stančić 2011, Dakovic 2000, Katsoulos 2016, Lemke 2001, Vekiru 2015, Li 2008, Armbruster 2001]

2.7 Serving Form

G-PUR is administered in stick-pack, but capsules or tablets may also be provided for future applications.

2.8 Serving Directions

Once daily, prior to a meal, pour the entire packet of contents into a glass of water (12 fl oz), stir and drink immediately. Do not take any medication or other supplements within 1 hour of drinking G-PUR. After 28 days of continual use, discontinue use for 2 days. Do not exceed recommended daily serving. Storage conditions: Store at room temperature; no direct sunlight.

2.9 Excluded Populations

Refrain from use in children. If you are pregnant, nursing, have a medical condition, are using medication, have an intolerance to silicium or aluminium compounds, have limited kidney function or have known chronic gastrointestinal disease, consult a healthcare professional before using.

2.10 GRAS of Alumosilicates

Comparisons to silicate substances generally recognized as safe (GRAS) defined by the FDA are reasonable with the sodium alumosilicate (CFR: 182.2727, as well known as Zeolite A [Hera 2004, Common Chemistry 2012]) and the hydrated sodium calcium alumosilicate (CFR: 182.2729; a subtype of zeolite A) as these minerals - beside a comparable composition - hold a similar crystal structure. Both analogues however are synthetic and have differing oxide ratios. The sodium aluminum silicates have Na_2O:Al_2O_3:SiO_2 oxide ratios of approx. 1:1:3, whereas Clinoptilolite has approx. 1:10:64. Considering the remaining alkaline and earth-alkaline elements that compose the Clinoptilolite not solely Na_2O, it results in a (K_2O+Na_2O+CaO):Al_2O_3:SiO2 ratio of 1:1.5:9. The structural

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relationships are given by cage-like structures of both the synthetic alumosilicates, and G-Science purified Clinoptilolite, forming porous crystals, which allow an uptake and exchange of ‘guest’ ions. A comparison to the other GRAS silicates cannot be drawn due to different chemical (no or insufficient aluminium like for example calciumsilicate, CAS 1344-95-2) and structural properties. [Baerlocher 2007, Lobo 2003]

The main difference between the synthetic GRAS silicates and G-Science purified Clinoptilolite is that Clinoptilolite is of natural origin, contains not only sodium but also potassium, calcium and magnesium, and the stability in acid environment is very high, compared to synthetic analogues.

3 Supportive information

3.1 Chemistry/Identity

3.1.1 Physical Properties of Clinoptilolite

The physical properties for Clinoptilolite from the Slovakian quarry are summarized in Table 1.

Items	Description
CAS No.	12173-10-3
Color/Form	grey-green
Odor	none
Physical state at 20°C	solid
Solubility in water	none
Melting point	1340°C
Flow temperature	1420°C
Ignition temperature	settled dust – neg. up to 600°C raised dust – neg. up to 800°C
Flash point	up to 600°C neg.
Explosive limits (volume % in air)	non-explosive
Specific gravity	2200 - 2440 kg/m^3
Density	1600 - 1800 kg/m^3
Porosity	24 - 32%
Pore diameter	0.4 nm (4 Å)
Mohs hardness	1.5 - 2.5
Specific surface (BET)	30 - 60 m^2/g
Thermal stability	up to 400°C

Table 1 Physical properties of Clinoptilolite from the Slovakian quarry [IZA 2010, Vatalová 2016]

3.1.2 Dissolution/pH stability

Clinoptilolite is not soluble in water and is highly resistant to acid environments, which is very important if applied to the human body. A comprehensive study of the effect of pH on the chemical stability of Clinoptilolite was conducted by Li et al. The authors exposed Clinoptilolite samples to aqueous solutions of nitric acid at a pH range between 1 to 5 for durations of 2, 12, 24, 48, 72, or 144 hours. The pH was maintained constant throughout the duration of the experiment. At each time-point samples of the supernatant were measured for concentrations of the major elements Al^{3+}, Si^{4+}, Na^{+}, and K^{+} for evidence of acid leaching using inductively coupled plasma mass-spectrometry (ICP-MS). Samples treated at each pH value for 144 hours also were evaluated for evidence of qualitative changes in chemical structure or mineralogical morphology using XRD, energy dispersive X-ray fluorescence (ED-XRF) and transmission electron microscopy (TEM). The authors found that no significant quantities of Al^{3+}, Si^{4+}, Na^{+}, and K^{+} were leached out of the material at pH values of ≥ 2. X-ray diffraction patterns demonstrated that the crystallinity of Clinoptilolite is highly resistant to pH as no effects on the major peaks within the X-ray diffraction pattern were observed even at a pH of 1 over 144 hours. The high stability of the material also was reflected in the absence of significant changes in TEM (transmission electron microscopy) morphology at pH ≥ 2. Findings from this study demonstrate that dissolution of G-PUR, during gastric transit, is not expected. [Li 2008]

3.1.3 G-PUR Raw Material

The G-PUR raw material is obtained exclusively from the Nižný Hrabovec volcanic tuff deposit, in eastern Slovakia. The deposit is characterized by the stable occurrence of Clinoptilolite (mostly K and Ca) of extremely high purity and homogeneity. The raw material, before being used for manufacturing G-PUR, is inspected optically for Mn/Fe-oxide crusts and tested by elemental analysis (ICP-MS) for inorganic impurities; the qualitative identity of the raw material is assured by X-ray diffraction (XRD) and analysis of the ion-exchange capacity (IEC). A typical chemical composition is summarized in Table 2.

Parameter	%
SiO_2	65 - 75
Al_2O_3	9.5 - 14
CaO	2.0 - 5.6
MgO	< 1.3
Na_2O	0.2 - 2.0
K_2O	1.5 - 4.5

Table 2 Typical major elemental composition of G-PUR raw material

3.1.4 Quality Control/Identity Verification of G-PUR

It is important to emphasize that a complete characterization of any Clinoptilolite before use in the biomedical field is a prerequisite. Clinoptilolite raw materials may be ‘‘different’’ depending on where they are mined and they cannot be proposed for any use as being identical. Moreover, standardization is necessary and suitable criteria must be defined and followed for each specific application. These must include the mineral composition and absence of undesired minerals, the exact particle size distribution and milling technique, the elemental composition with emphasis on heavy metal content and the functionality by measuring the ion exchange capacity. [Colella 2011]

The quality and identity of G-PUR is established by the following specific analytical characteristics:

- Determination of the elemental composition. Method: ICP-MS after pressure acid digestion.

- Determination of the crystalline constituents. Method: X-ray diffraction.

- Determination of the particle size distribution. Method: laser diffraction.

- Characteristic reaction: determination of the specific ion exchange capacity (by ion chromatography).

Identification via NMR, UV or mass spectrum is not possible and/or conclusive due to the specific properties of Clinoptilolite.

3.2 Manufacturing Process

All incoming lots of raw material are inspected optically for impurities and Mn/Fe- oxide crusts. The raw material is then processed with the G-Science proprietary, patented purification process to minimize the – naturally occurring – impurities of the raw material. The processing results in the desired depletion of the unwanted impurities. No mineralogical differences regarding the crystalline structure are observed between the un-processed raw material and the purified material (G-PUR). Similarly, the purification process alters the ion exchange capacity of the material, a qualitative and very important characteristic, insignificantly.

At the end of the production process, G-PUR is milled to a mean particle size of 3.1 µm (d50). The special milling technique, unlike techniques that use a grinding media like a ball mill, guarantees minimal damage to the crystal structure of G-PUR. Additionally, it adds almost no contamination to the milled product, as there is no wear of grinding balls etc. All

necessary energy is delivered by clean compressed air, the particles are micronized by particle-particle collisions. The mean particle size of 3 microns ensures a maximum efficacy concerning the active surface of G-PUR. Finally, G-PUR is processed to an ultimate heating step. The bulk is filled into food grade plastic bins, sealed, shipped to the US and packaged into stick pack by a certified contract manufacturer.

3.2.1 Process Controls

Manufacturing of G-PUR is performed according to processes defined in so called standard operating procedures. All stages of production, as well as, all media that get in contact with G-PUR during production are of certified, high-grade quality. The production process is closely monitored by a certified quality-management system and a highly sophisticated lab with validated, state-of-the-art chemical, microbiological and mineralogical analysis devices and methods, to verify compliance for all relevant quality parameters of the final product.

3.2.2 Quality Management

Documentation of the entire production is supervised and controlled by an ISO 9001 certified quality management system. During the production process, more than 30 checklists are filled out, consistently documenting the whole production process, from the incoming goods inspection to the shipping. Traceability down to the exact position of the origin of the raw material in the quarry can be guaranteed for every batch of G-PUR. Production equipment has been fully qualified with a group of external specialists to fulfil all regulatory requirements that apply.

3.2.3 Shelf Life

G-PUR consists of a mineral that is absolutely stable against normal degradation. To guarantee microbiological stability, the humidity is strictly controlled as part of the product specifications, as well as, testing the absence of E.coli and limiting the maximum cfu (colony forming unit) per dose. Microbiological control is not only restricted to the product itself, but also performed for the surrounding environment (air, surface etc.) and the media (compressed air, water) that comes into contact with G-PUR. Furthermore, G-PUR offers little to no nutritional value for growth of microorganisms. A stability study has been performed and resulted in a minimum shelf life of 3 years.

4 Intended use – Health Benefits*

4.1 Binding of Dietary Cholesterol

4.1.1 Preface

According to published scientific literature, zeolites do adsorb sterols. Zeolites have a particular affinity for molecules with a permanent dipole, and sterols have a dipole due to the -OH group in the ring system. Ground Clinoptilolite bears a charged surface of several hundred square feet per gram, and polar molecules that are big enough to prevent them from entering the pores can be adsorbed onto this surface. The complex with cholesterol shows a very high stability, as described by Berezin et al. In an artificial matrix, adsorption of cholesterol by zeolite appeared to be irreversible. Adsorption proceeded until there was either no sterol left in solution or the zeolite was saturated. Furthermore, treating the saturated zeolite with fresh hexane removed no sterol; hot ethanol had to be used for desorption. Though the Zeolite described by Berezin wasn't Clinoptilolite but a synthetic Zeolite, structural similarities justify the extrapolation to Clinoptilolite.* [Berezin 2001, Colella 2011, Mumpton 1985]

4.1.2 Animal data

In a study with rabbits, a significant interaction could be detected between dietary cholesterol and G-PUR.* 12 New Zealand white rabbits were divided into 4 groups (n = 3) and were fed according to the following scheme:

Group	Days 1 - 7	Days 8 - 21
1	Normal diet	Normal diet
2	Cholesterol diet + 1.5% Chol	Normal diet
3	Cholesterol diet + 1.5% Chol	Cholesterol diet + 1.5% Chol
4	Cholesterol diet + 1.5% Chol	Cholesterol diet + 1.5% Chol + 10% Clinoptilolite

Table 3 Study group assignment

The control-diet (ssniffV2333-000) contained very little cholesterol (0.4 g per kg feed based on dry weight); whereas the cholesterol diet was made up of a special feed (ssniff-pellets, 4 mm, SM K-H, item S8078-S020) with a comparable concentration of dietary fibres (13.3% compared to 14.9% in the control diet), the addition of 7% peanut oil, and 1.5% cholesterol.

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Study group 1 received the normal feed (control diet) during the whole test period. Group 2 received the cholesterol diet on days 1 to 7, then on days 8-21, group 2 was switched back to the normal diet (control diet). Groups 3 and 4 received the cholesterol diet throughout the entire study period, with group 4 also receiving 10% clinoptilolite (by weight) as a supplement to the cholesterol diet from days 8-21. Researchers collected blood samples from the animals on days 1, 5, 8, 10, 12, 19.

After an administration period of 21 days, the animals were sedated then euthanized by means of pentobarbital-Na. The researchers conducted histological examinations of the animals, included the assessment of the lungs, heart, small intestine (incl. Peyer patches), liver, spleen and kidneys. In addition, a collective faecal sample was taken for a period of several days and, inter alia, was analysed for the cholesterol content. Below are shown the results of the serum analysis on day 21 of the study:

Parameter	Normal Diet	Cholest. Diet + Normal Diet from Day 8	Cholest. Diet + Clinopt. from Day 8	Cholesterol Diet
HDL (mg/dl)	44.5	175.6	126.0	562.0
SD (mg/dl)	4.6	48.6	32.2	241.5
LDL (mg/dl)	24.5	650.9	538.1	1708.2
SD (mg/dl)	5.6	153.0	90.9	656.4
Trigly (mg/dl)	47.8	88.8	106.2	224.1
SD (mg/dl)	3.8	33.7	52.3	92.6
Cholest (mg/dl)	67.3	753.3	622.2	2335.0
SD (mg/dl)	1.4	168.2	96.5	929.6

Table 4 Cholesterol/LDL/HDL and Triglyceride serum concentrations of all study groups on day 21 (mean & standard deviation SD)

Based on the comparison of group 4 with the group 1 control and group 2 which switched back to a normal diet on day 8, it is obvious that the addition of Clinoptilolite to the feed reduces the bioavailability of dietary cholesterol and/or the dietary cholesterol resorption significantly and subsequently influences the biochemical markers of cholesterol supplementation.* LDL, HDL and Triglycerides did not increase as much in group 4 due to

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the supplementation of Clinoptilolite compared to the high Cholesterol diet of group 3 without the Clinoptilolite supplementation.*

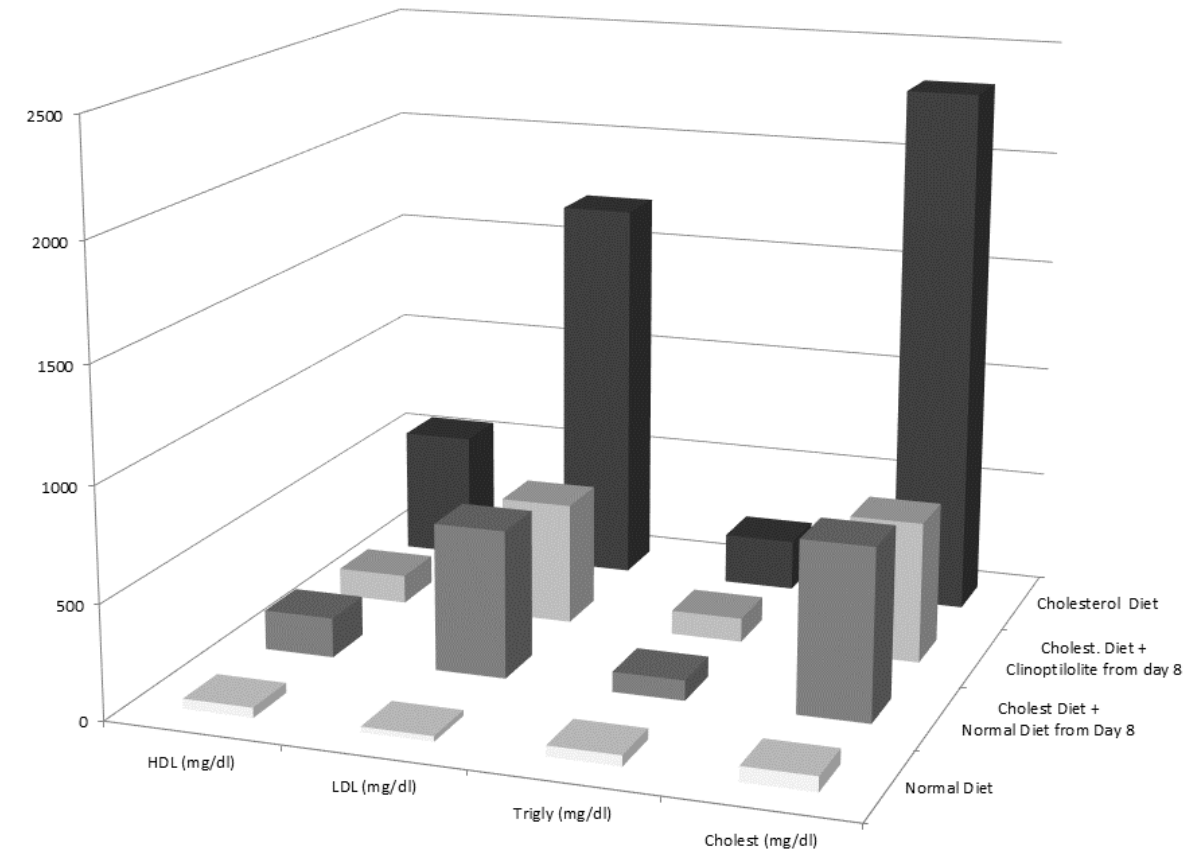

Figure 4 Cholesterol/LDL/HDL and Triglyceride serum concentrations of all study groups on day 21

The illustrated cholesterol/LDL/HDL and/or triglyceride concentrations of group 2 which received normal feed after day 8 and group 4 which received the feed fortified with cholesterol plus the 10% Clinoptilolite supplementation, cannot be differentiated from each other with statistical significance ($p > 0.05$). With the addition of Clinoptilolite, the serum cholesterol markers did not appear to rise despite the increased cholesterol consumption, suggesting the Clinoptilolite was not allowing the dietary cholesterol to be absorbed by the body. In other words, continuing with a high cholesterol diet with the addition of Clinoptilolite resulted in the reduced bioavailability of cholesterol and subsequently no further increase of the relevant serum parameters.* [Losert 2004]

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This is further substantiated as the cholesterol analysis from the faeces (data not shown) revealed that the analysis method, which normally involves an extraction step, could not recover all of the extra cholesterol that was applied with the fortified diet. This might be due to the very strong cholesterol-Clinoptilolite interaction that may even resist to desorption with solvents.* [Folch 1956, Lee 1994, Berezin 2001]

On the basis of the above results, a reduction of the bioavailability of dietary supplied cholesterol by addition of Clinoptilolite to a high cholesterol diet is supported.*

4.1.3 Lab data

The following G-Science lab data supports the idea that Clinoptilolite helps bind dietary cholesterol in the digestive tract (even in a high fat matrix) so it cannot be absorbed by the body, as a very strong interaction between cholesterol and Clinoptilolite is formed that is extremely stable.*

Laboratory tests were conducted to study the interaction of Clinoptilolite with cholesterol in a vegetable oil matrix doped with cholesterol. Cholesterol content was determined before and/or after incubation of the vegetable oil matrix doped with Clinoptilolite.

total cholesterol quantity [mg]	adsorbed by Clinoptilolite [mg]	adsorbed percentage [%]
23.3	11.5	49.3
46.5	19.1	41.1
116.3	30.8	26.5
232.7	39.9	17.2
465.3	69.4	14.9
2326.7	322.9	13.9

Table 5 In-vitro cholesterol adsorption per 1g G-PUR

The results clearly show that even in a complex, high-fat matrix there is a significant interaction between G-PUR and cholesterol.*

In order to verify the robustness of the adsorption of cholesterol on Clinoptilolite, in other complex matrices, experiments were carried out with bovine serum that was brought into contact with Clinoptilolite and incubated at 37°C. Bovine serum contains a high amount of

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protein, lipids, carbohydrates, amino acids and electrolytes. After subsequent separation of the Clinoptilolite, relevant clinical parameters were analyzed from the Clinoptilolite/serum samples, as well as from a control without Clinoptilolite, in order to examine the specific influence of Clinoptilolite in this matrix.

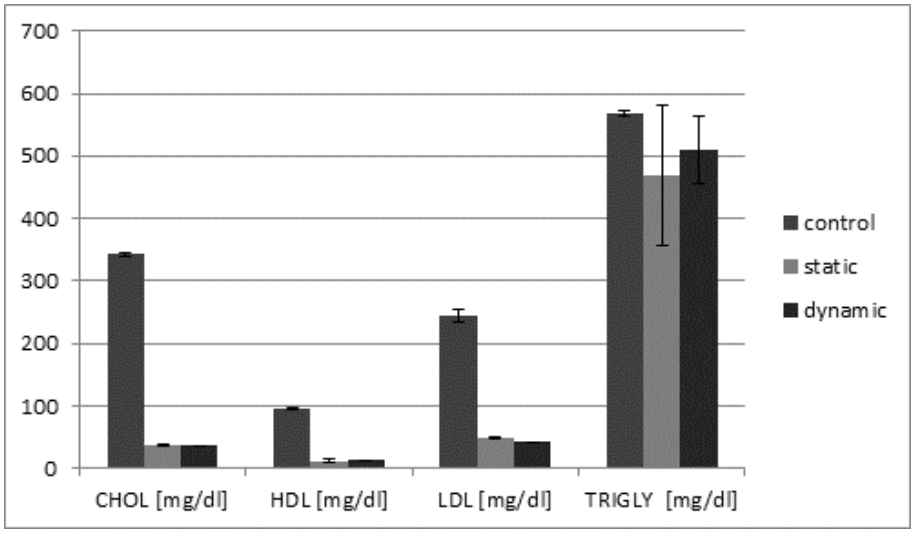

Figure 5 Lipid concentrations after incubation of bovine serum with G-PUR

For the parameters total cholesterol, HDL and LDL, a statistically significant reduction in serum concentration was found in the serum that had been exposed to Clinoptilolite when compared to the control bovine serum sample. This could be observed for a static, as well as a dynamic model with regular mixing of the sample by means of a rotator. These results clearly show that specific adsorption of cholesterol by clinoptilolite occurs, even in a highly complex matrix like bovine serum.*

Due to the technical feasibility, both experiments were carried out with significantly bigger particle sizes than those of G-PUR. Taking into consideration that the effect of G-PUR to bind cholesterol is mainly a surface effect, it could be expected that with smaller particle size/larger surface area this effect is even more pronounced for finer particle sizes.*

Berezin conducted in vitro studies to analyze the interaction of zeolites with sterols. An artificial mixture of different sterols was mixed with zeolite and the interaction was examined. In particular for cholesterol, Berezin was able to prove the amount of cholesterol in the

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mixture was reduced after mixing with zeolite. Furthermore, within the closely analogous sterols, the affinity to cholesterol was the greatest. The interaction between the sterols and the adsorbent was so strong that desorption of the adsorbed sterols with fresh Hexane was not possible and succeeded only with the addition of hot ethanol. Stability of the sterol after contact with zeolite was verified by subsequent analysis of the sample medium.*

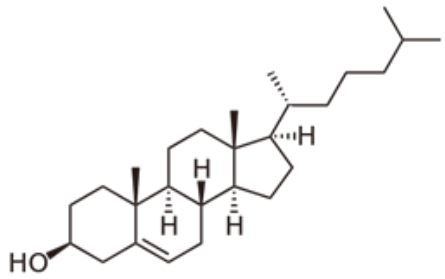

Figure 6 Cholesterol structural formula

The fact that zeolite can adsorb sterols is due to the affinity of the zeolite for molecules with a permanent dipole, as is inherent for instance with sterols of the OH group. This can also be extrapolated to the structurally similar Clinoptilolite that also has a charged surface and a high affinity to adsorb substances with a dipole.* [Mumpton 1985, Sprynsky 2008, Colella 2011, Berezin 2001]

Summarized, the totality of in-vivo and in-vitro data undoubtedly suggests that addition of G-PUR to diet might significantly reduce bioavailability of dietary cholesterol in the digestive tract.*

4.2 Binding of Heavy Metals*

4.2.1 Animal Data

Pond et al. evaluated the effect of Clinoptilolite and a synthetic Zeolite (NaA) against Cadmium- induced anaemia in swine. For 31 days, swine aged 4-5 weeks were administered a diet that contained 94 ppm Cd (WHO guideline for drinking water is 3 ppb) as $CdCl_2$. The Clinoptilolite administered at a dose of 3% (w/w) to feed originated from Castle Creek, Idaho, and consisted of 66% Clinoptilolite. Particle size was smaller than 50 mesh or < 300 microns. Liver Cd concentration was increased dramatically ($p < 0.01$) by dietary Cd,

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while haematocrit and haemoglobin were significantly reduced after 28 days of Cd administration, showing that the dietary Cd was highly bioavailable. Livers of pigs fed Clinoptilolite and Cd had significantly lower Cd concentrations than those of pigs fed Cd alone ($p < 0.01$).*

There was also a tendency for Clinoptilolite to lower the concentration of Cd in the kidney, but this effect failed to reach statistical significance. Another observation made indicated the Fe content did not decrease in swine consuming Clinoptilolite with the Cd diet. This supports the theory that some of the dietary Cd may have been adsorbed to the Clinoptilolite matrix in accordance with the known ion-binding capacity. Its protection from Cd absorption implies maintenance of appreciable integrity of the crystal structure and Cd binding capacity into the duodenum where most Fe absorption occurs. The failure of Clinoptilolite to affect plasma concentrations of urea-N, K, Na or Mg suggests no major shift in plasma electrolyte compounds due to the administration of Clinoptilolite.* [Pond 1983]

The fact that in this study Clinoptilolite administration has not been able to significantly lower the Cd accumulation in the kidney may be due to the very high concentration of Cd in the feed. Besides that, Clinoptilolite clearly prevented dietary Cd from being absorbed in a pig animal model.*

The binding of heavy metals with the subsequent reduction of their bioavailability is described by Beltcheva et al. in two publications. Reduction of bioavailability was examined after an administration period of 90 days via the lead concentration of different organs of mice and concurrent administration of drinking water with 0.005 M of lead nitrate solution with the addition of Clinoptilolite in feed. The lead concentration in the examined organs (carcass, liver, kidneys, bones) and also the feces could be reduced by between 77% and 90% with the addition of Clinoptilolite, implying that Clinoptilolite reduced the bioavailability of the dietary lead. Additionally, results of the feces analysis suggest that a large amount of the Pb administered was bound to the Clinoptilolite in feed and could not be recovered with the analytical method used. As the digestion mixture for analyzing organs consisted of nitric and perchloric acid without the addition of hydrofluoric acid, it seems quite reasonable that feces samples with a high content of inorganic Clinoptilolite could only be partially extracted instead of completely dissolved, resulting in much lower recovery rates. This further substantiates the theory that interaction between heavy metals and Clinoptilolite can be very strong, significantly reducing bioavailability in the digestive tract.* [Beltcheva 2012, Topashka-Ancheva 2012]

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In another study, ICR mice were held under identical conditions and administered lead in their diet, with some groups also receiving supplementary clinoptilolite. After 90 days of treatment, a 2.3-fold reduction of the occurrence of chromosomal aberrations was observed in the mice that consumed a diet fortified with lead and received clinoptilolite supplementation when compared to mice that consumed the lead-fortified diet but did not receive clinoptilolite; one by a 2.5-fold increase of the mitotic index, one by a 1.5 factor increase of normal erythrocytes, and one by a 1.3 factor increase in body weight. On day 90, the mean kidney Pb concentration in group 4 (receiving Pb & Clinoptilolite) was 11-fold lower than that in group 3 (receiving only Pb). Respectively, on day 90, the Pb concentration in bones of the mice from group 4 was 4.3-fold lower compared to Pb concentration in bones of mice from group 3, clearly indicating that less dietary Pb was bioavailable in group 4.*

Chromosomal aberrations, involving gross alterations of genetic material, have been considered a sensitive endpoint for detecting genotoxic effects induced by heavy metals and toxic chemicals. Thus, the study of cytogenetical status is considered highly relevant in the human context. In the experiment described above, the increased percentage of aberrant cells in the mice from group 3 is likely due to the very high concentration of bioavailable Pb.* [Pottier 2013, Garcia-Leston 2010]

Micronuclei in erythrocytes of peripheral blood were observed in the specimens from groups 3 and 4. In group 4, they appeared sporadically mainly before day 45. This fact indicates that the main part of Pb entering in the mouse organism via drinking water has been captured by the Clinoptilolite sorbent, and thus, the Pb quantity absorbed by the intestine mucosa and utilized by the organism at the cellular level had been significantly reduced. *

In summary, chromosome aberrations, lowered mitotic index, pathologically changed erythrocytes, diminished erythropoiesis, and reduced body weight gain of the Pb-exposed laboratory mice demonstrated a well-expressed toxicological stress due to the chronic exposure to bioavailable Pb. The structure of chromosomes and red blood cells as well as the mitotic index and erythropoiesis was significantly improved by Clinoptilolite supplementation. The time course of the body weight in the Clinoptilolite and Pb supplemented animals did not differ significantly from that in the control mice. The authors concluded these differences were a result of reduced amounts of Pb entering the blood, organs, and bone marrow of the Clinoptilolite-supplemented mice due to the high ion exchange capacity of the administered

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Clinoptilolite – suggesting that bioavailability of Pb has been significantly reduced by the supplementation of Clinoptilolite.* [Beltcheva 2012, Topashka-Ancheva 2012]

Due to their ion-exchange capacity, Pb bioavailability and Pb resorption could be reduced by adding Clinoptilolite to the diet, to maintain a healthy body system.* [Papaioannou 2005]

4.2.2 Lab Data

Numerous studies suggest that Clinoptilolite has a high affinity for heavy metals, and therefore, can be used to treat different matrices (wastewater, soil, drinking water etc.) to reduce the concentration of bioavailable heavy metals. [Shasheem 2012, Sharifipour 2015, Wingenfelder 2005, Erdem 2004, Ghasemi-Fasaei 2012, Kragović 2012, Minceva 2007, Elizalde-González 2001a/b, Faghihian 1999, Vaca Mier 2001, Delkash 2015, Puschenreiter 2005, Chojnacki 2004, Dursun 2007]

Buasri et al. investigated the ability of Clinoptilolite to remove lead from aqueous solutions. The results clearly showed that adsorption onto Clinoptilolite is a very rapid process, even in an acidic pH environment at elevated temperature. At a concentration of 2 g Clinoptilolite per 100 ml, even at pH 2 adsorption capacities reached > 30 mg lead per g Clinoptilolite for a very coarse grain size of 75-150 µm. For a concentration of 100 ppm lead, adsorption onto Clinoptilolite was complete after 30 minutes contact time. Coarser particle size resulted in a decrease in adsorption capacity. When compared to other adsorbents like clay, organic adsorbents or activated carbon, Clinoptilolite adsorption capacity was comparable and within the same order of magnitude.* [Buasri 2008]

Given the fact that G-PUR has a much finer particle size than the 75-150 µm used, effects may be even faster with G-PUR compared to those observed by Buasri. Adsorption efficacy at pH 2 is important to further verify that effects are also taking place in an acidic environment.*

In a study of Petrakakis et al. the leachability of lead (Pb^{2+}) from natural Clinoptilolite of Greek origin, batch-loaded with lead, was investigated according to standard procedures. Subsequently, the influence of pH, initial load of lead in Clinoptilolite, agitation rate, temperature and particle size on kinetics of lead leaching was also studied. Fractions 1.4-2 and 5-6 mm were used in the study. Sorption was fast during the first few hours and equilibrium was practically attained after 24 h. The loaded samples had 12-103 mg Pb^{2+}/ g Clinoptilolite. For leaching experiments, samples used contained typically 30 mg Pb^{2+}/ g Clinoptilolite. At pH1 (HCl), more than 18% of the lead could be leached from the (pre-loaded) Clinoptilolite. At

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higher pH, leaching rates dropped below 1%, showing high stability of the lead-Clinoptilolite complex at pH 2 or higher. Lead solubilization experiments were also performed with water (tap or deionized) at 30°C for 24 h and in all cases less than 0.02% of the Pb initially loaded was detected in the solution. Particle size did not appear to significantly alter the leachability of lead from pre-loaded Clinoptilolite.* [Petrakakis 2007]

These results suggest the lead-Clinoptilolite complex can be characterized by a very high stability in an environment at pH > 3. Due to the high pH in the human intestine, release of the adsorbed Pb within the intestine seems highly unlikely.*

Batch adsorption kinetic and isotherm studies were conducted by Payne et al. to compare and evaluate different types of adsorbents for lead ion removal from aqueous media. The effects on lead ion absorption from pH changes, competing ions, and temperature increases were also investigated. Besides natural Clinoptilolite, several other natural and synthetic adsorbents were investigated. Natural Clinoptilolite had a surface area of 40 m^2/g and a Si:Al ratio of 6:1. The relative rate for lead ion adsorption was: 13X > Chabazite > Clinoptilolite > 5A > activated carbon. Clinoptilolite Pb removal rate increased from 40% to > 80% if pH was raised from pH 2 to > pH 3 and was always > 80% for pH > 3. Ionic competition reduced lead ion removal by the zeolites: compared to DI water with an adsorption rate of almost 80%, addition of 0.01 M KNO_3 or 0.1 M KNO_3 reduced effectivity to 50% and 37% respectively. Increasing temperature from 23°C to 35°C significantly improved adsorption performance for clinoptilolite.* [Payne 2004]

Despite the fact that increase in temperature also increase the adsorbent performance, it is worth noting that even for a matrix with elevated levels of other cations like potassium, there was still a significant interaction between Clinoptilolite and lead, resulting in a reduction of available Pb in solution.*

Tests under standardized lab conditions, as well as verification of these results in animal models, indicate that G-PUR might interact with heavy metals, resulting in stable complexes that can significantly reduce bioavailability of dietary heavy metals in the digestive tract.*

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4.3 Reduction of the Bioavailability of Mycotoxins*

Mycotoxins are metabolic byproducts of molds which – even at a very low dose – can be toxic to invertebrates. Many regulatory authorities throughout the world have established low tolerances for mycotoxin contamination in food and feed in order to provide an adequate protection against the potential negative effects of these toxins in animals and humans. This is highly important as, due to their very high stability, even processing of food (i.e. cooking) does not destroy most of the mycotoxins, so they may accumulate in various sources and find their way into the human diet. [Bullermann 2007, Bosco 2012, Marin 2013]

4.3.1 Clinical Data*

An application of clay minerals for detoxification and/or reduction of the bioavailability of aflatoxins has been shown to be safe in phase 1 and phase 2 human studies. The calcium-montmorillonite used in the study is also an aluminosilicate, like Clinoptilolite, but it belongs to the group of phyllosilicates. Both substances consist mainly of the elements aluminum and silicium, but are different in their structure. However, concerning their principal sorptive properties and their basic affinity for aflatoxins, the materials are comparable.*

In a 3-month, double-blind and placebo-controlled, phase II clinical trial in adults, dosages of 1.5 g (LD) or 3.0 g (HD) of the clay material per day showed a statistically significant reduction of biomarkers of the aflatoxin burden of the test subjects. AFM1 levels in urine samples were analyzed, as well as AFB1 albumin adduct in serum. Levels of urinary AFM1 and serum AFB1 albumin adduct have shown significant correlation with dietary intake of aflatoxins. AFB1 albumin adduct is also the most reliable molecular biomarker for studying human exposures to Aflatoxins. About 2% of the ingested AFB1 is reported to be covalently bound to serum albumin. For both the low and the high dose group, AFB1 albumin adducts in serum significantly decreased after 3 month. This delay is probably attributable to the long half-life of albumin, which is estimated to be approximately 3 weeks in normal and healthy people. However, statistically significant decreases in median AFM1 levels were observed at 3 months after intervention ($p < 0.05$). While the median AFM1 level was comparable between the placebo (PL) group and the LD group, a reduction rate of 58.7% in the median AFM1 level was found between the HD group and the PL group ($p < 0.05$). Due to the sorptive properties of the silicate it was able bind the aflatoxin occurring in food within the digestive tract and thus withdraw it from the metabolism of the test subjects. A very wide range of AFM1 levels (from undetectable to 13.3 ng/mg creatinine) was observed in the study participants. The authors

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stated this may be attributed to seasonal changes in food contamination in the region, as well as genotypic or phenotypic variations in AF-metabolizing enzymes and individual susceptibility. Nevertheless, significant dose–time interaction effects associated with reduced urinary AFM1 levels in this study confirmed the efficacy of the clay test substance. No significant differences could be detected concerning hematological parameters, liver and kidney function or electrolyte concentration. Sporadically there were differences in the serum parameters, which nevertheless were within in the normal physiological range and did not follow a dose-dependent trend. Side effects associated with the administration were not observed.* [Afriyie-Gyawu 2008, Phillips 2008, Wang 2008, Wang 2005]

The route of administration (oral) and the mechanism of action (adsorption on a silicate that stays inside the digestive tract) are comparable to those described in animal studies with Clinoptilolite.* [Gallo 2010, Masoera 2009, Bulut Albayrak 2012, Grenier 2015, Oguz 2000b]

4.3.2 Animal Data

In a study by Ortatatli et al., chickens were administered a mixture of several Aflatoxins (consisting mainly of Aflatoxin B1) in feed (50 ppb or 100 ppb total aflatoxin) for 41 days. A test group of chickens received the contaminated feed as well as Clinoptilolite supplementation at concentration of 1.5 % (w/w). Macroscopic changes could not be observed in any group, as the levels of AFB1 applied were relatively low, which is in agreement with observations of other authors conducting similar studies. Nevertheless, addition of Clinoptilolite to the 100 ppb group partially but significantly decreased both the incidence of affected broilers and the severity of the lesions in the organs investigated. The beneficial effect of Clinoptilolite in this study might be attributed to the high affinity of Clinoptilolite for AFB1 and the resulting reduction in bioavailability of the dietary aflatoxins. Subsequently, AFB1 couldn't be absorbed in the gastrointestinal tract and effects associated with bioavailable AFB1 were less pronounced. * [Ortatatli 2005]

Oguz et al drew comparable results with studies regarding the effect of Clinoptilolite on chickens. According to the author, one approach to solve the problem of AF in the feed has been to use nonnutritive and inert adsorbents in the diet to bind AF and reduce their absorption from the gastrointestinal tract. These adsorbents must not be absorbed from the gastrointestinal tract and must have the ability to bind physically with chemical substances,

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precluding their absorption. Besides others, Clinoptilolite has been used as such an adsorbent in animal feeding. In the study described, Clinoptilolite incorporated into the diet at 1.5 and 2.5 % was evaluated for its ability to bind AF and reduce the deleterious effects of 2.5 mg total aflatoxin (more than 75% Aflatoxin B1) per kg diet on broiler chickens from 1 to 21 days of age. In total 360 broiler chicks were divided into six equal treatment groups (control, AF, Clinoptilolite (1.5 %), AF plus Clinoptilolite (1.5 %), Clinoptilolite (2.5 %) and AF plus Clinoptilolite (2.5 %)). When compared to controls, AF treatment significantly decreased serum total protein, albumin, inorganic phosphorus, uric acid, total cholesterol, haematocrit, red blood cell counts, mean corpuscular volume, haemoglobin, thrombocyte counts and percentage of monocyte counts, and increased values of white blood cell and heterophil counts. The addition of Clinoptilolite (1.5 %) to the AF-containing diet improved the decrease in thrombocyte counts caused by AF, most likely by decreasing the bioavailability of AF. Furthermore, by addition of Clinoptilolite (1.5 %) to the AF-containing diet and subsequent reduction of the bioavailable AF, the AF-related changes in red blood cell counts, haematocrit, haemoglobin, white blood cell counts, percentage of lymphocytes and monocyte counts were intermediately ameliorated.* [Oguz 2000a]

Safameher showed that changes of the serum parameters in chickens, caused by the addition of aflatoxins, are significantly reduced with the feeding of 2% Clinoptilolite. According to the author, Clinoptilolite (CLN) as an inert sorbent reduces the absorption of dietary mycotoxins from the gastrointestinal tract. Besides others, Clinoptilolite is preferred as a binding agent due to its high binding capacity against AF and its reducing effect on AF absorption from the gastrointestinal tract. In the actual study, Clinoptilolite was added to diets containing Aflatoxins (AF) for 41 days. A total of 480, one-day-old broiler chicks (Ross 308) was divided into 6 treatments, each consisting of 80 chicks: control; basal diet prepared with uncontaminated diet, control containing AF (0.5 mg/kg), control containing AF (1 mg/kg), control containing Clinoptilolite (20 g/kg), control containing AF (0.5 mg/kg) + Clinoptilolite (20 g/kg) and control containing AF (1 mg/kg) + Clinoptilolite (20 g/kg). Chickens were monitored daily and then body weight and feed consumption were recorded. Plasma enzymatic and non-enzymatic parameters showed progressive changes in birds examined 21 or 42 days after dietary aflatoxin treatments. Compared to controls, the AF treatment significantly ($p < 0.05$) decreased body weight gain, feed intake, serum total protein, albumin, cholesterol, uric acid, calcium, phosphorus, alkaline phosphatase (ALP) while the enzymatic parameters Lactate

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Dehydrogenase (LDH) and aspartate amino transferase (AST) increased. Decreases in serum parameters caused by AF was significantly ($p < 0.05$) ameliorated by Clinoptilolite. A similar increase was obtained in feed intake and body weight gain by adding Clinoptilolite to the AF-containing diet ($p<0.05$). The addition of Clinoptilolite to the AF-containing diet decreased the severity of negative effects of absorbed dietary AF. The results suggest that Clinoptilolite positively influenced several parameters due to aflatoxin not entering the body but being bound to Clinoptilolite in the digestive tract.* [Safameher 2008]

Milk is prone to Aflatoxin contamination as there is a carry-over from feed to milk at a rate of 1-6%. Katsoulos et al showed under field conditions that by adding 1% (w/w) of Clinoptilolite to feed of dairy cattle for a period of seven days, there is a clear and significant reduction of the Alfatoxin level in the milk produced. Fifteen commercial Greek dairy herds with AFM1 concentrations in bulk tank milk ≥0.05 μg/kg (caused by consuming feed rations naturally contaminated with AFM1) were selected. Participating farms had to meet the inclusion criteria for the difference between bulk tank milk AFM1 concentration at the initial measurement and at the onset of the experiment (3–10 days later) not being more than 10 %. Bulk tank milk AFM1 was determined prior to the onset and on day 7 of the experiment. The duration of 7 days was considered to be an adequate study period given that it is about double the clearance time after the removal of aflatoxins of the dairy cattle diet which is about 3–4 days. Clinoptilolite was added in the total mixed rations of all farms at the rate of 200 g per animal per day, throughout this period. Besides the addition of Clinoptilolite, rations remained unchanged during the whole experiment. Two different particle sizes of Clinoptilolite were used: one was below 150 μm and another below 800 μm (mean particle size of G-PUR is 3 μm). Especially for the finer Clinoptilolite particle size, there was a linear correlation between the Aflatoxin levels before and after 7 days of continuous Clinoptilolite application. For the 15 different farms that were included in the study, the mean Aflatoxin level reduction in milk by addition of Clinoptilolite to the feed for one week was more than 50% of the initial Aflatoxin level before Clinoptilolite administration and reached a statistical significance of $p < 0.001$. Furthermore, there was a significant and strong linear correlation among the milk AFM1 concentrations on Days 0 and 7 ($R = 0.95$, $P < 0.001$, $r^2 = 0.899$). The authors state that the results obtained prove efficacy of dietary administration of Clinoptilolite under field conditions and that Clinoptilolite is effective in reducing the milk concentration of AFM1. The criteria of farm selection were set in order to ensure, as much as possible, that the milk AFM1 concentration

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on day 0 represents a stable situation of high milk AFM1 and that the reductions detected on day 7 are due to the infeed inclusion of Clinoptilolite.* [Katsoulos 2016]

The average relative reduction of milk AFM1 recorded in this study, although not comparable with other experiments due to different study design, was similar to those achieved with other aluminosilicate mycotoxin binders; Diaz et al. observed that calcium and sodium bentonite products reduce milk AFM1 concentrations by 31 % to 65 %, most likely due to reduction of bioavailability. Kutz et al. observed that two hydrated sodium calcium aluminosilicates decreased the AFM1 in milk by 45 and 48 %.* [Diaz 2004, Kutz 2009]

This may also be used as additional evidence that studies which used other silicates like bentonite or hydrated sodium calcium aluminosilicates (HSCAS) may also be applicable for Clinoptilolite, as the mechanism of action is comparable, as both substances are quite inert and show a high affinity for AF.*

Furthermore, it clearly indicates that finer particle sizes are more effective in adsorbing dietary aflatoxins than coarser particle sizes. A smaller particle size provides a larger surface that is available for interaction with the polar mycotoxins such as aflatoxins. [Papaioannou 2005] Particle sizes used in the study of Katsoulos were in a range below 150 µm and 800 µm, whereas G-PUR has a mean particle size of only 3 µm, suggesting even higher efficacy in binding dietary aflatoxins than the particle sizes used by Katsoulos et al.*

In a study by Parlat et al, Japanese quail chicks from 10 to 45 days of age received a diet fortified with aflatoxins (mainly consisting of AFB1 and AFB2) and supplemented with Clinoptilolite (when various poultry species were fed diets with different concentrations of aflatoxins, poults and goslings appeared to be the most sensitive, quail were most vulnerable, and domestic chicks were most resistant). The experimental design consisted of 4 dietary treatments: basal diet; basal diet plus 2 mg AF/kg diet; basal diet plus 50 g Clinoptilolite, and basal diet plus 2 mg AF plus 50 g Clinoptilolite /kg diet. Basal diet consisted mainly of maize, barley, soybean meal, sunflower seed meal, fishmeal and vegetable oil. Researchers reported that cumulative food consumption was reduced by 14% among the quail chicks fed on the AF-containing diet, however the reduction was only 6% for chicks consuming the AF plus Clinoptilolite diet. Similarly, overall BWG was reduced by 27% in the AF-containing group, but by only 8% in the AF plus Clinoptilolite group. In the study, a significant improvement was

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observed in all of the investigated performance parameters when comparing the group consuming the AF diet with the group consuming the AF plus Clinoptilolite diet ($P < 0.05$).*

The authors of the publication from Parlat et al stated their observations suggest the addition of Clinoptilolite as an adsorbent to the diet may be an encouraging approach to overcome the problems associated with aflatoxin effects in poultry. Clinoptilolite binds the AF and reduces their absorption from the gastrointestinal tract. Adsorbents like Clinoptilolite, not being absorbed from the gastrointestinal tract, must have the ability to bind physically with aflatoxins, precluding their absorption. Zeolites have been used for this purpose. These are generally inert and non-toxic to animals and have a capacity to bind aflatoxins. This study suggests that – even for very high concentrations of aflatoxins – Clinoptilolite is able to reduce the bioavailability of dietary AF, even in a complex matrix like the chicken diet applied, which consisted of many ingredients that can also be found in a complex human diet.* [Parlat 1999]

4.3.3 Lab Data

For most regulated mycotoxins, G-Science performed in-vitro experiments to provide data for the specific toxin-Clinoptilolite interaction. The adsorption capacity of Clinoptilolite for the relevant toxins was determined in 3 different matrices, starting with a good solvent for the toxin (methanol 70%), water or physiological saline solution and simulated gastric juice. Depending on the type of toxin, particular strong interactions between Clinoptilolite and the toxin could be detected.*

mycotoxin	interaction with Clinoptilolite
Aflatoxin B1	+++
Zearalenone	+
T2-Toxin	+
Citrinin	++
Fumonisin B1	+++
Ochtratoxin A	+
Deoxynivalenol	-

Table 6 Interaction of G-PUR with various mycotoxins in-vitro [G-Science, unpublished]

These results are in line with relevant scientific publications:

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Dakovic et al. analyzed the interaction between aflatoxin B1 and G2, and Clinoptilolite. Particle size of Clinoptilolite used was below 63 µm. Kinetics of Aflatoxin B1 and G2 adsorption was analyzed at pH 2 and pH 7, with a time interval of 5 min to 48h. The composition of the electrolytes in the study material was similar to that of gastric juice in animals, and the temperature was 37°C. For both toxins, the authors observed a rapid adsorption process, with most of the toxin being adsorbed within the first few minutes. In addition, adsorption was characterized by the authors to be almost irreversible. For all experiments, at different pH levels and aflatoxin concentrations, adsorption of aflatoxins G2 and B1 on Clinoptilolite was always greater than 80%.* [Dakovic 2000]

In a study by Lemke et al., several adsorbents were tested for their Aflatoxin binding in a gastro-intestinal experimental model with 2 hours incubation at pH 1.3, followed by 2 hours at pH 7 with pancreatin and bile salts. Clinoptilolite was able to bind about 40% of the available Aflatoxin B1 in a simulated GI fluid (Clinoptilolite bound 7 mM/kg in a setup with 5 ml of a 8 µg/ml AFB1 solution and 1 mg Clinoptilolite). More than 50% of the bound Aflatoxin could not be recovered even by organic solvents (methanol and chloroform), as the adsorption strength of the Clinoptilolite-Aflatoxin complex is very high.* [Lemke 2001]

This further substantiates the hypothesis that the Aflatoxin-Clinoptilolite complex is of extraordinary stability, not releasing the bound Aflatoxin despite of the matrix.*

Bočarov-Stančić et al. have used an in vitro model for the evaluation of the binding capabilities of several natural binders and six different mycotoxins. For the adsorption experiments the ability to bind mycotoxins was evaluated in a buffered electrolyte solution at different pH levels. Concentration of the mycotoxins ranged from 0.2 to 2 mg/l. Though a relatively course particle size has been used compared to G-PUR (d50 ~ 13 µm compared to G-PUR with 3 µm), most of the mycotoxins analyzed could be adsorbed by Clinoptilolite. Only Ochratoxin A and Diacetoxy-scirpenol did not interact with Clinoptilolite at neither pH.* [Bočarov-Stančić 2011]

In a study of Vekiru et al., “intensified conditions” were used to analyze the efficacy of several sorbents. In an in-vitro experiment, a very high concentration of Aflatoxin B1 (4 mg/l) in

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different liquid matrices (buffer or gastric juice) were put into contact with relatively low (0.02%) concentrations of different adsorbents. Even in these extreme conditions, there still was an interaction between Clinoptilolite and Aflatoxin B1, adsorbing between 6.4% and 21.9% of the toxin. In a second experiment, broiler chicks were exposed to spiked feed (at an Aflatoxin concentration of 2 mg per kg feed) and different adsorbents at 0.5% (w/w). In this setup, Clinoptilolite was not effective to impact the effects of Aflatoxin B1, what might be due to the fact that the Clinoptilolite concentration was quite low and Aflatoxin concentration was extremely high.* [Vekiru 2015]

Tomasevic-Canovic et al. evaluated the affinity of different cation-exchanged forms of Clinoptilolite for aflatoxin B1 in vitro. The solution from which the adsorption was to be examined was chosen to simulate the gastric juice of animals and contained: 0.1 mol/dm^3 HCl and 0.05 mol/dm^3 NaCl. The experiment was carried out at pH 3.8 and at a temperature of 37 ºC. A certain amount of AFB1 (200 µg) was added to 100 cm^3 electrolyte and an aliquot (0.4 cm^3) was taken for the determination of the total toxin concentration present in the solution. Then, 1 g of mineral adsorbent was added to the contaminated electrolyte solution. At the end of the reaction time (2 h), the concentration of non-adsorbed AFB1 was determined in the supernatant. Natural and Ca- rich Clinoptilolite showed a very high chemisorption index comparable to the montmorillonite-clay analysed. Authors concluded that Clinoptilolite adsorbed a substantial amount of aflatoxin B1 and the presented in vitro data clearly demonstrate that, at a concentration of 200 µg per g of adsorbent, all of the mineral adsorbents used greatly reduce bioavailability of AFB1. Ratio of AFB1 and adsorbent correspond to 1 mg/kg of AFB1 in the diet, if the adsorbent is incorporated into the diet at a level of 0.5 %.* [Tomasevic 2001]

The totality of evidence demonstrated in the numerous scientific and peer reviewed publications, together with the G-PUR lab data and the human study published by Afriyie-Gyawu, Phillips and Wang clearly suggests that G-Clinoptilolite binds and reduces bioavailability of dietary mycotoxins.*

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5 Data and Supportive Information to Determine the Safety of G-PUR

5.1 Comprehensive Safety Profile for G-PUR

Data and information relevant to the safety of the G-PUR (purified Clinoptilolite) were obtained from product specific toxicity studies, and where applicable published studies identified during a comprehensive review of the scientific literature. Studies were selected from the literature search on the basis of their relevance to the safety of G-PUR. They were also selected based upon quantitative and qualitative similarity of the test material studied in relation to the chemical composition of G-PUR and in consideration of scientifically sound study methodologies conducted in accordance with international standards.

G-PUR is not soluble in water, and it is generally accepted that tectosilicate structures with high silicium to aluminum ratios are highly stable to acid degradation. No loss in crystal structure as determined using X-ray diffraction, energy dispersion-X-ray fluorescence, and transmission electron microscopy, and no change in metal composition of the mineral, as determined using inductively coupled plasma mass spectrometry (ICP-MS), were observed at pH values ≥ 2. [Li 2008]

In vivo studies using porcine models (FDA considers pigs to be the most appropriate animal model for the human digestive tracts [U.S. FDA, 2011]) have demonstrated that dissolution of the aluminosilicate structure of Clinoptilolite will not occur during gastro-intestinal passage in humans, as no evidence of aluminum bioavailability can be identified in pigs administered Clinoptilolite in the feed at significant dietary levels of up to 2%. [Pond 1989; Papaioannou 2002; Alexopoulos 2007]

The high stability of Clinoptilolite minerals to acid digestion are in contrast to other silicates (e.g., synthetic Zeolite A) which are subject to partial dissolution at the physiological pH of the stomach (pH ~2). Silicate minerals such as synthetic Zeolite A have an aluminium:silicium ratio of approximately 1:1, and the higher concentration of low-energy aluminium-silicium bonding throughout the crystal structure of these minerals renders these minerals susceptible to acid hydrolysis. These differences in the chemical stability of Clinoptilolite and other silicates are relevant to the safety assessment of G-PUR as toxicity studies conducted in rodents and dogs administered various chemically related silicates (i.e., Zeolite A) have identified lesions in the kidneys and bladder [HERA, 2004; WHO FAS No. 5 – JECFA, 1974, Cefali 1995]

These lesions can be identified within 90 days of treatment and are associated with accumulation of silicium in the kidneys and presence of non-staining crystalline material

deposited in the renal pelvis and urine, an observation that is attributed to absorption of small amounts of silicium-compounds from the gastrointestinal tract after dissociation of sodium aluminum silicate to sodium, aluminum and SiO_4 [HERA, 2004]. Accordingly, observations of bladder and kidney toxicity in animals administered silicate compounds with a low silicium to aluminum ratio (i.e., Zeolite A) were not considered relevant to the safety of G-Science purified Clinoptilolite.

Clinoptilolite minerals are permitted for consumption in the European Union under EC Directive 93/42/EEC, and the current and historical consumption patterns of these products in the European marketplace also were considered relevant to the safety determination. These products are authorized for consumption at use levels between 2 to 10 g per day for repeated daily consumption by the general population. Comprehensive chemical analyses of Clinoptilolite products legally permitted for sale in the European marketplace since 2006 were conducted using X-ray diffraction and ICP-MS. A comparison of the X-ray diffraction profiles and elemental composition of G-Science Clinoptilolite to other Clinoptilolite minerals authorized for consumption under EC Directive 93/42/EEC demonstrated these products were qualitatively and quantitatively comparable to G-PUR raw material, and in-fact have a very high probability of sharing the same geological origin for sourcing of the raw material.

Findings from controlled studies identified in the literature evaluating the dietary consumption of Enterex, a natural Clinoptilolite mineral consumed in the EU, were reviewed for potential reports of adverse effects. Short-term studies conducted in over 500 subjects administered Enterex at levels of up to 10 g per day did not result in notable test article related adverse effects of clinical significance. [Rodriguez-Fuentes 1997] G-Science has conducted comprehensive studies comparing the qualitative and quantitative chemical composition of Clinoptilolite minerals originating from the Tasajeras deposit in Cuba (source of Clinoptilolite used in Enterex) and have demonstrated that the source material is chemically similar to G-PUR raw material.

5.2 Patented Purification Process

Certain contaminants that are always present in natural Clinoptilolite (despite its origin) may pose a significant risk if applied to humans.

In a study with weaned pigs, Fokas et al. determined the retention coefficient of Pb from a natural, not purified Clinoptilolite of Greek origin. Twelve pigs were divided into two groups, one group received a diet that contained 2% of a Greek Clinoptilolite that contained elevated amounts of Pb (46 ppm). The authors found that liver lead content significantly increased in

the group that received the natural Clinoptilolite of Greek origin, though other organs did not show a statistically significant change. [Fokas 2004]

The study performed by Petrakakis et al. clearly showed that lead becomes available and may be leached from Clinoptilolite at certain pH levels. In batch reactor experiments with pH and temperature within physiological ranges, significant amounts of lead could be leached from Clinoptilolite. [Petrakakis 2007]

This clearly emphasizes the necessity to carefully choose the specific source from which Clinoptilolite is mined, and the need for a purification process that minimizes all contaminants to the lowest possible concentrations. G-Sciences understands the value and challenges inherent with Clinoptilolite and developed a proprietary process to remove natural contaminants from G-PUR to ensure it is both safe and efficacious.

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