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SILICIUM PRE-CLINICAL TRIAL

Title	Silicium concentrations after absorption from Solid orally dosed Silicium
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Contract Giver	Sil'innov Courcelles (Belgium)
Product	Solid Oral Silicium dosage forms

SIGNATURES

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1 INTRODUCTION

Due to the development of a **solid source** of Si, there was a need to understand the mode of action (solubility, absorbability, bioavailability) of the solid source of Si (Mesoporosil®) and compare also to the Preblend Mesoporosil® making sure there is no influence of the other ingredients. For these reasons a preclinical trial has been performed.

The scope of this study is to confirm **activated solubility** from in-vitro trials (lab), and **absorbability** from ex-vivo data (Ussing - Sprague Dawley rats). This will be confirmed in-vivo towards a **bio-available** source of Si. The study will execute a PK study in rats (280g female Sprague Dawley) to confirm the in-vitro results and compare the in-vivo silicium distribution from **solid silicium** from Eytelia.

After careful consultation and investigation, concentrations (the below table) were chosen to stay with the daily used dosage providing similar %Si element. In this study we used therefore 30mg for the solid Mesoporosil®, equivalent to 14mg theoretical Si element (47% of SiO2) for both the Mesoporosil and Preblend Mesoporosil®

A. Blanco, no dosage

B. Mesoporosil®

	m (**mg**)	BW (kg)
Si -> 14mg	27,4	70
	0,110	0,28

C. Preblend Mesoporosil®

Mesoporosil	m (**mg**)	BW (kg)
Si ->14mg	30	70
	0,120	0,28

- **Plasma** was collected at the following timepoints:
⇨ 0 (before test item administration), 0.5, 1, 2, 4, and 6 hours post dose.
- **Urine** was collected at the following timepoints:
⇨ 0 (before administration), 4 and 8 hours post dose.

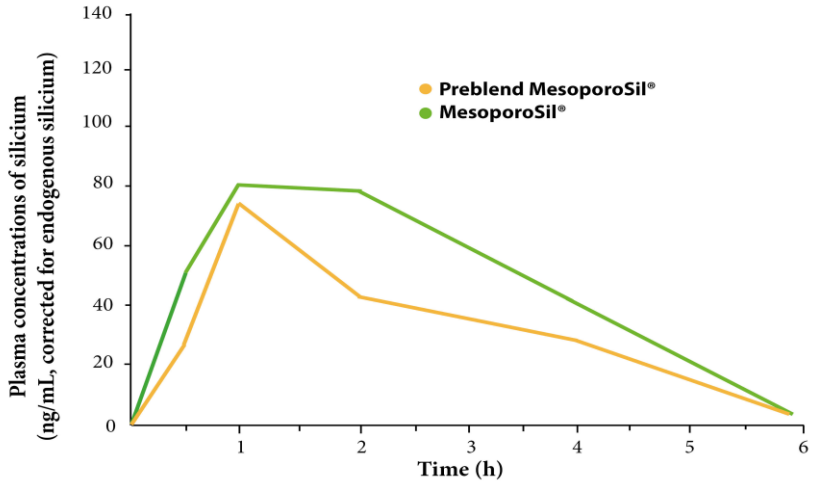
The full study protocol and all data can be consulted during a company visit, and after signing an NDA.

Samples were analyzed for silicium concentrations by ICP-MS. Working methods were developed including system suitability testing before each run.

2 SILICIUM CONCENTRATIONS IN PLASMA AND URINE

2.1 SILICUM CONCENTRATIONS IN PLASMA

A clear trend can be seen for the silicum plasma concentrations, with a peak concentration 1h after administration and returning to baseline levels after 6hrs. **We can assume this is the action of absorbability from activated silicium during the 6hrs.** The concentration-levels of both the Mesoporosil® and the Preblend Mesoporosil® formulations show comparable trend in uptake highest AUC of around 274 ng.h/mL.



Parameter	Summarized pharmacokinetic parameters of silicium (plasma)	
	Preblend Mesoporosil®	Mesoporosil®
Cmax ng/mL	73,00	87,33
AUC_{6h}, ng.h/mL	266,6	274.6

PK PARAMETERS

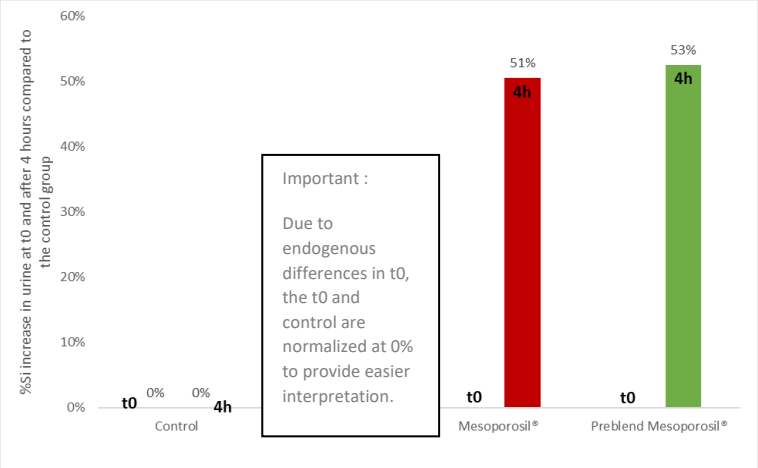
The PK analysis was performed on plasma concentrations per animal in each dose group. In Phoenix® WinNonlin® NCA model (Model Type: Plasma (200-202), Dose Type: Extravascular) was applied.

What we can conclude from these data is that both formulations lead to comparable systemic exposure, based on both C_{max} and AUC.

Software used for PK Analysis - The non-compartmental PK analysis was performed using the validated application Phoenix® WinNonlin® version 8.1 (Copyright ©1998 - 2018, Certara L.P., USA). In addition, Microsoft Office Standard version 2019 was used for reporting and calculation purposes.

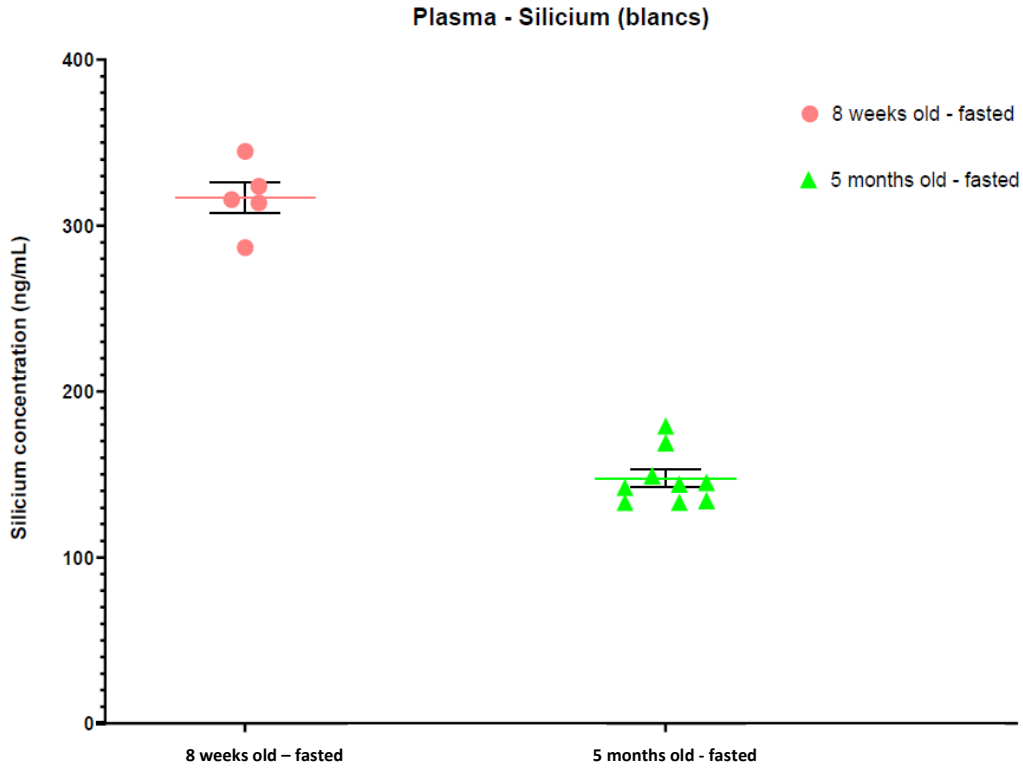
2.2 SILICIUM CONCENTRATIONS IN URINE

Compared to the control, comparable urinary Si excretion was found for Mesoporosil® (+51%) over the same 4h period compared to Preblend Mesoporosil® (53%)



3 EXTRA STUDY : IMPACT OF THE EFFECT OF AGE ON ENDOGENOUS PLASMA LEVELS

Endogenous silicium concentrations can be significant in young aged people. For investigational purposes an extra study was requested to receive confirmation trough Sprague-Dawley rats. In order to better understand and control this in future experiments, the effect of rat age was therefore evaluated. The rats were housed similar to the large study and plasma samples were also prepared and analyzed in an identical matter. Overall, it can be stated that younger rats have significantly higher endogenous silicium in their plasma (up to twice as much when compared to older rats).



4 OVERAL CONCLUSION

- Both Mesoporosil® and Preblend Mesoporosil® solid silicium formulations induce expected concentrations of silicium in urine and serum, being dosed at equal concentrations.

- The development of a solid source of Si, was creating the need to understand if the mode of action (solubility, absorbability, bioavailability) found in-vitro and ex-vivo, would be confirmed in-vivo. The scope of this study was to confirm the solubility (**activated**) from in-vitro trials, and absorbability (**absorbed**) (from ex-vivo data (Ussing - Sprague Dawley rats). With this study the solubility and absorbability is confirmed **in Vivo**. The solid Mesoporosil is providing an increase of around **50%** in urine excreted silicium being defined as bioavailable (**assimilated**) source of Si.

  These results allows us to develop the new concept of the "**Triple A" score.**

  "Activated, Absorbed and Assimilated"

- Extra knowledge was developed towards the confirmation of **age dependant** endogenous silicium concentrations. It is confirmed that age is of great importance. For preclinical studies, requesting older rats is of great importance to take into account for future studies.

Supported by :

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