

# Phenotypic characterization of panelists as selection criterion

Karolin Höhl, Heidelberg; Mechthild Busch-Stockfisch, Lauenburg

## Summary

The phenotype of perceiving propylthiouracil (PROP) as either bitter or tasteless (so-called "PROP status") correlates with other sensory parameters, including sensitivity to other tastes or food preference. The present article investigates whether knowledge of the phenotypic PROP status can provide relevant information on the suitability for analytical test procedures. 82 female students without sensory training were classified as PROP non-tasters (PNTs,  $n = 22$ ), PROP medium-tasters (PMTs,  $n = 39$ ) or PROP super-tasters (PSTs,  $n = 21$ ) and the sensitivity to sucrose and caffeine was determined. 45 subjects from all of these three sensitivity groups were then given one week of sensory training (intervention group). The remaining 37 test subjects from all three groups received no intervention (control). The sucrose and caffeine sensitivity of the intervention and control groups were checked at two time points at an interval of 6 months.

The results show that the initial differences in caffeine sensitivity in the three sensitivity groups could be eliminated by sensory training, as well as by experience or habituation. Even after an interval of 6 months, caffeine sensitivity did not return to the original value.

Thus the phenotypic PROP status is essentially irrelevant to the formation of sensory panels. After sensory training and/or experience and habituation, PNTs and PMTs can achieve the same test sensitivity to sweet and bitter as PSTs.

**Keywords:** sensory science, analytical tests, threshold measurement, training, receptor types

## Introduction

Sensory abilities – including taste sensitivity to sweet, sour, bitter, and umami, and perception of the intensity and recognition of different odors – differ between different individuals and within the same individual in the course of the day and year, depending on hormonal fluctuations [1] and environmental influences such as temperature, air pressure and light intensity [2–4].

Various factors influencing sensory discrimination, e. g. age, state of health, psychological factors, and genetic susceptibility, have already been published [5]. On this basis (■■■) ERNÄHRUNGS UMSCHAU 12/2015,

p. 216 ff.), the present article now examines whether and to what extent genetic susceptibility influences individual suitability to be a sensory test person (TP). For about the last 85 years, sensory studies have paid great attention to the effects of genetic susceptibility on the perception of the bitterness of a specific group of thioureas (e.g. phenylthiocarbamide [PTC] or propylthiouracil [PROP]). This originated in a chance discovery by A. L. Fox in the 1930s [6, 7]. The genetic foundation of these phenotypic differences – the ability or inability to perceive the bitter taste of PROP and/or PTC solutions (the "PROP status") – has now been identified. This has been found to involve substitutions of key amino acids in the bitter receptor *TAS2R38* and to lead to receptor variants with different sensitivities to substances with an isothiocyanate or thioamide group [8–12]. However, the *TAS2R38* receptor variant only explains about 50–85 % of the phenotypic PROP status [13]. At the phenotypic level, three different groups can be distinguished: PROP non-tasters (PNTs), PROP medium-tasters (PMTs) and PROP super-tasters (PSTs), who react to different concentrations of the substance with different sensitivity (PNTs < PMTs < PSTs). Five different combinations of substituted amino acids on the receptor can be distinguished by genotype (AVI, PAV, AAI, AAV and PVI)<sup>1</sup>, of which

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<sup>1</sup> The letters stand for the amino acids alanine (A), valine (V), isoleucine (I) and proline (P). The G-protein-coupled PROP receptor consists of about 333 amino acids; its function is modified by substitution of these four amino acids at positions 49, 262 and 296 [9].

the most frequent are AVI (so-called “non-taster amino acid sequence”) and PAV (so-called “taster amino acid sequence”) [9, 13–15]. The phenomenon of “super-tasters” can only be described at a phenotypic level; its genotypic explanation is unknown hitherto. Many studies have examined the effect of phenotypic differences on other sensory parameters (e.g. perception and assessment of other tastes [16, 17]) and on food habits [18]. There have however not yet been any adequate studies on whether these links between PROP status and sensitivity to other tastes can influence the practice of sensory science. Is it possible that determining the phenotypic PROP status at the start of the recruitment process can help to shorten the selection and training process, by selecting more sensitive TPs at the start? In other words, do PSTs possess generally greater and almost comprehensive sensory sensitivity and are they therefore more suitable for analytical test procedures than PNTs?

The first article investigated the effect of sensory training on sweet and bitter perception [5]. This is now complemented by measurements of the PROP status of the test persons (TP). It was examined whether the taste sensitivity differs between PROP types in untrained TPs and whether these differences are maintained during training. It would then be possible to select TPs during the recruitment process who possessed greater inherent taste sensitivity, and this would shorten this protracted and expensive process.

## Materials & methods

Details of the methods can be found in the first article, including information on the study group, study design, tasting rules, structure of sensory training, test methods to determine taste sensitivity, and statistics (■ ■ ■ ■ ■ ERNÄHRUNGS UMSCHAU

|        | Test Substance                     | Chemical Formula                                 | Molecular Weight (g/mol) | Concentration Series |           |           |
|--------|------------------------------------|--|--------------------------|----------------------|-----------|-----------|
| salty  | sodium chloride <sup>a</sup>       | NaCl   | 58.44                    | Verdünnung           | g/L       | mol/L     |
|        |                                    |  |                          | D1                   | 58.44     | 1.00      |
|        |                                    |  |                          | D2                   | 18.70     | 0.320     |
|        |                                    |  |                          | D3                   | 5.84      | 0.100     |
|        |                                    |  |                          | D4                   | 1.87      | 0.032     |
|        |                                    |  |                          | D5                   | 0.58      | 0.010     |
| bitter | 6-propyl-2-thiouracil <sup>b</sup> | C <sub>7</sub> H <sub>10</sub> N <sub>2</sub> OS | 170.23                   | dilution             | g/L       | mol/L     |
|        |                                    |  |                          | D1                   | 0.54460*  | 0.0032    |
|        |                                    |  |                          | D2                   | 0.17023** | 0.0010    |
|        |                                    |  |                          | D3                   | 0.05447   | 0.00032   |
|        |                                    |  |                          | D4                   | 0.01702   | 0.0001    |
|        |                                    |  |                          | D5                   | 0.00545   | 0.000032  |
|        |                                    |  |                          | D6                   | 0.00170   | 0.00001   |
|        |                                    |  |                          | D7                   | 0.00055   | 0.0000032 |
|        |                                    |  |                          | D8                   | 0.00017   | 0.000001  |

Tab. 1: Concentrations of the test substances sodium chloride (NaCl) and propylthiouracil (PROP)

\* Stock solution to prepare D1, D3, D5 and D7

\*\* Stock solution to prepare D2, D4, D6 and D8

<sup>a</sup> Merck KGaA, Darmstadt; <sup>b</sup> Fluka, Sigma-Aldrich Chemie GmbH, Steinheim

D = dilution

12/2015, pp. 216 ff.) [5]. Some additional information is given below.

### Test substances

In order to determine the phenotypic PROP status, five highly concentrated sodium chloride solutions (NaCl) and eight highly concentrated solutions with propylthiouracil (PROP) were prepared one day before tasting (♦ Table 1). These were prepared with lightly warmed deionized water in 1 liter graduated flasks.

### Determination of the phenotypic PROP status

The PROP status was determined in three steps:

- The **stimulus threshold for PROP** was determined with a two-alternative forced-choice test (2AFC) [19]. The stimulus threshold of PROP tasters was assumed to be less than 0.1 mmol/L, so that the detection limit of PNTs was above this limit.

- In order to subdivide the PROP tasters into PMTs and PSTs, two different **NaCl/PROP intensity ratios** (PROP quotients 1 and 2)<sup>2</sup> were calculated from the five NaCl and five PROP samples with concentrations above the threshold (D1–D5, ♦ Table 1; using a 15 cm visual analogue scale, VAS; left scale end = “not at all salty/bitter”; right scale end = “as salty/bitter as I have ever perceived”, [20]). The assessments on the VAS were transformed into values between 1 and 5 ((intensity assessment

<sup>2</sup> PROP quotient 1 = [(p1/n1)+(p2/n2)+(p3/n3)+(p4/n4)+(p5/n5)]/5

PROP quotient 2 = (p1+p2+p3+p4+p5)/(n1+n2+n3+n4+n5)

p1–5 = PROP intensity assessment from the VAS; n1–5 = NaCl intensity assessment from the VAS

If there is evidence that the classification of the PROP status is biased by outliers (PROP quotient 1 is relatively susceptible to outliers), PROP quotient 2 is also included in the classification.

|      | N  | PROP threshold |       | PROP quotient 1 |       | PROP quotient 2 |       |
|------|----|----------------|-------|-----------------|-------|-----------------|-------|
|      |    | M (mmol/L)     | s     | M               | s     | M               | s     |
| PNTs | 22 | 0.286          | 0.716 | 0.684           | 0.084 | 0.614           | 0.094 |
| PMTs | 39 | 0.047          | 0.668 | 0.996           | 0.132 | 0.937           | 0.139 |
| PSTs | 21 | 0.019          | 0.376 | 1.501           | 0.235 | 1.348           | 0.207 |
| F    |    | 20.540         |       | 151.111         |       | 129.755         |       |
|      |    | ***            |       | ***             |       | ***             |       |

Tab. 2: PROP threshold tests and the two PROP quotients separated by PROP status (PROP non-tasters [PNTs], PROP medium-tasters [PMTs] and PROP super-tasters [PST])

M = arithmetic mean; s = standard deviation (calculated from the original values in log(mmol/L, see article 1, material and methods, statistic); \*\*\* = significant: p ≤ 0.001

in cm/15) x 4]+1), so that an unambiguous calculation of the PROP quotients was then possible. PNTs, PMTs and PSTs were classified on the basis of the 25<sup>th</sup> and 75<sup>th</sup> percentiles of the PROP quotients. These borderline values correspond to the assumed 1/4, 1/2, 3/4-distribution of the PROP status in the study group [21, 22].

c. Stepwise classification of the PROP status using the results of a and b.

were highly significant differences in the parameters in the three PROP groups and this indicates that the classification method used here leads to maximal differences between the groups. Contrary to expectations, there were even highly significant differences between PSTs and PMTs in the PROP threshold value, in spite of the fact that previous studies [21, 23] considered that the threshold test was unsuitable for differentiating between these PROP types.

### Taste thresholds depend on the PROP status of untrained TPs

The stimulus and recognition thresholds for **sweetness in sucrose** did not significantly differ between the three PROP groups, although the numerical value appears to be lower for the PSTs than for the other groups:

- Stimulus threshold for sweet: PNTs = 1.84 mmol/L; PMTs = 1.85 mmol/L; PSTs = 1.42 mmol/L;

- Recognition threshold for sweet: PNTs = 14.96 mmol/L; PMTs = 13.55 mmol/L; PSTs = 9.77 mmol/L.

However, the three PROP groups differed significantly with respect to the detection (p = 0.007; F = 5.40) and recognition (p = 0.01; F = 4.89) of the **bitter caffeine**:

- Stimulus threshold for bitter: PNTs = 0.33 mmol/L; PMTs = 0.39 mmol/L; PSTs = 0.29 mmol/L;
- Recognition threshold for bitter: PNTs = 0.85 mmol/L; PMTs = 0.72 mmol/L; PSTs = 0.46 mmol/L.

The different taste sensitivity of the PROP types for other tastes may be concentration dependent. Thus two other studies found that PSTs were always more sensitive to highly concentrated solutions of sucrose, sodium chloride, quinine [24, 25], tartaric acid, and iron(II) sulphate [25]. Moreover CHANG et al. [26] found a significant correlation between PROP sensitivity and sweet (sucrose) and bitter (quinine) solutions, although they used much higher concentrations (up to 1 mol/L) to determine thresholds.

## Results and discussion

### PROP status in untrained TPs

Using the above method, the total group (N = 82) was classified into 22 PNTs (26.8 %), 39 PMTs (47.6 %) and 21 PSTs (25.6 %). The results for the PROP threshold determination and NaCl/PROP intensity quotients are classified as PNTs, PMTs and PSTs, as shown in ♦ Table 2. There

### Training effects and the PROP status (t<sub>0</sub> vs. t<sub>1</sub>)

After study time point t<sub>0</sub>, TPs were randomly assigned to the control or intervention groups. In order to ensure that the three PROP types were evenly distributed, it was necessary to consider the PROP status of the TPs. Therefore the TPs were assigned to the intervention or control group, as shown in ♦ Table 3. Significant differences in the **stimulus and recognition thresholds for sweet samples** between t<sub>0</sub> and t<sub>1</sub> (reductions in the stimulus and/or recognition threshold) were only found in intervention PMTs (stimulus threshold at t<sub>1</sub> = 1.18 mmol/L; p = 0.009; t = 2.89; recognition threshold at t<sub>1</sub> = 6.31 mmol/L; p = 0.01; t = 2.70). Thus, this study

|                    | PNTs |        | PMTs |        | PSTs |        | overall |       |
|--------------------|------|--------|------|--------|------|--------|---------|-------|
|                    | N    | %      | N    | %      | N    | %      | N       | %     |
| Control group      | 10   | 27 %   | 17   | 46 %   | 10   | 27 %   | 37      | 100 % |
|                    |      | 45.5 % |      | 43.6 % |      | 47.6 % |         |       |
| Intervention group | 12   | 27 %   | 22   | 49 %   | 11   | 24 %   | 45      | 100 % |
|                    |      | 54.5 % |      | 56.4 % |      | 52.4 % |         |       |
| overall            | 22   | 26.8 % | 39   | 47.6 % | 21   | 25.6 % | 82      | 100 % |
|                    |      | 100 %  |      | 100 %  |      | 100 %  |         | 100 % |

Tab. 3: PROP status (PROP non-tasters [PNTs], PROP medium-tasters [PMTs] and PROP super-tasters [PST]) and study groups (N = 82)

group detected and recognized the sweet taste of sucrose significantly earlier after than before training.

The **stimulus threshold for the bitter caffeine taste samples** was only significantly reduced for the control PMTs ( $t_1 = 0.34$  mmol/L;  $p = 0.02$ ;  $t = 2.58$ ; cf. ♦ Figure 1). In contrast, the **recognition of the bitter taste** (cf. ♦ Figure 2) in the threshold test improved significantly for the control PNTs (recognition threshold at  $t_1 = 0.54$  mmol/L;  $p = 0.04$ ;  $t = 2.46$ ), intervention PNTs (recognition threshold at  $t_1 = 0.44$  mmol/L;  $p = 0.007$ ;  $t = 3.42$ ) and intervention PMTs (recognition threshold at  $t_1 = 0.46$  mmol/L;  $p = 0.03$ ;  $t = 2.43$ ). Both PST groups (control and intervention) maintained their taste sensitivity for sweet and bitter at the same level as at  $t_0$ . It was interesting to note that the effect of experience, as noted in the first article [5], was also demonstrable when the PROP status was included. Thus the PMTs and PNTs in the control group benefited from their growing experience in dealing with sensory principles and exhibited significant improvements in the bitter caffeine threshold test. **In this way, as a result of training and experience, all PROP types achieved the same taste sensitivity for caffeine and the differences in sensitivity that were demonstrated without training were levelled off.**

We are only aware of one other study which compared the test performance and precision of trained and untrained TPs with allowance for the PROP status [27]. But in contrast to the present data, this recommended that the PROP status should be used as a selection criterion for panel recruitment. In the study of DE WIJK et al., untrained PSTs could better differentiate the product properties of vanilla desserts than could untrained PNTs. Then untrained PSTs achieved a similarly good result to trained TPs (the trained panel

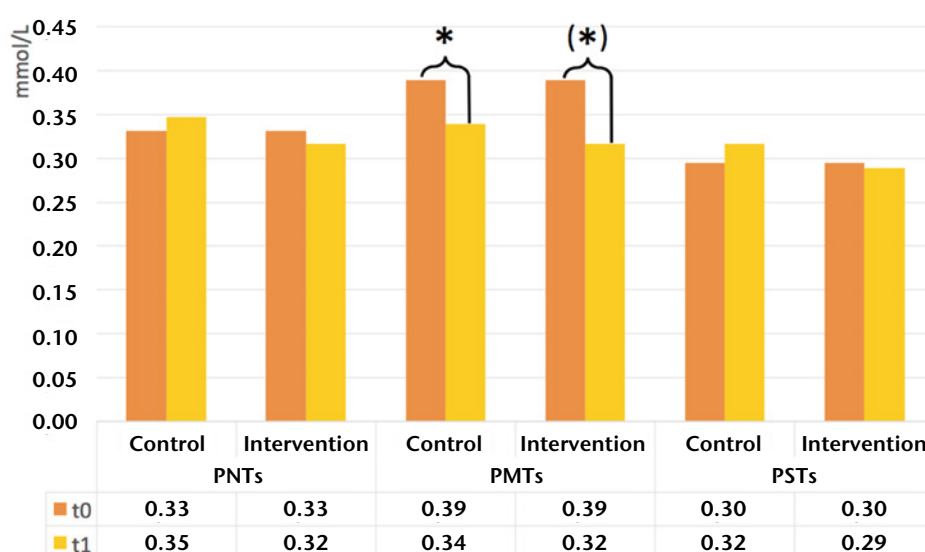


Fig. 1: Stimulus threshold for bitter (caffeine) for PROP non-tasters (PNTs), PROP medium-tasters (PMTs) and PROP super-tasters (PSTs): comparison between study time points  $t_0$  and  $t_1$  (after sensory training [intervention] or a one week pause [untrained = control])

PNTs control: n = 10; PNTs intervention: n = 11; PMTs control: n = 16; PMTs intervention: n = 20; PSTs control: n = 9; PSTs intervention: n = 10 (\*) = trend ( $p \leq 0.10$ ); \* = significant:  $p \leq 0.05$

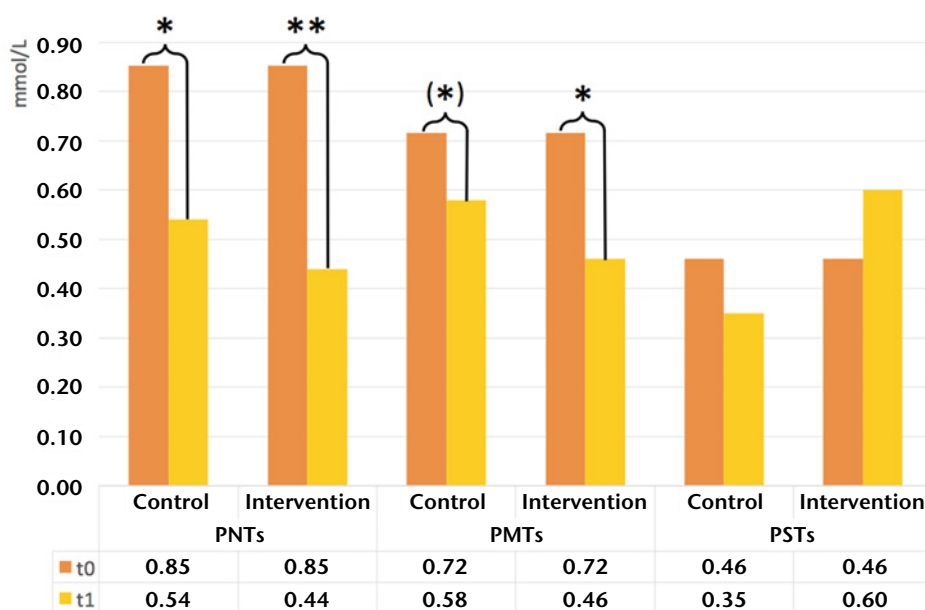


Fig. 2: Recognition threshold for bitter (caffeine) for PROP non-tasters (PNTs), PROP medium-tasters (PMTs) and PROP super-tasters (PSTs): comparison between study time points  $t_0$  and  $t_1$  (after sensory training [intervention] or a one week pause [untrained = control])

PNTs control: n = 10; PNTs intervention: n = 11; PMTs control: n = 16; PMTs intervention: n = 20; PSTs control: n = 9; PSTs intervention: n = 10 (\*) = trend ( $p \leq 0.10$ ); \* = significant:  $p \leq 0.05$

contained only PMTs and PSTs). However, DE WIJK et al. [27] failed to investigate whether the test performance of the less taste sensitive untrained PNTs could be changed

by sensory training. In an initial step, this was demonstrated in the present study for the perception of basic tastes.

### Taste sensitivity of the PROP groups over time ( $t_0$ , $t_1$ and $t_2$ )

Training effects are evident as an improvement in taste sensitivity between the time points  $t_0$ ,  $t_1$  and  $t_2$ . These were found for the sweet and bitter recognition threshold for the following groups:

#### *Recognition threshold for the sweet sucrose samples*

The intervention PNTs significantly improved their recognition of the sweet sucrose taste, both between  $t_0$  and  $t_2$  ( $p = 0.04$ ;  $t = 2.31$ ) and between  $t_1$  and  $t_2$  ( $p = 0.04$ ;  $t = 2.36$ ). The recognition threshold at  $t_2$  was 4.57 mmol/L. This means that there was an additional reduction in the recognition threshold at  $t_2$  in spite of the sensory pause of 29 weeks.

The intervention PMTs significantly improved their recognition of the sweet taste at  $t_2$  in comparison to  $t_0$  ( $p = 0.007$ ;  $t = 3.12$ ). The recognition threshold at  $t_2$  was 5.01 mmol/L. For this group, whose sensitivity had already been advanced by training, no difference was found between  $t_1$  and  $t_2$ , so that their taste sensitivity for sweet remained at the level of  $t_1$ , even with the pause of 29 weeks without participation in sensory tests.

#### *Recognition threshold for the bitter caffeine samples*

The intervention PMTs exhibited a significant reduction in the bitter recognition threshold between  $t_0$  and  $t_2$  ( $p = 0.01$ ;  $t = 2.84$ ); the value at  $t_2$  was 0.4 mmol/L. They therefore sustained the low level attained by training even after a relatively protracted sensory pause. Deterioration in the sensitivity to sucrose or caffeine was equivalent to an increase in taste thresholds.

No such changes were found in any group over the three time points. In general, the PSTs exhibited the most stable taste sensitivity over the three time points. Their initial low level

for taste sensitivity was maintained over  $t_1$  and  $t_2$ . However, training reduced the initial differences between PNTs, PMTs and PSTs with respect to the bitter taste of caffeine.

### Limitations

This study examined a highly homogenous study group, consisting of young female students of European origin. In addition, the study concentrated on two of five basic tastes. In order to confirm our results on the effects of training and experience and how these lead to a levelling off of the effects of the phenotypic PROP status, additional studies would be necessary with male and/or older TPs; these should also cover the other basic tastes (salty, sour, umami), as well as complex stimuli (e.g. beverages and foods).

### Application and outlook

As our studies and conclusions are inconsistent with those of DE WILK et al. [27], additional investigations must be carried out to clarify whether there are TPs with a phenotypic "general and comprehensive sensory sensitivity" probably unrelated to the substance PROP [28]. This might serve to shorten the selection procedure for panel formation, or to generate additional information about TPs which might complement the assessment and comparison of test results. For example, two recent studies have demonstrated a correlation between polymorphism of the umami receptor and the intensity assessment for the other basic taste types [29], as well as with an overall taste sensitivity (OTS)-parameter. This is calculated from the taste sensitivity to various reference substances and correlates very well with general taste sensitivity [30]. It has long been discussed to what extent the phenomenon of "super-tast-

ing" can be explained by relatively high densities of fungiform taste papillae on the tongue, with more intense innervation [31]. It must also be investigated to what extent the time point of the evaluation of PROP status influences phenotypic expression. For example, it has been shown that trained TPs are only made up of PSTs and PMTs [27]. Unfortunately, the authors failed to discuss whether the PNTs were "sorted out" during the training process, or whether, as a consequence of the sensory training, the TPs employed other parameters to evaluate PROP intensity and therefore restricted their allocation to PSTs and PMTs.

### Conclusion

There are ethical and medical reservations about the broad use of phenotypic PROP screening in practical sensory science. Quite apart from this, our data indicate that the initial differences between PNTs, PMTs and PSTs with respect to taste sensitivity to caffeine can be balanced by training, experience and habituation. It follows that determining the PROP status at the start of the selection process provides no additional benefit by shortening this protracted and tedious process.

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#### Conflict of Interest

The authors declare no conflict of interest.

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**Dr. Karolin Höhl**

Dr. Rainer Wild-Stiftung  
Mittelgewannweg 10, 69123 Heidelberg  
E-Mail:  
karolin.hoehl@gesunde-ernaehrung.org

**Prof. Dr. Mechthild Busch-Stockfisch**

Weingarten 23, 21481 Lauenburg  
E-Mail:  
mechthild.busch-stockfisch@haw-hamburg.de

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