

## Microbiological risk assessment of the nicotine product ZYN

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## Abstract

(b) (4) have performed a risk assessment in order to evaluate possible risks in terms of microbial growth and toxin production in ZYN<sup>®</sup> products, tobacco derived nicotine products. The ingredients used are food grade and can thus be considered initially safe. Measured water activities on fresh samples were very low, below (b) (4), and under these conditions no microorganisms are able to grow during storage. To ascertain microbial safety, the cans were put open in constant temperature and humidity for (b) (4). In this conservative approach, all observed levels of  $a_w$  were still below the point where any growth can occur. Moreover, the concentration of nicotine is most likely sufficient to prevent growth of relevant contaminant microorganisms. To conclude, in terms of microbial safety, no concerns with the ZYN<sup>®</sup> products were identified.

## Assignment

A risk assessment was performed by (b) (4) in order to evaluate possible risks in terms of microbial growth and toxin production in ZYN<sup>®</sup> products, a tobacco derived nicotine product. (see product name table 2, Appendix 1)

## Risk assessment

Potential microbial risks with the products were evaluated in three different respects:

1. **Impact from Product composition** (The contents of the pouches).
2. **Impact from Water activities** (Possible microbial growth at the very low observed water activities).
3. **Nicotine as an inhibitor of microbial growth** (Does the antimicrobial agent nicotine in itself prevent microbial growth?).

### Impact from Product composition

ZYN<sup>®</sup> product portfolio have two different levels of nicotine (3 mg and 6 mg) added as nicotine bitartrate. There are 15 different aromas, and "Smooth" with no aroma added (see Table 2, Appendix 1). Other ingredients are principally similar for all products and consist of the following:

(b) (4)

The pouches are packed in cans containing 15 individual pouches of 400 mg each.

All added ingredient are food grade and can thus be considered safe for human consumption. The aroma suppliers have either supplied documentation of estimated microbial content or supplied a non-microbiological testing statement. As neither of the additives are expected to be problematic in terms of microbial safety, all products are considered microbial safe. These

facts do not rule out the possibility of microbial propagation or contamination during mixing and manufacturing. However, considering the nature of the components, microorganisms are not likely to propagate rapidly to large numbers as there are no easily available nutrients because available carbon and nitrogen are bound to complex molecules not degradable by most microorganisms. In other terms there are no simple carbohydrates, proteins or other easily accessible carbon or nitrogen sources available.

Water activities

As the pouches were considered dry when first examined, a logical assessment was to estimate the moisture in terms of water activity to verify whether or not any microbe can grow. With a water activity meter ((b) (4)), used and calibrated according to manufacturer's instructions, activities were measured on four different ZYN® products. Samples were taken directly on materials from the can, both as unbroken pouches but also using the pouch contents without the pouch paper. The water activities were also measured on materials taken from cans that have been stored opened (b) (4) in a chamber with fixed temperature ((b) (4)) and relative humidity ((b) (4)). Results are listed in Table 1. Based on the very similar chemistry in all different products, the results based on the four tested products are representative for all 32 products. Calculated water activities ranging from (b) (4).

Table 1. Estimated water activities on a limited set of ZYN® pouches

Product	Whole pouch	Internal content	Whole pouch after (b) (4)	Internal content after (b) (4)
Spearmint 3 mg	(b) (4)			
Peppermint 3 mg				
Smooth 3 mg				
Smooth 6 mg				

Very few microorganisms can grow below aw 0.81 and the organism known to growth at the lowest water activity is the mould *Xeromyces bisporus*, which grows at aw 0.622. This means that even in a very conservative view it can be stated that there is no possibility for any harmful microorganism to grow. The great variation between estimated water activities indicates that the activities cannot be estimated with accuracy, most likely explained by the fact that the instrument and the surrounding working environment is not fully optimized to measure such low activities. However, as the highest estimated value, (b) (4) is far from what is required for growth of even the most xerotolerant organism, the "no growth" statement can be given without reservation.

As the water activity also was tested with similar results when the can had been open for (b) (4) (b) (4) in a realistic indoor environment, strengthens the conclusion that microbes cannot grow under recommended storage.

<sup>1</sup> Beuchat, L.R. Influence of water activity on growth, metabolic activities and survival of yeasts and molds. Journal of Food Protection (1983) 46:135-141.  
<sup>2</sup> Leong, S-L. L., Lantz, H., Pettersson, O.V., Frisvald, J.C., Thrane, U., Heipieper, H.J., Dijksterhuis, J., Grabherr, M., Pettersson, M., Tellgren-Roth, C. & Schnürer, J. Genome and physiology of the ascomycete filamentous fungus *Xeromyces bisporus*, the most xerophilic organism isolated to date. Environmental Microbiology (2015) 17:496-513.



## Nicotine as an inhibitor of microbial growth

It has been reported that nicotine in itself has antimicrobial activities<sup>3,4</sup>. According to a first study by Pavia et al. 2000, there is a strong growth inhibitory effect at 250 µg/ml of all tested organisms, eight common bacteria and one yeast. In a second study, Zaidi et al. 2012, a concentration of 200 µg/ml resulted in inhibition of three out of eight tested bacteria but in that methodology inhibition was monitored as clearing zones on agar plates instead of reduced growth rate in liquid cultures.

For the risk assessment of the ZYN<sup>®</sup> products, we decided to calculate whether or not the total nicotine content is sufficient to inhibit growth of harmful microorganisms. We performed our calculation on the lower nicotine concentration of 3 mg. To be able to compare the nicotine contents of the ZYN<sup>®</sup> products and the above-mentioned studies, we also made the assumption that one pouch is dissolved in (b) (4) to a final volume of (b) (4), which we considered a worst-case dilution scenario. This mean that the final concentration of nicotine is then 3.0 mg/ml, i.e. (b) (4) than the inhibiting concentration of 250 µg/ml.

There is a lack of data regarding antimicrobial properties of nicotine but considering the comparatively high levels of nicotine in the ZYN<sup>®</sup> products a substantial growth inhibition of microorganisms can be expected.

The observed difference in pH between the study by Pavia et al. (pH 7.4) and the ZYN<sup>®</sup> products (pH (b) (4)) is not expected to have any impact on the antimicrobial effect due to the chemistry of nicotine.

## Final conclusion

In terms of microbial safety, all assessed products within the ZYN<sup>®</sup> portfolio are considered safe under proposed and realistic storage conditions. The conclusion is mainly based on the extreme dryness that prevents growth of all known organisms. Other factors indicating product safety are food grade ingredients and antimicrobial properties of nicotine.

(b) (4)

Performed by

(b) (6)

<sup>3</sup> Pavia, C.S., Pierre, A. & Nowakowski, J. Antimicrobial activity of nicotine against a spectrum of bacterial and fungal pathogens. *Journal of Medical Microbiology* (2000) 49:674-675.

<sup>4</sup> Zaidi, M.I, Wattoo, F.H., Wattoo, M.H.S., Tirmizi, S.A. & Salman, S. Antibacterial activities of nicotine and its zinc complex. *African Journal of Microbiology Research* (2012) 6:5134-5137.

## Appendix 1

Table 2 Product name and SKU number

Product name	SKU-number
ZYN Cool Mint 3 mg	8105
ZYN Cool Mint 6 mg	8106
ZYN Peppermint 3 mg	8107
ZYN Peppermint 6 mg	8108
ZYN Spearmint 3mg	8109
ZYN Spearmint 6mg	8110
ZYN Wintergreen 3 mg	8111
ZYN Wintergreen 6 mg	8112
ZYN Coffee 3 mg	8124
ZYN Coffee 6 mg	8125
ZYN Cinnamon 3 mg	8128
ZYN Cinnamon 6 mg	8129
(b) (4)	(b) (4)
ZYN Citrus 3 mg	8122
ZYN Citrus 6 mg	8123
(b) (4)	(b) (4)
ZYN Smooth 3 mg	8134
ZYN Smooth 6 mg	8135
(b) (4)	(b) (4)
ZYN Chill 3 mg	8140
ZYN Chill 6 mg	8141
(b) (4)	(b) (4)