Medium/Long Term Variability of Moist Smokeless Tobacco Products from the United States Marketplace

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Study Plan

- The purpose of the study was to examine the temporal variability of harmful and potentially harmful constituents (HPHC) in moist smokeless tobacco (MST) products
- Twenty products comprising 70% of the US market were collected at seven-time points over a three-year period
- Analyzed for FDA's abbreviated list of smokeless tobacco HPHCs
  - NNN, NNK, nicotine (free and total), benzo[a]pyrene, acetaldehyde, formaldehyde, crotonaldehyde, arsenic, and cadmium[1,2]. All analytes are expressed on an as-is basis.
- Samples were frozen (-20° C) when received and then removed from the freezer and analyzed together to minimize the effect of temporal analytical method variability from product variability
- Three replicates were conducted per sample for each HPHC

1. Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke Under Section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act. Draft Guidance. March 2012.
2. NAT, NAB, pH, moisture and portion weight for portioned products are not part of FDA's abbreviated list of smokeless tobacco HPHCs. However, these items were also measured and reported for a more comprehensive characterization of the products.

Study Products

Product Name	Prod. Abbr.
American Snuff Company, LLC	
Grizzly Natural Fine Cut	Grzly FC Natl
Grizzly Straight Long Cut	Grzly LC Strt
Grizzly Wintergreen Long Cut	Grzly LC WG
Grizzly Wintergreen Pouch	Grzly Pch WG
Kodiak Wintergreen Long Cut	Kodiak LC WG
National Tobacco Company, L.P.	
Stokers Wintergreen Long Cut	Stokers LC WG
Swedish Match North America LLC	
Longhorn Wintergreen Long Cut	Lnghrn LC WG
Swisher International, Inc.	
Kayak Wintergreen Long Cut	Kayak LC WG

Product Name	Prod. Abbr.
U.S. Smokeless Tobacco Products LLC	
Copenhagen Mint Long Cut	Cphgn LC Mnt
Copenhagen Mint Pouch	Cphgn Pch Mnt
Copenhagen Natural Fine Cut	Cphgn FC Natl
Copenhagen Natural Long Cut	Cphgn LC Natl
Copenhagen Natural Pouch	Cphgn Pch Nat
Copenhagen Straight Long Cut	Cphgn LC Strt
Copenhagen Wintergreen Long Cut	Cphgn LC WG
Red Seal Wintergreen Long Cut	RedSeal LC WG
Skoal Mint Pouch	Skoal Pch Mnt
Skoal Straight Long Cut	Skoal LC Strt
Skoal Wintergreen Fine Cut	Skoal FC WG
Skoal Wintergreen Long Cut	Skoal LC WG

- Sample selection: we selected the top 17 market share products of MST available in the U.S. (based on volume share as of 04/28/2019), augmented with the top product from each of three smaller manufacturers for a total of 20 MST products.
- Third party trademarks are the property of their respective owners, are used for reference only, and are not intended to suggest any affiliation.

Quantification/Assessment of Sample-to-Sample Variability

- Statistical significance
  - Proportion of analyte differences that are statistically significant across timepoints
- Estimation of sample-to-sample standard deviations
  - Essentially equivalent to the range (after scaling*). Since the range seems more intuitive and this measure was roughly equivalent, only the range is shown.
- Calculation of the range of the values for each product relative to the mean value
  - (max-min)/average – this illustrates how different the analytes of the same product can be at two timepoints
- Comparison of product ranges to QC monitor ranges

* The expected value of the range with seven data points is approximately 2.7*StdDev. These matched very closely when the range and the standard deviation were both calculated from the same data set.

Statistically Significant Differences and Range

Analyte	Significant (%)	N Significant	N Not Significant	Avg Range % (max-min)/avg[1]
NNN	85%	17	3	51.0%
NNK	95%	19	1	85.2%
NAT	80%	16	4	48.7%
NAB	90%	18	2	55.5%
Nicotine	90%	18	2	16.6%
Nicotine (Unprotonated)[2]	85%	17	3	63.2%
Acetaldehyde	80%	16	4	116.4%
Crotonaldehyde[3]	NA	NA	NA	NA
Formaldehyde	55%	11	9	39.8%
Benzo[a]pyrene	70%	14	6	29.0%
Arsenic	50%	10	10	21.8%
Cadmium	75%	15	5	18.9%
Moisture	75%	15	5	2.7%
pH[4]	75%	15	5	0.36
Portion Weight	50%	2	2	4.0%

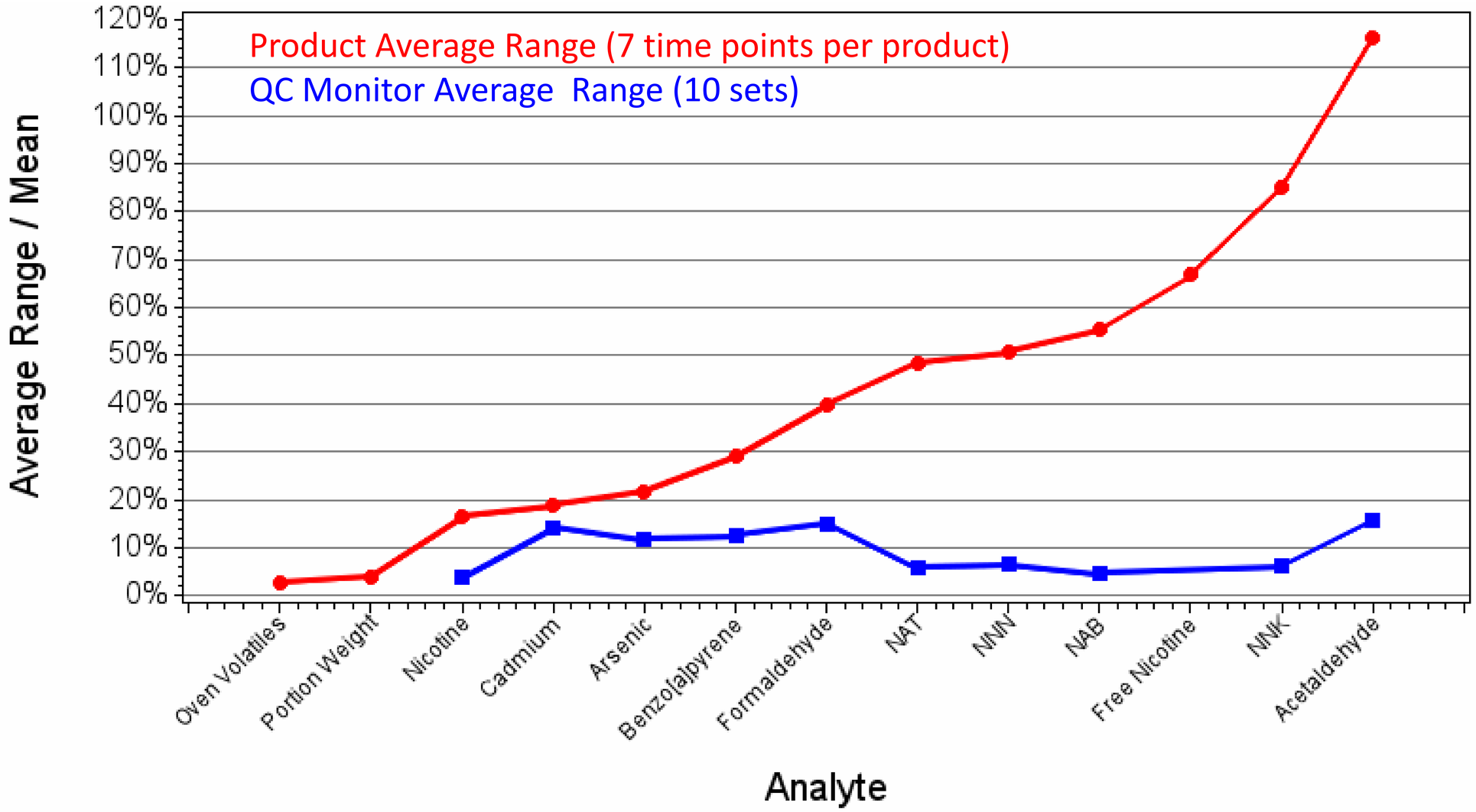
1. Averaged over the 20 products.

2. Unprotonated/free nicotine was calculated using the Henderson-Hasselbalch equation.

3. There were too few crotonaldehyde values above the LOQ for the calculations to be meaningful.

4. pH range was expressed as (max-min). It was not divided by the mean because pH is not a proportional scale.

Average Range for Each Analyte

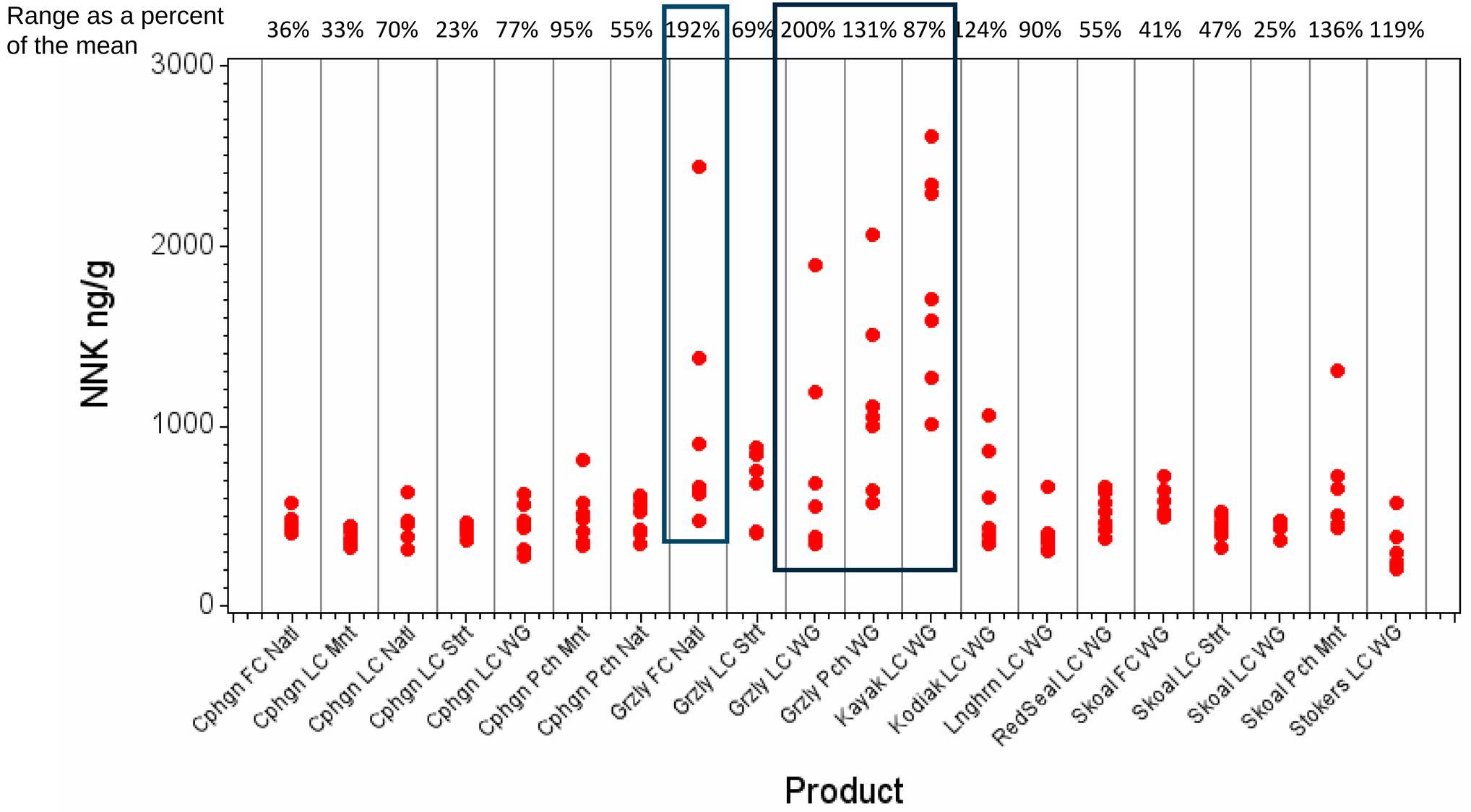


For reference, the product averages were compared to QC monitors run with the products. These results confirm that the temporal variability that is observed in the products reflects real differences, not just analytical variability.

Key Findings

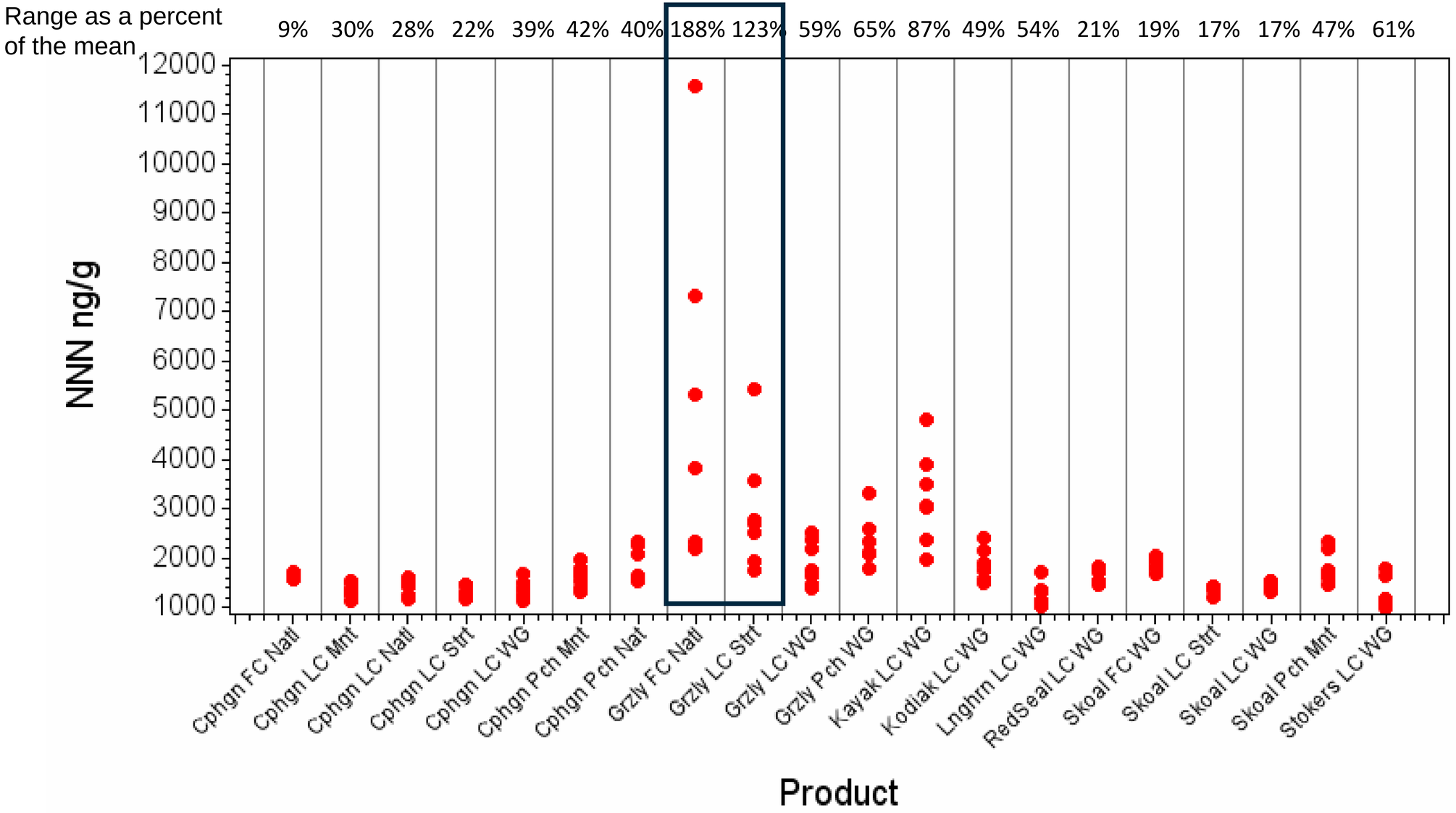
- The HPHCs were generally quite variable from time period to time period
  - TSNAs, unprotonated nicotine, and acetaldehyde were particularly variable
- Manufacturing characteristics such as moisture, pH and pouch weight were reasonably consistent from time period to time period though they were often statistically significantly different

NNK, Averaged by Time Point



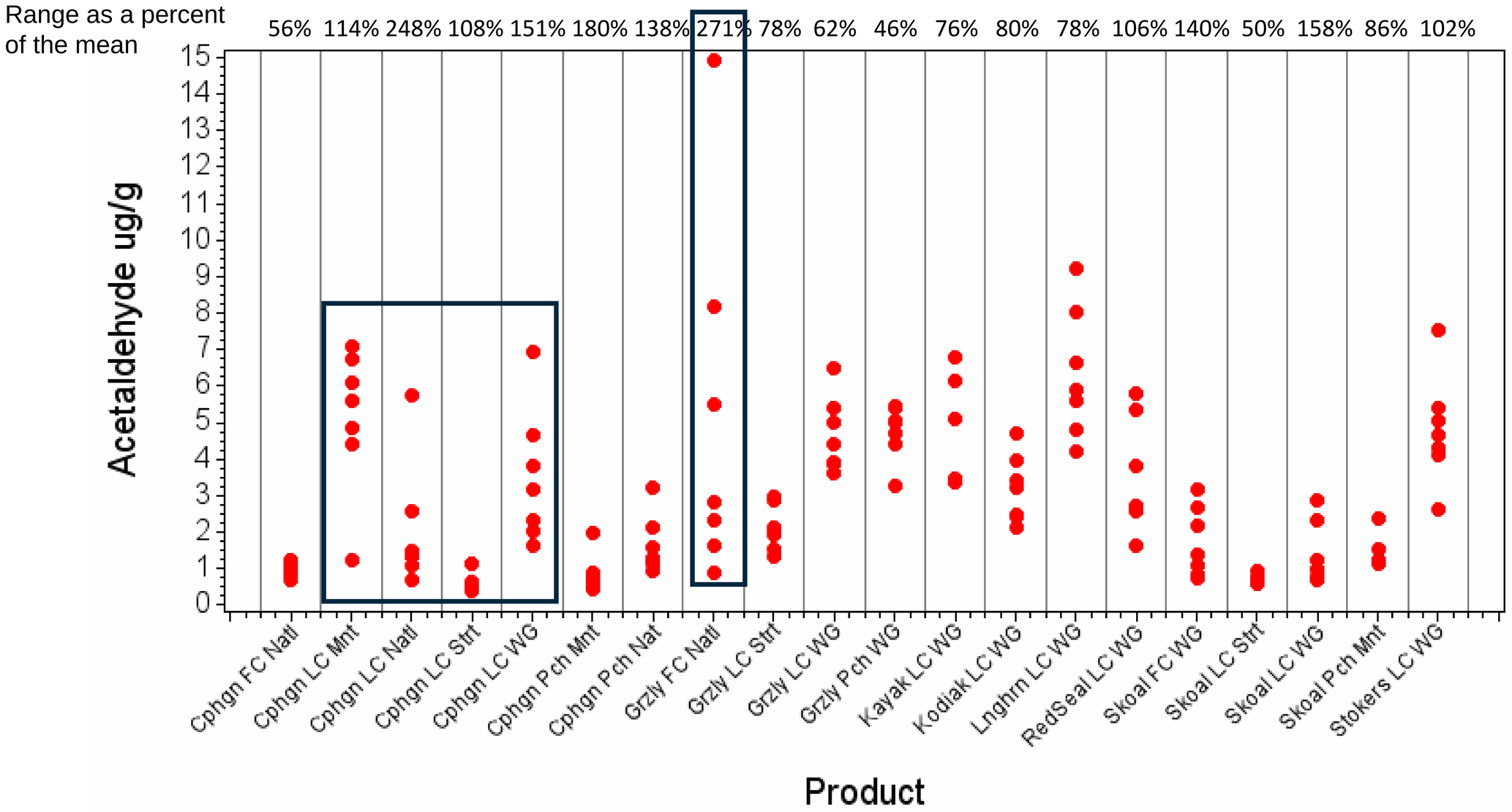
TSNAs are notoriously variable over time. Some of the larger ranges are highlighted.

NNN, Averaged by Time Point



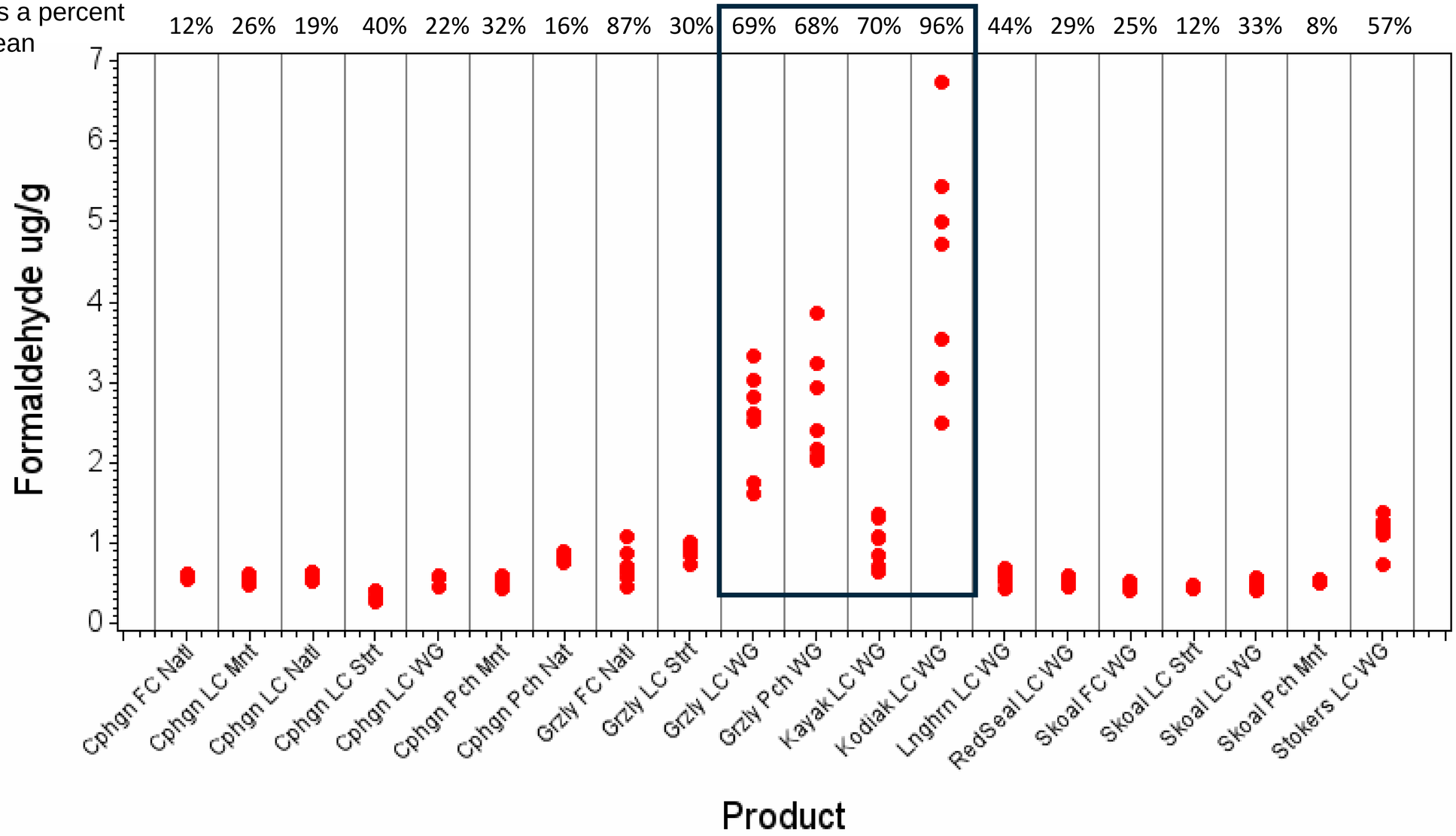
TSNAs are notoriously variable over time. Some of the larger ranges are highlighted.

Acetaldehyde, Averaged by Time Point



Acetaldehyde was more variable than I anticipated. Some of the larger ranges are highlighted.

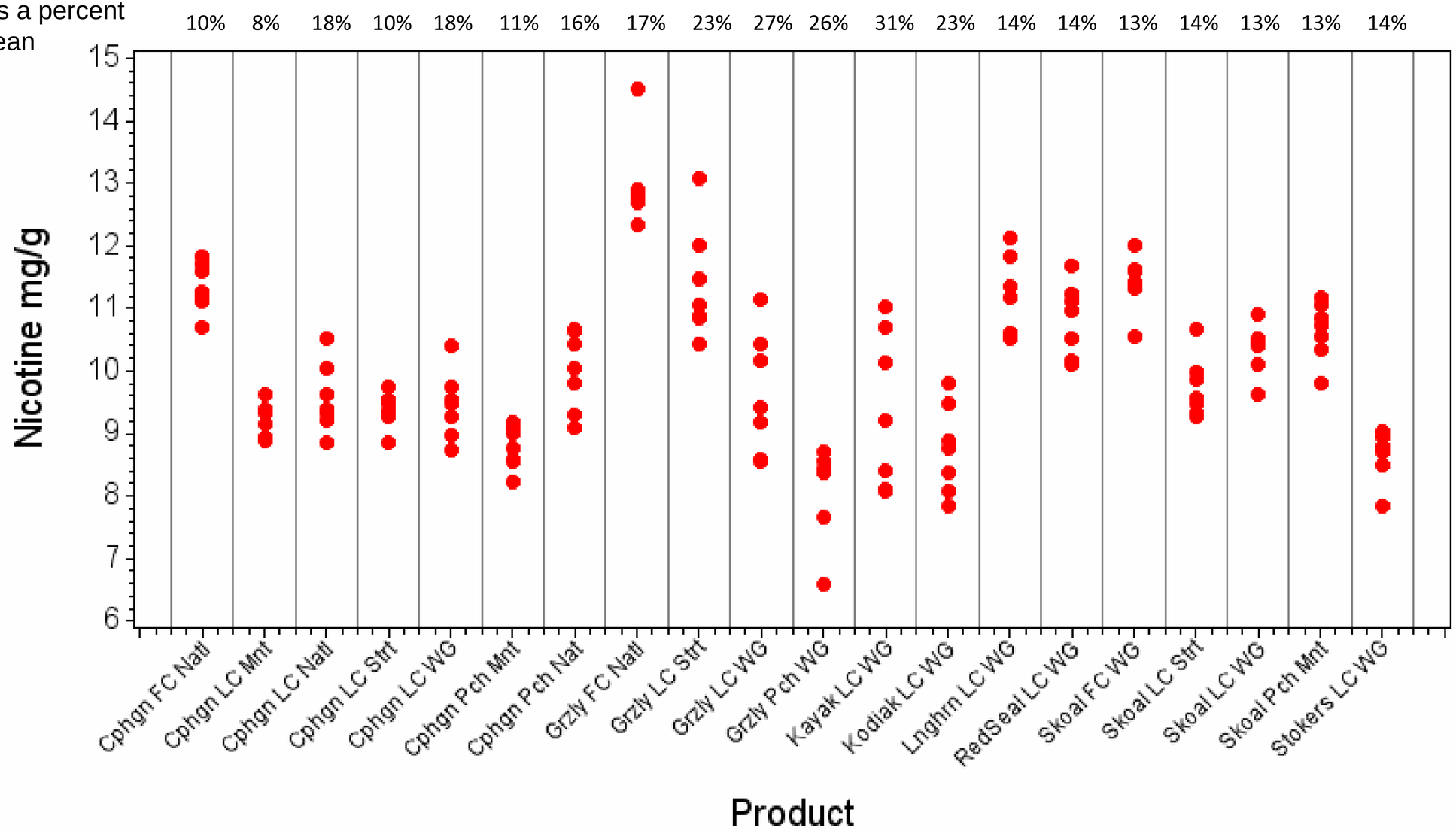
Formaldehyde, Averaged by Time Point



Formaldehyde was in some instances quite variable.
Some of the larger ranges are highlighted.

Nicotine, Averaged by Time Point

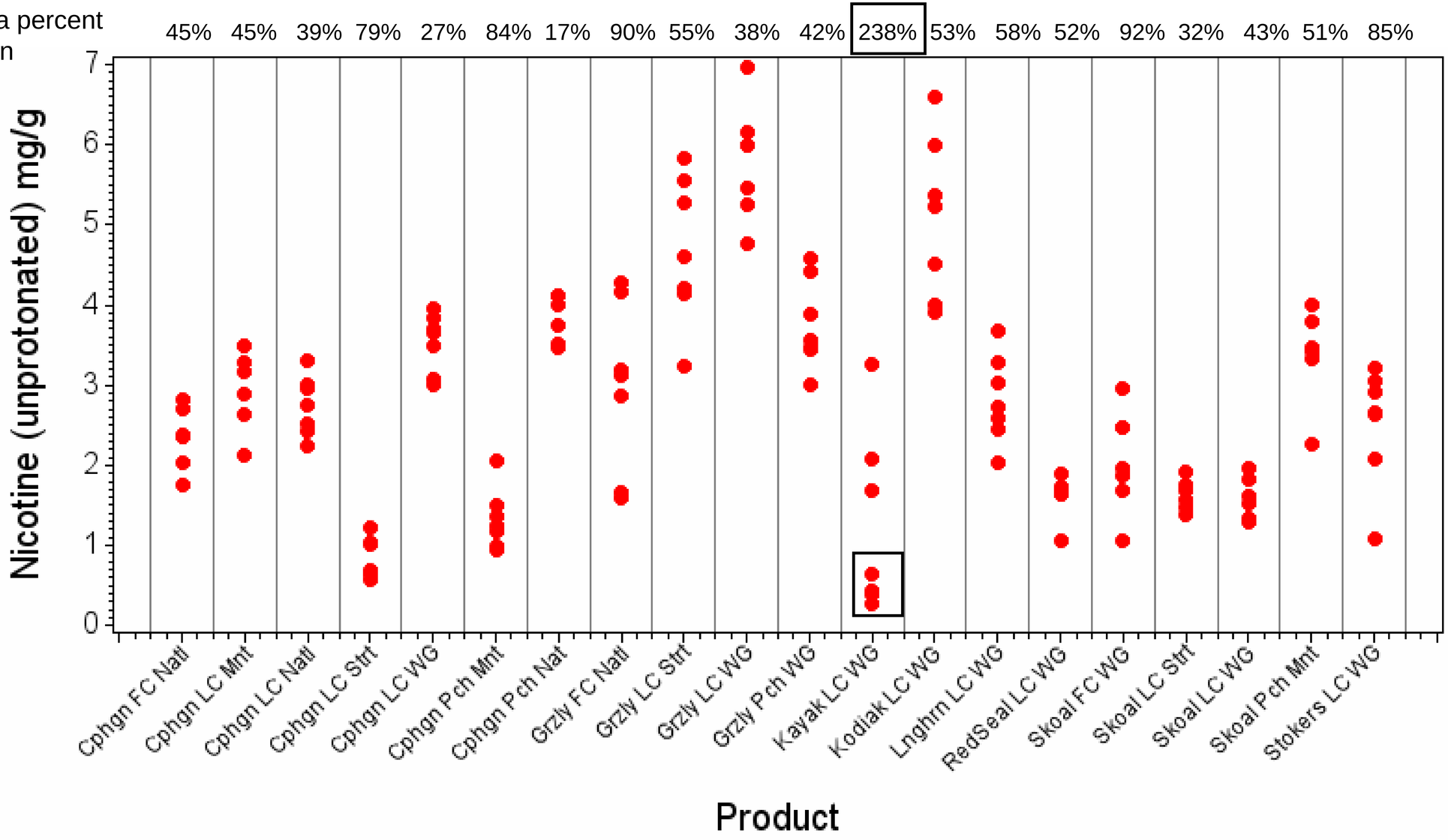
Range as a percent of the mean



Nicotine varied statistically significantly in most cases but less so than many of the other HPHCs

Nicotine (Unprotonated), Averaged by Time Point

Range as a percent of the mean



This has a wider range than nicotine because of the sensitivity to pH. The range for the product with the largest range could be exaggerated because of a possible pH design change.

Implications of Findings

- Large HPHC variability in products produced at different times would greatly complicate compliance with a performance standard
- Uncertainty in product characterizations must consider both temporal variability in the product as well as temporal and lab-to-lab variation in the analytical method
- Replicates of a single sample at a single point in time provide very limited benefit for product characterization
  - The common statistical formula for standard error ($\sigma_{se} = \sqrt{\sigma^2/n} = \sigma/\sqrt{n}$) assumes that the data values are statistically independent – they are not independent when all test results come from the same batch or are tested in the same lab in a short timespan
  - Proper calculation from multiple replicates from the same lab and the same batch is $\sigma_{se} = \sqrt{\sigma_B^2 + \sigma_L^2 + \sigma^2/n}$, where σ_B^2 and σ_L^2 are the batch-to-batch and lab-to-lab variabilities, respectively. This normally asymptotes after no. reps=n=2 or 3.

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