Tobacco and Tobacco Products Analytes Sub-Group

Technical Report

2021 Nicotine Pouches Collaborative Study

October 2021

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1. Summary

At the virtual CORESTA Tobacco and Tobacco Products Analytes Sub-Group (TTPA) meeting held on October 13, 2020, the Sub-Group initiated a collaborative study for the determination of nicotine, pH, moisture (oven volatiles), tobacco-specific nitrosamines (TSNAs), water activity, metals, carbonyls, and benzo[a]pyrene (B[a]P) in nicotine pouches. The intent of this study was to update the scopes of the applicable CORESTA Recommended Methods (CRMs) to also include nicotine pouches and to provide repeatability (r) and reproducibility (R) results and z-scores to support laboratory accreditation. Seventeen laboratories participated in the study.

The results of the study demonstrate that the CRMs for nicotine, pH, moisture, TSNAs, water activity, metals, carbonyls and B[a]P are suitable for the analysis of nicotine pouches and the TTPA recommends an update of the eight CRMs to include nicotine pouches.

2. Introduction

Nicotine pouches, or white pouches, are oral tobacco products that contain tobacco derived nicotine, but not tobacco leaf. Currently there are no standardized methods available for the analysis of nicotine pouches even though there are regulatory reporting requirements. At the virtual CORESTA TTPA meeting held on October 13, 2020, the group agreed to conduct a collaborative study to support scope expansion of the nicotine, pH, moisture, TSNAs, water activity, metals, carbonyls and B[a]P CRMs to also include nicotine pouches.

2.1 Objective

The participating laboratories were to provide analytical results for nicotine, pH, moisture, four TSNAs (N-Nitrosonornicotine (NNN), N-Nitrosoanatabine (NAT), N-Nitrosoanabasine (NAB) and 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)), water activity, metals (arsenic, cadmium), carbonyls (formaldehyde, acetaldehyde, crotonaldehyde), and B[a]P. This work was conducted using the CRMs referenced in Section 3.

The study provided an assessment of intra- and inter-laboratory variability for the analysis of nicotine pouches using the applicable CRMs, and thereby allowed for a determination of the suitability of the CRMs for the analysis of nicotine pouches. Data were collected from the participating laboratories and statistically evaluated in basic conformance with the recommendations of ISO 5725-2:1994 and ISO/TR 22971:2005. Additionally, z-scores were calculated as a measure of each laboratory's performance as compared to the results of other laboratories.

It was not possible to calculate repeatability and reproducibility values for analytes below the limit of detection. In order to scientifically demonstrate that the CRMs are fit for use, one laboratory conducted fortification studies for these analytes.

3. Organisation

3.1 Participants

A list of the participating laboratories is provided in Table 1. Not all laboratories provided data for all analyses. The laboratories are listed in alphabetical order. Letter codes were assigned to each laboratory and do not correspond to the order in the table below.

Table 1: List of Participating Laboratories

Participating Laboratories
Altria Client Services LLC, United States
American Snuff Company, United States
C.I.T. Montepaz S.A., Uruguay
Enthalpy Analytical, Richmond, United States
Essentra – Jarrow, United Kingdom
Eurofins Food & Feed Testing Sweden AB, Sweden
Global Laboratory Services, United States
Imperial Brands, Reemtsma, Germany
JTI Oekolab, Austria
Labstat International ULC, Canada
Japan Tobacco Inc., Leaf Tobacco Research Center, Japan
R.J. Reynolds Tobacco Company, United States
Shanghai New Tobacco Product Research Institute, China
Swedish Match North America, United States
Swedish Match Northern Europe, Sweden
University of Kentucky, United States
Zhengzhou Tobacco Research Institute of CNTC, China

3.2 Protocol

The protocol is provided in Appendix A and specific details from the protocol are described below:

3.2.1 Sample Shipment

The samples listed in Table 2 were distributed by Swedish Match and RJR. Samples were shipped at ambient conditions in their original cans. Upon receipt, the laboratories were requested to store the samples at approximately −20 °C. Laboratories were requested to conduct the study in November through February and report data by March 1, 2021.

Table 2: Sample Identification

Sample Name	Description	Moisture (OV) (%)	Nicotine Strength (mg/portion)	Portion Weight (grams)	Quantity/can (pouches)
NP1	Nicotine pouch	5	3	0,4	20
NP2	Nicotine pouch	40	11	0,8	21
NP3	Nicotine pouch	15	4	0,45	15
NP4	Nicotine pouch	32	4	0,6	15

3.2.2 Within Laboratory Sample Preparation

The laboratories were directed to place unopened samples held at -20 °C in a refrigerator for a minimum of 24 hours to ensure water had fully equilibrated within the product. Samples could then be removed from the refrigerator for a minimum of 2 hours prior to opening for analysis. The samples were not to be opened during equilibration to ambient temperature. Once samples were equilibrated to ambient temperature, the samples were to be stored at approximately 4 °C for up to one week if the analyses were not conducted immediately. Handling requirements for the nicotine pouches are described below:

The nicotine pouches shall be cut into 2 halves, and the filler and pouch added directly to the extraction or digestion vessel. Both the filler and pouch material were to be analyzed.

Replicates for water activity and oven volatiles shall be taken from a freshly opened can where intact pouches are analyzed.

3.2.3 Sample Analysis and Data Reporting

The participating laboratories were instructed to conduct triplicate replicate analyses (individual weighting) using the latest versions of the following CRMs:

- CRM N° 62 - Determination of Nicotine in Tobacco and Tobacco Products by Gas Chromatographic Analysis
- CRM N° 69 - Determination of pH in Tobacco and Tobacco Products
- CRM N° 72 - Determination of Tobacco Specific Nitrosamines in Tobacco and Tobacco Products by Liquid Chromatography - Tandem Mass Spectrometry
- CRM N° 76 - Determination of Moisture Content (Oven Volatiles) of Tobacco and Tobacco Products
- CRM N° 82 - Determination of Benzo[a]pyrene in Tobacco Products by GC-MS
- CRM N° 86 - Determination of Select Carbonyls in Tobacco and Tobacco Products by UHPLC-MS/MS
- CRM N° 88 - Determination of Water Activity of Tobacco and Tobacco Products
- CRM N° 93 - Determination of Selected Metals in Tobacco Products by ICP-MS

Participating laboratories were requested to document any deviations from the protocol and the CRMs and submit the deviations with their results. As stated in the protocol, data submitted with significant deviations from the applicable CRM were excluded from the r & R portion of the study. Deviations reported by the laboratories are listed below as well as if the data were excluded from the study.

- Lab E: Minor deviations. One pouch of each sample was extracted by 1 ml NaOH and 10 ml MTBE, instead of 7 ml NaOH and 50 ml MTBE as stated in CRM N° 62. These results were included in the study.
- Lab H: Minor deviations. Added hydrogen peroxide after nitric acid and prior to microwave digestion for the analysis of metals as described in CRM N° 93. These results were included in the study.
- Lab K: Minor deviations. 2 M NaOH instead of 5 M NaOH, as stated in CRM N° 62. 1,000 ml (600 ng/ml) internal standard (IS) was added instead of 0,300 ml (2000 ng/ml) IS. 10 mM ammonium acetate. pH 4,7 (acetic acid) as mobile phase A and 0,1 % formic acid in acetonitrile as mobile phase B, instead of water (mobile phase A) and 0,1 % (v/v) acetic acid in methanol (mobile phase B), as stated in CRM N° 72. Oven temperature (99 ± 1) °C instead of (100 ± 1) °C as stated in CRM N° 76. Standards

were made using nitric and hydrochloric acids instead of only nitric as specified in the CRM N° 93. The samples were digested in 5 ml water/4 ml nitric acid/1 mL hydrogen peroxide versus 10 ml of concentrated nitric acid as specified in the CRM N° 93. An amount of 1 mL of HCl was added to the sample post microwave digestion, this step is not included in the CRM ° 93. The sample was brought to a final sample volume of 50 mL of water versus 100 mL as specified in the CRM N° 93. Methanol was added as a carbon effect minimizer during analysis, this step is not included in the CRM N° 93. These results were included in the study.

- Lab N: Major deviations. Sample preparation with methanol/water (50/50. v/v) instead of ammonium acetate as stated in CRM N° 72. Calibration standards were prepared with methanol/water (50/50, v/v) instead of acetonitrile/water and ammonium acetate. These results were excluded from the study.
- Lab Q: 5 mL of a 2N NaOH solution was used instead of 7 mL of 5N NaOH solution as described in CRM N° 62. The results for nicotine analysis were included in the study. Cyclohexane was used instead of methanol as stated in CRM N° 82 and NH2/Si cartridges were used for the clean-up instead of polymeric reversed phase SPE cartridges. The results for B[a]P were excluded from the study.

All test results were to be reported on an as-is basis to the four significant figures, with no correction for moisture content. The study results and the comments were to be sent by e-mail to the study coordinators (Rozanna Avagyan and Karl Wagner).

4. Data – Raw

The full data set for the study is provided in Appendix B. The results are presented on an as-is basis, without correction for moisture. Each analysis includes three replicates. Not all laboratories provided data for all analyses.

As mentioned above, data sets were removed from the r & R portion of the study due to significant deviations from the study protocol or if the data were identified as outlying data. Those data are included in Appendix B but were eliminated prior to the r & R analysis.

5. Data – Statistical Analysis

The statistical analysis was conducted in basic conformance with ISO 5725-2:1994 and ISO/TR 22971:2005. A summary of the results from outlier detection and the calculated results for repeatability (r) and reproducibility (R) are given below in sections 5.1 and 5.2, respectively. Even though ISO 5725-2:1994 does not suggest calculation of z-scores, z-scores are presented in section 5.3 so that the participating laboratories would have an additional measure of their performance compared to their peers. Raw data plots that include all replicates, without removal of outliers, are shown in Appendix C.

5.1 Exclusion of Outliers

The data for several analytes were often or always below reporting limits. Repeatability and reproducibility could not be calculated for TSNAs, B[a]P, crotonaldehyde, arsenic, cadmium, and for acetaldehyde for NP3 and NP4 because too few reported results were available (between 0 and 5 data sets were available for those analytes).

Where the r & R calculations were possible, procedures outlined in ISO 5725-2:1994 and ISO/TR 22971:2005 were generally used for the exclusion of outliers. An adaptation of

Levene's Test was used for eliminating laboratories with overly large repeatability standard deviations and Grubbs' Test was used to eliminate laboratories with outlying mean values.

ISO 5725(2) also recommends the use of Mandel's h and k plots. Mandel's h statistic is the same as the statistic used in Grubbs' Test. Similarly, Mandel's k statistic, associated with within lab standard deviation, is statistically equivalent to the c-value calculated in Cochran's Test

$$k = \sqrt{n_{labs} \times c}$$

However, the critical values associated with Mandel's h and k statistics do not make allowance for multiple testing and can therefore, give a false impression of statistical significance. Thus, Mandel's h and k statistics do not add fundamentally new information and may lead to incorrect conclusions. For those reasons, we do not include Mandel's h and k plots.

The intent of ISO 5725-2:1994 is to eliminate outliers that exceed a 1 % critical value. This was accomplished by an adaptation of Levene's Test[1]. The Levene's and Grubbs' outliers are provided in the table below

Table 3: Outliers

Analyte	Product	Levene's Outlier Lab	Grubbs' Outlier Lab
Formaldehyde	NP1	–	P
Acetaldehyde	NP1	–	P
Water Activity	NP2	–	A
pH	NP3	–	A
OV	NP3	–	F
Water Activity	NP3	–	I, Q
pH	NP4	–	A

The (-) symbol indicates an outlier was not detected.

5.2 Calculation of Repeatability (r) and Reproducibility (R)

After removal of outlying data based on numerical data consistency methods (Grubbs' Test and Levene's Test), the final repeatability and reproducibility (r & R) results were calculated. The r & R results are shown in the table below. The r & R results reflect both laboratory variability and product consistency.

Table 4: Repeatability (r) and Reproducibility (R) Results

Parameter	Product	N° of Labs*	Mean	Repeatability		Reproducibility	
				r	%r	R	%R
Nicotine (mg/g)	NP1	13	7,39	0,469	6,3 %	1,53	20,7 %
Nicotine (mg/g)	NP2	13	13,24	0,759	5,7 %	2,59	19,5 %
Nicotine (mg/g)	NP3	13	8,61	0,685	8,0 %	1,85	21,5 %
Nicotine (mg/g)	NP4	13	6,60	0,441	6,7 %	2,02	30,6 %
OV (%)	NP1	14	2,84	0,241	8,5 %	0,80	28,3 %

[1] The approach is discussed in detail by Michael Morton in "Within-Laboratory Variance Outlier Detection: An Alternative to Cochran's Test" in Beiträge zur Tabakforschung International, Vol 27 No. 7, pp135-144.

Parameter	Product	N° of Labs*	Mean	Repeatability		Reproducibility	
				r	%r	R	%R
OV (%)	NP2	14	38,06	1,396	3,7 %	2,10	5,5 %
OV (%)	NP3	13	12,75	1,082	8,5 %	1,97	15,4 %
OV (%)	NP4	14	31,67	1,326	4,2 %	1,56	4,9 %
pH	NP1	16	8,25	0,106	NA	0,37	NA
pH	NP2	16	8,31	0,024	NA	0,23	NA
pH	NP3	15	7,57	0,053	NA	0,19	NA
pH	NP4	15	6,08	0,052	NA	0,14	NA
Aw	NP1	10	0,390	0,041	10,5 %	0,20	50,7 %
Aw	NP2	9	0,875	0,012	1,4 %	0,02	2,3 %
Aw	NP3	8	0,848	0,021	2,4 %	0,06	7,2 %
Aw	NP4	10	0,939	0,012	1,3 %	0,03	3,4 %
Formaldehyde (µg/g)	NP1	8	12,36	0,975	7,9 %	2,99	24,2 %
Formaldehyde (µg/g)	NP2	9	1,62	0,201	12,4 %	1,22	75,3 %
Formaldehyde (µg/g)	NP3	9	1,05	0,207	19,8 %	0,35	32,9 %
Formaldehyde (µg/g)	NP4	9	1,43	0,132	9,2 %	0,51	35,6%
Acetaldehyde (µg/g)	NP1	7	0,179	0,040	22,3 %	0,17	95,8 %
Acetaldehyde (µg/g)	NP2	9	0,249	0,102	41,2 %	0,42	169,7 %

*This is the number of laboratory data sets with reported values after removal of outliers.
NA = Not applicable since pH is not a proportional scale. It is not appropriate to calculate %r or %R.

5.3 Calculation of Z-Scores

Although calculation of z-scores is not suggested in ISO 5725-2:1994, z-scores were calculated so that the participating laboratories could compare their results to those of their peers. As mentioned above, data sets were removed from the r & R portion of the study due to significant deviations from the study protocol or if the data were identified as outlying data. Those data are included in the calculation of z-scores.

The z-scores were calculated using methods suggested in ISO 13528: 2005. The calculations were based on Algorithm A given in Annex C of ISO 13528: 2005. It is expected that most of the data should fall within the range of ±2, and that laboratories having values with $|z|>3$ should be treated as an "action signal" to investigate laboratory performance. Final summary tables of z-scores are presented in the table below and are shown graphically in Appendix D.

Table 5: Z-Scores

Lab	Product	Nicotine	Moisture	pH	Water Activity	Formaldehyde	Acetaldehyde
A	NP1	–	1,20	-0,58	-0,01	–	–
B	NP1	–	–	-2,10	–	-0,26	–
C	NP1	–	–	-0,29	–	-0,35	-0,70
D	NP1	-0,25	2,19	1,05	1,49	–	–
E	NP1	-0,46	-0,25	0,20	-0,61	–	–
F	NP1	0,25	-0,06	0,31	–	–	–

Lab	Product	Nicotine	Moisture	pH	Water Activity	Formaldehyde	Acetaldehyde
G	NP1	0,52	0,10	-1,07	1,42	-0,81	-0,82
H	NP1	-2,32	-0,85	0,42	-0,31	1,42	-0,48
I	NP1	-0,92	-1,26	1,47	-0,88	–	–
J	NP1	1,40	1,46	–	–	–	–
K	NP1	1,12	-1,06	-0,31	-0,78	0,35	0,94
L	NP1	0,40	-0,01	1,59	0,46	-0,13	0,22
M	NP1	–	0,31	0,22	–	–	–
N	NP1	-0,79	–	-0,27	–	0,91	-0,91
O	NP1	-0,82	-0,20	0,57	–	0,37	0,25
P	NP1	0,98	-0,80	-0,87	-0,84	-4,52	4,11
Q	NP1	0,07	-0,09	-0,85	0,06	–	–
A	NP2	–	-0,20	-1,86	4,19	–	–
B	NP2	–	–	-1,13	–	0,45	-0,18
C	NP2	–	–	-0,61	–	1,16	-1,32
D	NP2	-0,53	0,28	-1,38	-0,67	–	–
E	NP2	-0,27	1,98	0,16	1,24	–	–
F	NP2	0,40	-1,56	-0,04	–	–	–
G	NP2	0,81	0,10	1,29	-0,04	-0,28	-0,23
H	NP2	-2,86	-0,45	0,40	-0,02	1,09	-0,46
I	NP2	-1,25	-0,49	1,90	-1,45	–	–
J	NP2	1,43	0,09	–	–	–	–
K	NP2	0,92	0,35	0,15	0,51	-1,01	1,69
L	NP2	0,06	0,11	-0,12	-0,42	0,21	0,67
M	NP2	–	-1,51	0,49	–	–	–
N	NP2	-0,22	–	0,71	–	-0,28	0,93
O	NP2	-0,79	1,62	0,32	–	0,16	-0,78
P	NP2	0,89	-0,35	-0,77	-0,42	-3,57	-0,13
Q	NP2	0,08	0,54	0,53	-0,23	–	–
A	NP3	–	1,37	-4,43	1,00	–	–
B	NP3	–	–	1,36	–	-0,03	–
C	NP3	–	–	-0,24	–	0,51	–
D	NP3	0,22	0,94	-1,12	-0,43	–	–
E	NP3	0,05	-0,57	-0,18	0,34	–	–
F	NP3	0,64	-5,26	-0,14	–	–	–
G	NP3	-0,27	-0,68	2,20	0,76	-0,40	–
H	NP3	-2,87	0,02	0,19	0,23	0,78	–
I	NP3	-0,99	0,66	-0,70	-2,28	–	–

Lab	Product	Nicotine	Moisture	pH	Water Activity	Formaldehyde	Acetaldehyde
J	NP3	1,63	-0,07	–	–	–	–
K	NP3	0,83	0,88	0,21	0,36	-1,21	–
L	NP3	1,03	0,75	-0,32	0,07	-0,03	–
M	NP3	–	0,26	0,75	–	–	–
N	NP3	-0,44	–	0,62	–	-1,31	–
O	NP3	-0,33	-0,48	0,70	–	0,28	–
P	NP3	0,35	-0,08	-1,35	0,67	1,40	–
Q	NP3	-1,07	-1,97	0,21	-7,13	–	–
A	NP4	–	0,97	-7,90	2,87	–	–
B	NP4	–	–	0,51	–	1,01	–
C	NP4	–	–	0,35	–	-0,30	–
D	NP4	0,07	0,74	-0,63	-0,75	–	–
E	NP4	-1,28	0,77	-1,25	0,22	–	–
F	NP4	0,49	-2,60	-1,14	–	–	–
G	NP4	0,48	-0,74	3,04	0,84	-1,03	–
H	NP4	-2,64	-0,60	0,59	-0,09	-0,18	–
I	NP4	-0,98	-0,98	-0,56	-1,81	–	–
J	NP4	1,49	-0,29	–	–	–	–
K	NP4	0,78	1,12	0,30	0,19	-1,29	–
L	NP4	0,52	-0,05	-0,46	0,08	-0,10	–
M	NP4	–	-1,18	1,13	–	–	–
N	NP4	-0,38	–	0,37	–	0,23	–
O	NP4	-0,58	0,92	-0,48	–	2,12	–
P	NP4	0,61	0,38	0,36	0,47	0,17	–
Q	NP4	0,27	0,42	0,90	-0,96	–	–

The (–) symbol indicates the laboratory did not submit data for that sample analysis.

6. Validation by fortification experiments

In this collaborative study, some of the analytes (TSNAs, carbonyls, B[a]P and metals) were either reported by only a few laboratories in some products and/or found at levels below or near the limits of quantification. Consequently, the reproducibility limits for these analytes could not be calculated due to insufficient number of data sets or the reproducibility limits were found to be poor, as the analyte levels found were mostly below the limits of quantification. For this reason, a limited validation, involving repeatability and accuracy was performed by one participating laboratory.

Laboratory fortified matrix spikes were used to determine if the analytical method accurately measures the concentration of the analytes in the presence of sample matrix components. The investigated sample types were NP1, NP2, NP3 and NP4. The CRMs specified in this study were followed except that know quantities of analyte were added prior to the extraction process. Each sample was fortified at one level and prepared in six replicates. The average value (n=6) of analyte determined in the unfortified matrix was used for the unfortified sample concentration in the equation below. The mean % recovery, standard deviation, and % relative standard deviation (%RSD) for each analyte are listed in Table 6.

$$\text{Accuracy (\%)} = \frac{C_{\text{fortified}} - C_{\text{unfortified}}}{C_{\text{theoretical}}} \times 100$$

$C_{fortified}$ = concentration determined experimentally for the fortified sample
$C_{unfortified}$ = concentration determined experimentally for the unfortified sample
$C_{theoretical}$ = the theoretical concentration of the spike in the resulting sample

The relative standard deviation for the analytes in the fortified samples was in the range 1 % – 11 % RSD. The accuracy for the analytes in the fortified samples was in the range 82 % – 120 % and fell within the range of 80 % – 120 % of the target value, except for crotonaldehyde in one product (NP1) where the recovery was 62 %. These experiments are summarized in the table below and the full data set is provided in Appendix E.

Table 6: Summary of fortification experiments

Parameter	Fortified amount		NP1	NP2	NP3	NP4
NNN	1,009 ng	Mean Recovery (%)	106	103	103	105
		Repeatability (RSD%)	1,5	1,2	0,9	1,5
NNK	1,006 ng	Mean Recovery (%)	95	95	95	95
		Repeatability (RSD%)	1,0	1,2	1,0	1,1
NAT	1,006 ng	Mean Recovery (%)	103	103	103	101
		Repeatability (RSD%)	2,0	0,6	1,2	1,3
NAB	0,2642 ng	Mean Recovery (%)	98	96	96	94
		Repeatability (RSD%)	1,6	1,0	1,7	1,8
B[a]P	2,500 ng	Mean Recovery (%)	87	95	91	90
		Repeatability (RSD%)	3,5	4,1	1,7	3,7
Formaldehyde	5,000 µg	Mean Recovery (%)	82	115	115	89
		Repeatability (RSD%)	9,1	7,1	2,4	6,1
Acetaldehyde	5,000 µg	Mean Recovery (%)	82	119	120	92
		Repeatability (RSD%)	6,6	11	3,8	6,2
Crotonaldehyde	1,000 µg	Mean Recovery (%)	62	86	101	80
		Repeatability (RSD%)	7,5	8,7	3,7	5,9
Arsenic	0,1000 µg	Mean Recovery (%)	95	97	104	103
		Repeatability (RSD%)	4,2	4,4	5,1	5,5
Cadmium	0,4000 µg	Mean Recovery (%)	96	99	98	98
		Repeatability (RSD%)	1,8	3,7	3,3	4,0

7. Data Interpretations

Generally, both the %r and %R in this study were acceptable for most of the analytes. For some analytes, e.g. formaldehyde and acetaldehyde, the %R was poor, due to insufficient number of data sets or low levels found in the products. The %r and %R from this study were compared with the %r and %R in the corresponding CRMs and were in line with those obtained in the CRMs for most of the analytes, see Appendix F. Similar to what was observed in the CRMs, low analyte levels result in high %r and %R. This could be seen for all analytes, and especially for formaldehyde and acetaldehyde that were detected at low levels in the products. The relatively high %R for water activity for NP1 could not entirely be explained by this trend, although high %R were also observed for CRP 3.1, which has low moisture content. A plausible cause for this might be that portion products with low moisture content are sensitive to sample handling, e.g. repeatedly opening the containers, leaving the containers open and that there also might be differences from container to container.

The repeatability and accuracy data from the fortification study met the acceptance criteria and demonstrated that the methods are able to quantify those analytes but with an unknown level of confidence since no statistical data evaluation from the collaborative study data could be performed. The low recovery of crotonaldehyde in NP1 might be a result of that acetaldehyde-d4 was used as internal standard, instead of crotonaldehyde-DNPH-d3 (as stated in the CRM) and did not compensate for the matrix effects (ion suppression) in this matrix.

8. Recommendations

The results of this study demonstrate that the CRMs for nicotine, TSNAs, water activity, pH, moisture, metals, carbonyls and B[a]P are suitable for the analysis of nicotine pouches and it is therefore recommended that the scope of the eight CRMs are updated to include these matrices.

APPENDIX A: Protocol

CORESTA TOBACCO and TOBACCO PRODUCTS ANALYTES SUB-GROUP

Project Title: 2021 Nicotine Pouches Collaborative Study

Type of Document: Study Protocol

Date: November 24, 2020

Written by: Johan Patring

Confidentiality Notice: All data submitted by participating laboratories will be coded and kept confidential.

1. Introduction

Nicotine pouches are gaining market share and are also included on the CORESTA 5-year strategy roadmap. Currently there are no standardized methods available for the analysis of nicotine pouches even though there are regulatory reporting requirements. At the virtual CORESTA Tobacco and Tobacco Products Analytes Sub-Group (TTPA) meeting held on October 13, 2020, the group agreed to conduct a collaborative study to determine if existing CORESTA Recommended Methods (CRMs) that were developed for the analysis of tobacco and traditional (unburned) tobacco products are fit for the analysis of nicotine pouches.

2. Objective

The participating laboratories are to provide analytical results for four different nicotine pouches using the current versions of the applicable CRMs that were originally developed for the analysis of traditional unburned tobacco products such as smokeless tobacco, cigarette and cigar filler. The analyses include nicotine, pH, moisture (oven volatiles), tobacco-specific nitrosamines (N-Nitrosonornicotine (NNN), N-Nitrosoanatabine (NAT), N-Nitrosoanabasine (NAB) and 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) or TSNAs), water activity, metals (arsenic, cadmium), carbonyls (formaldehyde, acetaldehyde, crotonaldehyde), and benzo[a]pyrene (B[a]P). This study will provide an assessment of intra- and inter-laboratory variability for the analysis of nicotine pouches using the applicable CRMs and thereby allow for the determination of the suitability of the CRMs for the analysis of nicotine pouches. Data generated with methods that differ from the respective CRMs will be excluded from the study results.

The data will be statistically evaluated in basic conformance with the recommendations of ISO 5725-2 to assess within and between laboratory variability. Additionally, z-scores will be calculated in order to allow laboratories to compare their results to those of other laboratories. Results will be presented at the spring 2021 TTPA meeting and in a technical report.

3. Time schedule and Data Reporting

The study timeline is shown in Table 1**.**

Table 1: Study timeline

Date	Activity
November, 2020	Participants to be sent materials (nicotine pouches)
November-February, 2020-2021	Laboratories conduct the study
March 1, 2021	Laboratories submit results by this date
April, 2021	Discuss results at spring TTPA SG meeting

4. Participating Laboratories

The laboratories listed in Table 2 have kindly agreed to take part in the study. Other laboratories are encouraged to participate and should notify Rozanna Avagyan and Karl Wagner of their interest to participate.

Table 2: Participating laboratories

Participating Laboratories	Nicotine CRM 62/87 MTBE	TSNA CRM 72	pH CRM 69	OV CRM 76	Water Activity CRM 88	Metals CRM 93	Carbonyls CRM 86	B(a)P CRM 82
Altria, United States	X	X	X	X	X	X	X	X
American Snuff Company, United States	X	X	X	X	X			X
British American Tobacco, Brazil		X	X	X		X		X
C.I.T. Montepaz S.A., Uruguay	X	X	X	X			X	X
Enthalpy Analytical, LLC, Richmond, United States		X		X		X	X	
Essentra, United Kingdom		X	X	X				X
Eurofins Food & Feed Testing Sweden AB, Sweden		X	X				X	
Global Laboratory Services, Inc., United States	X	X	X	X	X	X	X	X
Imperial Brands, Reemtsma, Germany	X	X	X		X	X	X	X
Japan tobacco, Leaf Tobacco Research Center, Japan	X	X	X	X				
JTI Ökolab, Vienna, Austria		X	X			X	X	
KT&G, South Korea	X	X	X	X	X	X		
Labstat ULC, Canada	X	X	X	X	X	X	X	X
National Institute of Standards and Technology, United States	X	X	X					X
Novasina, United States					X			
RJ Reynolds Tobacco Company, United States	X	X	X	X	X	X	X	X

Participating Laboratories	Nicotine CRM 62/87 MTBE	TSNA CRM 72	pH CRM 69	OV CRM 76	Water Activity CRM 88	Metals CRM 93	Carbonyls CRM 86	B(a)P CRM 82
Shanghai New Tobacco Product Research Institute, China	X	X	X	X			X	
Swedish Match NE, Sweden	X	X	X	X	X	X	X	X
Swedish Match North America, United States	X	X	X	X	X			
Swisher International, United States	X	X	X	X	X			
University of Kentucky, United States	X	X	X	X	X			X
Zhengzhou Tobacco Research Institute of CNTC, China			X	X	X			

5. Samples

The samples listed in Table 3 will be distributed by the providing Swedish Match and RJR.

- NP1 and NP2 will be sent through Rozanna Avagyan
- NP3 and NP4 will be sent by Jannell Rowe

Each participating laboratory will need to provide their shipping address, person to whom delivery should be made. and any special delivery information to Rozanna Avagyan. Rozanna will inform the laboratories of the actual shipping date and tracking information so that the receiving laboratories can prepare for receipt of the samples. Samples will be shipped at ambient conditions. Laboratories should not submit data if they question the integrity of the samples they received.

Upon receipt, the samples must immediately be stored in the refrigerator at approximately 4 ^{o}C. If samples will not be analysed within 1-week, they shall be stored in a freezer at approximately -20 °C until the analyses are performed. Unused test samples should be retained at -20 °C until after the study data has been evaluated by the project leader.

Table 3: Study samples

Sample Name	Moisture (OV) (%)	Nicotine Strength (mg/portion)	Portion Weight (g)	Quantity/can
NP1	5	3	0.4	20 pouches/can; total 8 g
NP2	40	11	0.8	21 pouches/can; total 16.8 g
NP3	15	4	0.45	15 pouches/can; total 6.75 g
NP4	32	4	0.6	15 pouches/can; total 9 g

6. Analysis

6.1 Methods

The latest versions of the following CRMs shall be used for all analyses. Data collected with other methods, or with methods that have significant deviations from the CRMs will not be included in the study.

- CRM N° 62. Determination of Nicotine in Tobacco and Tobacco Products by Gas Chromatographic Analysis
- CRM N° 69. Determination of pH in Tobacco and Tobacco Products
- CRM N° 72. Determination of Tobacco Specific Nitrosamines in Tobacco and Tobacco Products by Liquid Chromatography - Tandem Mass Spectrometry
- CRM N° 76. Determination of Moisture Content (Oven Volatiles) of Tobacco and Tobacco Products
- CRM N° 82. Determination of Benzo[a]pyrene in Tobacco Products by GC-MS
- CRM N° 86. Determination of Select Carbonyls in Tobacco and Tobacco Products by UHPLC-MS/MS
- CRM N° 93. Determination of Selected Metals in Tobacco Products by ICP-MS
- CRM N° 88. Determination of Water Activity of Tobacco and Tobacco Products

6.2 Sample Equilibration

Samples held at -20 °C shall be placed unopened in a refrigerator for a minimum of 24 hours to ensure water has fully equilibrated within the product. Samples shall be removed from the refrigerator a minimum of 2 hours prior to opening for analysis. The samples shall not be opened during the time the samples are equilibrating to ambient temperature. Once samples are equilibrated to ambient temperature, the samples shall be stored at approximately 4 ^{o}C for up to one week if the analyses will not be conducted immediately.

6.3 Replicates

Conduct three (3) independent replicate analyses for each sample. The replicates shall be determined from independent tobacco extractions or measurements.

6.4 Sample Preparation and Analysis

6.4.1 Oven Volatiles

Remove the nicotine pouches from the manufacturer's packaging just prior to analysis. Add a sufficient number of nicotine pouches to the OV tins to reach the target weight specified in CRM N° 76. Conduct three (3) independent replicate analyses for each sample.

6.4.2 Water Activity

Remove the nicotine pouches from the manufacturer's packaging just prior to analysis. Add a sufficient number of nicotine pouches to the water activity sample cup to reach the level specified by the water activity meter instructions. The nicotine pouches container shall be kept sealed unless removing pouches. Proceed with the analysis and the remaining replicates immediately. Conduct three (3) independent replicate analyses for each sample.

6.4.3 Nicotine, pH, TSNAs, B[a]P, Carbonyls, and Metals

The nicotine pouches shall be cut into 2 halves, and the filler and pouch paper added directly into the extraction vessel. Both the filler and pouch paper shall be analysed.

Note: the sample must be shaken sufficiently vigorously to separate the tobacco from the pouch material during sample preparation for each of the analytical methods.

7. Statistical Analysis

The data will be statistically evaluated in basic conformance with the recommendations of ISO 5725-2:2019 to assess within and between laboratory variability. Z-scores will be calculated in order to allow laboratories to compare their results to those of other laboratories. Repeatability and reproducibility values will be calculated or, alternatively, guidance levels will be provided if there are too few laboratories.

For analytes below the limit of detection, it will not be possible to calculate repeatability and reproducibility values. In order to scientifically demonstrate that the CRMs are fit for use, one laboratory (Swedish Match) will conduct fortification studies.

8. Data Reporting

The provided data report spreadsheet with the results of the analysis and applicable comments, shall be sent by e-mail to Rozanna Avagyan and Karl Wagner.

- Data shall be reported in the units and to the four significant figures specified in the Data Reporting Sheet.
- If data are below a Limit of Quantitation (LOQ), but above the Limit of Detection (LOD), report the estimated analytical result and note that the analytical result is an estimate.
- If data are below the Limit of Detection (LOD) report the numerical value of the LOD preceded by the "<" symbol. For example, <0.3 ng/g.
- For TSNAs, if data are below the calibration range, but there is clearly a signal with a signal-to-noise ratio that exceeds 10:1, estimate the concentration and make a note in the data reporting sheet.
- All test results shall be reported as is (with no correction for moisture content).
- Specify the method used for the analysis of nicotine (CRM 62 MTBE or hexane).
- Report any relevant deviations from the most current version of the CRM.

9. Tabulation and Presentation of the Data

The data will be coded by laboratory number rather than laboratory identity. The code will be provided to the respective participating laboratory along with the tabulated data. Results will be presented at the spring 2021 TTPA meeting and in a technical report.

APPENDIX B: Raw Data

Full Data Set (results are presented on an as-is basis)

Lab code	Product	Nicotine	OV	pH	Water activity	NNK	NNN	NAT	NAB	B[a]P	Formaldehyde	Acetaldehyde	Crotonaldehyde	Arsenic	Cadmium
		mg/g	%		Aw	ng/g	ng/g	ng/g	ng/g	ng/g	µg/g	µg/g	µg/g	ng/g	ng/g
A	NP1		3.280	8.18	0.3898										
A	NP1		3.147	8.18	0.3922										
A	NP1		3.076	8.17	0.3851										
A	NP2		38.43	8.16	0.9099										
A	NP2		37.80	8.16	0.9126										
A	NP2		37.47	8.15	0.9131										
A	NP3		14.02	7.25	0.8717										
A	NP3		13.44	7.24	0.8793										
A	NP3		13.53	7.23	0.8827										
A	NP4		32.80	5.72	0.9625										
A	NP4		31.16	5.73	0.9603										
A	NP4		32.25	5.72	0.9651										
B	NP1			7.98		<LOD	<LOD	<LOD	<LOD	<LOD	11.48	<LOD	<LOD		
B	NP1			7.99		<LOD	<LOD	<LOD	<LOD	<LOD	11.99	<LOD	<LOD		
B	NP1			7.95		<LOD	<LOD	<LOD	<LOD	<LOD	11.71	<LOD	<LOD		
B	NP2			8.21		<LOD	<LOD	<LOD	<LOD	<LOD	1.875	0.2331*	<LOD		
B	NP2			8.21		<LOD	<LOD	<LOD	<LOD	<LOD	1.754	0.2047*	<LOD		
B	NP2			8.23		<LOD	<LOD	<LOD	<LOD	<LOD	1.839	0.2120*	<LOD		
B	NP3			7.65		<LOD	<LOD	<LOD	<LOD	<LOD	1.056	<LOD	<LOD		
B	NP3			7.66		<LOD	<LOD	<LOD	<LOD	<LOD	1.020	<LOD	<LOD		
B	NP3			7.65		<LOD	<LOD	<LOD	<LOD	<LOD	1.063	<LOD	<LOD		
B	NP4			6.10		<LOD	<LOD	<LOD	<LOD	<LOD	1.600	<LOD	<LOD		
B	NP4			6.08		<LOD	<LOD	<LOD	<LOD	<LOD	1.577	<LOD	<LOD		
B	NP4			6.09		<LOD	<LOD	<LOD	<LOD	<LOD	1.612	<LOD	<LOD		

Lab code	Product	Nicotine	OV	pH	Water activity	NNK	NNN	NAT	NAB	B[a]P	Formaldehyde	Acetaldehyde	Crotonaldehyde	Arsenic	Cadmium
		mg/g	%		Aw	ng/g	ng/g	ng/g	ng/g	ng/g	µg/g	µg/g	µg/g	ng/g	ng/g
C	NP1			8.213		<LOD	<LOD	<LOD	<LOD		11.84	0.1440	0.0326*	<LOD	<LOD
C	NP1			8.213		<LOD	<LOD	<LOD	<LOD		11.12	0.1260	0.0316*	<LOD	<LOD
C	NP1			8.218		<LOD	<LOD	<LOD	<LOD		11.85	0.1398	0.0317*	<LOD	<LOD
C	NP2			8.257		<LOD	<LOD	<LOD	<LOD		2.014	0.0365*	0.0313*	<LOD	<LOD
C	NP2			8.260		<LOD	<LOD	<LOD	<LOD		2.048	0.0357*	0.0332*	<LOD	<LOD
C	NP2			8.262		<LOD	<LOD	<LOD	<LOD		2.045	0.0326*	0.0318*	<LOD	<LOD
C	NP3			7.531		<LOD	<LOD	<LOD	<LOD		1.169	<LOD	0.0263*	<LOD	<LOD
C	NP3			7.533		<LOD	<LOD	<LOD	<LOD		1.209	<LOD	0.0260*	<LOD	<LOD
C	NP3			7.553		<LOD	<LOD	<LOD	<LOD		0.9559	0.0382*	0.0299*	<LOD	<LOD
C	NP4			6.090		<LOD	<LOD	<LOD	<LOD		1.379	<LOD	0.0371*	<LOD	<LOD
C	NP4			6.061		<LOD	<LOD	<LOD	<LOD		1.392	<LOD	0.0327*	<LOD	<LOD
C	NP4			6.098		<LOD	<LOD	<LOD	<LOD		1.337	<LOD	0.0378*	<LOD	<LOD
D	NP1	7.321	3.510	8.35	0.5149	<LOD	<LOD	<LOD	<LOD	<LOD					
D	NP1	7.331	3.460	8.45	0.5063	<LOD	<LOD	<LOD	<LOD	<LOD					
D	NP1	7.218	3.370	8.38	0.5019	<LOD	<LOD	<LOD	<LOD	<LOD					
D	NP2	13.03	38.29	8.20	0.8735	<LOD	<LOD	<LOD	<LOD	<LOD					
D	NP2	12.78	38.19	8.20	0.8735	<LOD	<LOD	<LOD	<LOD	<LOD					
D	NP2	12.89	38.17	8.19	0.8647	<LOD	<LOD	<LOD	<LOD	<LOD					
D	NP3	8.843	13.07	7.48	0.8141	<LOD	<LOD	<LOD	<LOD	<LOD					
D	NP3	8.674	13.54	7.47	0.8105	<LOD	<LOD	<LOD	<LOD	<LOD					
D	NP3	8.844	13.48	7.48	0.8058	<LOD	<LOD	<LOD	<LOD	<LOD					
D	NP4	6.688	32.43	6.06	0.9346	<LOD	<LOD	<LOD	<LOD	<LOD					
D	NP4	6.670	31.71	6.04	0.9307	<LOD	<LOD	<LOD	<LOD	<LOD					
D	NP4	6.751	31.80	6.02	0.9293	<LOD	<LOD	<LOD	<LOD	<LOD					
E	NP1	7.505	2.694	8.300	0.3417	<LOD	<LOD	<LOD	<LOD	<LOD					
E	NP1	7.413	2.766	8.270	0.3416	<LOD	<LOD	<LOD	<LOD	<LOD					
E	NP1	6.634	2.810	8.270	0.3418	<LOD	<LOD	<LOD	<LOD	<LOD					

Lab code	Product	Nicotine	OV	pH	Water activity	NNK	NNN	NAT	NAB	B[a]P	Formaldehyde	Acetaldehyde	Crotonaldehyde	Arsenic	Cadmium
		mg/g	%		Aw	ng/g	ng/g	ng/g	ng/g	ng/g	µg/g	µg/g	µg/g	ng/g	ng/g
E	NP2	13.18	39.08	8.310	0.8804	<LOD	<LOD	<LOD	<LOD	<LOD					
E	NP2	13.03	39.61	8.340	0.8857	<LOD	<LOD	<LOD	<LOD	<LOD					
E	NP2	13.11	39.26	8.320	0.8942	<LOD	<LOD	<LOD	<LOD	<LOD					
E	NP3	8.498	11.88	7.550	0.8330	<LOD	<LOD	<LOD	<LOD	<LOD					
E	NP3	8.544	12.38	7.530	0.8549	<LOD	<LOD	<LOD	<LOD	<LOD					
E	NP3	9.028	12.60	7.550	0.8527	<LOD	<LOD	<LOD	<LOD	<LOD					
E	NP4	5.616	32.48	6.020	0.9386	<LOD	<LOD	<LOD	<LOD	<LOD					
E	NP4	5.546	31.80	6.000	0.9350	<LOD	<LOD	<LOD	<LOD	<LOD					
E	NP4	6.290	31.70	6.020	0.9460	<LOD	<LOD	<LOD	<LOD	<LOD					
F	NP1	7.492	2.907	8.309		<LOD	<LOD	<LOD	<LOD						
F	NP1	7.577	2.849	8.284		<LOD	<LOD	<LOD	<LOD						
F	NP1	7.585	2.678	8.293		<LOD	<LOD	<LOD	<LOD						
F	NP2	13.65	37.63	8.308		<LOD	<LOD	<LOD	<LOD						
F	NP2	13.50	35.98	8.306		<LOD	<LOD	<LOD	<LOD						
F	NP2	13.78	37.46	8.307		<LOD	<LOD	<LOD	<LOD						
F	NP3	8.985	8.771	7.533		<LOD	<LOD	<LOD	<LOD						
F	NP3	9.020	9.095	7.562		<LOD	<LOD	<LOD	<LOD						
F	NP3	9.040	9.018	7.544		<LOD	<LOD	<LOD	<LOD						
F	NP4	6.854	29.90	6.020		<LOD	<LOD	<LOD	<LOD						
F	NP4	7.103	32.05	6.021		<LOD	<LOD	<LOD	<LOD						
F	NP4	6.979	30.21	6.013		<LOD	<LOD	<LOD	<LOD						
G	NP1	7.699	2.828	8.10	0.5041	<LOD	<LOD	<LOD	<LOD	0.1623*	11.57	0.1351	<LOD	<LOD	<LOD
G	NP1	7.711	2.760	8.13	0.5027	<LOD	<LOD	<LOD	<LOD	0.1656*	10.67	0.1446	<LOD	<LOD	0.6399*
G	NP1	7.659	2.978	8.10	0.5002	<LOD	<LOD	<LOD	0.6082*	0.1450*	10.60	0.1001	<LOD	<LOD	<LOD
G	NP2	13.90	38.80	8.42	0.8741	<LOD	<LOD	<LOD	<LOD	0.2293*	1.459	0.2400	<LOD	<LOD	<LOD
G	NP2	13.96	37.71	8.41	0.8744	<LOD	<LOD	<LOD	<LOD	0.2225*	1.747	0.1809	<LOD	<LOD	<LOD
G	NP2	14.05	37.79	8.42	0.8792	<LOD	<LOD	<LOD	<LOD	0.2053*	1.599	0.2038	<LOD	<LOD	<LOD

Lab code	Product	Nicotine	OV	pH	Water activity	NNK	NNN	NAT	NAB	B[a]P	Formaldehyde	Acetaldehyde	Crotonaldehyde	Arsenic	Cadmium
		mg/g	%		Aw	ng/g	ng/g	ng/g	ng/g	ng/g	µg/g	µg/g	µg/g	ng/g	ng/g
G	NP3	8.413	11.23	7.71	0.8537	<LOD	<LOD	<LOD	<LOD	0.1987*	1.029	<LOD	<LOD	<LOD	<LOD
G	NP3	8.692	12.34	7.72	0.8710	<LOD	<LOD	<LOD	<LOD	0.2180*	0.929	<LOD	<LOD	<LOD	<LOD
G	NP3	8.444	13.07	7.71	0.8757	<LOD	<LOD	<LOD	<LOD	0.2718*	1.045	<LOD	<LOD	<LOD	<LOD
G	NP4	7.087	31.45	6.23	0.9432	<LOD	<LOD	<LOD	<LOD	0.3279*	1.224	0.0815*	<LOD	<LOD	<LOD
G	NP4	6.769	31.36	6.20	0.9434	<LOD	<LOD	<LOD	<LOD	0.3199*	1.298	0.0644*	<LOD	<LOD	<LOD
G	NP4	7.060	31.46	6.17	0.9489	<LOD	<LOD	<LOD	<LOD	0.7050	1.205	<LOD	<LOD	<LOD	<LOD
H	NP1	6.142	2.532	8.25	0.3650	<LOD	<LOD	<LOD	<LOD	<LOD	14.0400	0.16240	<LOD	<LOD	<LOD
H	NP1	6.252	2.630	8.30	0.3676	<LOD	<LOD	<LOD	<LOD	<LOD	14.3800	0.16030	<LOD	<LOD	<LOD
H	NP1	6.249	2.605	8.38	0.3653	<LOD	<LOD	<LOD	<LOD	<LOD	13.9200	0.14440	<LOD	<LOD	<LOD
H	NP2	10.86	38.19	8.34	0.8746	<LOD	<LOD	<LOD	<LOD	<LOD	2.0810	0.12150	<LOD	<LOD	<LOD
H	NP2	11.27	37.73	8.34	0.8729	<LOD	<LOD	<LOD	<LOD	<LOD	1.9880	0.17970	<LOD	<LOD	<LOD
H	NP2	10.99	37.31	8.35	0.8808	<LOD	<LOD	<LOD	<LOD	<LOD	1.9710	0.21270	<LOD	<LOD	<LOD
H	NP3	7.265	12.65	7.56	0.8430	<LOD	<LOD	<LOD	<LOD	<LOD	1.2590	<LOD	<LOD	<LOD	<LOD
H	NP3	6.592	12.92	7.57	0.8411	<LOD	<LOD	<LOD	<LOD	<LOD	1.1280	<LOD	<LOD	<LOD	<LOD
H	NP3	7.387	12.55	7.58	0.8411	<LOD	<LOD	<LOD	<LOD	<LOD	1.0480	<LOD	<LOD	<LOD	<LOD
H	NP4	5.018	31.57	6.10	0.9353	<LOD	<LOD	<LOD	<LOD	<LOD	1.3900	0.0379*	<LOD	<LOD	<LOD
H	NP4	4.849	31.80	6.10	0.9391	<LOD	<LOD	<LOD	<LOD	<LOD	1.4210	0.0612*	<LOD	<LOD	<LOD
H	NP4	4.890	31.05	6.08	0.9373	<LOD	<LOD	<LOD	<LOD	<LOD	1.3570	0.0538*	<LOD	<LOD	<LOD
I	NP1	6.916	2.554	8.42	0.2839	<LOD	<LOD	<LOD	<LOD						
I	NP1	6.649	2.542	8.43	0.3485	<LOD	<LOD	<LOD	<LOD						
I	NP1	7.260	2.323	8.50	0.3300	<LOD	<LOD	<LOD	<LOD						
I	NP2	12.19	37.87	8.46	0.8659	<LOD	<LOD	<LOD	<LOD						
I	NP2	11.89	37.30	8.47	0.8670	<LOD	<LOD	<LOD	<LOD						
I	NP2	12.89	37.98	8.47	0.8588	<LOD	<LOD	<LOD	<LOD						
I	NP3	8.149	13.05	7.51	0.7457	<LOD	<LOD	<LOD	<LOD						
I	NP3	7.876	13.36	7.49	0.7268	<LOD	<LOD	<LOD	<LOD						
I	NP3	8.325	13.08	7.52	0.6938	<LOD	<LOD	<LOD	<LOD						

Lab code	Product	Nicotine	OV	pH	Water activity	NNK	NNN	NAT	NAB	B[a]P	Formaldehyde	Acetaldehyde	Crotonaldehyde	Arsenic	Cadmium
		mg/g	%		Aw	ng/g	ng/g	ng/g	ng/g	ng/g	µg/g	µg/g	µg/g	ng/g	ng/g
I	NP4	6.251	31.30	6.05	0.9248	<LOD	<LOD	<LOD	<LOD						
I	NP4	5.750	31.26	6.06	0.9254	<LOD	<LOD	<LOD	<LOD						
I	NP4	6.030	31.43	6.02	0.9171	<LOD	<LOD	<LOD	<LOD						
J	NP1	8.200	3.168												
J	NP1	8.169	3.218												
J	NP1	8.074	3.338												
J	NP2	14.21	38.08												
J	NP2	14.81	38.48												
J	NP2	14.37	37.72												
J	NP3	8.982	12.69												
J	NP3	9.823	12.53												
J	NP3	9.889	12.72												
J	NP4	7.682	31.84												
J	NP4	7.535	31.50												
J	NP4	7.684	31.44												
K	NP1	8.023	2.554	8.200	0.3237	<LOD	<LOD	<LOD	<LOD	<LOD	12.61	0.2928*	0.0277*	1.323*	0.5074*
K	NP1	7.989	2.493	8.201	0.3280	<LOD	<LOD	<LOD	<LOD	<LOD	12.23	0.2745*	0.0279*	<LOD	0.6779*
K	NP1	8.001	2.540	8.236	0.3330	<LOD	<LOD	<LOD	<LOD	<LOD	12.94	0.2693*	0.0267*	1.742*	0.6482*
K	NP2	14.40	38.38	8.318	0.8799	0.9818*	0.6182*	<LOD	<LOD	<LOD	1.292	0.5713	0.0273*	2.461*	0.4827*
K	NP2	14.04	38.95	8.329	0.8857	0.9514*	0.7231*	<LOD	<LOD	<LOD	1.398	0.4908*	0.0275*	1.222*	<LOD
K	NP2	13.73	37.44	8.321	0.8761	0.8782*	0.6417*	<LOD	<LOD	<LOD	1.451	0.4838*	0.0287*	1.981*	<LOD
K	NP3	9.281	13.32	7.582	0.8386	<LOD	<LOD	<LOD	<LOD	<LOD	0.8912	<LOD	0.0231*	1.675*	<LOD
K	NP3	9.185	13.30	7.564	0.8562	<LOD	<LOD	<LOD	<LOD	<LOD	0.8846	<LOD	0.0240*	2.321*	<LOD
K	NP3	8.899	13.33	7.568	0.8480	<LOD	<LOD	<LOD	<LOD	<LOD	0.9315	<LOD	0.0235*	1.737*	0.2846*
K	NP4	7.155	32.24	6.082	0.9308	<LOD	<LOD	<LOD	<LOD	<LOD	1.177	<LOD	0.0185*	<LOD	<LOD
K	NP4	7.249	32.10	6.095	0.9422	<LOD	<LOD	<LOD	<LOD	<LOD	1.254	<LOD	0.0184*	2.254*	<LOD
K	NP4	7.102	32.04	6.065	0.9459	<LOD	<LOD	<LOD	<LOD	<LOD	1.159	<LOD	0.0181*	<LOD	<LOD

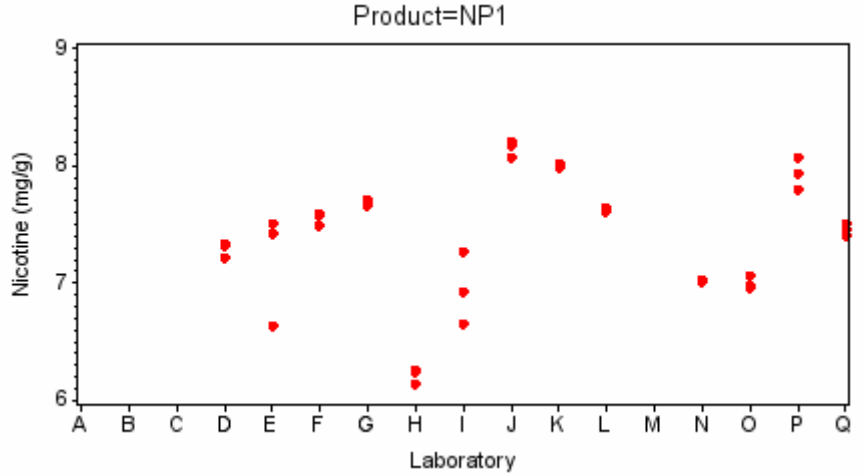
Lab code	Product	Nicotine	OV	pH	Water activity	NNK	NNN	NAT	NAB	B[a]P	Formaldehyde	Acetaldehyde	Crotonaldehyde	Arsenic	Cadmium
		mg/g	%		Aw	ng/g	ng/g	ng/g	ng/g	ng/g	µg/g	µg/g	µg/g	ng/g	ng/g
L	NP1	7.614	2.888	8.511	0.4575	0.5493*	<LOD	<LOD	<LOD	<LOD	11.53	0.2381*	<LOD	3.398*	<LOD
L	NP1	7.623	2.845	8.496	0.4156	0.6522*	<LOD	<LOD	<LOD	<LOD	12.04	0.1977*	<LOD	1.743*	<LOD
L	NP1	7.649	2.747	8.390	0.4060	0.5921*	<LOD	<LOD	<LOD	<LOD	12.17	0.2139*	0.0003*	1.277*	<LOD
L	NP2	13.47	38.21	8.309	0.8719	1.4139*	<LOD	<LOD	<LOD	<LOD	1.766	0.3124*	0.0039*	1.550*	0.1211*
L	NP2	13.37	38.68	8.312	0.8757	1.1025*	<LOD	<LOD	<LOD	<LOD	1.767	0.3977	<LOD	1.552*	0.0233*
L	NP2	13.28	37.43	8.279	0.8705	0.5839*	<LOD	<LOD	<LOD	<LOD	1.716	0.3488	0.0026*	1.569*	0.1405*
L	NP3	9.214	13.03	7.540	0.8349	0.7945*	<LOD	<LOD	<LOD	<LOD	1.142	0.0422*	<LOD	0.7010*	<LOD
L	NP3	9.225	13.26	7.529	0.8298	0.7509*	<LOD	<LOD	<LOD	<LOD	0.9164	0.0347*	<LOD	0.7879*	<LOD
L	NP3	9.255	13.39	7.531	0.8370	0.5965*	<LOD	<LOD	<LOD	<LOD	1.077	0.0424*	0.0010*	0.5957*	<LOD
L	NP4	7.011	31.75	6.055	0.9404	0.4157*	<LOD	<LOD	<LOD	<LOD	1.396	0.0833*	0.0016*	0.9460*	<LOD
L	NP4	6.969	31.83	6.036	0.9362	0.4250*	<LOD	<LOD	<LOD	<LOD	1.415	0.0861*	<LOD	0.8269*	<LOD
L	NP4	7.017	31.47	6.051	0.9393	0.4096*	<LOD	<LOD	<LOD	<LOD	1.398	0.0670*	0.0002*	1.285*	<LOD
M	NP1		2.9187	8.26		<LOD	<LOD	<LOD	<LOD						
M	NP1		2.9238	8.30		<LOD	<LOD	<LOD	<LOD						
M	NP1		2.9028	8.29		<LOD	<LOD	<LOD	<LOD						
M	NP2		37.0977	8.35		<LOD	<LOD	<LOD	<LOD						
M	NP2		36.9887	8.35		<LOD	<LOD	<LOD	<LOD						
M	NP2		37.0820	8.35		<LOD	<LOD	<LOD	<LOD						
M	NP3		12.8873	7.60		<LOD	<LOD	<LOD	<LOD						
M	NP3		12.8373	7.60		<LOD	<LOD	<LOD	<LOD						
M	NP3		12.9188	7.63		<LOD	<LOD	<LOD	<LOD						
M	NP4		31.3563	6.12		<LOD	<LOD	<LOD	<LOD						
M	NP4		31.0359	6.11		<LOD	<LOD	<LOD	<LOD						
M	NP4		31.3775	6.12		<LOD	<LOD	<LOD	<LOD						
N	NP1	7.025		8.183		<LOD	<LOD	<LOD	<LOD	<LOD	13.23	0.1192	<LOD	<LOD	<LOD
N	NP1	7.001		8.173		<LOD	<LOD	<LOD	<LOD	<LOD	13.22	0.1191	<LOD	<LOD	<LOD
N	NP1	7.006		8.295		<LOD	<LOD	<LOD	<LOD	<LOD	13.70	0.1181	<LOD	<LOD	<LOD

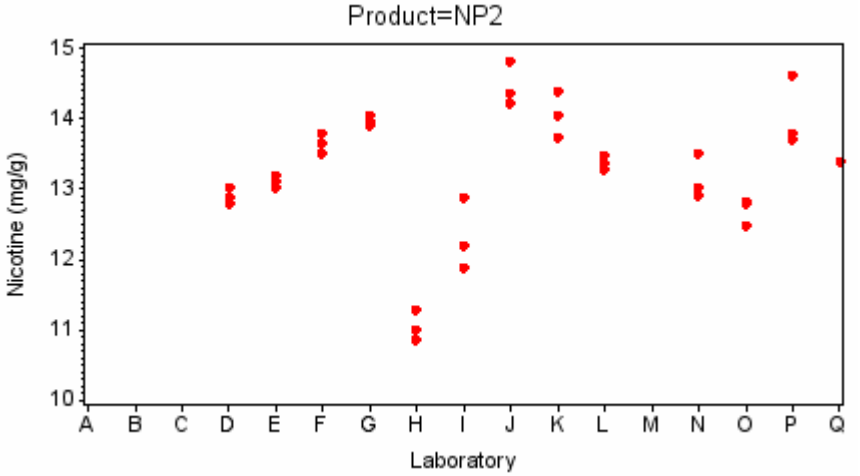
Lab code	Product	Nicotine	OV	pH	Water activity	NNK	NNN	NAT	NAB	B[a]P	Formaldehyde	Acetaldehyde	Crotonaldehyde	Arsenic	Cadmium
		mg/g	%		Aw	ng/g	ng/g	ng/g	ng/g	ng/g	µg/g	µg/g	µg/g	ng/g	ng/g
N	NP2	12.91		8.369		<LOD	<LOD	<LOD	<LOD	<LOD	1.625	0.4643	<LOD	<LOD	<LOD
N	NP2	13.02		8.361		<LOD	<LOD	<LOD	<LOD	<LOD	1.524	0.3518	<LOD	<LOD	<LOD
N	NP2	13.51		8.375		<LOD	<LOD	<LOD	<LOD	<LOD	1.649	0.3663	<LOD	<LOD	<LOD
N	NP3	8.397		7.618		<LOD	<LOD	<LOD	<LOD	<LOD	0.8898	<LOD	<LOD	<LOD	<LOD
N	NP3	8.380		7.581		<LOD	<LOD	<LOD	<LOD	<LOD	0.8850	<LOD	<LOD	<LOD	<LOD
N	NP3	8.486		7.602		<LOD	<LOD	<LOD	<LOD	<LOD	0.8951	<LOD	<LOD	<LOD	<LOD
N	NP4	6.364		6.128		<LOD	<LOD	<LOD	<LOD	<LOD	1.508	0.1675	<LOD	<LOD	<LOD
N	NP4	6.405		6.065		<LOD	<LOD	<LOD	<LOD	<LOD	1.515	0.1082	<LOD	<LOD	<LOD
N	NP4	6.454		6.058		<LOD	<LOD	<LOD	<LOD	<LOD	1.361	0.0907	<LOD	<LOD	<LOD
O	NP1	6.981	2.825	8.30		<LOD	<LOD	<LOD	<LOD		12.68	0.2067	<LOD		
O	NP1	6.950	2.786	8.37		<LOD	<LOD	<LOD	<LOD		12.85	0.2236	<LOD		
O	NP1	7.056	2.705	8.32		<LOD	<LOD	<LOD	<LOD		12.35	0.2264	<LOD		
O	NP2	12.78	39.39	8.33		0.6872*	0.4951*	<LOD	<LOD		1.796	0.1084	<LOD		
O	NP2	12.82	39.39	8.34		0.6976*	0.5227*	<LOD	<LOD		1.657	0.1318	<LOD		
O	NP2	12.47	38.47	8.34		0.7447*	0.4995*	<LOD	<LOD		1.748	0.1193	<LOD		
O	NP3	8.449	12.69	7.65		0.4090*	<LOD	<LOD	<LOD		1.054	<LOD	<LOD		
O	NP3	8.441	11.60	7.58		0.4162*	<LOD	<LOD	<LOD		1.085	<LOD	<LOD		
O	NP3	8.553	12.78	7.59		0.3726*	<LOD	<LOD	<LOD		1.111	<LOD	<LOD		
O	NP4	6.249	31.67	6.07		<LOD	<LOD	<LOD	<LOD		1.829	<LOD	<LOD		
O	NP4	6.290	32.81	6.03		<LOD	<LOD	<LOD	<LOD		1.710	<LOD	<LOD		
O	NP4	6.289	31.67	6.04		<LOD	<LOD	<LOD	<LOD		1.827	<LOD	<LOD		
P	NP1	8.070	2.460	8.12	0.3210	<LOD	<LOD	<LOD	<LOD		5.740	0.5096*	<LOD	2.228*	1.608*
P	NP1	7.790	2.620	8.11	0.3280	<LOD	<LOD	<LOD	<LOD		5.570	0.4614*	<LOD	4.579*	1.525*
P	NP1	7.930	2.730	8.18	0.3230	<LOD	<LOD	<LOD	<LOD		5.770	0.6916*	<LOD	4.091*	0.9431*
P	NP2	14.60	37.95	8.24	0.8690	<LOD	<LOD	<LOD	<LOD		0.5730	0.2325*	<LOD	3.544*	1.097*
P	NP2	13.70	37.90	8.25	0.8740	<LOD	<LOD	<LOD	<LOD		0.6400	<LOD	<LOD	3.905*	0.8586*
P	NP2	13.80	37.57	8.25	0.8750	<LOD	<LOD	<LOD	<LOD		0.6080	0.2183*	<LOD	4.418*	0.9207*

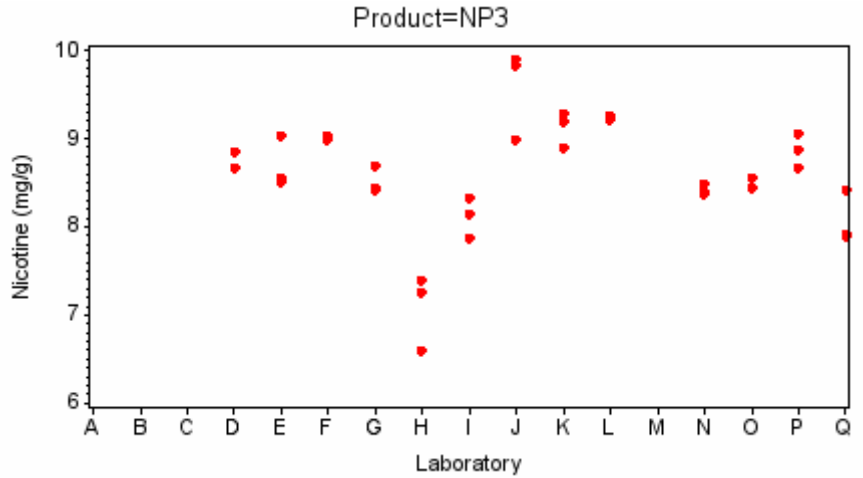
Lab code	Product	Nicotine	OV	pH	Water activity	NNK	NNN	NAT	NAB	B[a]P	Formaldehyde	Acetaldehyde	Crotonaldehyde	Arsenic	Cadmium
		mg/g	%		Aw	ng/g	ng/g	ng/g	ng/g	ng/g	µg/g	µg/g	µg/g	ng/g	ng/g
P	NP3	9.050	13.18	7.47	0.8650	<LOD	<LOD	<LOD	<LOD		1.210	<LOD	<LOD	3.034*	<LOD
P	NP3	8.860	12.30	7.50	0.8640	<LOD	<LOD	<LOD	<LOD		1.230	<LOD	<LOD	2.854*	<LOD
P	NP3	8.670	12.43	7.41	0.8580	<LOD	<LOD	<LOD	<LOD		1.220	<LOD	<LOD	<LOD	<LOD
P	NP4	7.030	31.94	6.09	0.9460	<LOD	<LOD	<LOD	<LOD		1.460	<LOD	<LOD	2.464*	0.569*
P	NP4	6.960	31.92	6.10	0.9410	<LOD	<LOD	<LOD	<LOD		1.420	<LOD	<LOD	3.447*	<LOD
P	NP4	7.190	31.68	6.06	0.9390	<LOD	<LOD	<LOD	<LOD		1.470	0.3216*	<LOD	3.891*	0.9828*
Q	NP1	7.511	2.827	8.125	0.3996	<LOD	<LOD	<LOD	<LOD	<LOD				7.609*	<LOD
Q	NP1	7.453	2.834	8.145	0.4046	<LOD	<LOD	<LOD	<LOD	0.0568*				9.635*	<LOD
Q	NP1	7.405	2.744	8.15	0.3794	<LOD	<LOD	<LOD	<LOD	0.1267*				7.195*	<LOD
Q	NP2	13.39	38.73	8.35	0.8762	<LOD	<LOD	<LOD	<LOD	0.0382*				4.899*	<LOD
Q	NP2	13.38	38.53	8.365	0.8754	<LOD	<LOD	<LOD	<LOD	<LOD				4.628*	<LOD
Q	NP2	13.39	37.89	8.345	0.8713	<LOD	<LOD	<LOD	<LOD	0.0372*				4.522*	<LOD
Q	NP3	7.917	11.39	7.56	0.488	<LOD	<LOD	<LOD	<LOD	0.0543*				<LOD	<LOD
Q	NP3	7.892	11.35	7.58	0.4929	<LOD	<LOD	<LOD	<LOD	<LOD				6.649*	<LOD
Q	NP3	8.409	11.16	7.575	0.4936	<LOD	<LOD	<LOD	<LOD	0.0976*				6.965*	<LOD
Q	NP4	6.762	31.87	6.1	0.9358	<LOD	<LOD	<LOD	<LOD	<LOD				<LOD	<LOD
Q	NP4	6.878	31.99	6.11	0.9237	<LOD	<LOD	<LOD	<LOD	<LOD				<LOD	<LOD
Q	NP4	6.857	31.72	6.11	0.9297	<LOD	<LOD	<LOD	<LOD	0.0400*				<LOD	<LOD

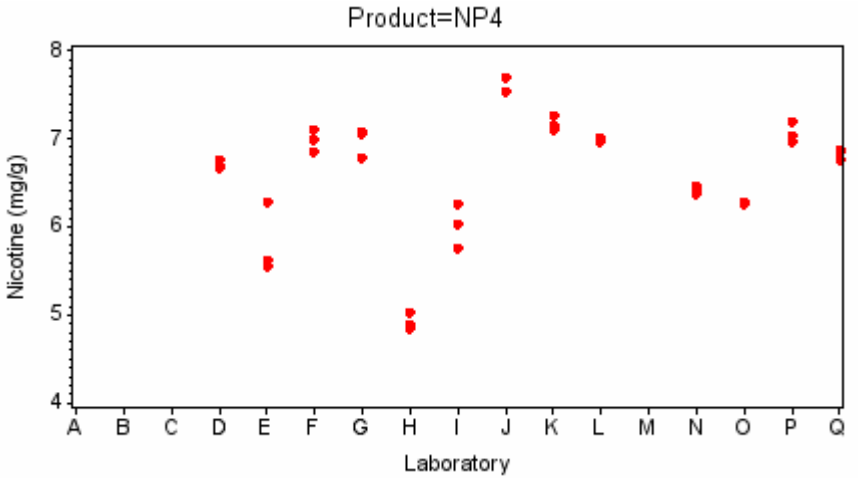
Analyte data not submitted for this study is indicated by grey cells.
The (*) symbol indicates that the value is below the limit of quantification, but above the limit of detection.
<LOD indicates that the value is below the limit of detection.

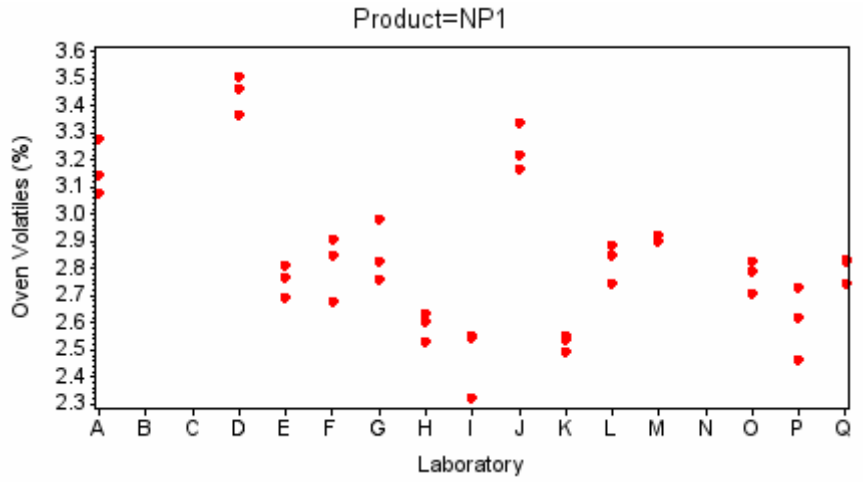
APPENDIX C: Raw Data Plots

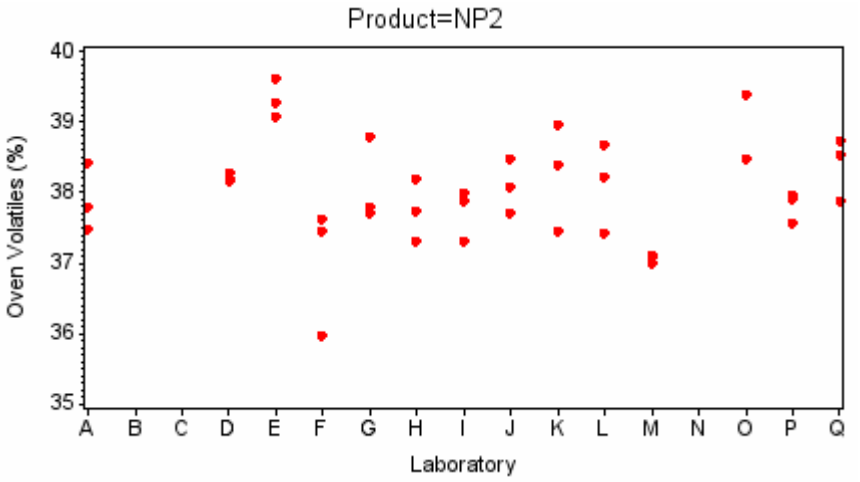


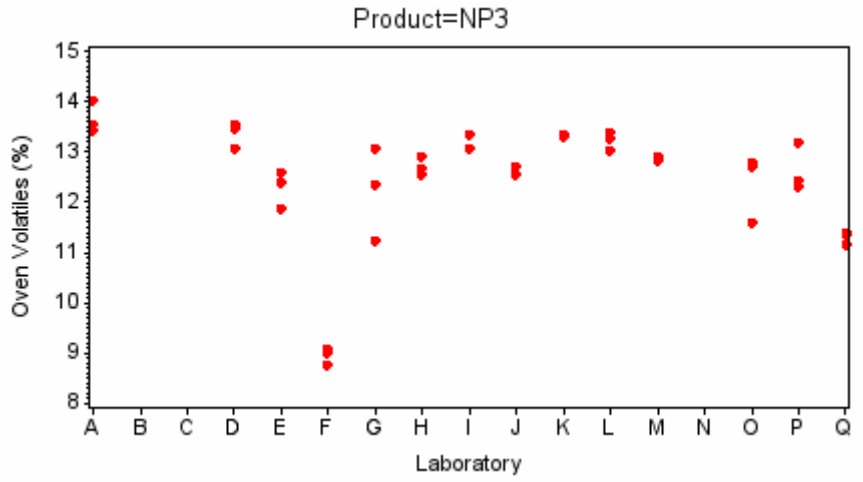


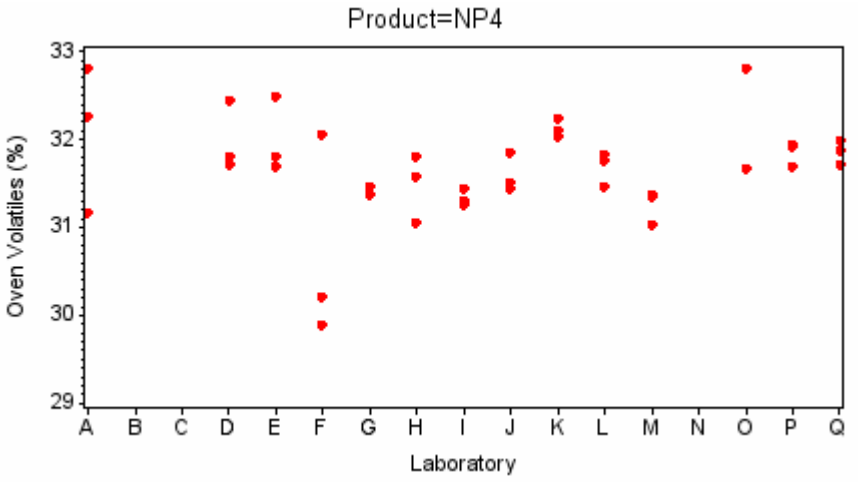





Product=NP1
Oven Volatiles (%)
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
A B C D E F G H I J K L M N O P Q
Laboratory


Product=NP2
Oven Volatiles (%)
40
39
38
37
36
35
A B C D E F G H I J K L M N O P Q
Laboratory


Product=NP3
Oven Volatiles (%)
15
14
13
12
11
10
9
8
A B C D E F G H I J K L M N O P Q
Laboratory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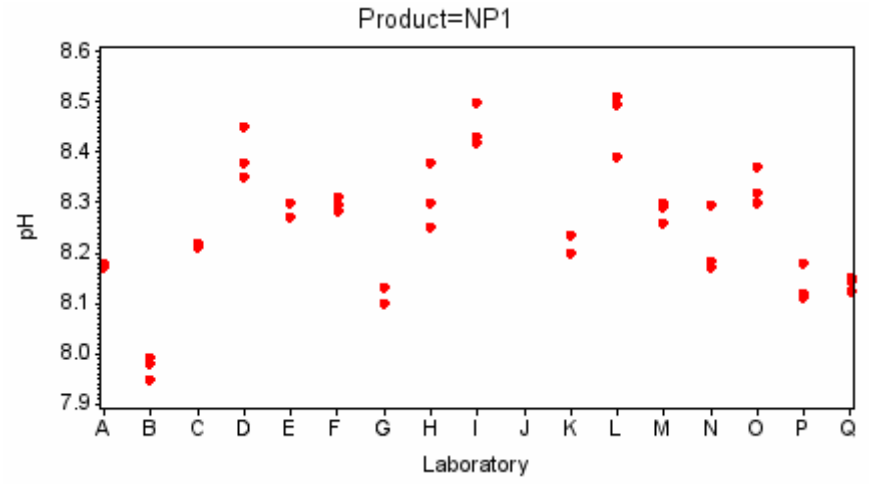
Oven Volatiles (%)
33
32
31
30
29
A B C D E F G H I J K L M N O P Q
Laboratory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pH
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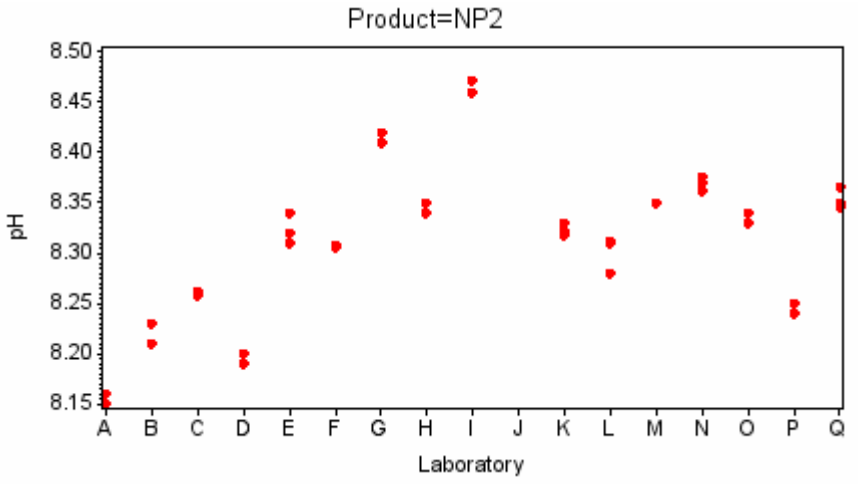
8.5
8.4
8.3
8.2
8.1
8.0
7.9
A B C D E F G H I J K L M N O P Q
Laboratory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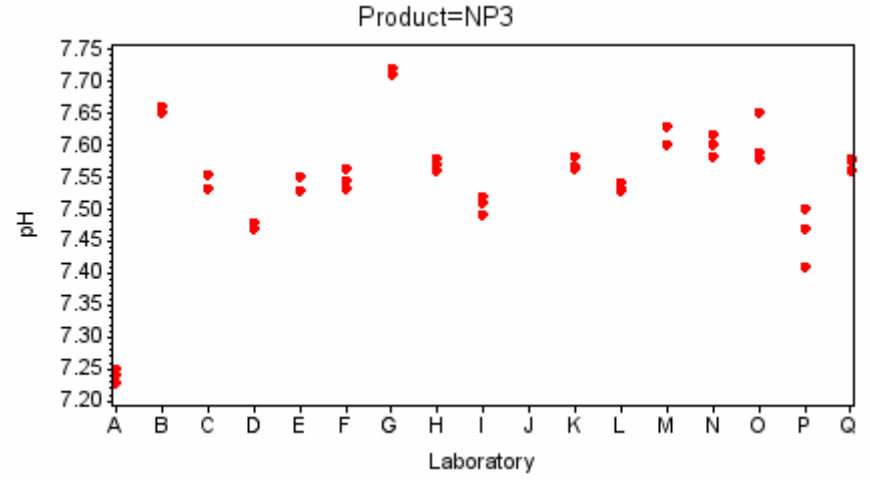
8.35
8.30
8.25
8.20
8.15
A B C D E F G H I J K L M N O P Q
Laboratory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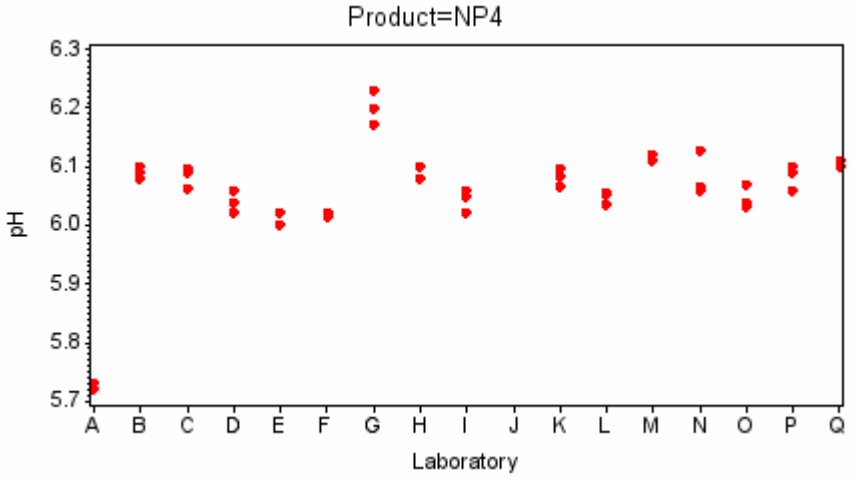
7.60
7.55
7.50
7.45
7.40
7.35
7.30
7.25
7.20
A B C D E F G H I J K L M N O P Q
Laboratory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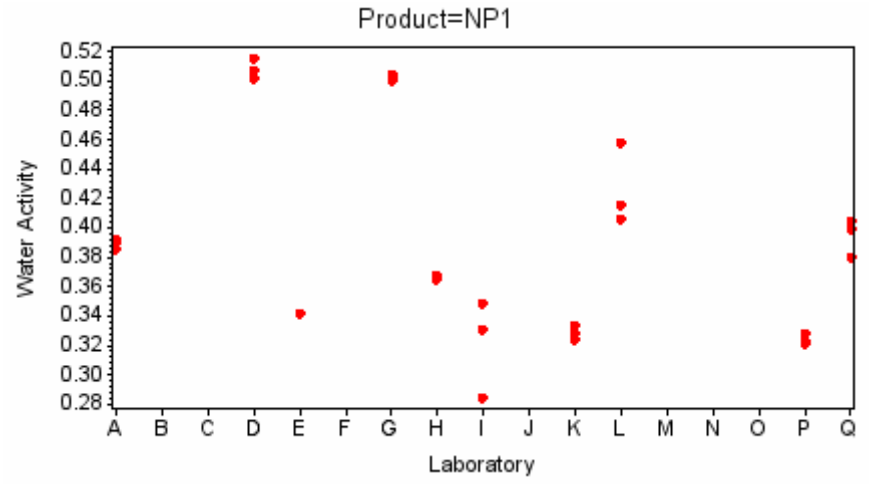
6.3
6.2
6.1
6.0
5.9
5.8
5.7
A B C D E F G H I J K L M N O P Q
Laboratory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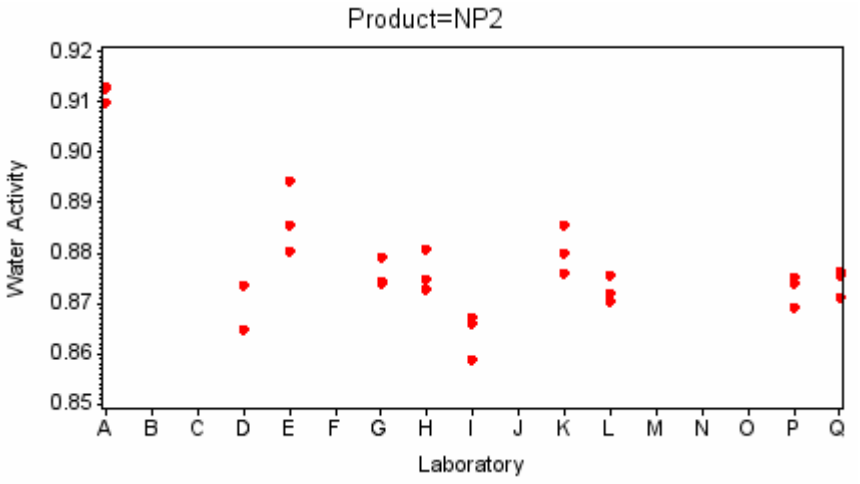
Water Activity
0.52
0.50
0.48
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
A B C D E F G H I J K L M N O P Q
Laboratory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0.90
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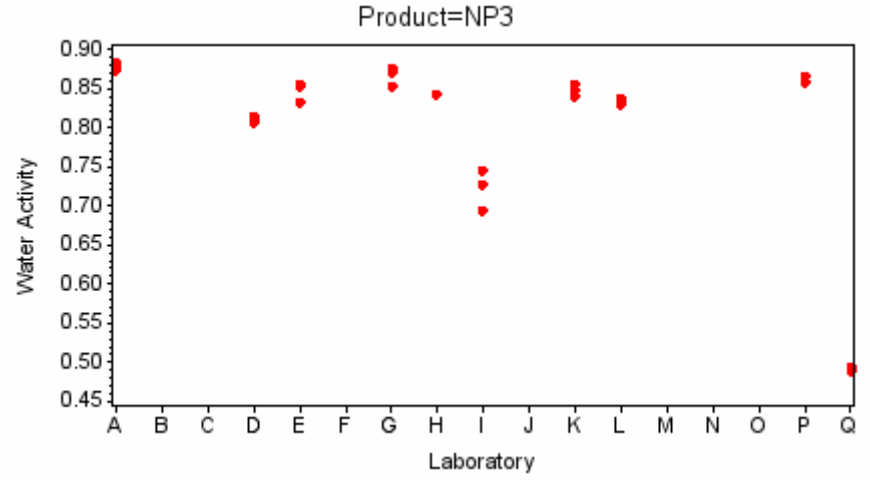
0.87
0.86
0.85
A B C D E F G H I J K L M N O P Q
Laboratory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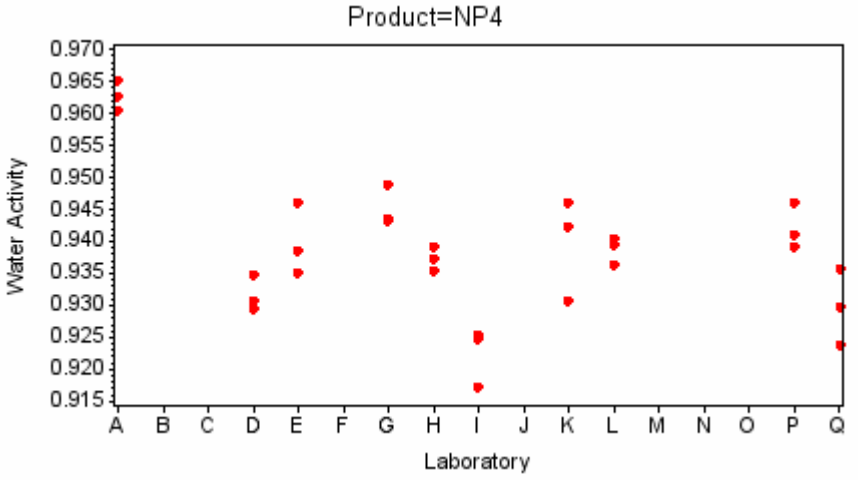
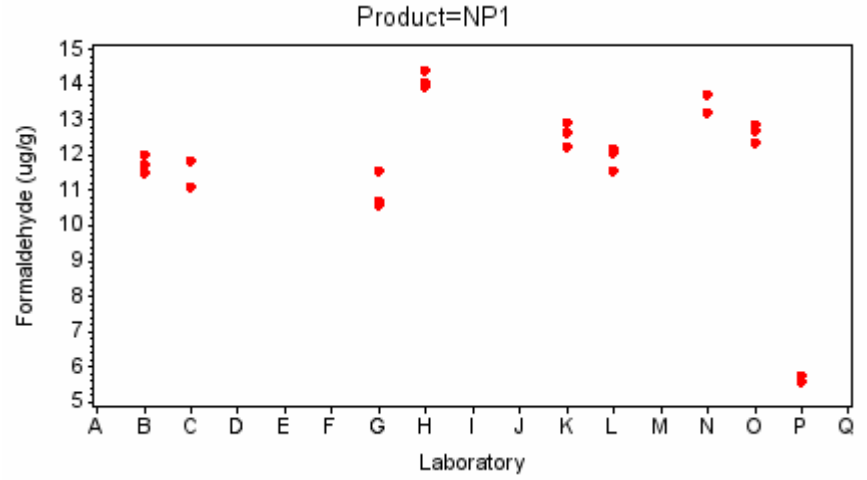
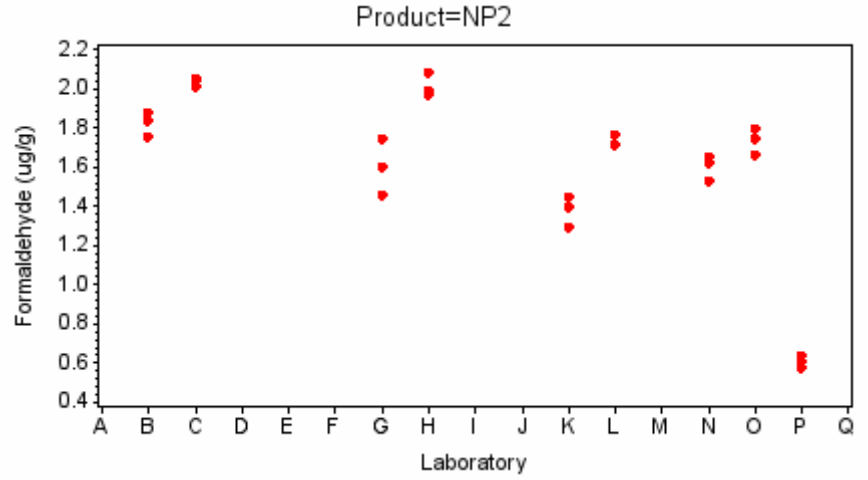
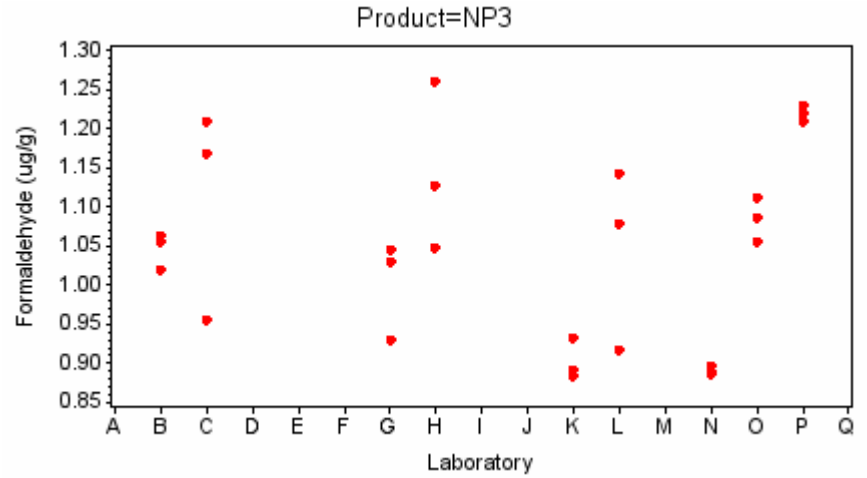
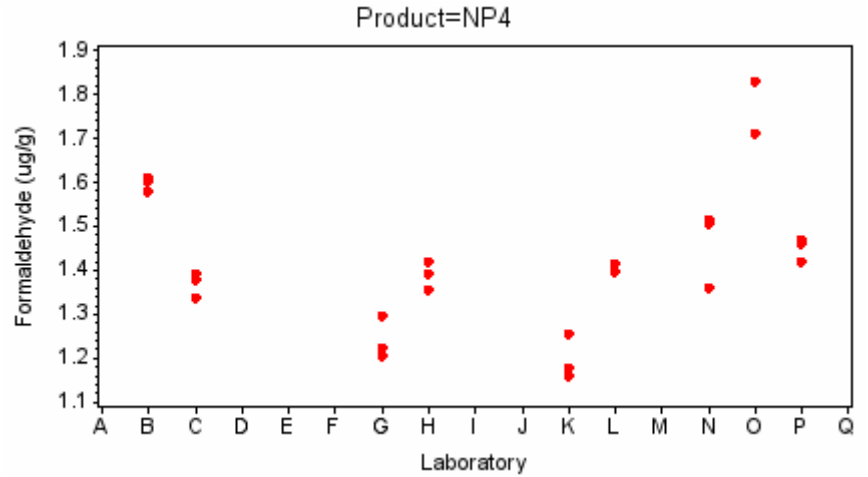
Water Activity
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
A B C D E F G H I J K L M N O P Q
Laboratory

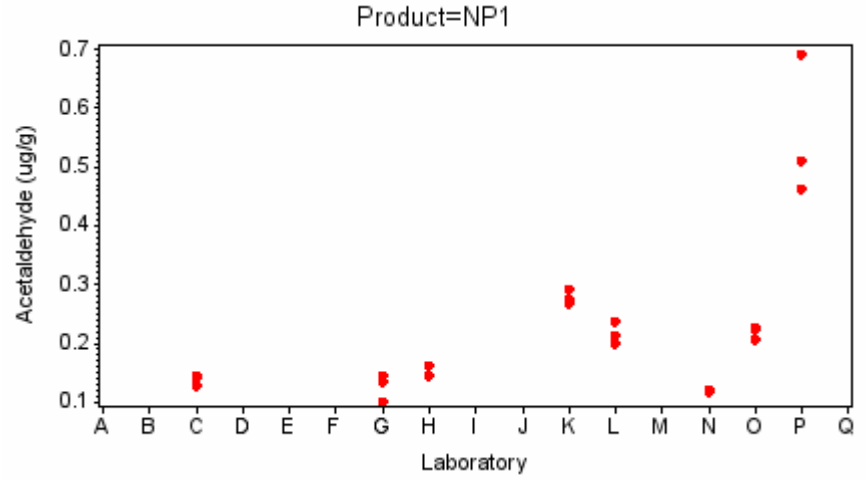

Product=NP4
Water Activity
0.970
0.965
0.960
0.955
0.950
0.945
0.940
0.935
0.930
0.925
0.920
0.915
A B C D E F G H I J K L M N O P Q
Laboratory

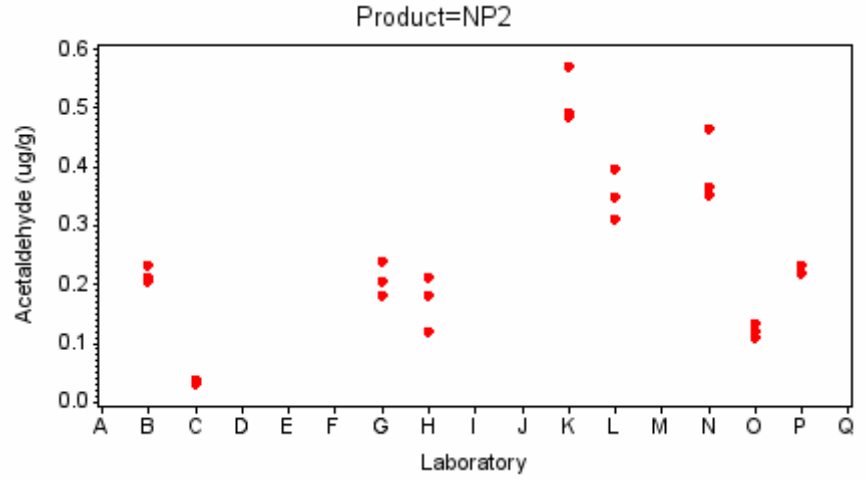

Product=NP1
Formaldehyde (ug/g)
15
14
13
12
11
10
9
8
7
6
5
A B C D E F G H I J K L M N O P Q
Laboratory

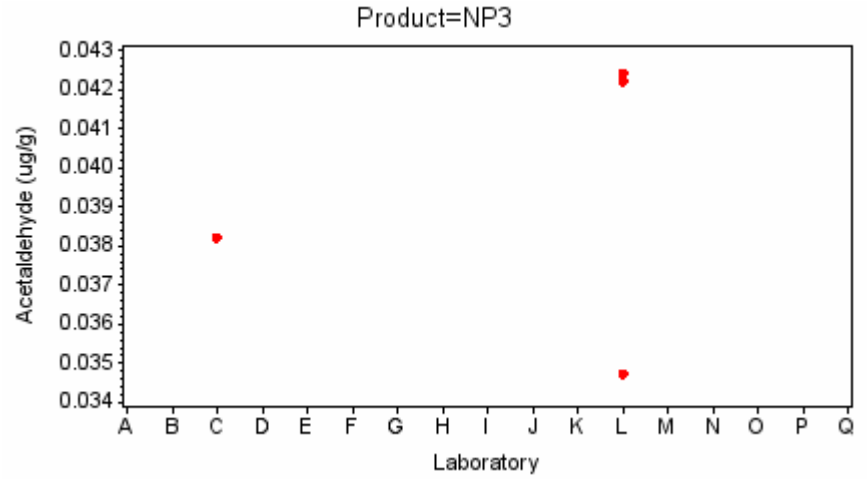

Product=NP2
Formaldehyde (ug/g)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
A B C D E F G H I J K L M N O P Q
Laboratory

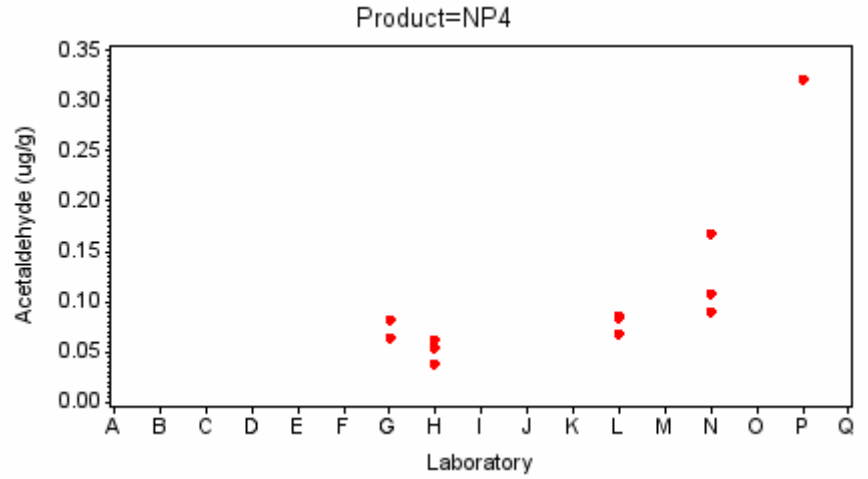

Product=NP3
Formaldehyde (ug/g)
1.30
1.25
1.20
1.15
1.10
1.05
1.00
0.95
0.90
0.85
A B C D E F G H I J K L M N O P Q
Laboratory


Product=NP4
Formaldehyde (ug/g)
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
A B C D E F G H I J K L M N O P Q
Laboratory

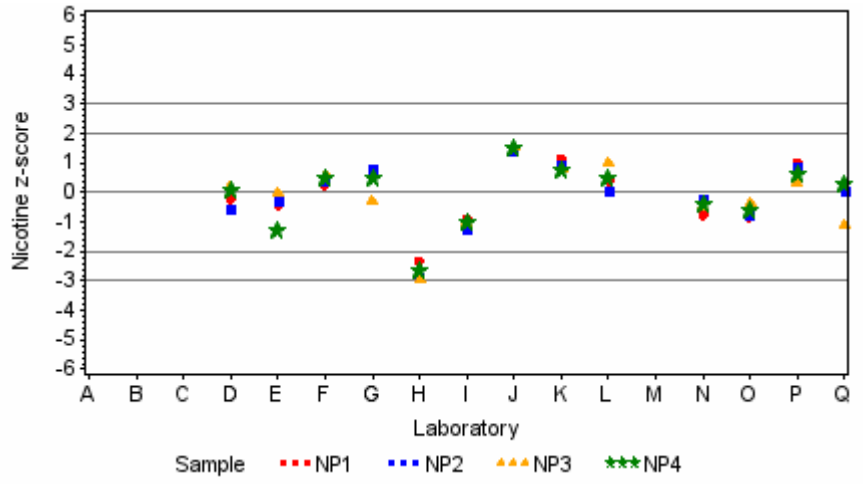

Product=NP1
Acetaldehyde (ug/g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
A B C D E F G H I J K L M N O P Q
Laboratory

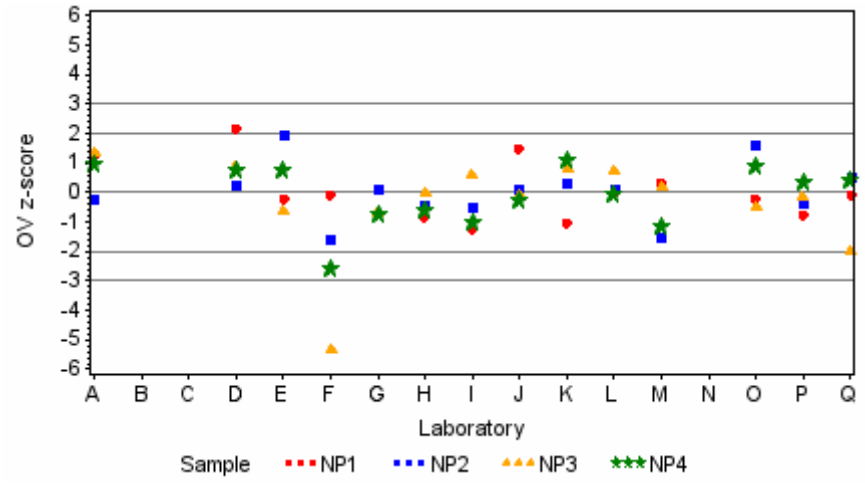

Product=NP2
Acetaldehyde (ug/g)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
A B C D E F G H I J K L M N O P Q
Laboratory

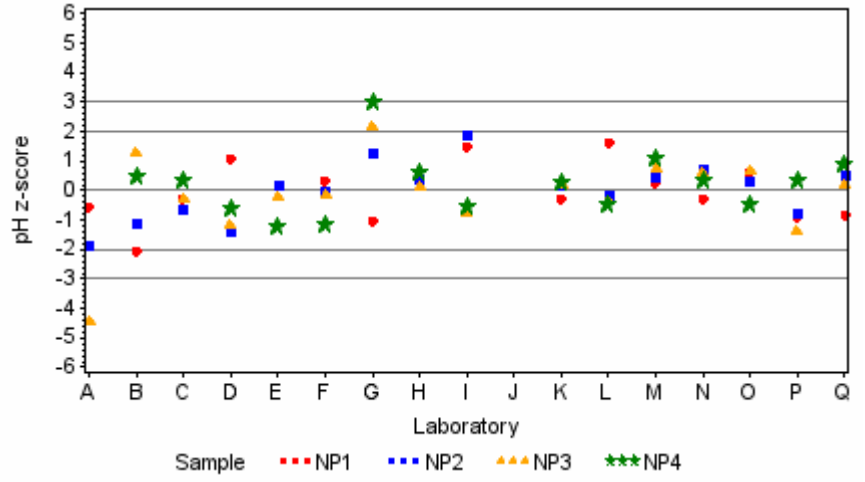

Product=NP3
Acetaldehyde (ug/g)
0.043
0.042
0.041
0.040
0.039
0.038
0.037
0.036
0.035
0.034
A B C D E F G H I J K L M N O P Q
Laboratory

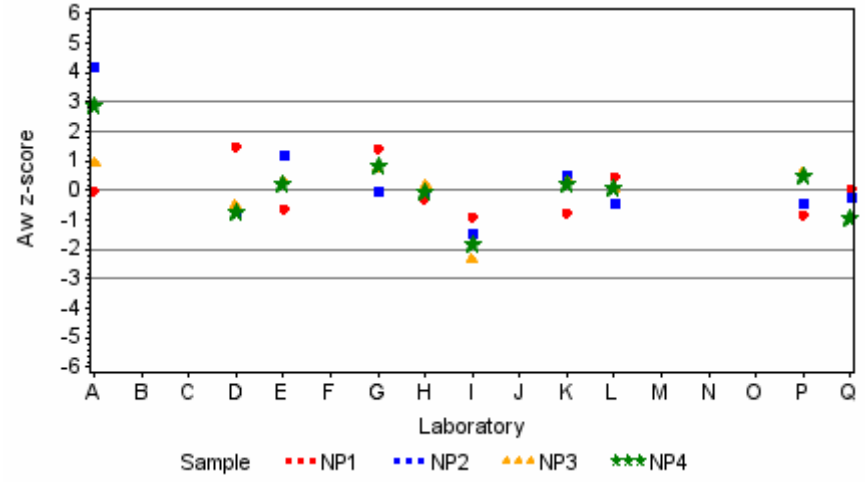

Product=NP4
Acetaldehyde (ug/g)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
A B C D E F G H I J K L M N O P Q
Laboratory

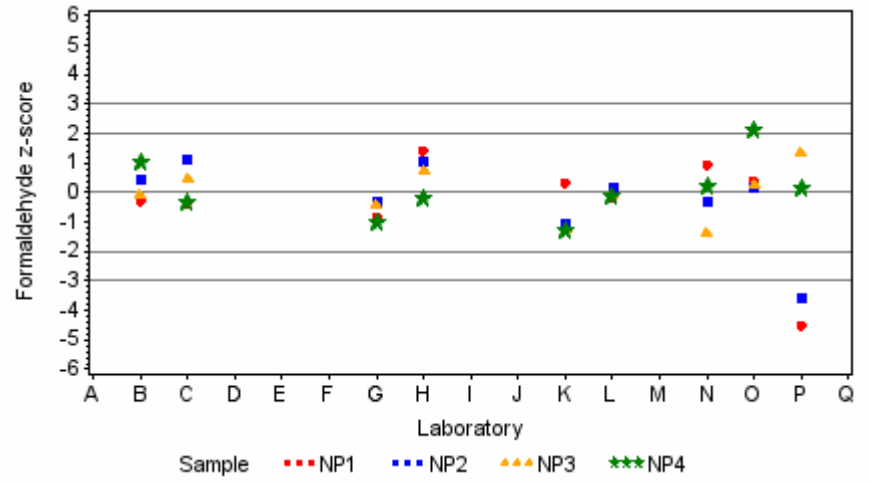
APPENDIX D: Z-Score Plots

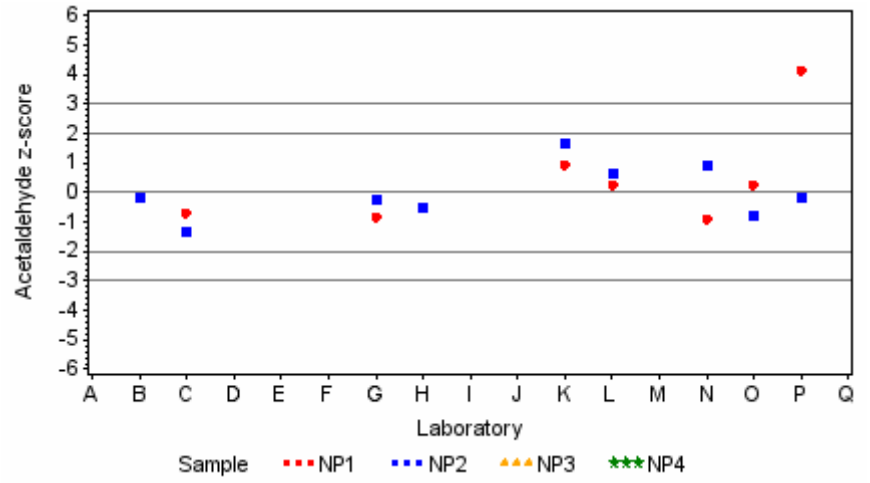









Formaldehyde z-score
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
A B C D E F G H I J K L M N O P Q
Laboratory
Sample NP1 NP2 NP3 NP4

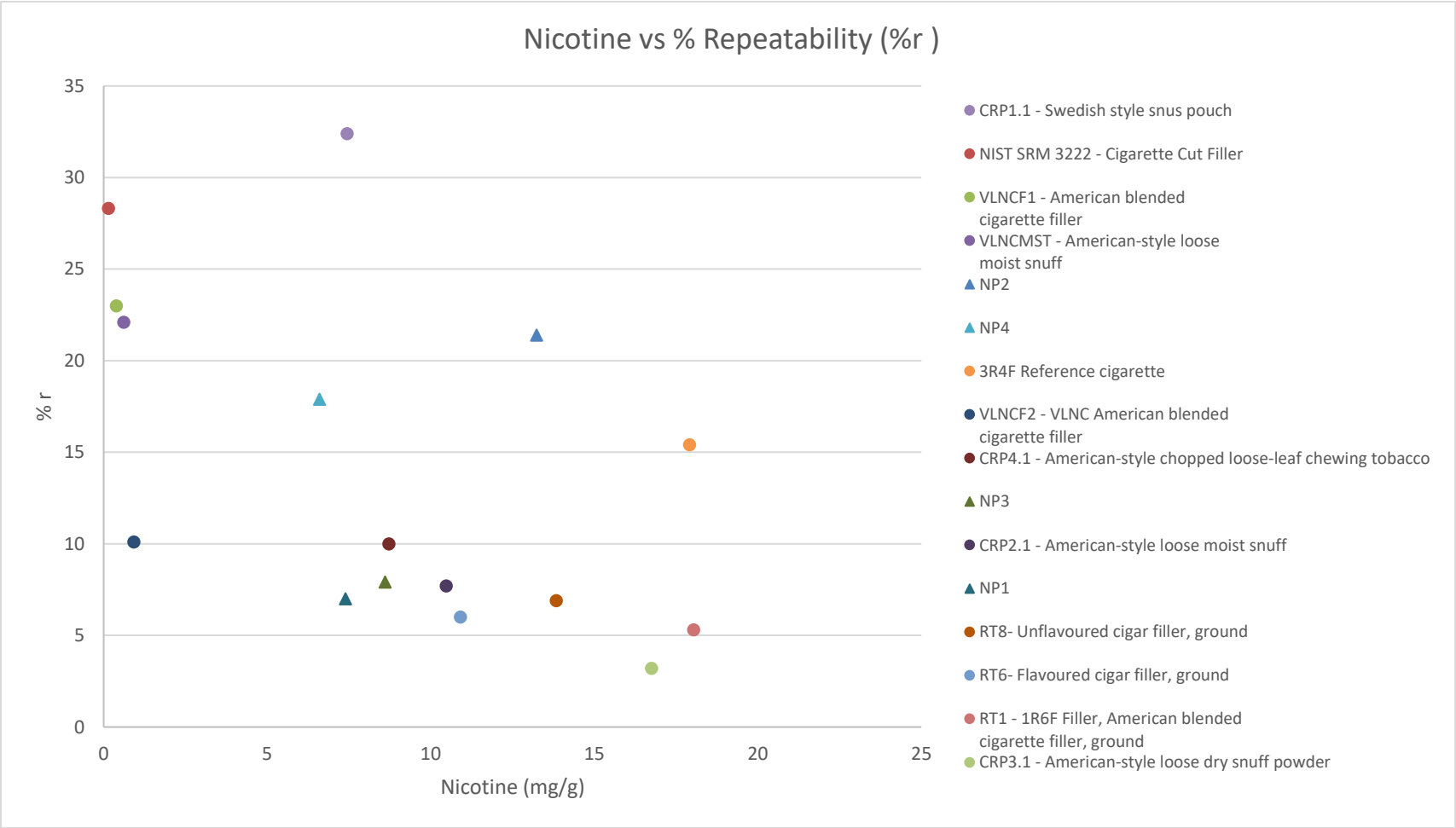

Acetaldehyde z-score
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
A B C D E F G H I J K L M N O P Q
Laboratory
Sample NP1 NP2 NP3 NP4

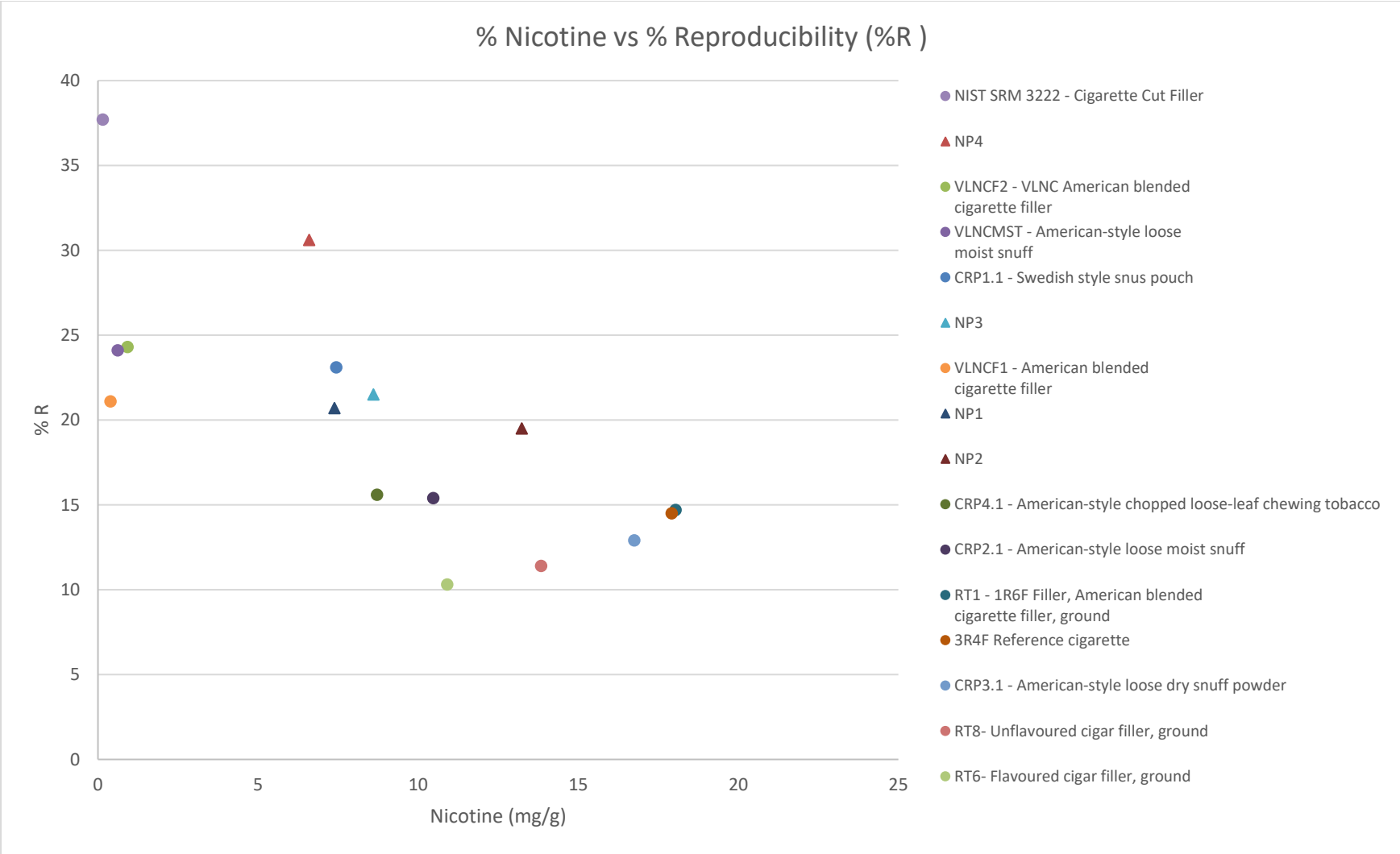
APPENDIX E: Raw Data – Fortification Study

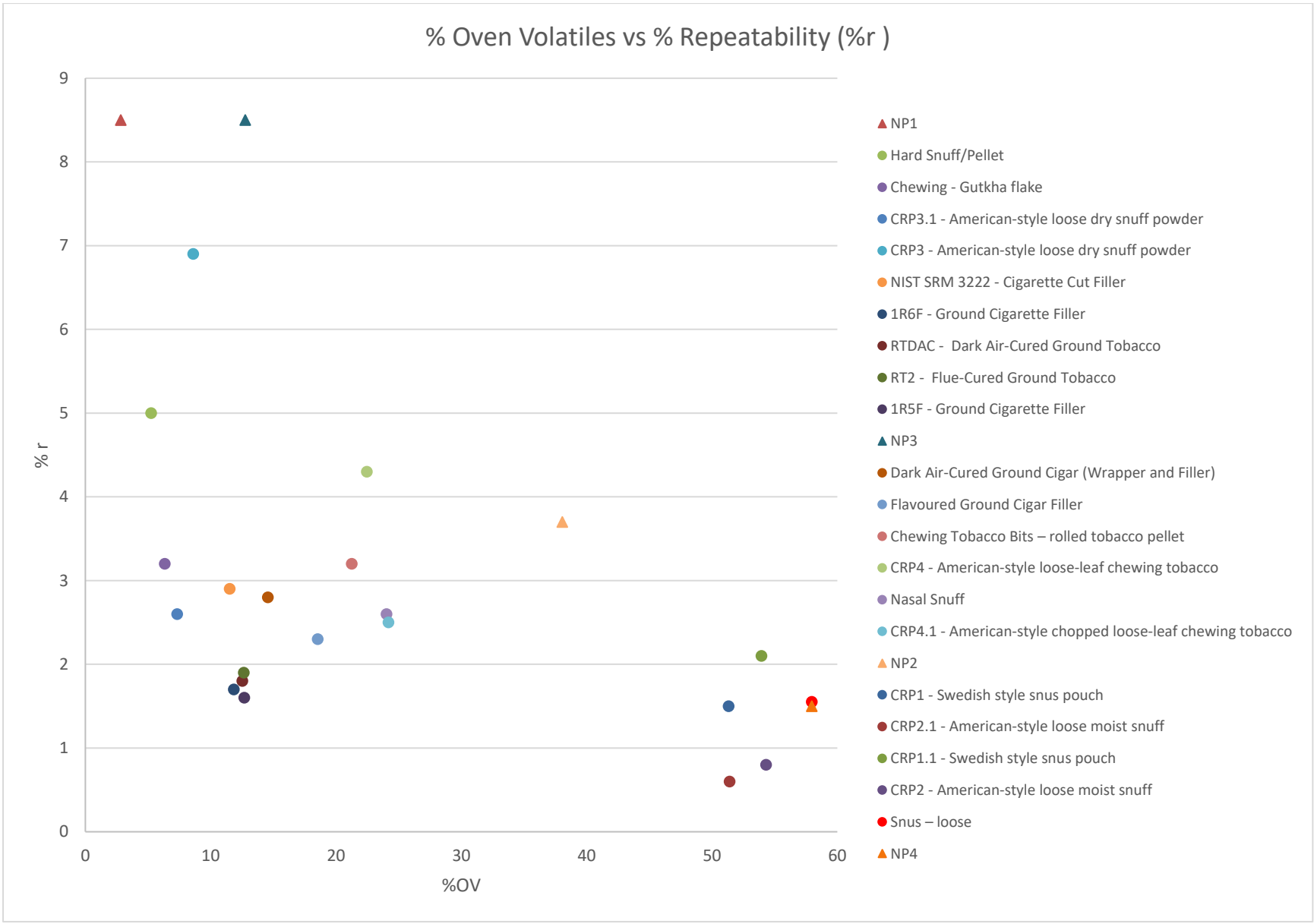
	Product	Replicates	NNK	NNN	NAT	NAB	B[a]P	Formaldehyde	Acetaldehyde	Crotonaldehyde	Arsenic	Cadmium
			ng/g	ng/g	ng/g	ng/g	ng/g	µg/g	µg/g	µg/g	ng/g	ng/g
unfortified	NP1	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12.91	0.3418	0.0114	<LOQ	<LOQ
unfortified	NP1	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12.32	0.3031	0.0102	<LOQ	<LOQ
unfortified	NP1	3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	9.308	0.3080	0.0084	<LOQ	<LOQ
unfortified	NP1	4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.56	0.2999	0.0087	<LOQ	<LOQ
unfortified	NP1	5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.73	0.2676	0.0079	<LOQ	<LOQ
unfortified	NP1	6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	13.36	0.1598	0.0085	<LOQ	<LOQ
unfortified	NP2	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.524	0.0483	0.0080	<LOQ	<LOQ
unfortified	NP2	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.386	0.0473	0.0092	<LOQ	<LOQ
unfortified	NP2	3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.447	0.0383	0.0096	<LOQ	<LOQ
unfortified	NP2	4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.147	0.1161	0.0080	<LOQ	<LOQ
unfortified	NP2	5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.404	0.0421	0.0096	<LOQ	<LOQ
unfortified	NP2	6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.358	0.0501	0.0082	<LOQ	<LOQ
unfortified	NP3	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.010	0.0000	0.0089	<LOQ	<LOQ
unfortified	NP3	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.9564	0.0000	0.0078	<LOQ	<LOQ
unfortified	NP3	3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.9959	0.0000	0.0089	<LOQ	<LOQ
unfortified	NP3	4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.003	0.0000	0.0092	<LOQ	<LOQ
unfortified	NP3	5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.059	0.0011	0.0078	<LOQ	<LOQ
unfortified	NP3	6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.971	0.0000	0.0079	<LOQ	<LOQ
unfortified	NP 4	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.423	0.0161	0.0059	<LOQ	<LOQ
unfortified	NP 4	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.357	0.0225	0.0058	<LOQ	<LOQ
unfortified	NP 4	3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.156	0.0189	0.0069	<LOQ	<LOQ
unfortified	NP 4	4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.150	0.0145	0.0056	<LOQ	<LOQ
unfortified	NP 4	5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.139	0.0185	0.0067	<LOQ	<LOQ
unfortified	NP 4	6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.118	0.0124	0.0051	<LOQ	<LOQ
Fortified amount			1.066	1.009	1.006	0.2642	2.5	5	5	1	0.1	0.4

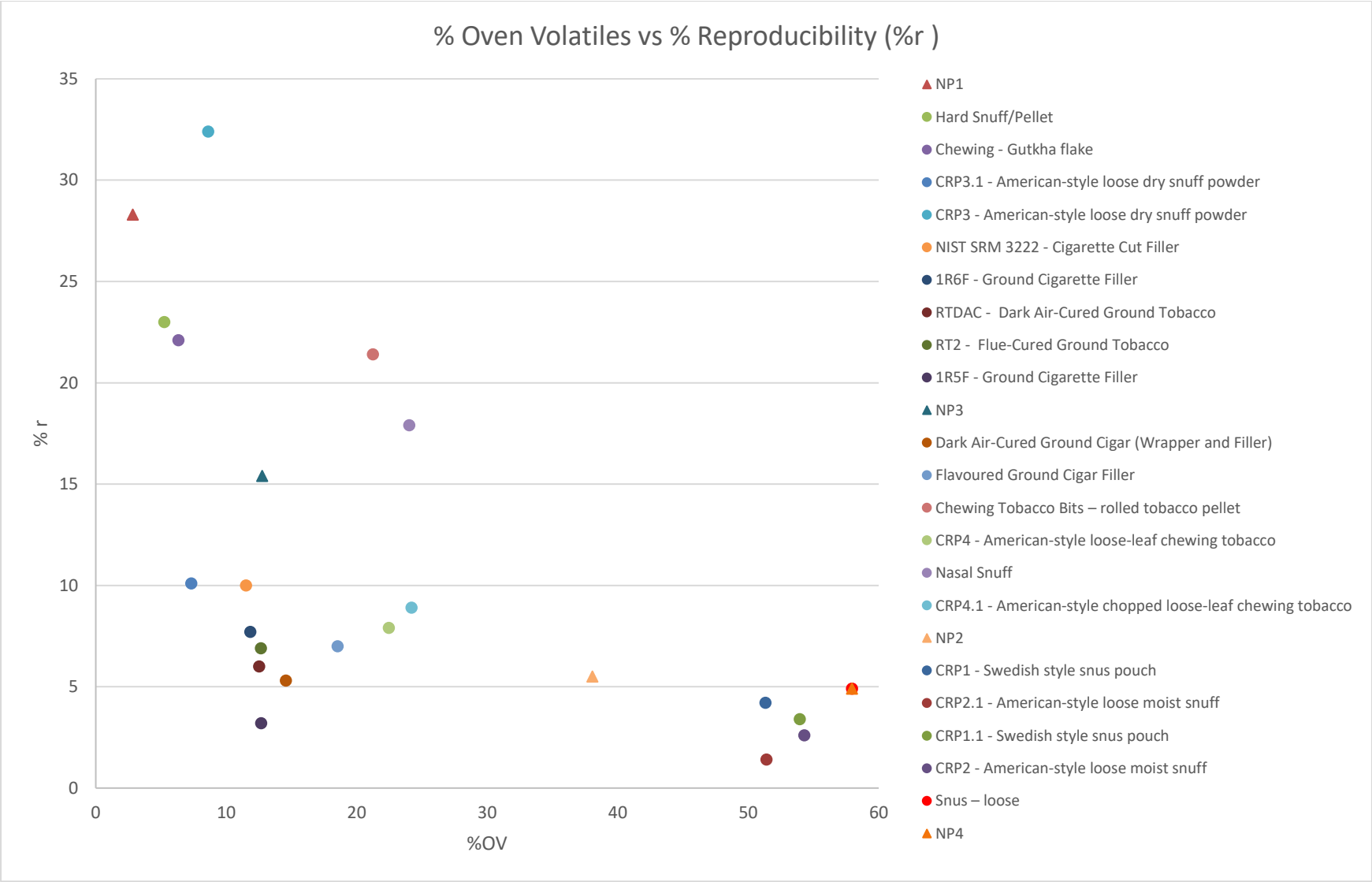
	Product	Replicates	NNK	NNN	NAT	NAB	B[a]P	Formaldehyde	Acetaldehyde	Crotonaldehyde	Arsenic	Cadmium
			ng/g	ng/g	ng/g	ng/g	ng/g	µg/g	µg/g	µg/g	ng/g	ng/g
fortified	NP1	1	1.029	1.063	1.009	0.2648	2.040	17.16	4.502	0.6650	0.0952	0.3880
fortified	NP1	2	1.008	1.088	1.043	0.2590	2.247	16.39	4.351	0.6166	0.0949	0.3890
fortified	NP1	3	1.014	1.058	1.037	0.2540	2.232	13.24	4.110	0.5456	0.0934	0.3720
fortified	NP1	4	1.011	1.055	1.031	0.2547	2.189	16.06	4.012	0.6336	0.0942	0.3790
fortified	NP1	5	1.031	1.087	1.013	0.2625	2.170	15.72	4.574	0.6372	0.1018	0.3810
fortified	NP1	6	1.013	1.054	1.066	0.2598	2.219	17.19	4.783	0.6795	0.0896	0.3890
fortified	NP2	1	1.007	1.049	1.030	0.2583	2.279	7.054	7.170	0.9957	0.0960	0.3920
fortified	NP2	2	1.005	1.028	1.026	0.2550	2.463	7.343	5.910	0.8449	0.0938	0.4220
fortified	NP2	3	1.034	1.055	1.037	0.2530	2.219	7.522	5.303	0.8314	0.0980	0.3880
fortified	NP2	4	1.004	1.032	1.042	0.2503	2.409	6.148	5.428	0.7698	0.0922	0.3990
fortified	NP2	5	1.009	1.047	1.029	0.2540	2.448	7.432	6.124	0.8901	0.1042	0.3790
fortified	NP2	6	1.024	1.027	1.033	0.2542	2.367	7.151	6.228	0.8618	0.0956	0.3970
fortified	NP3	1	1.014	1.040	1.036	0.2498	2.305	6.422	5.855	0.9485	0.1078	0.3780
fortified	NP3	2	1.009	1.042	1.043	0.2521	2.281	6.861	5.956	1.045	0.0952	0.3750
fortified	NP3	3	1.005	1.043	1.019	0.2618	2.239	6.803	6.030	1.024	0.1009	0.3940
fortified	NP3	4	1.008	1.035	1.048	0.2561	2.207	6.847	6.126	1.024	0.1098	0.3960
fortified	NP3	5	0.995	1.026	1.022	0.2523	2.300	6.735	6.065	1.049	0.1048	0.4010
fortified	NP3	6	1.027	1.054	1.045	0.2557	2.296	6.724	6.021	1.039	0.1059	0.4080
fortified	NP 4	1	1.023	1.062	1.016	0.2458	2.402	6.291	4.822	0.8069	0.1035	0.3700
fortified	NP 4	2	1.035	1.061	1.003	0.2497	2.265	5.745	4.532	0.7635	0.1043	0.3800
fortified	NP 4	3	1.014	1.042	1.017	0.2490	2.154	5.266	4.339	0.7262	0.1052	0.4010
fortified	NP 4	4	1.013	1.049	0.990	0.2430	2.251	5.578	4.690	0.7971	0.1014	0.3790
fortified	NP 4	5	1.007	1.073	1.025	0.2556	2.219	5.780	5.089	0.8664	0.0923	0.4050
fortified	NP 4	6	1.005	1.086	1.020	0.2459	2.214	5.487	4.360	0.8877	0.1092	0.4050

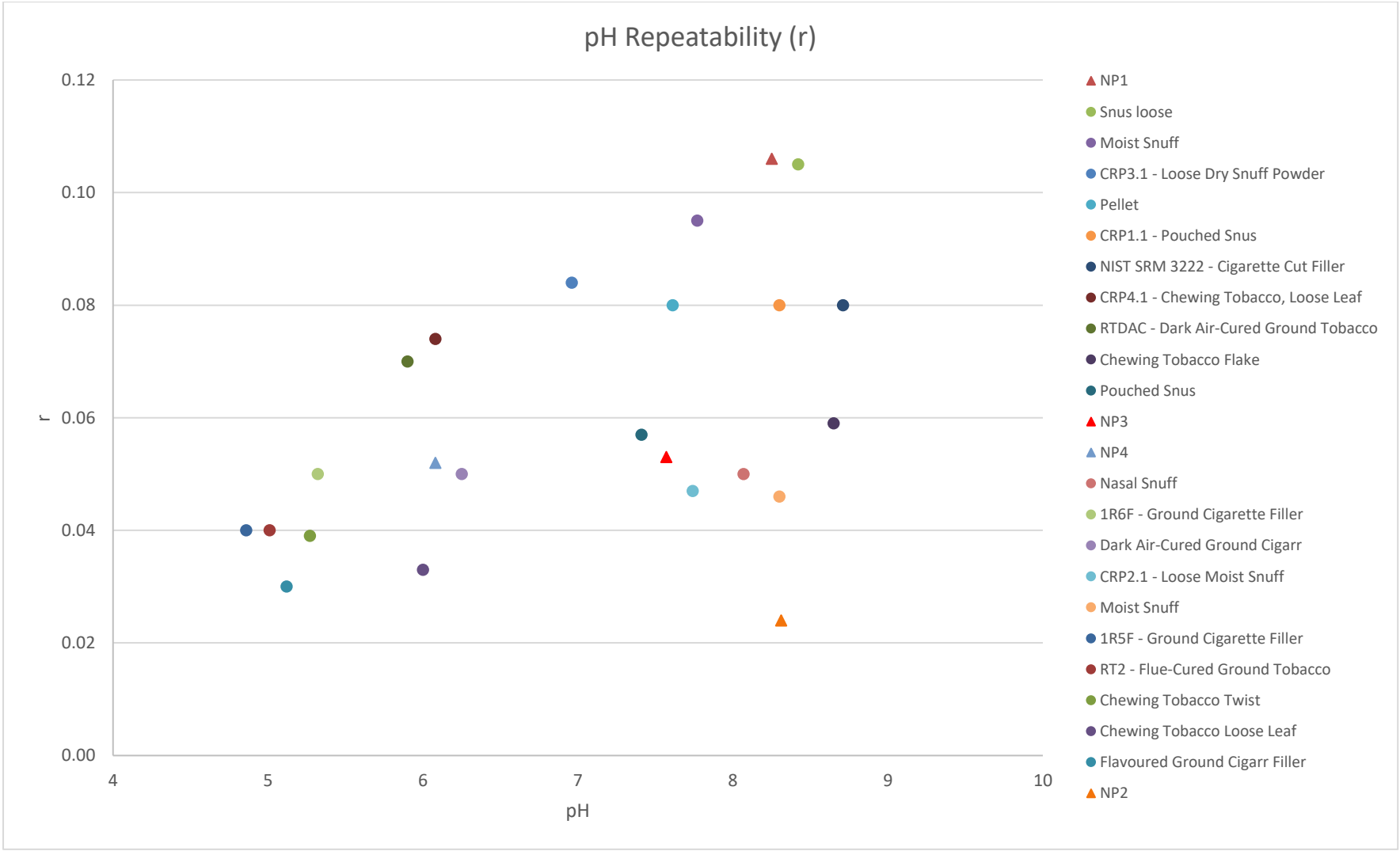
APPENDIX F: Comparison of %r and %R from this study and the respective CRMs

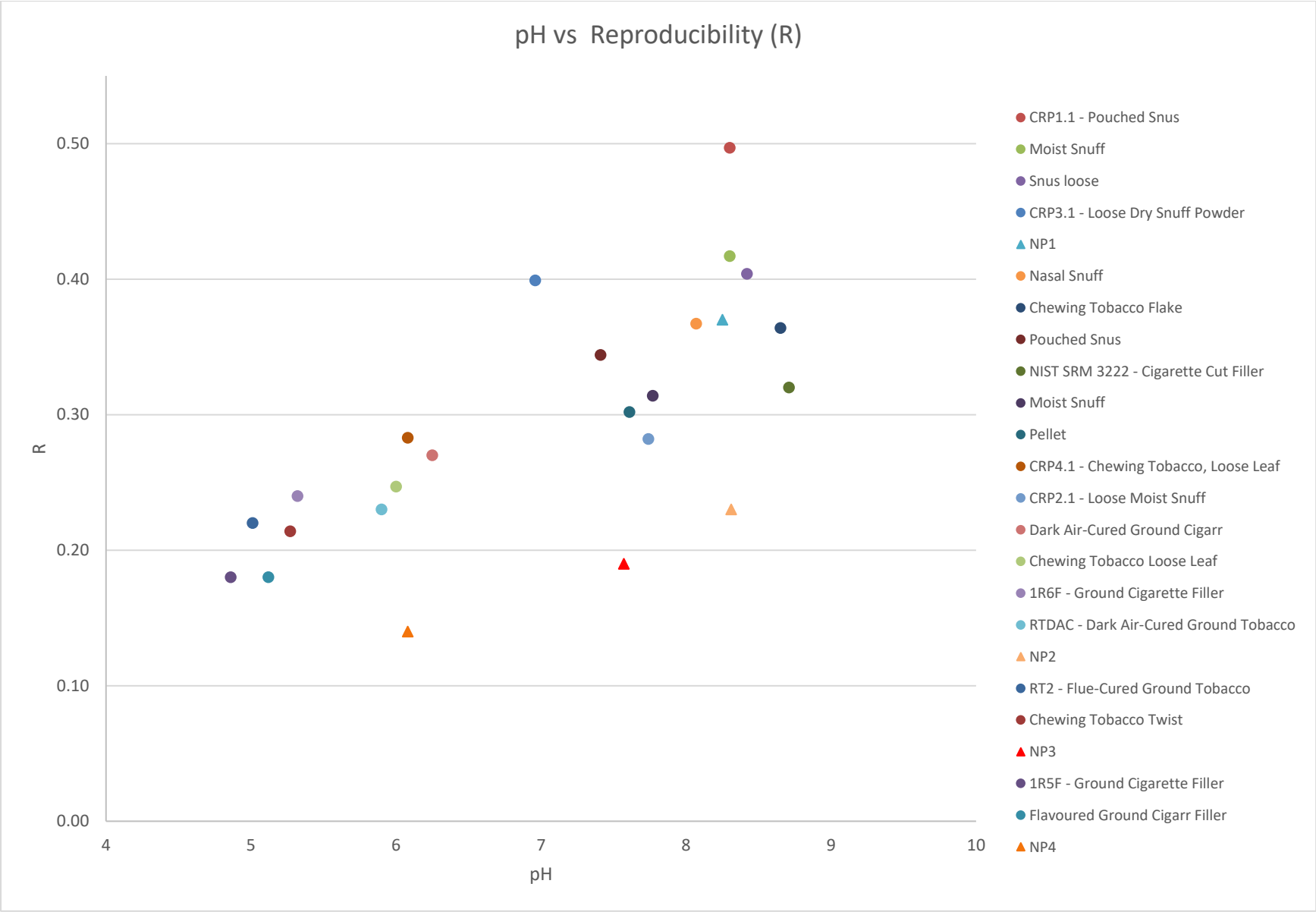


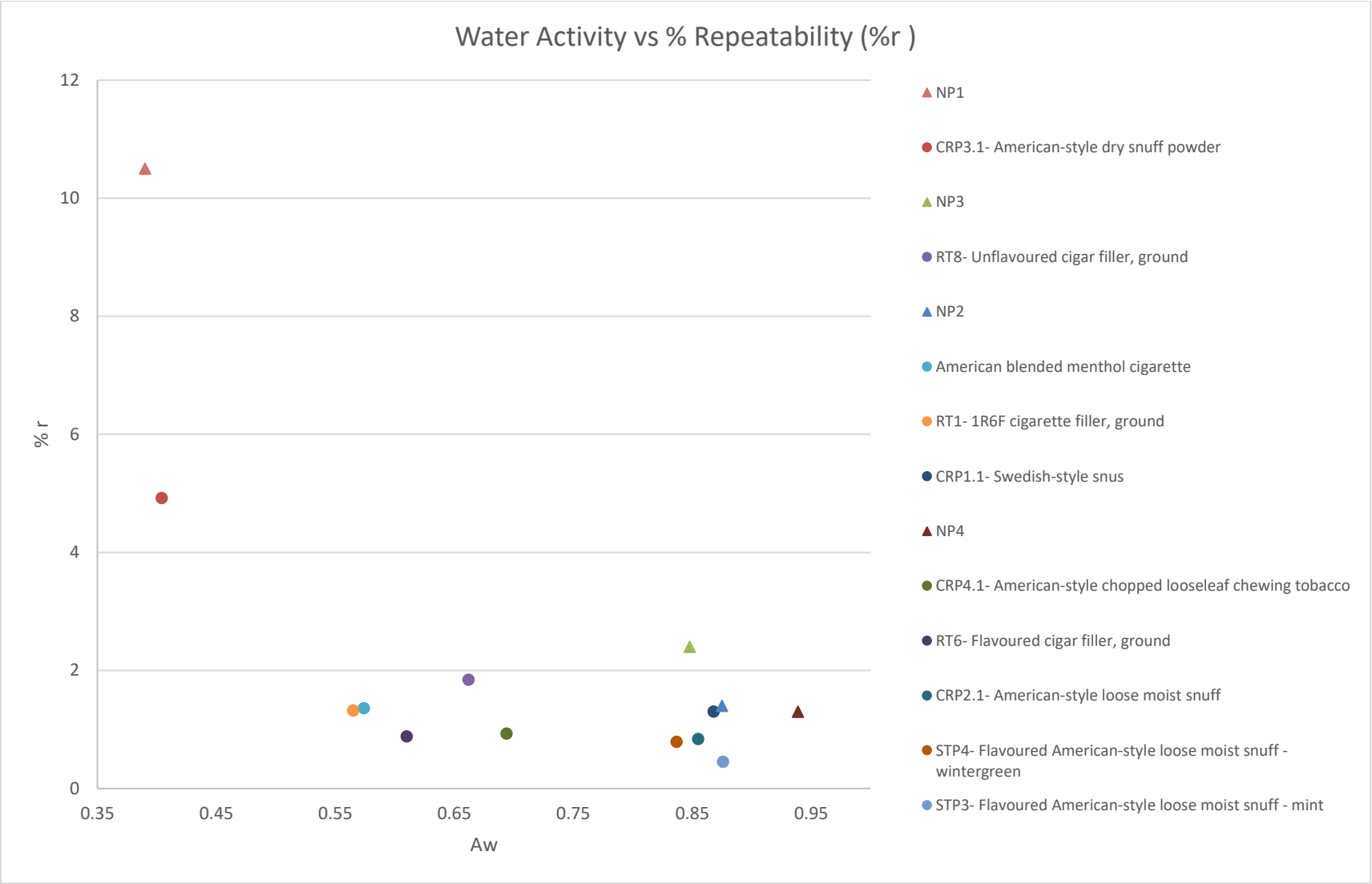

% Nicotine vs % Reproducibility (%R)
40
35
30
25
20
15
10
5
0
% R
0
5
10
15
20
25
Nicotine (mg/g)
NIST SRM 3222 - Cigarette Cut Filler
NP4
VLNCF2 - VLNC American blended cigarette filler
VLNCMST - American-style loose moist snuff
CRP1.1 - Swedish style snus pouch
NP3
VLNCF1 - American blended cigarette filler
NP1
NP2
CRP4.1 - American-style chopped loose-leaf chewing tobacco
CRP2.1 - American-style loose moist snuff
RT1 - 1R6F Filler, American blended cigarette filler, ground
3R4F Reference cigarette
CRP3.1 - American-style loose dry snuff powder
RT8- Unflavoured cigar filler, ground
RT6- Flavoured cigar filler, ground

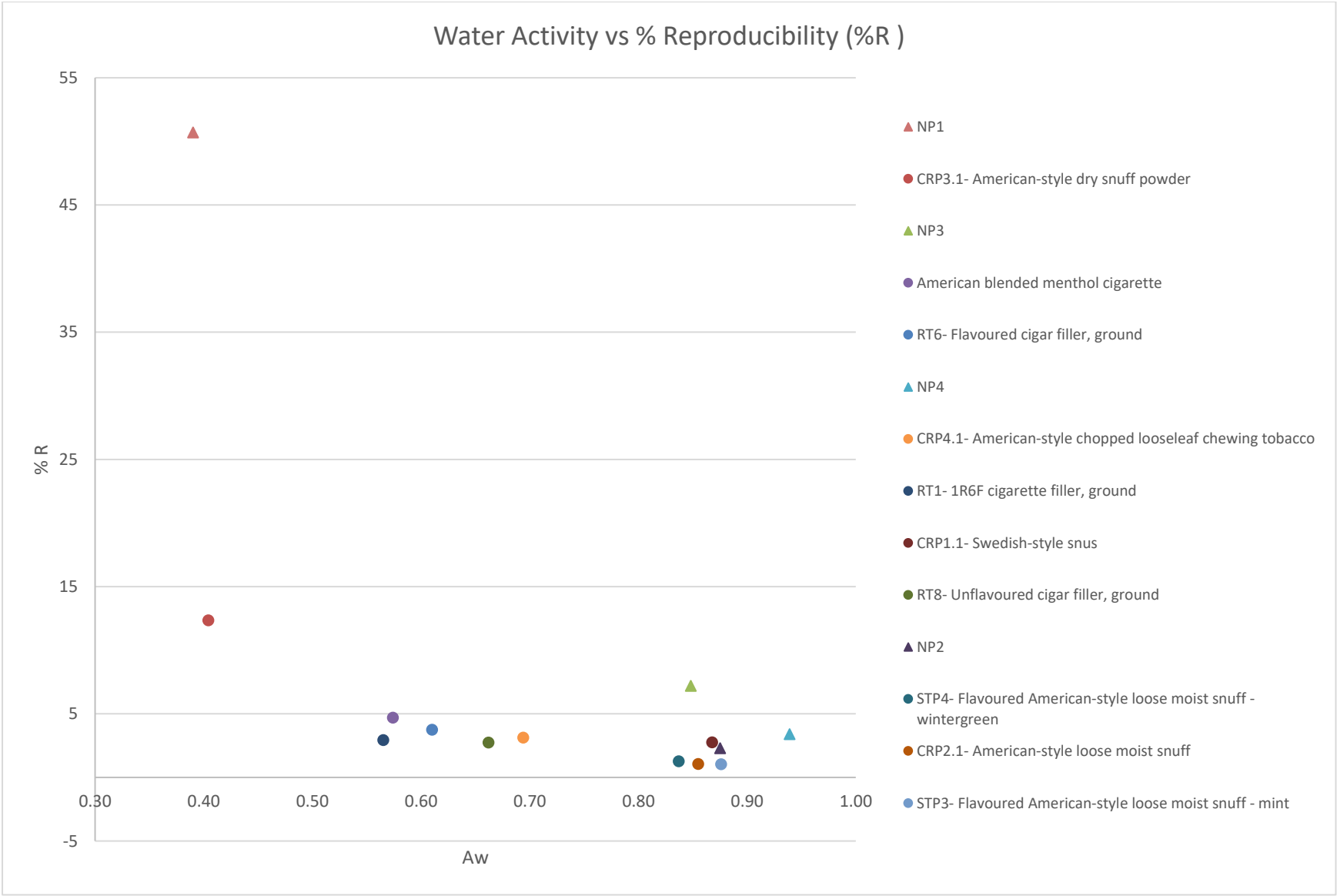

% Oven Volatiles vs % Repeatability (%r)
% r
%OV
0
1
2
3
4
5
6
7
8
9
0
10
20
30
40
50
60
NP1
Hard Snuff/Pellet
Chewing - Gutkha flake
CRP3.1 - American-style loose dry snuff powder
CRP3 - American-style loose dry snuff powder
NIST SRM 3222 - Cigarette Cut Filler
1R6F - Ground Cigarette Filler
RTDAC - Dark Air-Cured Ground Tobacco
RT2 - Flue-Cured Ground Tobacco
1R5F - Ground Cigarette Filler
NP3
Dark Air-Cured Ground Cigar (Wrapper and Filler)
Flavoured Ground Cigar Filler
Chewing Tobacco Bits – rolled tobacco pellet
CRP4 - American-style loose-leaf chewing tobacco
Nasal Snuff
CRP4.1 - American-style chopped loose-leaf chewing tobacco
NP2
CRP1 - Swedish style snus pouch
CRP2.1 - American-style loose moist snuff
CRP1.1 - Swedish style snus pouch
CRP2 - American-style loose moist snuff
Snus – loose
NP4

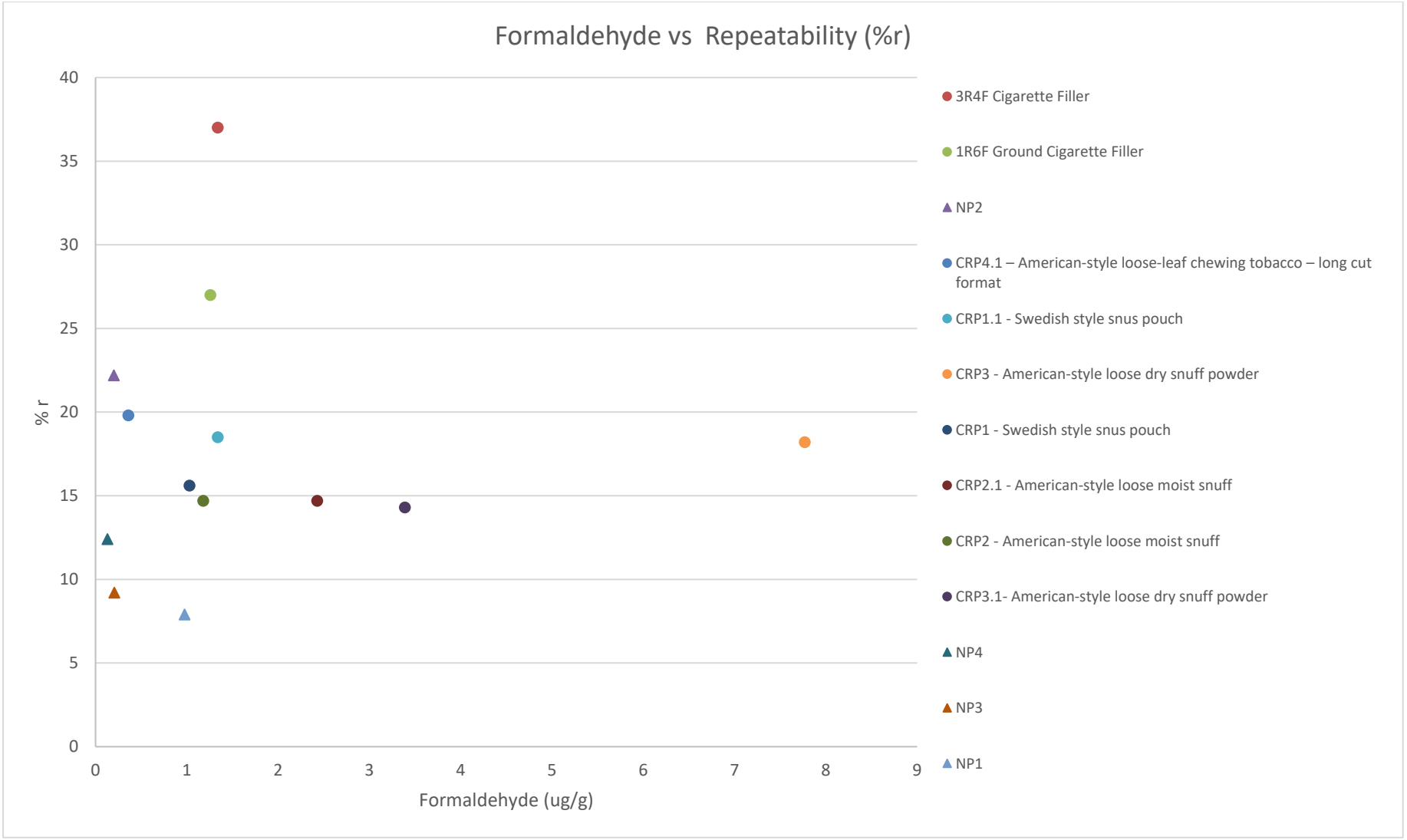

% Oven Volatiles vs % Reproducibility (%r)
35
30
25
20
15
10
5
0
% r
0
10
20
30
40
50
60
%OV
NP1
Hard Snuff/Pellet
Chewing - Gutkha flake
CRP3.1 - American-style loose dry snuff powder
CRP3 - American-style loose dry snuff powder
NIST SRM 3222 - Cigarette Cut Filler
1R6F - Ground Cigarette Filler
RTDAC - Dark Air-Cured Ground Tobacco
RT2 - Flue-Cured Ground Tobacco
1R5F - Ground Cigarette Filler
NP3
Dark Air-Cured Ground Cigar (Wrapper and Filler)
Flavoured Ground Cigar Filler
Chewing Tobacco Bits – rolled tobacco pellet
CRP4 - American-style loose-leaf chewing tobacco
Nasal Snuff
CRP4.1 - American-style chopped loose-leaf chewing tobacco
NP2
CRP1 - Swedish style snus pouch
CRP2.1 - American-style loose moist snuff
CRP1.1 - Swedish style snus pouch
CRP2 - American-style loose moist snuff
Snus – loose
NP4

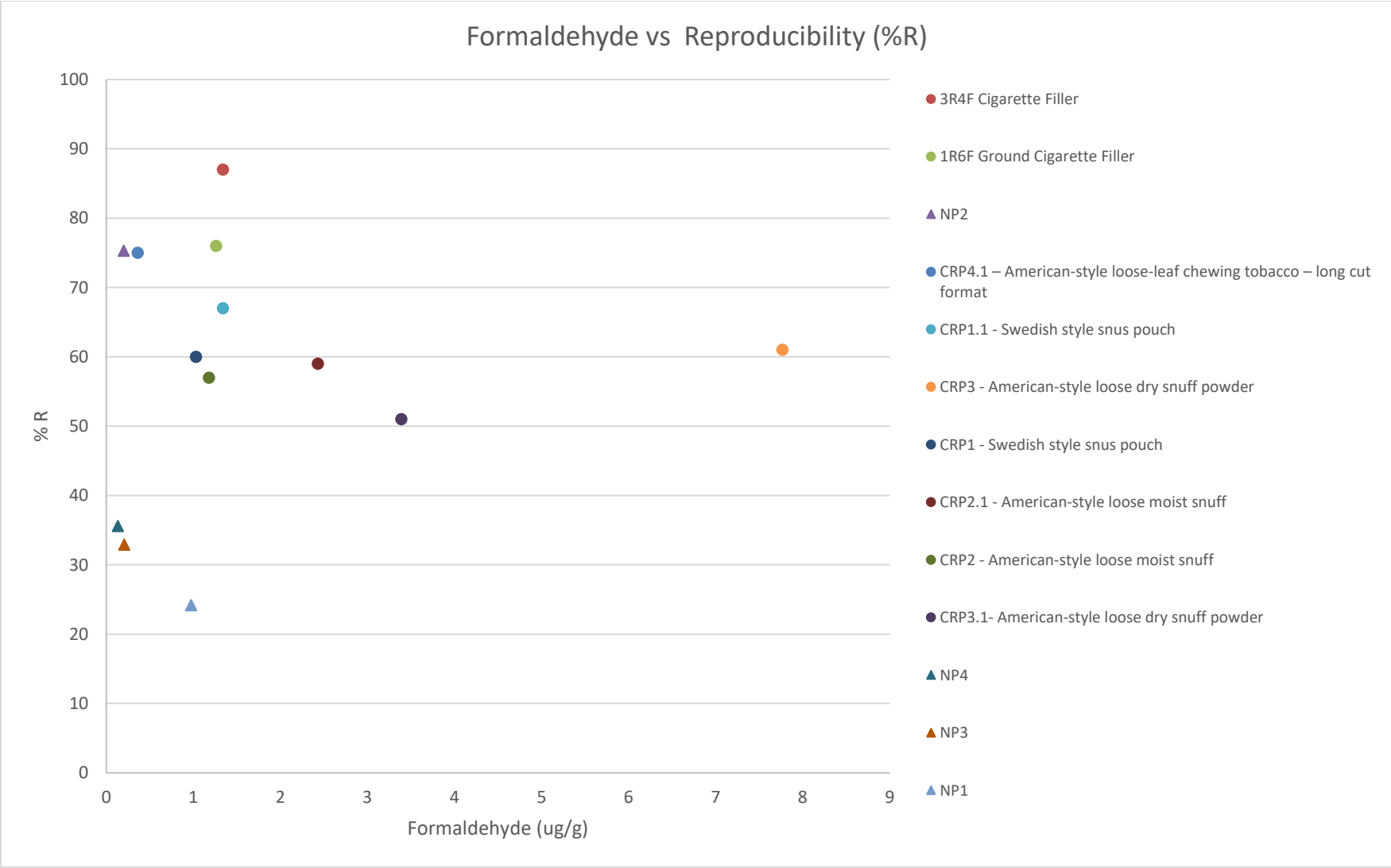

pH Repeatability (r)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
r
4
5
6
7
8
9
10
pH
NP1
Snus loose
Moist Snuff
CRP3.1 - Loose Dry Snuff Powder
Pellet
CRP1.1 - Pouched Snus
NIST SRM 3222 - Cigarette Cut Filler
CRP4.1 - Chewing Tobacco, Loose Leaf
RTDAC - Dark Air-Cured Ground Tobacco
Chewing Tobacco Flake
Pouched Snus
NP3
NP4
Nasal Snuff
1R6F - Ground Cigarette Filler
Dark Air-Cured Ground Cigarr
CRP2.1 - Loose Moist Snuff
Moist Snuff
1R5F - Ground Cigarette Filler
RT2 - Flue-Cured Ground Tobacco
Chewing Tobacco Twist
Chewing Tobacco Loose Leaf
Flavoured Ground Cigarr Filler
NP2

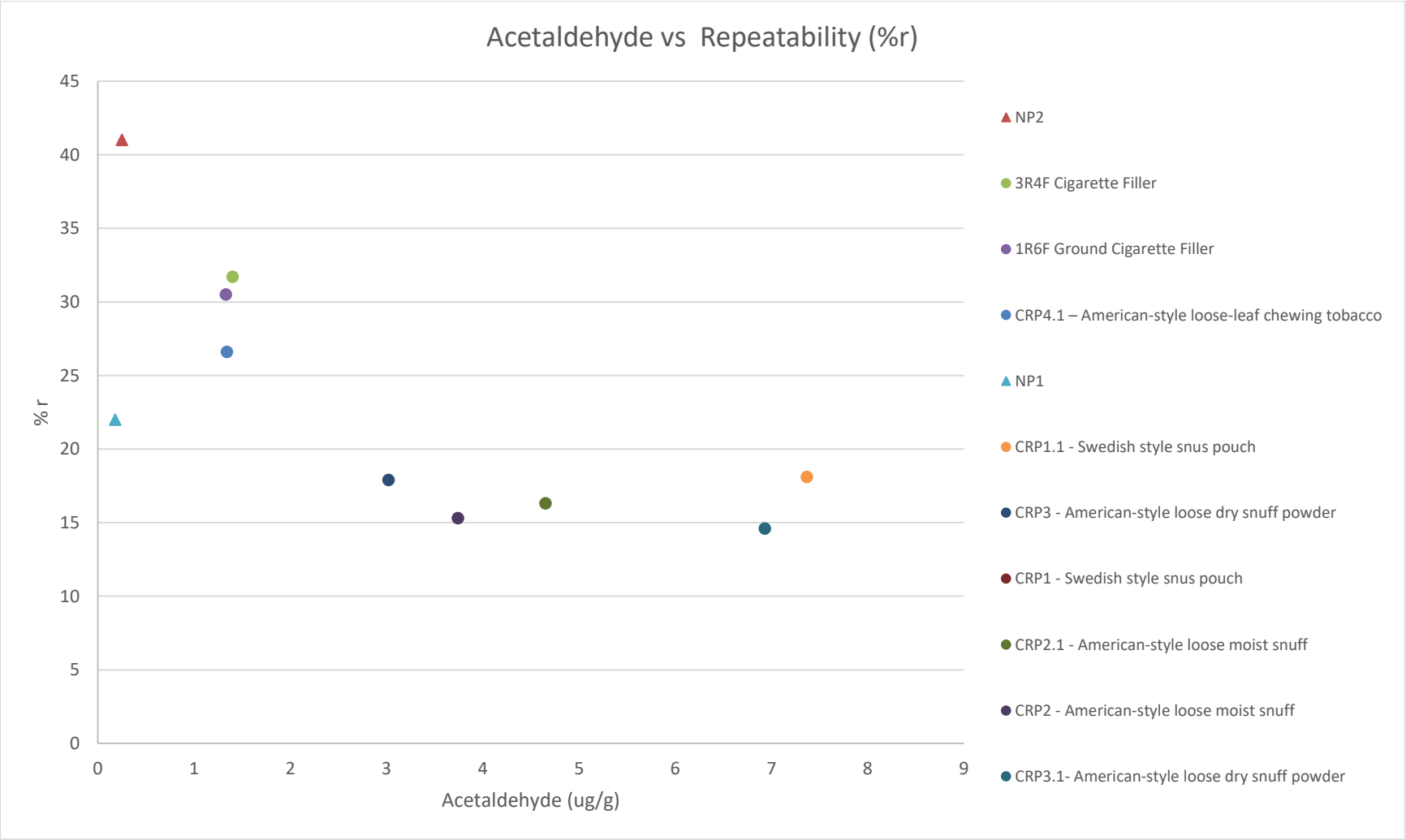

pH vs Reproducibility (R)
0.50
0.40
0.30
0.20
0.10
0.00
R
4
5
6
7
8
9
10
pH
CRP1.1 - Pouched Snus
Moist Snuff
Snus loose
CRP3.1 - Loose Dry Snuff Powder
NP1
Nasal Snuff
Chewing Tobacco Flake
Pouched Snus
NIST SRM 3222 - Cigarette Cut Filler
Moist Snuff
Pellet
CRP4.1 - Chewing Tobacco, Loose Leaf
CRP2.1 - Loose Moist Snuff
Dark Air-Cured Ground Cigarr
Chewing Tobacco Loose Leaf
1R6F - Ground Cigarette Filler
RTDAC - Dark Air-Cured Ground Tobacco
NP2
RT2 - Flue-Cured Ground Tobacco
Chewing Tobacco Twist
NP3
1R5F - Ground Cigarette Filler
Flavoured Ground Cigarr Filler
NP4

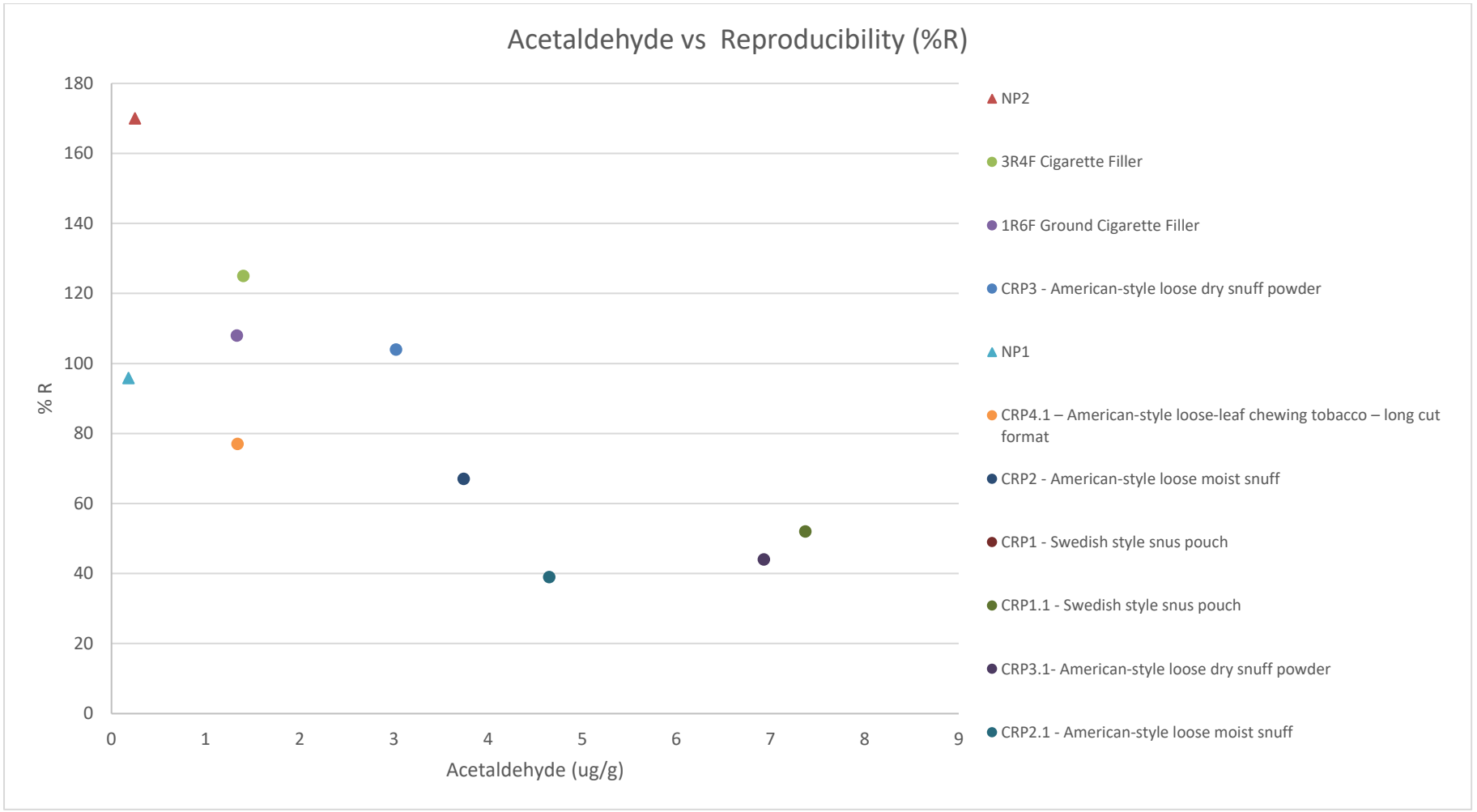

Water Activity vs % Repeatability (%r)
12
10
8
6
4
2
0
% r
0.35
0.45
0.55
0.65
0.75
0.85
0.95
Aw
NP1
CRP3.1- American-style dry snuff powder
NP3
RT8- Unflavoured cigar filler, ground
NP2
American blended menthol cigarette
RT1- 1R6F cigarette filler, ground
CRP1.1- Swedish-style snus
NP4
CRP4.1- American-style chopped looseleaf chewing tobacco
RT6- Flavoured cigar filler, ground
CRP2.1- American-style loose moist snuff
STP4- Flavoured American-style loose moist snuff - wintergreen
STP3- Flavoured American-style loose moist snuff - mint


Water Activity vs % Reproducibility (%R)
55
45
35
25
15
5
-5
% R
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
Aw
NP1
CRP3.1- American-style dry snuff powder
NP3
American blended menthol cigarette
RT6- Flavoured cigar filler, ground
NP4
CRP4.1- American-style chopped looseleaf chewing tobacco
RT1- 1R6F cigarette filler, ground
CRP1.1- Swedish-style snus
RT8- Unflavoured cigar filler, ground
NP2
STP4- Flavoured American-style loose moist snuff - wintergreen
CRP2.1- American-style loose moist snuff
STP3- Flavoured American-style loose moist snuff - mint


Formaldehyde vs Repeatability (%r)
% r
Formaldehyde (ug/g)
0
5
10
15
20
25
30
35
40
0
1
2
3
4
5
6
7
8
9
3R4F Cigarette Filler
1R6F Ground Cigarette Filler
NP2
CRP4.1 – American-style loose-leaf chewing tobacco – long cut format
CRP1.1 - Swedish style snus pouch
CRP3 - American-style loose dry snuff powder
CRP1 - Swedish style snus pouch
CRP2.1 - American-style loose moist snuff
CRP2 - American-style loose moist snuff
CRP3.1- American-style loose dry snuff powder
NP4
NP3
NP1


Formaldehyde vs Reproducibility (%R)
% R
Formaldehyde (ug/g)
0
10
20
30
40
50
60
70
80
90
100
0
1
2
3
4
5
6
7
8
9
3R4F Cigarette Filler
1R6F Ground Cigarette Filler
NP2
CRP4.1 – American-style loose-leaf chewing tobacco – long cut format
CRP1.1 - Swedish style snus pouch
CRP3 - American-style loose dry snuff powder
CRP1 - Swedish style snus pouch
CRP2.1 - American-style loose moist snuff
CRP2 - American-style loose moist snuff
CRP3.1- American-style loose dry snuff powder
NP4
NP3
NP1


Acetaldehyde vs Repeatability (%r)
45
40
35
30
25
20
15
10
5
0
% r
0
1
2
3
4
5
6
7
8
9
Acetaldehyde (ug/g)
NP2
3R4F Cigarette Filler
1R6F Ground Cigarette Filler
CRP4.1 – American-style loose-leaf chewing tobacco
NP1
CRP1.1 - Swedish style snus pouch
CRP3 - American-style loose dry snuff powder
CRP1 - Swedish style snus pouch
CRP2.1 - American-style loose moist snuff
CRP2 - American-style loose moist snuff
CRP3.1- American-style loose dry snuff powder


Acetaldehyde vs Reproducibility (%R)
180
160
140
120
100
80
60
40
20
0
% R
0
1
2
3
4
5
6
7
8
9
Acetaldehyde (ug/g)
NP2
3R4F Cigarette Filler
1R6F Ground Cigarette Filler
CRP3 - American-style loose dry snuff powder
NP1
CRP4.1 – American-style loose-leaf chewing tobacco – long cut format
CRP2 - American-style loose moist snuff
CRP1 - Swedish style snus pouch
CRP1.1 - Swedish style snus pouch
CRP3.1- American-style loose dry snuff powder
CRP2.1 - American-style loose moist snuff