Tobacco and Tobacco Products Analytes Sub-Group

Technical Report

Stability Study for the 2016 CORESTA Reference Products 2023 Analysis

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Study Coordinator and Author:
Rozanna Avagyan, Ph.D., Swedish Match Northern Europe

Co-Author and Statistical Analysis:
Michael Morton, Ph.D., Altria Client Services LLC

Sub-Group Coordinator:
Karl Wagner, Ph.D., Altria Client Services LLC

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1. Summary

At the hybrid CORESTA Tobacco and Tobacco Products Analytes Sub-Group (TTPA) meeting held on September 10, 2022, the Sub-Group initiated the fourth study to assess the stability of the four CORESTA Reference Products (CRPs) manufactured in 2016, and to provide repeatability (r) and reproducibility (R) results and z-scores to support laboratory accreditation. Sixteen laboratories participated in the study. The participating laboratories reported the levels of nicotine, pH, moisture (oven volatiles), and tobacco specific nitrosamines (TSNAs) in the CRPs using CORESTA Recommended Methods (CRMs). Tabulated data are presented along with a trend analysis, repeatability and reproducibility estimates, and z-scores.

The results from this 2023 stability analysis generally compared well to the results from 2016, 2019, and 2021[1,2,3]. The only statistically significant product trends differences from the 2016 and 2019 results were slight increases in moisture for CRP3.1 and a small pH drop in CRP1.1. These results are consistent with the trends seen with the CRPs produced in 2009[4]. The TTPA recommends that the stability of the 2016 CRPs continue to be monitored on a biennial basis.

2. Introduction

In November 2008, the Smokeless Tobacco Sub-Group (STS), now named Tobacco and Tobacco Product Analytes Sub-Group (TTPA), was established by recommendation of the CORESTA Scientific Commission. In 2009, the STS Working Group Three (WG3) cooperated to design and manufacture four CORESTA Reference Products (CRPs) referred to as CRP1, CRP2, CRP3, and CRP4. These products were intended as replacements for the Smokeless Tobacco Research Products: 2S3 (Moist Snuff), 1S2 (Dry Snuff) and 2S1 (Loose-leaf Chewing Tobacco), which were more than ten years old. The STS recommended monitoring the stability of the 2009 CRPs on an annual basis, by determining the levels of nicotine, pH, moisture (oven volatiles) and TSNAs using CRMs or draft CRMs. These studies were conducted through 2019 and demonstrated that the CRPs were stable when held at the recommended storage conditions of 20 °C[4].

In 2015 the supply of the 2009 CRPs decreased to the point that the STS decided to remanufacture the CRPs. Similar to the 2009 production, four CRPs were manufactured in 2016 and −included Swedish snus pouch (CRP1.1), American-style loose moist snuff (CRP2.1), American-style loose dry snuff powder (CRP3.1), and American-style chopped loose-leaf chewing tobacco (CRP4.1). CRP4.1 was produced in a chopped format to improve within package sample homogeneity as compared to the 2009 loose leaf format (CRP4). Even though the 2016 CRPs included the four product styles that were produced in 2009, it was anticipated the chemistry would differ between the two productions considering different tobaccos were used and different manufacturers produced three of the reference products. In 2016, an initial characterization of the 2016 CRPs was conducted that included nicotine, pH, moisture, TSNAs, ammonia and B[a]P using the applicable CRMs[1]. This report serves as the fourth assessment of the 2016 CRPs by reporting the levels of nicotine, pH, moisture, and TSNAs using the applicable CRMs.

The 2016 CRPs are stored at −20 °C and distributed by the North Carolina State University (NCSU) Tobacco Analytical Services Laboratory.

[1] TTPA Technical Report: CORESTA Reference Products – 2016 Analysis.
[2] TTPA Technical Report: CORESTA Reference Products – 2019 Analysis.
[3] TTPA Technical Report: CORESTA Reference Products – 2021 Analysis.
[4] TTPA Technical Report: CORESTA 2009 Reference Products – 2019 Analysis.

2.1 Objective

- This study was conducted to provide a stability assessment for the CRPs. The participating laboratories were to provide analytical results for nicotine, pH, moisture (oven volatiles), and TSNAs (N-nitrosonornicotine (NNN), N-nitrosoanatabine (NAT), N-nitrosoanabasine (NAB), 4-N-nitrosomethylamino-1-3-pyridyl-1-butanone (NNK)). Results were reported on an as-is basis and not corrected for moisture. Additionally, this study provides an assessment of inter-laboratory variability and laboratory proficiency.
- Data were collected from the participating laboratories and statistically evaluated in basic conformance with the recommendations of ISO 5725-5:1998. Additionally, z-scores were calculated as a measure of each laboratory's performance as compared to the results of other laboratories. The results from this year's stability evaluation were compared to the results from 2016, 2019, and 2021 via trend analysis using a t-test with $p<0.0125$ (the criterion for statistical significance). The 0.0125 criterion is based on adjusting the standard 0.05 criterion to account for the four reference products being compared for each analyte.

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, that do not correspond to the order below, were assigned to each laboratory.

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
Eurofins Food & Feed Testing Sweden AB, Sweden
Imperial Brands, Reemtsma, Germany
ITC Limited, India
JTI Oekolab, Austria
Japan Tobacco Inc, Leaf Tobacco Research Center, Japan
KT&G, South Korea
R.J. Reynolds Tobacco Company, United States
Scandinavian Tobacco Group, Belgium
Swedish Match North America, United States
Swedish Match, Sweden
Swisher International, United States
University of Kentucky, United States

3.2 Protocol

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

3.2.1 Sample Shipment

Laboratories were responsible for the CRPs from NCSU immediately before starting the study. Laboratories were requested to store the samples at approximately 4 °C upon receipt if the analyses would be conducted within one week or to store the samples at approximately −20 °C if the analyses would be delayed. Laboratories were requested to conduct the study during October 2022 to January 2023 and report data by February 1, 2023. The samples are identified in the table below.

Table 2: Sample Identification

Sample Type
CRP1.1 - Swedish style snus pouch
CRP2.1 - American-style loose moist snuff
CRP3.1 - American-style loose dry snuff powder
CRP4.1 - American-style chopped loose-leaf chewing tobacco

3.2.2 Within Laboratory Sample Preparation

The laboratories were directed to remove samples from the −20 °C freezer and place the unopened samples in a refrigerator for a minimum of 24 hours to ensure water was fully equilibrated. Samples could then be removed from the refrigerator for a minimum of 2 hours prior to opening for analysis. Once samples were opened, the samples could be stored in a tightly sealed container and stored at approximately 4 °C for up to one week. Handling requirements for the CRPs are described below:

- CRP1.1 for nicotine, TSNAs, and pH: The snus pouches shall be cut into 2 halves, and the tobacco and pouch added directly into the extraction vessel. Both the tobacco and pouch material shall be analyzed.

  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.

- CRP1.1 for moisture (OV): The snus pouches shall be added to the OV sample tin intact.
- CRP2.1, CRP3.1, and CRP4.1: These products shall be analyzed as-is, without grinding.

3.2.3 Sample Analysis and Data Reporting

The participating laboratories were instructed to conduct triplicate analyses for the following: nicotine, pH, moisture (oven volatiles), and TSNAs. The laboratories were requested to use the current versions of the following CRMs:

- CRM No. 62, Determination of Nicotine in Tobacco and Tobacco Products by Gas Chromatographic Analysis or CRM N° 87, Determination of Nicotine in Tobacco Products by GC/MS
- CRM No. 69, Determination of pH in Smokeless Tobacco Products
- CRM No. 72, Determination of Tobacco Specific Nitrosamines in Smokeless Tobacco Products by Liquid Chromatography - Tandem Mass Spectrometry

- CRM No. 76, Determination of Moisture Content (Oven Volatiles) of Smokeless Tobacco Products

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 identified below as well as if the data were excluded from the study.

- Lab A: Major deviations. In-house nicotine method. Extraction steps is based on Health Canada-Official Method: T-301, LC-MS/MS is used for separation and detection of nicotine instead of GC-FID. The results were excluded from the study.
- Lab B: Minor deviations. Nicotine: 0.2 g of CRP2.1 and CRP4.1 were weighted out and extracted by 0.5 ml 2 M NaOH and 5 ml MTBE. The results were included in the study.
- Lab C: Major deviations. Nicotine was downscaled: 0.7 ± 0.2 g sample, 5 mL 2 M NaOH, 35 mL MTBE, nicotine-d3 was used as the internal standard and GS-MS was used instead of GC-FID. The results were excluded from the study. Minor deviations. pH: CRP3.1 was weighed out with half the amount (1 g). TSNA: mobile phase A: 10 mM ammonium acetate, pH 4.7 (acetic acid), mobile phase B: 0.1 % formic acid in acetonitrile. OV: Oven 99 ± 1 °C, 3 h (+15 min). The results for pH, TSNAs, and OV were included in the study.
- Lab F: Minors deviations. Nicotine: 0.5 g of sample was weighed into a 50 ml plastic tube. 10 mL of distilled water, 20 mL of n-hexane, and 5 ml of 8 N NaOH were added to each sample. TSNA: 0.5 g of tobacco samples were weighed into a 50 ml plastic tube. 20 ml of 0.1 M ammonium acetate solution was added to each sample. Extracts were diluted 10 times with 0.1 M ammonium acetate solution. The results were included in the study.
- Lab G: Major deviations. TSNA: ISO/TS 22304:2008 Tobacco — Determination of tobacco specific nitrosamines — Method using alkaline dichloromethane extraction. GC-TEA instead of GC-FID. The results were excluded from the study.
- Lab K: Minor deviations. TSNA: 0.01 % aqueous acetic acid was used as mobile phase A instead of pure water - this was to resolve an NAT-d4 ISTD interference in the 1R6F tobacco used as a process control. The results were included in the study.
- Lab L: Minor deviations. TSNA: Flow rate is 0.450 mL/min, Injection volume is 5 µL, Mobile phase A is 10 mM ammonium acetate buffer, Mobile phase B is Acetonitrile with 0.1 % formic acid. The results were included in the study.
- Lab O: Minor deviations. TSNA: Internal standard double the concentration of CRM, column temperature 70 °C, mobile phase A 95 % 10 mM NH4OAc (pH 6.75) in 5 % acetonitrile, mobile phase B was 0.1 % acetic acid in acetonitrile, samples prepared at lower weights. Nicotine: MTBE: a smaller ratio of internal standard to extraction volume was used, a DB-35MS UI, 15 m × 0.25 mm id, 0.25 micron film thickness column instead of a J&W DB-WAX, (30 m × 0.25 mm I.D., 0.25 µm film thickness. The results were included in 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

The full data set for the study is listed 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. Raw data plots that include all replicates, including the results excluded for not following the CRMs, are shown in Appendix C.

5. Statistical Analysis

A statistical analysis was conducted in basic conformance with ISO 5725-5:1998. This procedure does not include outlier detection, but rather utilizes algorithms that are more resistant to the effects of outliers. A summary of the calculated results for repeatability (r) and reproducibility (R) are given below in sections 5.1. Even though it was not necessary for the objective of evaluating stability of the reference products, z-scores are presented in section 5.2 so that the participating laboratories would have an additional measure of their performance compared to their peers and to support their laboratory accreditation. Raw data plots that include all replicates, including the results excluded for not following the CRMs, are shown in Appendix C.

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

The estimated r & R values are shown in the table below and reflect both laboratory variability and product homogeneity.

Table 3: Repeatability (r) and Reproducibility (R) Limits

Parameter	Product	Mean	N° of Labs*	Repeatability		Reproducibility	
				r	% r	R	% R
Nicotine (mg/g)	CRP1.1	7,32	11	0,625	8,5 %	1,07	14,6 %
Nicotine (mg/g)	CRP2.1	10,44	11	0,267	2,6 %	0,78	7,5
Nicotine (mg/g)	CRP3.1	16,35	11	0,206	1,3 %	0,89	5,4 %
Nicotine (mg/g)	CRP4.1	8,79	11	0,222	2,5 %	0,77	8,8 %
NNN (µg/g)	CRP1.1	0,21	14	0,034	16,6 %	0,07	32,8 %
NNN (µg/g)	CRP2.1	3,66	14	0,138	3,8 %	0,94	25,8 %
NNN (µg/g)	CRP3.1	5,82	14	0,278	4,8 %	1,46	25,0 %
NNN (µg/g)	CRP4.1	3,57	14	0,176	4,9 %	0,77	21,4 %
NNK (µg/g)	CRP1.1	0,05	13	0,011	20,5 %	0,02	38,0 %
NNK (µg/g)	CRP2.1	2,13	14	0,123	5,8 %	0,49	22,9 %
NNK (µg/g)	CRP3.1	2,67	14	0,091	3,4 %	0,54	20,1 %
NNK (µg/g)	CRP4.1	0,81	14	0,049	6,1 %	0,21	26,5 %
NAT (µg/g)	CRP1.1	0,14	14	0,019	13,6 %	0,05	35,1 %
NAT (µg/g)	CRP2.1	4,06	14	0,212	5,2 %	1,16	28,5 %
NAT (µg/g)	CRP3.1	4,24	14	0,276	6,5 %	1,13	26,7 %
NAT (µg/g)	CRP4.1	1,55	14	0,083	5,4 %	0,46	29,5 %
NAB (µg/g)	CRP1.1	0,009	11	0,003	31,4 %	0,01	59,9 %
NAB (µg/g)	CRP2.1	0,27	14	0,013	4,8 %	0,08	30,7 %

Parameter	Product	Mean	N° of Labs*	Repeatability		Reproducibility	
				r	% r	R	% R
NAB (µg/g)	CRP3.1	0,30	14	0,013	4,1 %	0,08	27,8 %
NAB (µg/g)	CRP4.1	0,12	14	0,008	7,1 %	0,03	30,2 %
pH	CRP1.1	8,14	15	0,064	NA	0,33	NA
pH	CRP2.1	7,66	15	0,034	NA	0,20	NA
pH	CRP3.1	6,90	15	0,027	NA	0,28	NA
pH	CRP4.1	6,09	15	0,039	NA	0,24	NA
Moisture (%)	CRP1.1	53,4	14	1,85	3,5 %	3,66	6,9 %
Moisture (%)	CRP2.1	51,2	14	0,73	1,4 %	1,26	2,5 %
Moisture (%)	CRP3.1	8,30	13	0,29	3,5 %	1,34	16,1 %
Moisture (%)	CRP4.1	24,5	13	0,61	2,5 %	3,45	14,1 %

*This is the number of laboratory data sets reporting numerical values and after removal of laboratories with major CRM deviations.

NA = Since pH is not a proportional scale, it is not appropriate to calculate % r or % R.

The r&R values from this study are compared to the values obtained in the 2016, 2019, and 2021 studies in Table 4 and 5, respectively. The results from this study are generally comparable those in the prior years.

Table 4. Comparison of Repeatability Results

Parameter	Sample	% r 2016	% r 2019	% r 2021	% r 2023
Nicotine (mg/g)	CRP1.1	11,00 %	10,60 %	6,5 %	8,5 %
Nicotine (mg/g)	CRP2.1	5,00 %	2,80 %	1,6 %	2,6 %
Nicotine (mg/g)	CRP3.1	1,90 %	2,70 %	2,0 %	1,3 %
Nicotine (mg/g)	CRP4.1	7,90 %	1,50 %	2,5 %	2,5 %
Moisture (%)	CRP1.1	2,10 %	2,70 %	3,8 %	3,47 %
Moisture (%)	CRP2.1	0,60 %	1,40 %	1,6 %	1,42 %
Moisture (%)	CRP3.1	2,60 %	3,00 %	3,8 %	3,54 %
Moisture (%)	CRP4.1	2,50 %	3,10 %	2,8 %	2,51 %
NNN (µg/g)	CRP1.1	22,10 %	17,70 %	12,1 %	16,6 %
NNN (µg/g)	CRP2.1	6,30 %	6,30 %	6,5 %	3,76 %
NNN (µg/g)	CRP3.1	5,70 %	4,70 %	9,5 %	4,79 %
NNN (µg/g)	CRP4.1	5,80 %	8,50 %	10,6 %	4,9 %
NNK (µg/g)	CRP1.1	25,20 %	29,00 %	14,5 %	20,5 %
NNK (µg/g)	CRP2.1	10,20 %	5,20 %	8,8 %	5,76 %
NNK (µg/g)	CRP3.1	5,50 %	7,90 %	6,0 %	3,43 %
NNK (µg/g)	CRP4.1	11,40 %	12,40 %	11,9 %	6,1 %
NAT (µg/g)	CRP1.1	22,90 %	19,60 %	17,7 %	13,6 %
NAT (µg/g)	CRP2.1	8,80 %	6,80 %	7,2 %	5,21 %

Parameter	Sample	% r 2016	% r 2019	% r 2021	% r 2023
NAT (µg/g)	CRP3.1	2,70 %	6,20 %	4,4 %	6,50 %
NAT (µg/g)	CRP4.1	6,80 %	10,90 %	7,4 %	5,37 %
NAB (µg/g)	CRP1.1	35,90 %	30,60 %	26,7 %	31,4 %
NAB (µg/g)	CRP2.1	10,20 %	10,20 %	13,6 %	4,8 %
NAB (µg/g)	CRP3.1	10,80 %	12,80 %	11,6 %	4,1 %
NAB (µg/g)	CRP4.1	9,40 %	7,90 %	8,2 %	7,10 %

1. % r and % R are as a ratio to the mean value. pH is excluded, since it is not a ratio scale.

Table 5. Comparison of Reproducibility Results

Parameter	Sample	%R 2016	%R 2019	%R 2021	%R 2023
Nicotine (mg/g)	CRP1.1	16,80 %	17,70 %	13,6 %	14,6 %
Nicotine (mg/g)	CRP2.1	24,80 %	15,60 %	14,7 %	7,5 %
Nicotine (mg/g)	CRP3.1	12,20 %	11,50 %	8,1 %	5,4 %
Nicotine (mg/g)	CRP4.1	13,10 %	10,10 %	12,3 %	8,8 %
Moisture (%)	CRP1.1	3,40 %	3,00 %	4,7 %	6,86 %
Moisture (%)	CRP2.1	1,40 %	2,70 %	2,6 %	2,46 %
Moisture (%)	CRP3.1	10,10 %	20,10 %	15,0 %	16,1 %
Moisture (%)	CRP4.1	8,90 %	12,60 %	9,6 %	14,09 %
NNN (µg/g)	CRP1.1	35,80 %	30,40 %	30,7 %	32,8 %
NNN (µg/g)	CRP2.1	25,80 %	24,80 %	25,1 %	25,8 %
NNN (µg/g)	CRP3.1	27,30 %	31,20 %	31,5 %	25,0 %
NNN (µg/g)	CRP4.1	21,80 %	19,80 %	17,1 %	21,4 %
NNK (µg/g)	CRP1.1	56,60 %	71,20 %	31,7 %	38,0 %
NNK (µg/g)	CRP2.1	25,50 %	16,60 %	14,3 %	22,9 %
NNK (µg/g)	CRP3.1	22,60 %	16,90 %	17,5 %	20,1 %
NNK (µg/g)	CRP4.1	33,10 %	16,60 %	22,7 %	26,5 %
NAT (µg/g)	CRP1.1	50,70 %	25,90 %	23,9 %	35,1 %
NAT (µg/g)	CRP2.1	21,00 %	20,20 %	27,8 %	28,5 %
NAT (µg/g)	CRP3.1	20,20 %	17,00 %	24,3 %	26,7 %
NAT (µg/g)	CRP4.1	23,80 %	19,40 %	17,4 %	29,5 %
NAB (µg/g)	CRP1.1	63,20 %	59,60 %	52,5 %	59,9 %
NAB (µg/g)	CRP2.1	25,40 %	17,40 %	26,2 %	30,7 %
NAB (µg/g)	CRP3.1	28,00 %	18,40 %	21,8 %	27,8 %
NAB (µg/g)	CRP4.1	37,90 %	11,30 %	24,9 %	30,2 %

5.3 Calculation of Z-Scores

Although calculation of z-scores is not suggested in ISO 5725-5:1998, z-scores were calculated so that the participating laboratories could compare their results to those of their peers. The assigned value and standard deviation for proficiency assessment were calculated utilizing all data sets, including the results excluded from the r and R calculations for not following the CRMs. Absolute z-scores less than 2 are acceptable, absolute z-scores between 2 and 3 constitute a warning, and those above 3 should be investigated. The means and standard deviations used in the z-score calculations were from the algorithm A, a robust estimation procedure. Final summary tables of z-scores are presented below and graphs of the z-scores are shown in Appendix D.

Table 6: Z-Scores

Product	Lab	Nicotine	NNN	NNK	NAT	NAB	Moisture	pH
CRP1.1	A	0,57	0,78	0,07	0,08	--	0,83	-1,06
CRP1.1	B	-0,11	0,44	-0,38	1,29	0,88	-0,43	0,48
CRP1.1	C	-0,17	0,02	0,03	0,10	-0,17	-0,27	0,14
CRP1.1	D	1,36	-1,27	--	-1,15	--	-1,11	0,40
CRP1.1	E	-1,13	0,14	-0,74	0,21	-0,75	--	1,41
CRP1.1	F	-0,33	0,04	0,90	-0,08	--	0,24	0,25
CRP1.1	G	0,21	0,14	0,07	1,12	1,36	0,76	-0,79
CRP1.1	H	0,39	-0,74	-0,61	-0,66	-0,44	0,56	0,05
CRP1.1	I	–	0,59	0,53	0,44	-0,94	1,27	0,05
CRP1.1	J	-1,31	0,70	0,11	-0,24	2,41	-0,12	-2,68
CRP1.1	K	2,15	-0,45	-0,19	0,29	0,16	-8,07	-0,47
CRP1.1	L	0,33	-1,57	-2,13	-0,25	-1,05	0,59	1,04
CRP1.1	M	–	--	--	--	--	-0,04	-1,43
CRP1.1	N	-1,20	1,19	1,42	1,62	0,25	0,71	0,86
CRP1.1	O	-0,11	-1,30	-1,21	-1,29	-0,90	-5,93	0,57
CRP1.1	P	–	1,22	1,48	-1,36	0,10	--	--
CRP2.1	A	0,03	0,61	-0,99	0,35	1,17	2,04	-1,76
CRP2.1	B	-0,53	0,62	1,29	-0,60	1,23	-1,61	-0,43
CRP2.1	C	-0,02	-0,19	-0,09	0,46	0,66	0,79	0,71
CRP2.1	D	0,35	-0,83	-0,62	-0,56	-0,77	-0,36	0,11
CRP2.1	E	0,91	-0,23	-0,05	-0,06	-0,51	--	1,14
CRP2.1	F	0,06	0,14	0,84	0,48	0,72	-0,59	0,43
CRP2.1	G	-3,08	-0,32	0,34	-0,01	-1,22	-0,01	-1,05
CRP2.1	H	-0,56	-0,81	-1,13	-0,94	-0,66	1,11	-0,29
CRP2.1	I	–	1,00	1,17	0,94	0,47	-0,17	-0,10
CRP2.1	J	1,34	0,97	-0,08	-1,16	-0,78	-0,45	-0,67
CRP2.1	K	-0,96	-1,33	-0,67	0,13	0,00	-0,08	0,33
CRP2.1	L	-0,80	-0,87	-0,60	-0,96	-0,82	0,65	0,76

Product	Lab	Nicotine	NNN	NNK	NAT	NAB	Moisture	pH
CRP2.1	M	–	--	--	--	--	0,01	-1,33
CRP2.1	N	1,46	1,73	0,95	2,24	0,24	0,61	2,28
CRP2.1	O	0,22	-1,09	-1,33	-1,06	-1,02	-6,47	0,38
CRP2.1	P	–	0,83	0,95	2,02	1,30	--	--
CRP3.1	A	0,11	1,41	-1,29	0,70	1,36	--	-1,00
CRP3.1	B	0,49	0,57	1,49	2,08	1,86	-1,30	-0,73
CRP3.1	C	0,02	-0,06	0,04	0,33	0,75	0,12	0,91
CRP3.1	D	0,00	-0,61	-0,68	-0,60	-0,93	-0,76	-1,25
CRP3.1	E	1,59	-0,03	-0,34	-0,09	-0,31	--	0,64
CRP3.1	F	-0,52	0,52	0,83	-0,03	0,80	-1,03	-0,13
CRP3.1	G	-0,69	-0,23	-0,72	-0,65	0,21	0,41	-0,73
CRP3.1	H	-0,12	-0,46	-0,86	-1,10	-0,98	0,67	1,04
CRP3.1	I	–	1,22	1,09	1,07	0,07	-0,49	0,71
CRP3.1	J	1,44	-2,93	0,98	-0,67	0,42	-1,07	-0,63
CRP3.1	K	-0,77	-0,86	0,00	0,24	0,09	1,18	-0,33
CRP3.1	L	-0,95	-0,55	-0,46	-1,04	-0,60	1,92	-0,77
CRP3.1	M	–	--	--	--	--	0,20	-0,23
CRP3.1	N	0,84	2,10	1,10	1,83	0,04	0,01	1,85
CRP3.1	O	-1,35	-0,81	-0,90	-1,02	-1,56	0,57	1,01
CRP3.1	P	–	-0,12	-0,28	-0,16	-0,92	--	--
CRP4.1	A	0,19	0,15	-0,96	-1,27	-0,22	--	-0,92
CRP4.1	B	0,15	0,71	1,08	0,09	1,13	-1,34	-0,53
CRP4.1	C	-0,21	0,18	0,12	0,63	0,83	0,35	-0,45
CRP4.1	D	0,85	-0,92	-0,92	-0,48	-0,87	-0,67	-0,78
CRP4.1	E	-0,27	0,40	0,38	0,26	0,22	--	0,88
CRP4.1	F	-0,31	0,61	0,76	0,27	0,85	-0,54	0,18
CRP4.1	G	-2,52	0,21	0,44	-0,42	-1,55	0,86	-1,03
CRP4.1	H	-0,66	-0,75	-1,13	-0,95	-0,66	0,86	1,11
CRP4.1	I	–	0,97	0,45	0,94	0,44	-0,43	0,68
CRP4.1	J	1,34	-0,49	1,06	-1,05	-0,13	0,07	-1,58
CRP4.1	K	0,57	-0,73	-0,94	0,29	-0,05	-2,06	-0,14
CRP4.1	L	-1,20	-1,19	-0,90	-0,57	-0,92	1,44	-0,21
CRP4.1	M	–	--	--	--	--	-0,15	0,37
CRP4.1	N	1,41	3,20	0,92	3,51	0,91	0,76	3,14
CRP4.1	O	-0,37	-1,63	-1,13	-0,74	-1,20	0,30	0,84
CRP4.1	P	–	0,84	0,79	1,64	1,16	--	--

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

5.4 CRP Stability Assessment

The data were evaluated for stability by comparing the results of the testing across 2016, 2019, 2021, and 2023.

Table 7: Summary of Stability Analysis

Variable	Product	2016 Avg.	2019 Avg.	2021 Avg.	2023 Avg.	Slope	p-value
Nicotine (mg/g)	CRP1.1	7,55	7,30	7,53	7,32	-0,0199	0,3725
Nicotine (mg/g)	CRP2.1	10,64	10,21	10,65	10,44	-0,0147	0,5936
Nicotine (mg/g)	CRP3.1	17,11	16,48	16,83	16,35	-0,0822	0,0134
Nicotine (mg/g)	CRP4.1	8,92	8,77	8,95	8,79	-0,0110	0,5911
NNN (µg/g)	CRP1.1	0,191	0,199	0,201	0,207	0,0023	0,0313
NNN (µg/g)	CRP2.1	3,42	3,51	3,49	3,66	0,0325	0,0335
NNN (µg/g)	CRP3.1	5,58	5,77	5,68	5,82	0,0284	0,2703
NNN (µg/g)	CRP4.1	3,41	3,59	3,56	3,57	0,0215	0,1326
NNK (µg/g)	CRP1.1	0,0507	0,0493	0,0494	0,0515	0,0002	0,6453
NNK (µg/g)	CRP2.1	2,06	2,07	2,07	2,13	0,0106	0,1908
NNK (µg/g)	CRP3.1	2,51	2,59	2,56	2,67	0,0210	0,0216
NNK (µg/g)	CRP4.1	0,78	0,82	0,81	0,81	0,0041	0,2299
NAT (µg/g)	CRP1.1	0,14	0,14	0,14	0,14	0,0004	0,6483
NAT (µg/g)	CRP2.1	4,22	4,13	4,01	4,06	-0,0300	0,0855
NAT (µg/g)	CRP3.1	4,30	4,28	4,10	4,24	-0,0173	0,2908
NAT (µg/g)	CRP4.1	1,58	1,59	1,53	1,55	-0,0081	0,2027
NAB (µg/g)	CRP1.1	0,0086	0,0087	0,0085	0,0091	0,0000	0,6039
NAB (µg/g)	CRP2.1	0,269	0,267	0,267	0,271	0,0003	0,7955
NAB (µg/g)	CRP3.1	0,305	0,309	0,298	0,304	-0,0006	0,6237
NAB (µg/g)	CRP4.1	0,112	0,114	0,114	0,115	0,0005	0,3885
pH	CRP1.1	8,31	8,19	8,21	8,14	-0,0226	0,0002
pH	CRP2.1	7,73	7,68	7,71	7,66	-0,0086	0,0148
pH	CRP3.1	6,94	6,91	6,90	6,90	-0,0041	0,2769
pH	CRP4.1	6,07	6,07	6,10	6,09	0,0026	0,4598
Moisture (%)	CRP1.1	53,82	53,59	53,65	53,44	-0,0402	0,1824
Moisture (%)	CRP2.1	51,41	51,20	51,16	51,18	-0,0346	0,0353
Moisture (%)	CRP3.1	7,41	7,91	8,30	8,30	0,1393	<,0001
Moisture (%)	CRP4.1	24,17	24,00	24,13	24,51	0,0383	0,3779

1. P-values are declared statistically significant if $p < 0,0125$. 0,0125 is derived by dividing the nominal 0,05 level by 4 to account for testing multiplicity per analyte. The two values that are statistically significant are highlighted in the table.
2. The yearly averages and slopes were based on robust estimates including the data of all laboratories without major discrepancies in their conduct of the respective analytical methods.

Because of the large number of comparisons being made in Table 7, some consideration should be made to allow for testing multiplicity. Our practice for stability studies has been to adjust by analyte. That means a comparison is judged to be statistically significant if the p-value is less than 0,05 / 4 = 0,0125, as noted in the note to Table 7. The divisor of four is based on four comparisons being made per analyte, one for each CRP. The only two statistically significant slopes were pH for CRP1.1 and moisture for CRP3.1. Note that CRP2.1 showed a statistically significant moisture loss and CRP 3.1 showed a statistically significant moisture gain. The gain in moisture with CRP3.1 is consistent with what was previously seen with CRP3 produced in 2009 [5]. Graphs of the two statistically significant trends are shown in Figures 1 and 2. Each data point in the two graphs represents a lab average.

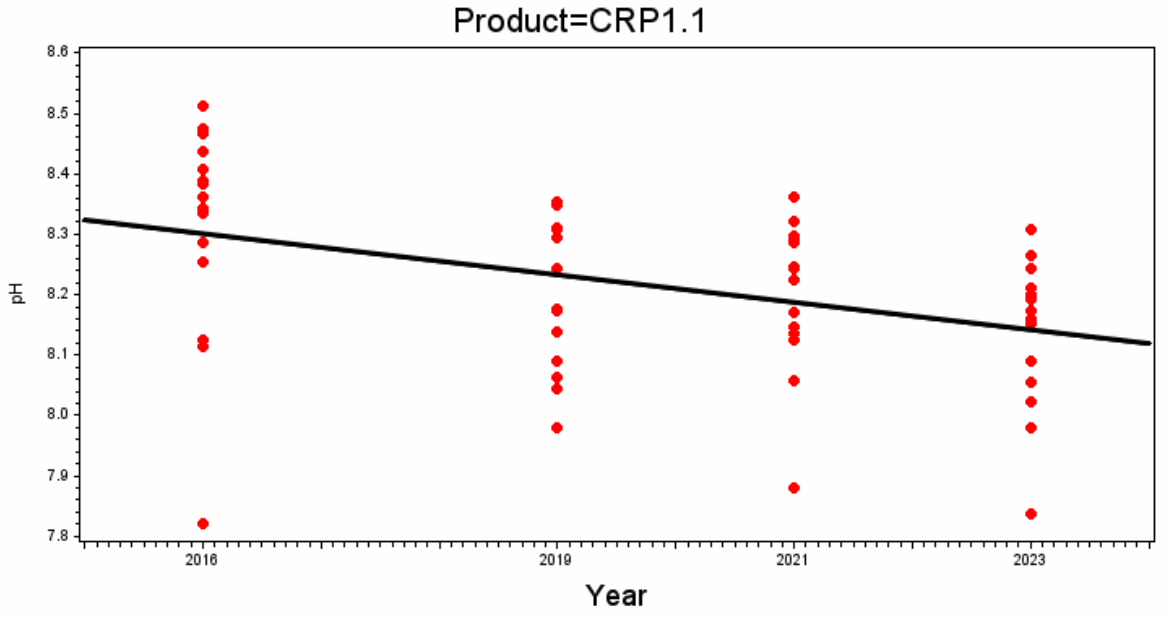


Figure 1. pH results for CRP1.1 for 2016, 2019, 2021, and 2023.

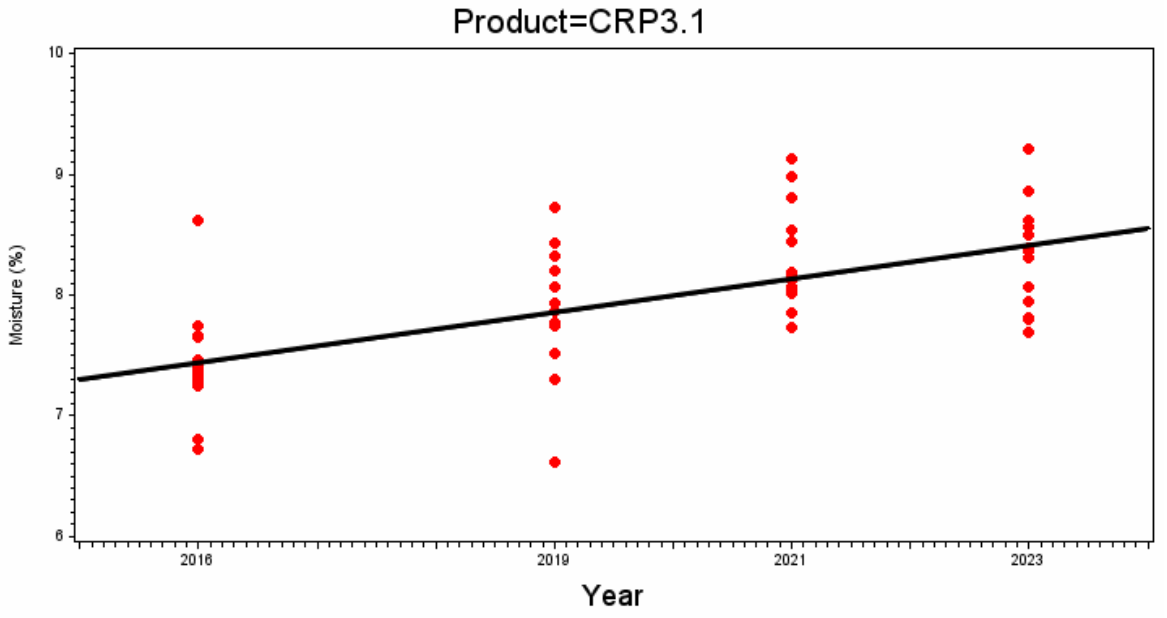


Figure 2. Moisture results for CRP3.1 for 2016, 2019, and 2021.

[5] TTPA Technical Report: CORESTA 2009 Reference Products - 2019 Analysis.

6. Data Interpretation

In terms of stability, moisture for CRP3.1 showed a statistically significant trend across the six years of testing. That increase was consistent with the pattern seen for CRP3 from the 2009 CRPs. The CRP3.1 moisture increase from the time of manufacture is approximately 0,9 %. pH for CRP 1.1 also showed a statistically significant trend, although the year-to-year differences are not large. The pH of CRP1.1 appeared to drop slightly from the initial 2016 testing and then to be relatively stable after the second time point. As mentioned earlier, results are presented on an as-is basis.

A comparison of the r & R results was presented in Tables 4 and 5. Generally speaking, the repeatability and reproducibility values calculated for this study were comparable to the corresponding values seen in the prior years.

7. Recommendations

The results of this study were consistent with those in studies of the 2009 CRPs. Most analytes and measures did not show statistically significantly time trends across the studies. The exception were moisture for CRP3.1 and pH for CRP1.1. Even though there were two statistically significant changes, the magnitude of the changes are small and would not affect use usefulness of these reference products. The TTPA recommends that the stability of the 2016 CRPs be continued to be monitored on a biennial basis.

APPENDIX A: Protocol

CORESTA TOBACCO and TOBACCO PRODUCTS ANALYTES SUB-GROUP

Project Title: Stability study of 2016 CORESTA Reference Products - 2023 Analysis

Type of Document: Collaborative Study Protocol

Date: September 26, 2022

Study Coordinator and Author: Rozanna Avagyan, Ph.D.

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

1. Introduction

In October 2015, Jeju, South Korea, the CORESTA Smokeless Tobacco Sub-Group (STS, now named Tobacco and Tobacco Products Analytes Sub-Group, TTPA) agreed to manufacture another batch of CORESTA Reference Products (CRPs) due to a limited supply of the 2009 CRPs. In 2016, the TTPA cooperated to design and coordinate the manufacture of four CORESTA Reference Products (CRPs) referred to as CRP1.1 (Swedish snus pouch), CRP2.1 (American-style loose moist snuff), CRP3.1 (American-style loose dry snuff powder), and CRP4.1 (American-style chopped loose-leaf chewing tobacco). CRP4.1 was produced in a chopped format in 2016 to improve within package sample homogeneity as compared to the 2009 loose leaf format. These products were intended as replacements for the 2009 CRPs.

In May 2016, the TTPA conducted an inter-laboratory study designed to characterize the four CRPs produced in 2016. Nineteen laboratories participated in the study. The participating laboratories reported the levels of nicotine, pH, moisture (oven volatiles), tobacco specific nitrosamines (N-Nitrosonornicotine (NNN), N-Nitrosoanatabine (NAT), N-Nitrosoanabasine (NAB) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or TSNAs), ammonia, and benzo[a]pyrene (B[a]P) in the CRPs using CORESTA Recommended Methods (CRMs). Even though the 2016 CRPs included the four product styles that were produced in 2009, different crop year tobaccos were used, and different manufacturers produced three of the reference products. Therefore, the chemistry differed in many instances between the 2009 and 2016 CRPs. The results of the 2016 study established a baseline for future biennial stability studies.

At the September 2022 Tobacco and Tobacco Products Analytes (TTPA) Sub-Group meeting, the group agreed to initiate the fourth stability study for the 2016 CRPs by determining nicotine, pH, moisture (oven volatiles), and TSNAs using the respective CORESTA Recommended Methods (CRMs).

The CRPs are stored at −20 °C and distributed by North Carolina State University (NCSU) Tobacco Analytical Services Laboratory.

2. Objective

The participating laboratories are to provide analytical results from the 2016 CRPs for pH, moisture (oven volatiles), nicotine, and TSNAs using the current versions of the applicable CRMs. This study will serve as the fourth assessment of the 2016 CRPs and provide an assessment of inter-laboratory variability using the applicable CRMs.

Data generated with methods that differ from the respective CRMs will be excluded from the study results. The stability of the products will be evaluated using regression analysis to test for time trends. Within and between laboratory variability will be evaluated in basic conformance with the recommendations of ISO 5725-2:2019. Additionally, z-scores will be calculated in order to allow laboratories to compare their results to those of other laboratories.

3. Time schedule and Data Reporting

Laboratories are urged to order the products immediately to ensure delays importing the reference products through customs does not impact the study timelines.

Table 1: Study timeline

Date	Activity
September – October 2022	Participants order CRPs
October 10, 2022	Distribute study protocol by this date
November 2022 – January 2023	Laboratories conduct the study
February 1, 2023	Laboratories submit results by this date
February 5 - April 2023	Conduct the statistical analysis
April, 2023	Discuss results at spring TTPA SG meeting

4. Samples

4.1 Samples

Each participating laboratory should request a minimum of 5 cans of the 2016 CRPs from North Carolina State University Tobacco Analytical Services Laboratory (TASL). Laboratories may use CRPs that they have onsite as long as the CRPs are unopened and have been maintained at the recommended storage conditions of -20 °C. Samples that have been opened or not stored at −20 °C should not be used for this study. The 2016 CRPs included in this study are shown below:

- CRP1.1, Snus Pouch
- CRP2.1, Moist Snuff
- CRP3.1, Dry Snuff
- CRP4.1, Chopped Loose Leaf

Shipments of the four CRPs may be obtained through Karen Andres at North Carolina State University (NCSU) Analytical Services Laboratory (https://strp.wordpress.ncsu.edu). Each participating laboratory will need to send their shipping address, person to whom delivery should be made, shipping account (FedEx International, DHS, UPS) arrangements and any special delivery information to Karen Andres. Karen 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.

4.2 Receipt

Upon receipt, the CRPs shall be immediately stored at the recommended long-term storage temperature of −20 °C. Samples analyzed within one week may be stored in the refrigerator at approximately 4 °C.

4.3 Within Laboratory Sample Preparation

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 and placed at laboratory conditions 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 °C for up to one week if the analyses will not be conducted immediately. Special handling requirements for the CRPs are described below:

- **CRP1.1 for nicotine, TSNAs, and pH:** The snus pouches shall be cut into 2 halves, and the tobacco and pouch added directly into the extraction vessel. Both the tobacco and pouch material shall be analyzed.

  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.
- **CRP1.1 for moisture (OV):** The snus pouches shall be added to the OV sample tin intact.
- **CRP2.1, CRP3.1, and CRP4.1:** These products shall be analyzed as-is, without grinding.

Refer to CORESTA Guide No. 11 *Technical Guide for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products* - May 2020 for additional handling instructions for the smokeless tobacco products.

5. Analysis

5.1 Replicates

Each participating laboratory shall conduct three independent replicates using the applicable CRMs.

5.2 Methods

The most recent versions of the following CRMs shall be used for the analyses. Data collected with other methods, or with methods that have significant deviations from the CRMs cannot be included in the study.

- CRM No. 69, Determination of pH in Smokeless Tobacco Products
- CRM No. 76, Determination of Moisture Content (Oven Volatiles) of Smokeless Tobacco Products
- CRM No. 62, Determination of Nicotine in Tobacco and Tobacco Products by Gas Chromatographic Analysis or CRM No. 87 - Determination of Nicotine in Tobacco Products by GC/MS
- CRM No. 72, Determination of Tobacco Specific Nitrosamines in Smokeless Tobacco Products by Liquid Chromatography - Tandem Mass Spectrometry

6. Statistical Analysis

The stability of the products will be evaluated using regression analysis to test for time trends. Within and between laboratory variability will be evaluated in basic conformance with the recommendations of ISO 5725-2:2019. Additionally, z-scores will be calculated in order to allow laboratories to compare their results to those of other laboratories.

7. Data Reporting

Report results in the Data Reporting Sheet that has been provided. The completed Data Reporting Sheet should be emailed to Rozanna Avagyan, and Karl Wagner.

- Data shall be reported in the units and number of significant figures specified in the Data Reporting Sheet.

- If data are below a Limit of Quantitation (LOQ), the LOQ shall be stated preceded by a "<" symbol.
- All test results shall be reported as-is (with no correction for moisture content).
- Specify the method used for the analysis of nicotine (CRM 87, CRM 62 MTBE, or CRM 62 Hexane).
- Report any relevant deviations from the most current version of the CRM.

8. 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 2023 TTPA meeting and a technical report will be drafted.

APPENDIX B: Raw Data

Results are presented on an as-is basis and outliers are included.

Lab Code	sample	replicate	Nicotine (mg/g)	NNK (µg/g)	NNN (µg/g)	NAT (µg/g)	NAB (µg/g)	Oven Volatiles (%)	pH
A	CRP1.1	1	7,580	0,0560	0,2290	0,1530	<0,0100	54,08	8,020
A	CRP1.1	2	7,710	0,0520	0,2200	0,1430	<0,0100	54,62	8,040
A	CRP1.1	3	7,230	0,0480	0,2220	0,1430	<0,0100	54,58	8,010
A	CRP2.1	1	10,25	1,950	3,940	4,200	0,3080	52,10	7,490
A	CRP2.1	2	10,65	2,020	3,810	4,180	0,3000	52,04	7,600
A	CRP2.1	3	10,47	1,950	3,790	4,170	0,3060	51,82	7,510
A	CRP3.1	1	16,22	2,340	6,260	4,290	0,3370	–	6,790
A	CRP3.1	2	16,46	2,400	6,520	4,530	0,3440	–	6,800
A	CRP3.1	3	16,45	2,500	6,690	4,610	0,3480	–	6,820
A	CRP4.1	1	8,840	0,7160	3,630	1,320	0,1030	–	6,010
A	CRP4.1	2	8,910	0,7560	3,640	1,370	0,1080	–	6,040
A	CRP4.1	3	8,780	0,7570	3,570	1,360	0,1210	–	5,970
B	CRP1.1	1	7,436	0,0483	0,2160	0,1607	0,0108	53,41	8,210
B	CRP1.1	2	7,504	0,0542	0,2047	0,1755	0,0097	52,88	8,230
B	CRP1.1	3	6,950	0,0460	0,2279	0,1682	0,0129	52,47	8,160
B	CRP2.1	1	10,18	2,314	3,860	3,696	0,3300	49,94	7,640
B	CRP2.1	2	10,39	2,278	3,980	3,964	0,3080	51,10	7,630
B	CRP2.1	3	10,40	2,464	3,706	3,806	0,2820	50,60	7,610
B	CRP3.1	1	16,42	3,062	6,134	4,954	0,3380	8,063	6,830
B	CRP3.1	2	16,57	2,748	6,202	4,766	0,3460	7,505	6,830
B	CRP3.1	3	16,46	2,994	5,924	5,260	0,3880	7,498	6,830
B	CRP4.1	1	8,883	0,8400	3,756	1,592	0,1300	22,98	6,040
B	CRP4.1	2	8,892	0,9260	3,692	1,552	0,1320	22,66	6,040
B	CRP4.1	3	8,728	0,9120	3,820	1,520	0,1240	22,98	6,040
C	CRP1.1	1	7,222	0,0522	0,2088	0,1489	0,0092	52,80	8,120
C	CRP1.1	2	7,298	0,0506	0,2006	0,1428	0,0090	53,17	8,150
C	CRP1.1	3	7,321	0,0525	0,2131	0,1481	0,0091	53,39	8,210
C	CRP2.1	1	10,47	2,125	3,584	4,219	0,2871	51,08	7,710
C	CRP2.1	2	10,58	2,118	3,584	4,215	0,2907	51,83	7,710
C	CRP2.1	3	10,28	2,124	3,608	4,236	0,2889	51,57	7,700
C	CRP3.1	1	16,42	2,667	5,783	4,388	0,3270	8,360	6,970
C	CRP3.1	2	16,15	2,655	5,759	4,325	0,3282	8,390	6,980
C	CRP3.1	3	16,49	2,665	5,797	4,310	0,3216	8,320	7,030
C	CRP4.1	1	8,856	0,8203	3,675	1,653	0,1247	24,76	6,050
C	CRP4.1	2	8,596	0,8200	3,582	1,618	0,1258	25,03	6,040
C	CRP4.1	3	8,795	0,8268	3,604	1,633	0,1233	25,04	6,050
D	CRP1.1	1	7,468	<0,1130	0,1802	0,1242	<0,0282	52,80	8,210

Lab Code	sample	replicate	Nicotine (mg/g)	NNK (µg/g)	NNN (µg/g)	NAT (µg/g)	NAB (µg/g)	Oven Volatiles (%)	pH
D	CRP1.1	2	8,041	<0,1220	0,1850	0,1235	<0,0303	52,22	8,181
D	CRP1.1	3	7,734	<0,1060	0,1758	0,1245	<0,0264	51,33	8,181
D	CRP2.1	1	10,55	2,008	3,379	3,826	0,2430	51,02	7,665
D	CRP2.1	2	10,50	2,055	3,408	3,887	0,2474	51,01	7,663
D	CRP2.1	3	10,54	2,042	3,374	3,805	0,2443	51,08	7,665
D	CRP3.1	1	16,32	2,512	5,541	3,994	0,2798	7,963	6,776
D	CRP3.1	2	16,35	2,543	5,492	3,993	0,2766	7,904	6,779
D	CRP3.1	3	16,37	2,532	5,508	3,993	0,2775	7,955	6,781
D	CRP4.1	1	9,072	0,7352	3,361	1,478	0,1023	23,50	6,017
D	CRP4.1	2	8,937	0,7569	3,351	1,458	0,1022	23,80	6,020
D	CRP4.1	3	8,990	0,7457	3,318	1,472	0,1017	23,77	6,018
E	CRP1.1	1	6,725	0,0478	0,2123	0,1513	0,0082	–	8,280
E	CRP1.1	2	7,188	0,0457	0,2074	0,1435	0,0072	–	8,320
E	CRP1.1	3	7,042	0,0480	0,2105	0,1511	0,0082	–	8,320
E	CRP2.1	1	10,43	2,118	3,572	4,076	0,2522	–	7,730
E	CRP2.1	2	11,54	2,130	3,623	4,018	0,2569	–	7,770
E	CRP2.1	3	10,02	2,141	3,543	3,985	0,2500	–	7,710
E	CRP3.1	1	16,77	2,588	5,775	4,217	0,2962	–	6,970
E	CRP3.1	2	16,85	2,604	5,777	4,203	0,2929	–	6,970
E	CRP3.1	3	16,76	2,585	5,840	4,134	0,2977	–	6,960
E	CRP4.1	1	8,843	0,8351	3,747	1,572	0,1146	–	6,150
E	CRP4.1	2	8,752	0,8506	3,618	1,558	0,1153	–	6,160
E	CRP4.1	3	8,610	0,8377	3,668	1,611	0,1196	–	6,170
F	CRP1.1	1	7,180	0,0567	0,2022	0,1450	<0,1500	53,59	8,220
F	CRP1.1	2	7,208	0,0574	0,2177	0,1508	<0,1230	53,87	8,150
F	CRP1.1	3	7,300	0,0566	0,2039	0,1343	<0,1140	53,72	8,150
F	CRP2.1	1	10,45	2,305	3,655	4,202	0,2955	51,00	7,680
F	CRP2.1	2	10,46	2,222	3,715	4,346	0,2870	50,88	7,700
F	CRP2.1	3	10,48	2,303	3,717	4,154	0,2898	50,95	7,680
F	CRP3.1	1	16,22	2,795	6,125	4,207	0,3249	7,774	6,890
F	CRP3.1	2	16,17	2,887	5,905	4,188	0,3360	7,838	6,890
F	CRP3.1	3	16,21	2,753	6,160	4,228	0,3199	7,827	6,890
F	CRP4.1	1	8,800	0,892	3,748	1,598	0,1279	23,93	6,110
F	CRP4.1	2	8,693	0,8386	3,771	1,558	0,1241	23,79	6,090
F	CRP4.1	3	8,683	0,8763	3,673	1,587	0,1229	23,85	6,100
G	CRP1.1	1	7,422	0,0510	0,2320	0,1200	0,0160	54,49	8,060
G	CRP1.1	2	7,141	0,0580	0,2100	0,2060	0,0110	53,85	8,050
G	CRP1.1	3	7,620	0,0470	0,1880	0,1690	0,0100	54,70	8,050
G	CRP2.1	1	9,721	2,163	3,326	3,922	0,2420	51,07	7,580
G	CRP2.1	2	9,706	2,140	3,560	4,065	0,2220	51,34	7,580

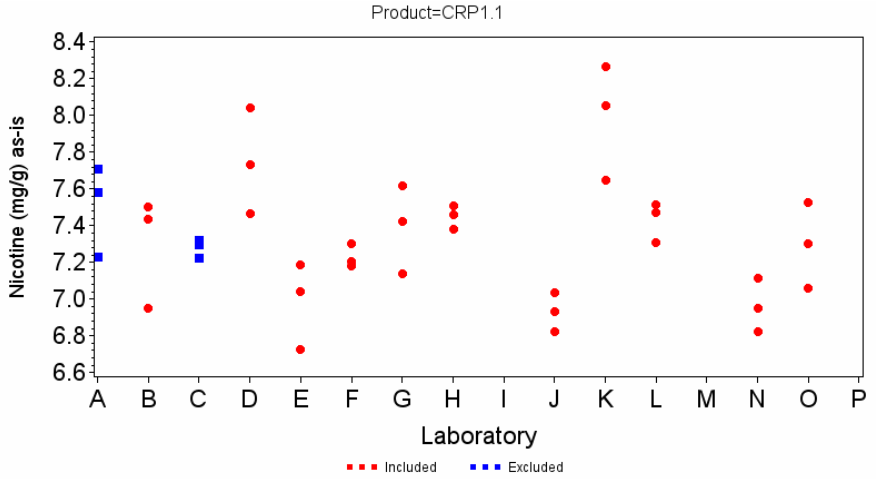
Lab Code	sample	replicate	Nicotine (mg/g)	NNK (µg/g)	NNN (µg/g)	NAT (µg/g)	NAB (µg/g)	Oven Volatiles (%)	pH
G	CRP2.1	3	9,750	2,280	3,757	4,150	0,2290	51,12	7,590
G	CRP3.1	1	16,21	2,264	5,622	4,037	0,3210	8,622	6,830
G	CRP3.1	2	16,13	2,653	5,638	4,051	0,3260	8,439	6,830
G	CRP3.1	3	16,13	2,6470	5,830	3,840	0,2840	8,414	6,830
G	CRP4.1	1	8,235	0,9210	3,810	1,502	0,0870	25,48	6,030
G	CRP4.1	2	8,147	0,7690	3,622	1,554	0,0950	25,52	5,960
G	CRP4.1	3	8,211	0,8480	3,453	1,379	0,0970	25,69	6,000
H	CRP1.1	1	7,382	0,0490	0,1926	0,1323	0,0087	54,55	8,130
H	CRP1.1	2	7,509	0,0485	0,1878	0,1288	0,0081	53,15	8,150
H	CRP1.1	3	7,461	0,0464	0,1940	0,1377	0,0088	54,63	8,170
H	CRP2.1	1	10,34	1,930	3,397	3,674	0,2467	51,37	7,630
H	CRP2.1	2	10,26	1,933	3,412	3,666	0,2481	51,81	7,640
H	CRP2.1	3	10,35	1,985	3,368	3,745	0,2501	51,68	7,640
H	CRP3.1	1	16,29	2,482	5,739	3,784	0,2777	8,580	7,010
H	CRP3.1	2	16,33	2,485	5,585	3,833	0,2738	8,610	7,000
H	CRP3.1	3	16,32	2,515	5,441	3,810	0,2780	8,650	7,010
H	CRP4.1	1	8,632	0,7398	3,439	1,415	0,1069	25,66	6,190
H	CRP4.1	2	8,667	0,7368	3,436	1,389	0,1046	25,35	6,160
H	CRP4.1	3	8,622	0,7165	3,280	1,388	0,1031	25,68	6,190
I	CRP1.1	1	–	0,0532	0,2197	0,1554	0,0075	54,50	8,150
I	CRP1.1	2	–	0,0536	0,2165	0,1489	0,0075	54,89	8,150
I	CRP1.1	3	–	0,0574	0,2227	0,1537	0,0074	55,47	8,150
I	CRP2.1	1	–	2,331	3,999	4,235	0,2907	51,02	7,650
I	CRP2.1	2	–	2,354	3,952	4,400	0,2787	51,41	7,650
I	CRP2.1	3	–	2,309	3,965	4,583	0,2805	50,90	7,650
I	CRP3.1	1	–	2,879	6,393	4,751	0,3099	7,988	6,960
I	CRP3.1	2	–	2,844	6,362	4,673	0,3031	8,173	6,980
I	CRP3.1	3	–	2,859	6,440	4,428	0,3058	8,044	6,980
I	CRP4.1	1	–	0,8566	3,785	1,604	0,1193	23,93	6,140
I	CRP4.1	2	–	0,8317	3,828	1,695	0,1197	24,12	6,140
I	CRP4.1	3	–	0,8522	3,848	1,745	0,1194	23,90	6,150
J	CRP1.1	1	7,036	0,0465	0,1974	0,1274	0,0130	53,85	7,870
J	CRP1.1	2	6,935	0,0544	0,2354	0,1507	0,0145	52,75	7,830
J	CRP1.1	3	6,821	0,0558	0,2327	0,1432	0,0162	53,29	7,810
J	CRP2.1	1	10,69	2,079	3,931	3,569	0,2251	50,88	7,560
J	CRP2.1	2	10,84	2,153	4,003	3,703	0,2551	50,97	7,640
J	CRP2.1	3	10,76	2,141	3,949	3,563	0,2540	51,15	7,630
J	CRP3.1	1	16,90	2,864	4,462	3,817	0,3174	7,828	6,850
J	CRP3.1	2	17,20	2,858	4,337	4,090	0,3141	7,829	6,840
J	CRP3.1	3	16,15	2,796	4,378	3,998	0,3173	7,735	6,830

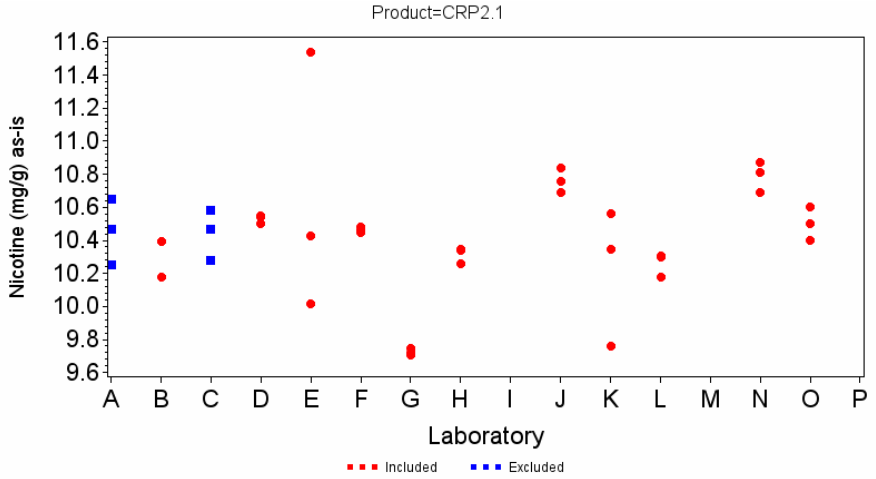
Lab Code	sample	replicate	Nicotine (mg/g)	NNK (µg/g)	NNN (µg/g)	NAT (µg/g)	NAB (µg/g)	Oven Volatiles (%)	pH
J	CRP4.1	1	9,179	0,9018	3,419	1,364	0,1128	24,50	5,940
J	CRP4.1	2	9,092	0,8960	3,308	1,364	0,1154	24,60	5,960
J	CRP4.1	3	9,078	0,8755	3,631	1,421	0,1074	24,68	5,950
K	CRP1.1	1	8,266	0,0458	0,1779	0,145	0,0095	43,08	8,080
K	CRP1.1	2	7,649	0,0524	0,2083	0,1540	0,0102	43,75	8,100
K	CRP1.1	3	8,053	0,0531	0,2069	0,1517	0,0097	44,64	8,090
K	CRP2.1	1	9,764	1,977	3,153	4,055	0,2671	51,34	7,690
K	CRP2.1	2	10,56	2,044	3,235	4,158	0,2649	50,99	7,670
K	CRP2.1	3	10,35	2,059	3,286	4,087	0,2739	51,11	7,680
K	CRP3.1	1	16,22	2,657	5,360	4,181	0,3025	8,573	6,850
K	CRP3.1	2	15,98	2,670	5,427	4,452	0,3173	8,982	6,890
K	CRP3.1	3	16,19	2,640	5,400	4,292	0,3005	9,009	6,870
K	CRP4.1	1	8,862	0,7452	3,414	1,596	0,1137	21,67	6,060
K	CRP4.1	2	8,945	0,7387	3,350	1,572	0,1129	22,04	6,100
K	CRP4.1	3	8,994	0,7514	3,413	1,585	0,1123	22,27	6,060
L	CRP1.1	1	7,306	0,0497	0,203	0,1446	0,0085	55,14	8,230
L	CRP1.1	2	7,514	0,0337	0,167	0,1450	0,0068	53,96	8,280
L	CRP1.1	3	7,472	0,0334	0,152	0,1311	0,0064	53,33	8,280
L	CRP2.1	1	10,30	2,034	3,326	3,696	0,2423	51,71	7,710
L	CRP2.1	2	10,30	2,063	3,405	3,681	0,2448	51,32	7,710
L	CRP2.1	3	10,18	2,017	3,393	3,690	0,2426	51,28	7,710
L	CRP3.1	1	16,10	2,553	5,561	3,802	0,2880	9,215	6,820
L	CRP3.1	2	16,09	2,585	5,432	3,813	0,2834	9,264	6,830
L	CRP3.1	3	16,06	2,571	5,638	3,871	0,2912	9,137	6,830
L	CRP4.1	1	8,525	0,7522	3,258	1,439	0,1004	26,26	6,080
L	CRP4.1	2	8,596	0,7561	3,350	1,488	0,1041	26,10	6,060
L	CRP4.1	3	8,419	0,7337	3,216	1,437	0,1004	26,45	6,060
M	CRP1.1	1	–	–	–	–	–	53,59	7,980
M	CRP1.1	2	–	–	–	–	–	53,16	7,980
M	CRP1.1	3	–	–	–	–	–	53,42	7,980
M	CRP2.1	1	–	–	–	–	–	50,89	7,550
M	CRP2.1	2	–	–	–	–	–	51,32	7,560
M	CRP2.1	3	–	–	–	–	–	51,34	7,580
M	CRP3.1	1	–	–	–	–	–	8,340	6,890
M	CRP3.1	2	–	–	–	–	–	8,360	6,880
M	CRP3.1	3	–	–	–	–	–	8,480	6,870
M	CRP4.1	1	–	–	–	–	–	24,52	6,140
M	CRP4.1	2	–	–	–	–	–	24,45	6,110
M	CRP4.1	3	–	–	–	–	–	24,00	6,100
N	CRP1.1	1	7,115	0,0650	0,2550	0,1810	0,0100	54,04	8,240

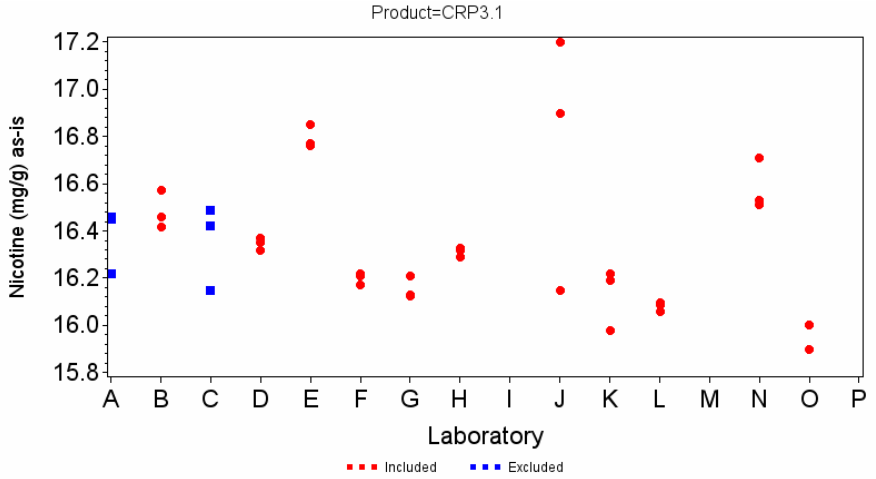
Lab Code	sample	replicate	Nicotine (mg/g)	NNK (µg/g)	NNN (µg/g)	NAT (µg/g)	NAB (µg/g)	Oven Volatiles (%)	pH
N	CRP1.1	2	6,823	0,0580	0,2250	0,1730	0,0120	55,15	8,240
N	CRP1.1	3	6,954	0,0570	0,2170	0,1680	0,0080	53,65	8,250
N	CRP2.1	1	10,88	2,334	4,217	4,879	0,2800	51,29	7,820
N	CRP2.1	2	10,70	2,287	4,228	4,977	0,2760	51,69	7,820
N	CRP2.1	3	10,81	2,263	4,171	4,844	0,2720	51,28	7,810
N	CRP3.1	1	16,51	2,848	6,894	4,837	0,3040	8,330	7,090
N	CRP3.1	2	16,53	2,851	6,878	4,887	0,3100	8,430	7,090
N	CRP3.1	3	16,71	2,884	6,706	4,978	0,3020	8,150	7,080
N	CRP4.1	1	9,260	0,8660	4,400	2,0390	0,1240	25,10	6,360
N	CRP4.1	2	9,092	0,8850	4,375	2,104	0,1240	25,34	6,350
N	CRP4.1	3	9,047	0,8920	4,376	2,059	0,1290	25,87	6,350
O	CRP1.1	1	7,530	0,0405	0,1670	0,1140	<0,0078	48,23	8,200
O	CRP1.1	2	7,060	0,0477	0,1870	0,1250	0,0075	46,61	8,220
O	CRP1.1	3	7,300	0,0449	0,1850	0,1260	0,0076	44,29	8,210
O	CRP2.1	1	10,50	1,840	3,280	3,600	0,2400	48,79	7,690
O	CRP2.1	2	10,40	1,990	3,330	3,700	0,2350	46,28	7,690
O	CRP2.1	3	10,60	1,920	3,300	3,650	0,2370	50,77	7,670
O	CRP3.1	1	15,90	2,460	5,390	3,900	0,2600	8,460	6,990
O	CRP3.1	2	16,00	2,500	5,440	3,790	0,2620	8,520	7,010
O	CRP3.1	3	16,00	2,500	5,430	3,820	0,2580	8,720	7,010
O	CRP4.1	1	8,730	0,7360	3,170	1,410	0,0970	25,22	6,150
O	CRP4.1	2	8,680	0,7390	3,130	1,410	0,0981	24,70	6,160
O	CRP4.1	3	8,720	0,7170	3,190	1,470	0,0976	24,70	6,160
P	CRP1.1	1	–	0,0650	0,2440	0,1310	0,0110	–	–
P	CRP1.1	2	–	0,0590	0,2330	0,1200	0,0090	–	–
P	CRP1.1	3	–	0,0570	0,2220	0,1100	0,0090	–	–
P	CRP2.1	1	–	2,266	3,921	4,776	0,3080	–	–
P	CRP2.1	2	–	2,367	3,993	4,961	0,3140	–	–
P	CRP2.1	3	–	2,251	3,836	4,709	0,3040	–	–
P	CRP3.1	1	–	2,549	5,648	4,118	0,2800	–	–
P	CRP3.1	2	–	2,627	5,793	4,222	0,2760	–	–
P	CRP3.1	3	–	2,633	5,812	4,136	0,2780	–	–
P	CRP4.1	1	–	0,9450	3,954	1,822	0,1370	–	–
P	CRP4.1	2	–	0,8240	3,631	1,772	0,1230	–	–
P	CRP4.1	3	–	0,8450	3,781	1,769	0,1270	–	–

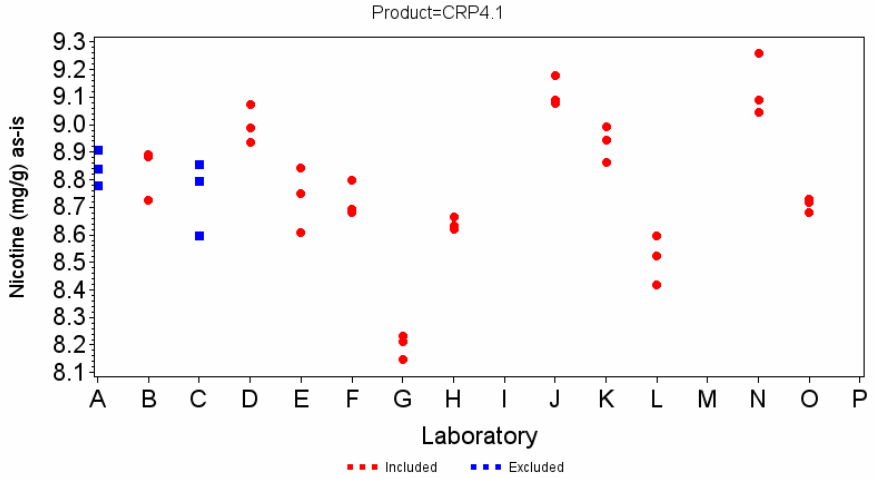
The (–) symbol indicates the laboratory did not submit data for that sample analysis.

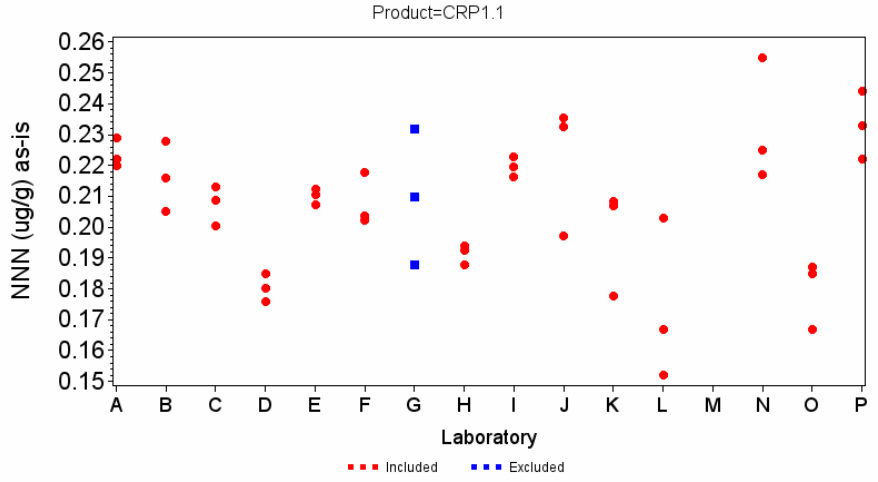
APPENDIX C: Raw Data Plots







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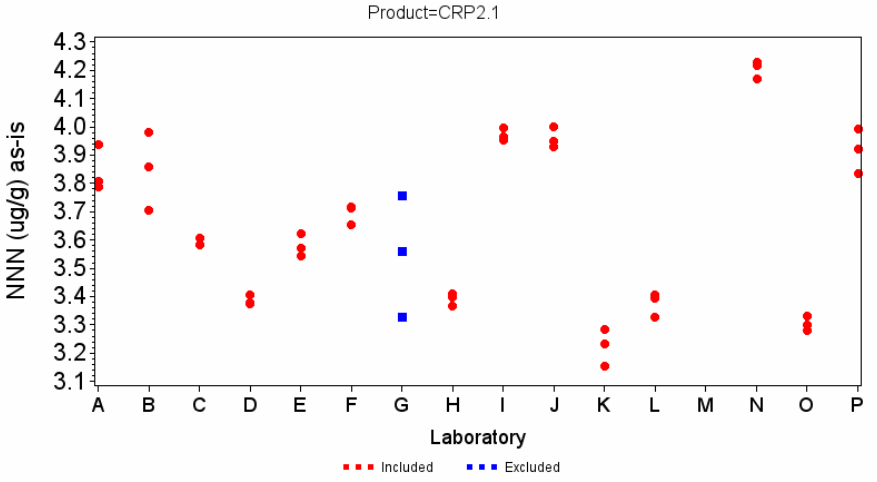
NNN (ug/g) as-is
0.26
0.25
0.24
0.23
0.22
0.21
0.20
0.19
0.18
0.17
0.16
0.15
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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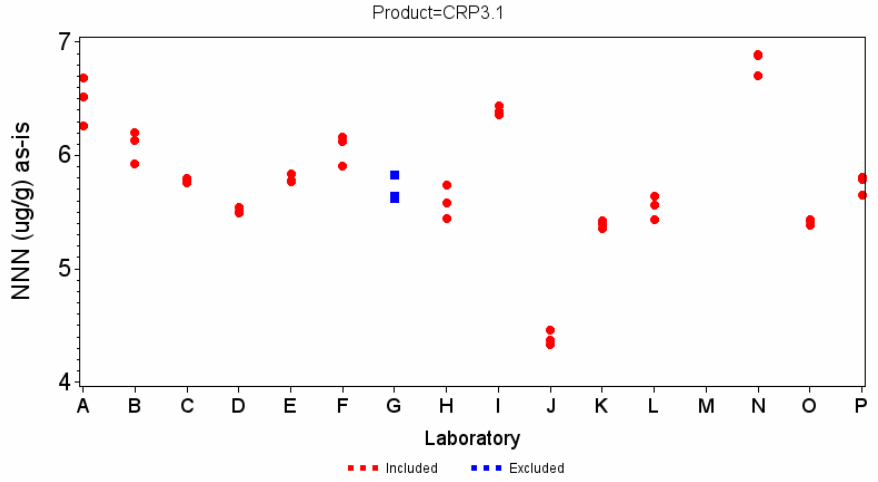
NNN (ug/g) as-is
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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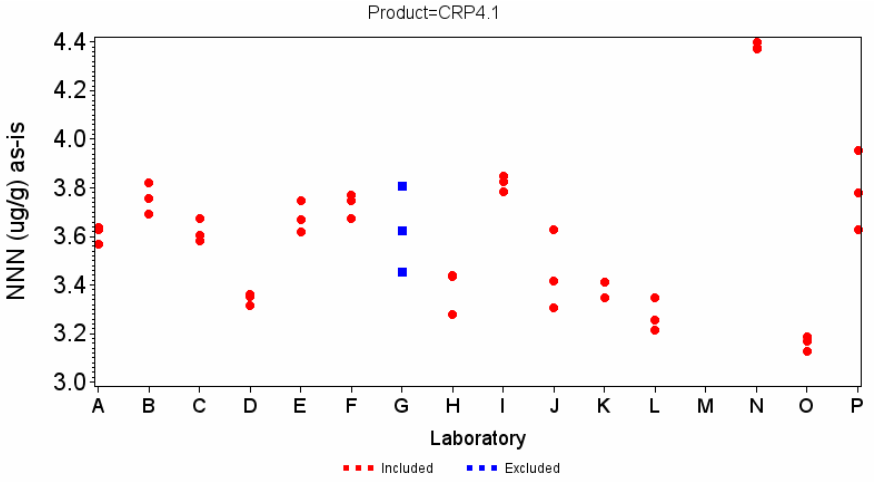
NNN (ug/g) as-is
7
6
5
4
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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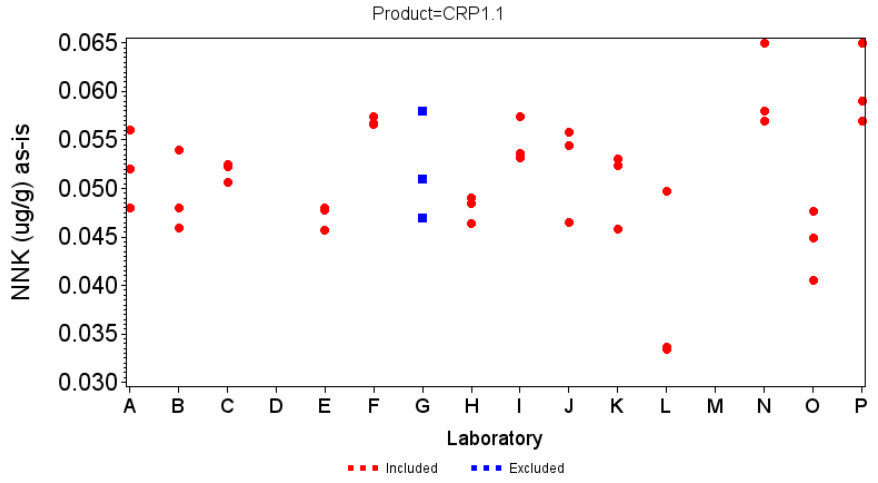
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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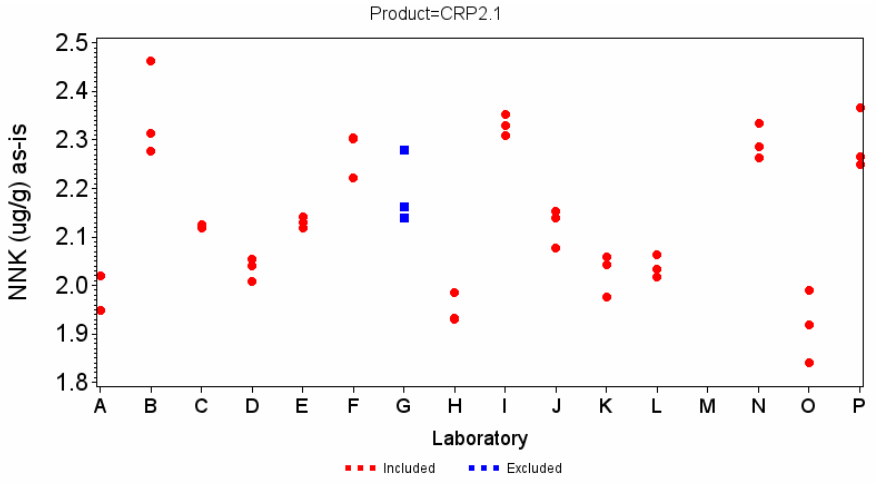
0.060
0.055
0.050
0.045
0.040
0.035
0.030
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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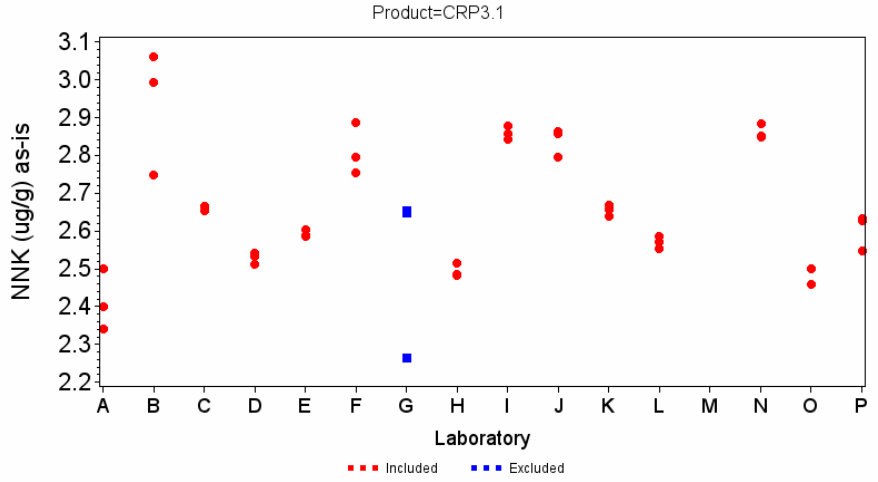
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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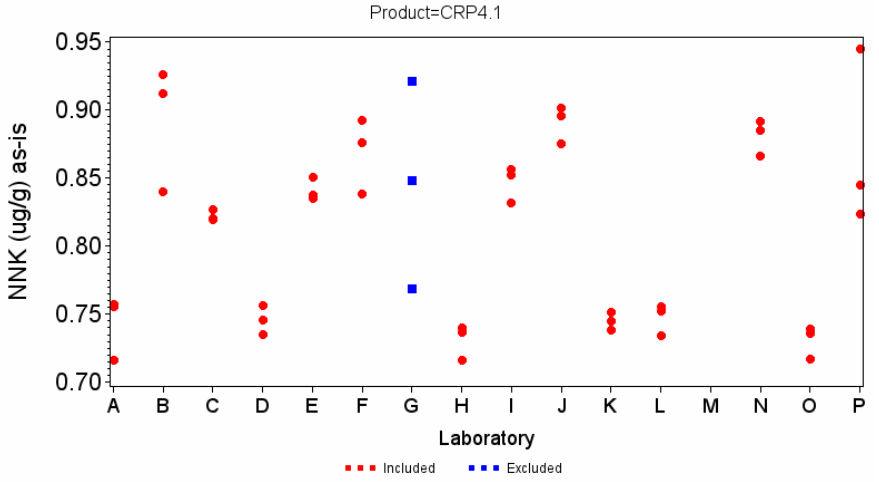
NNK (ug/g) as-is
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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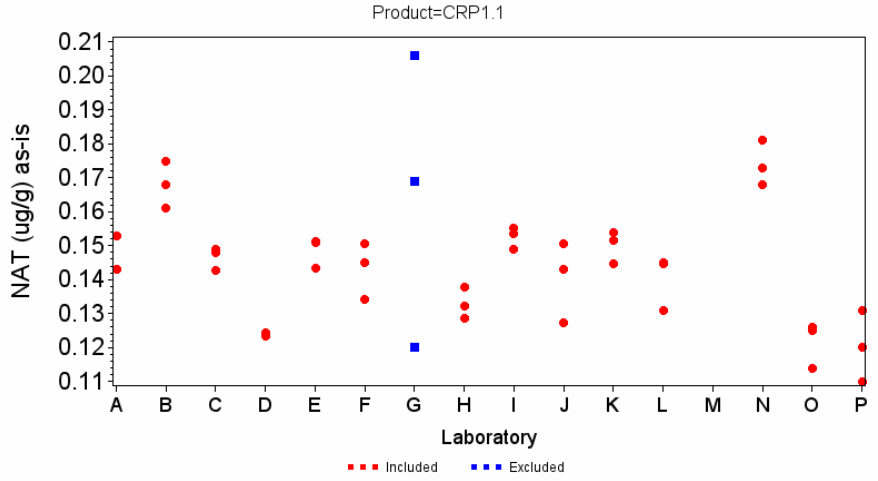
0.90
0.85
0.80
0.75
0.70
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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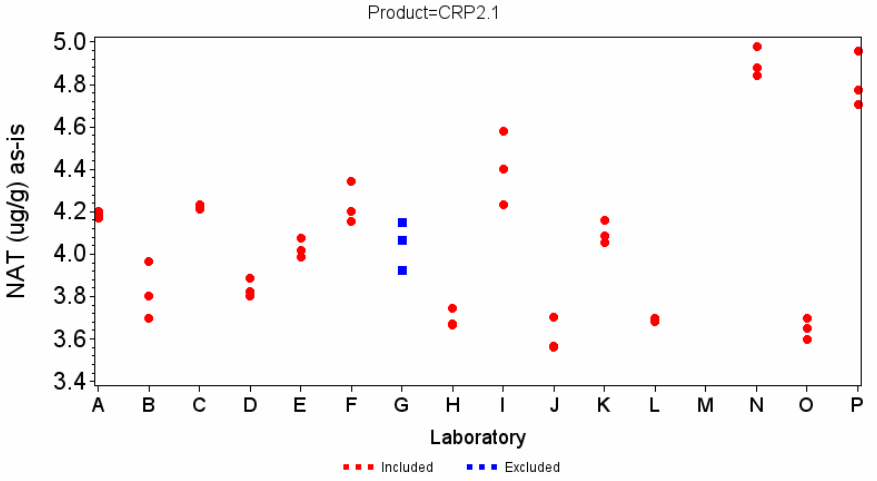
NAT (ug/g) as-is
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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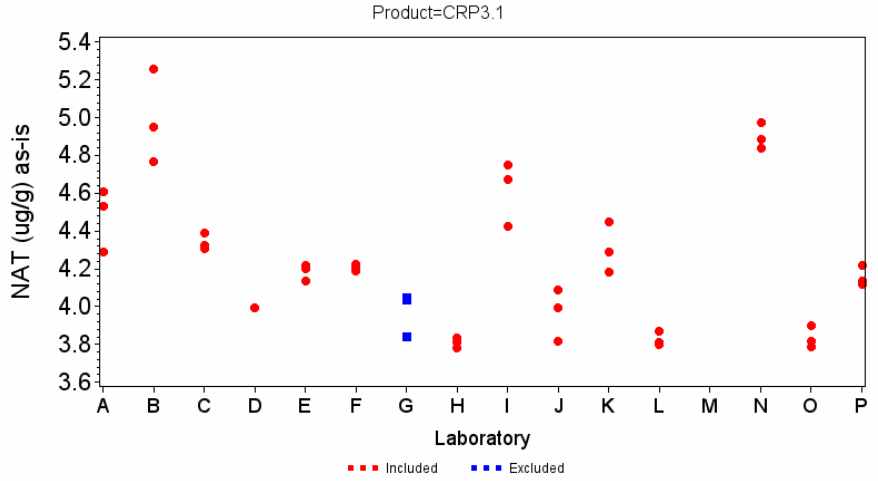
NAT (ug/g) as-is
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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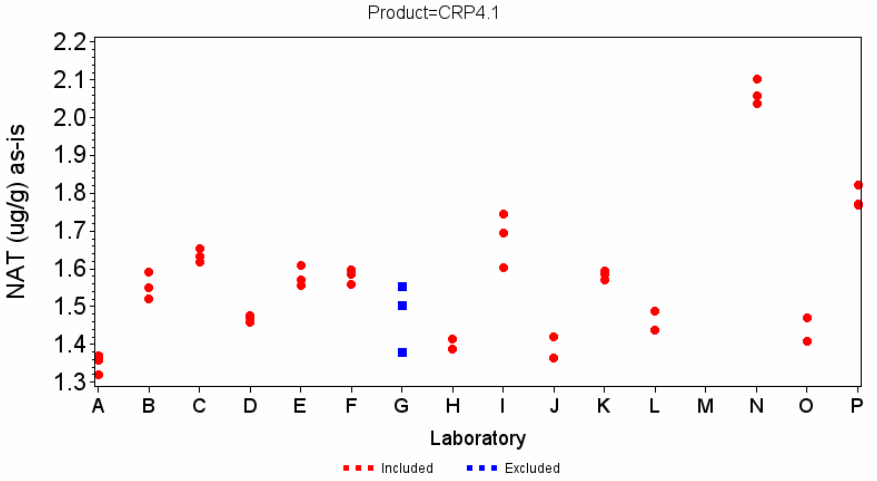
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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NAT (ug/g) as-is
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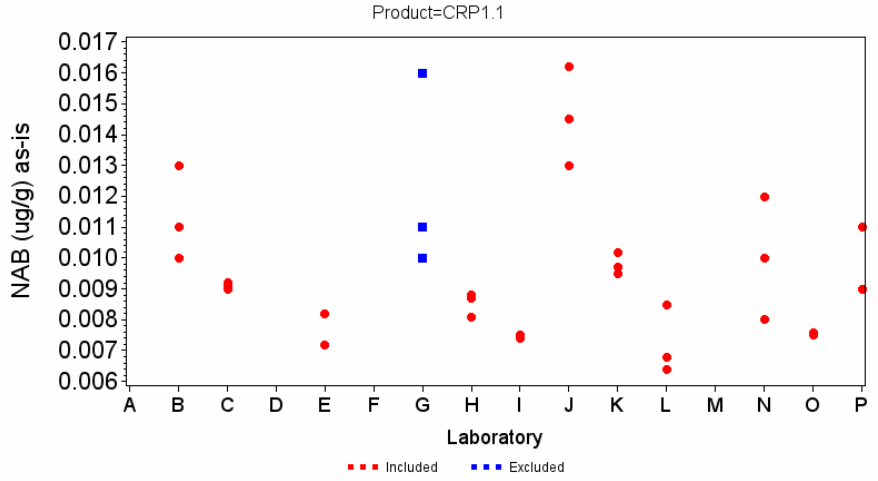
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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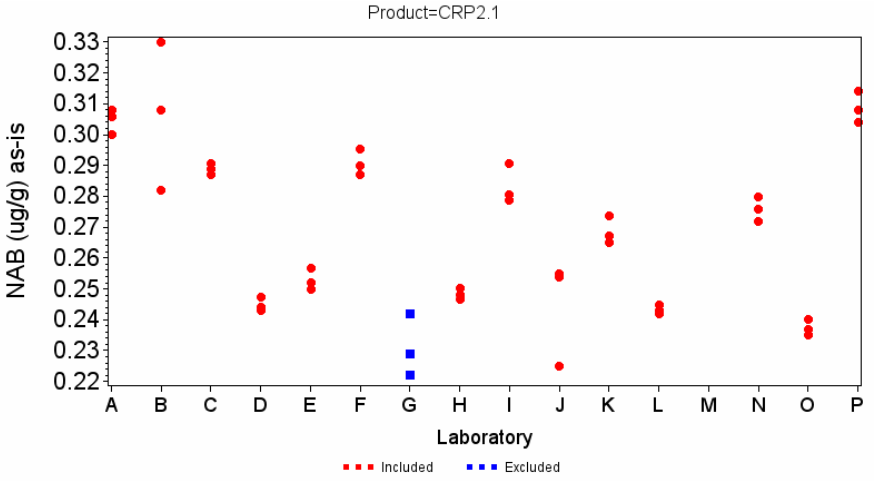
NAB (ug/g) as-is
0.017
0.016
0.015
0.014
0.013
0.012
0.011
0.010
0.009
0.008
0.007
0.006
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded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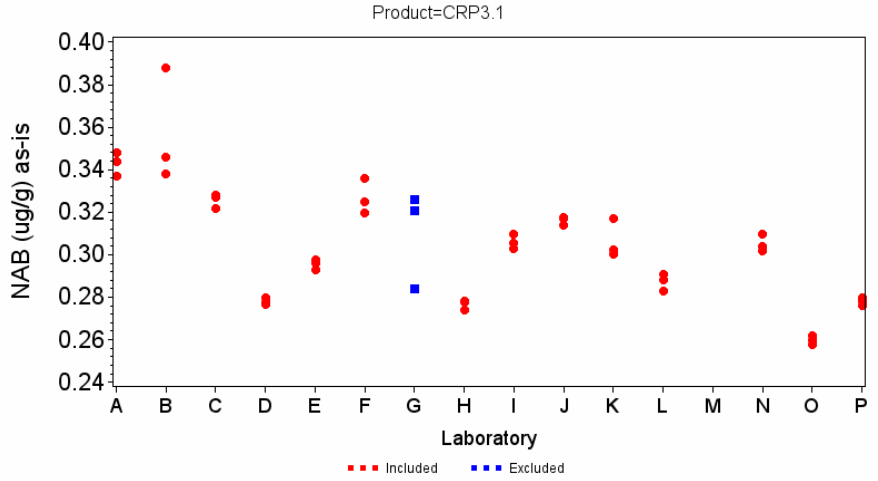
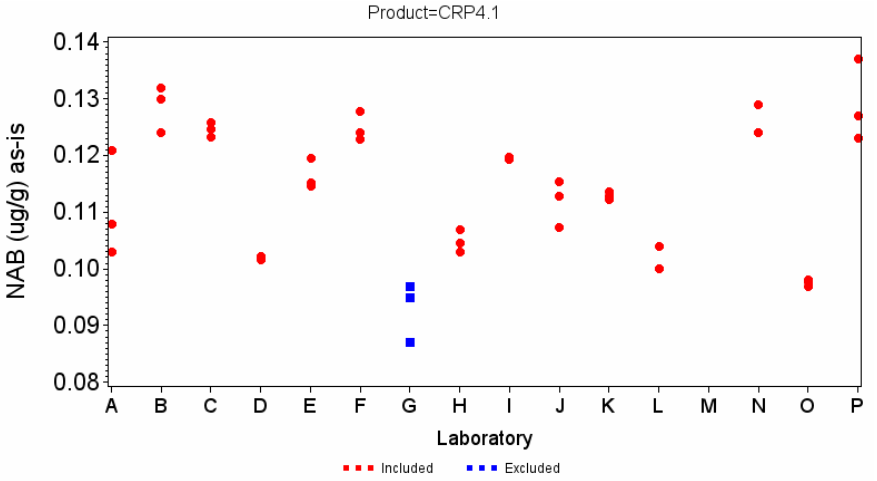
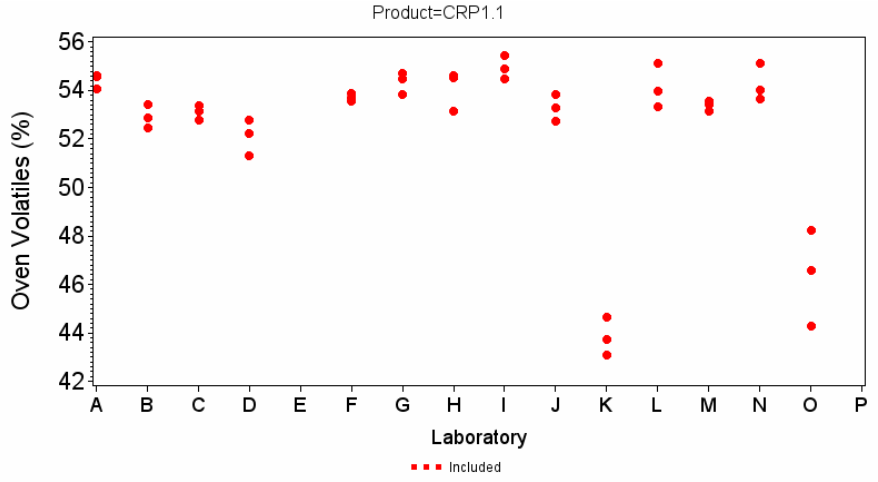
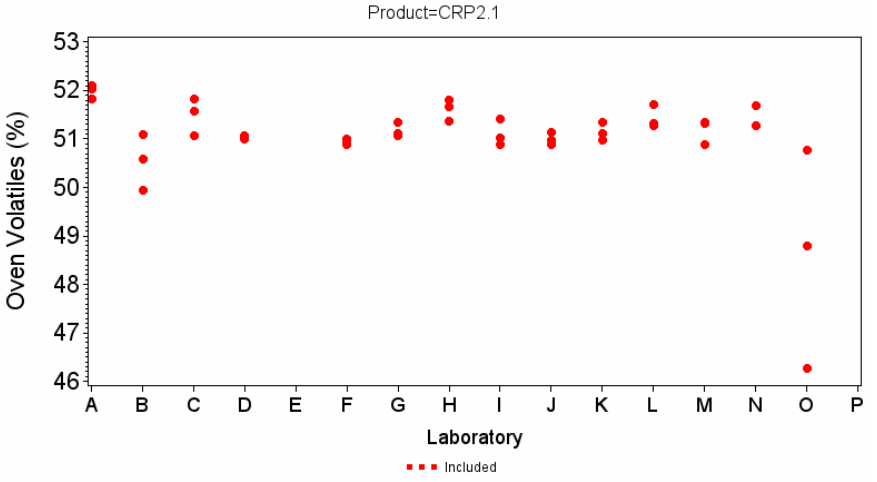
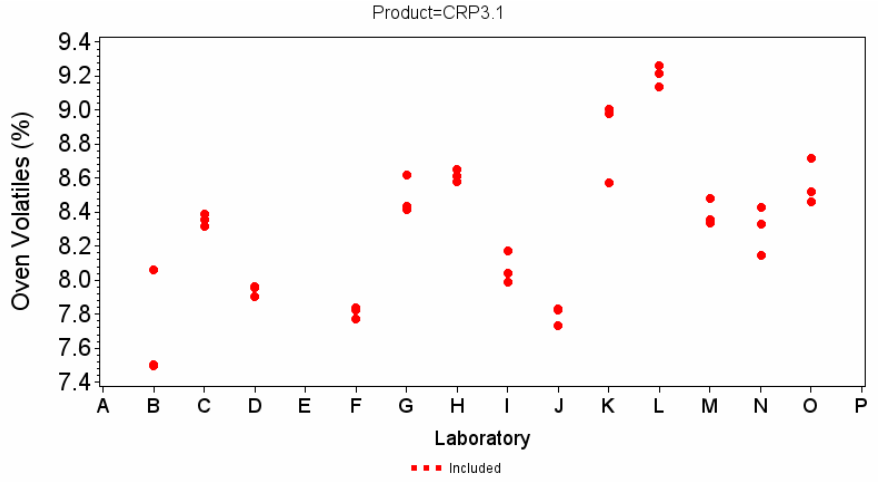
0.32
0.31
0.30
0.29
0.28
0.27
0.26
0.25
0.24
0.23
0.22
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded

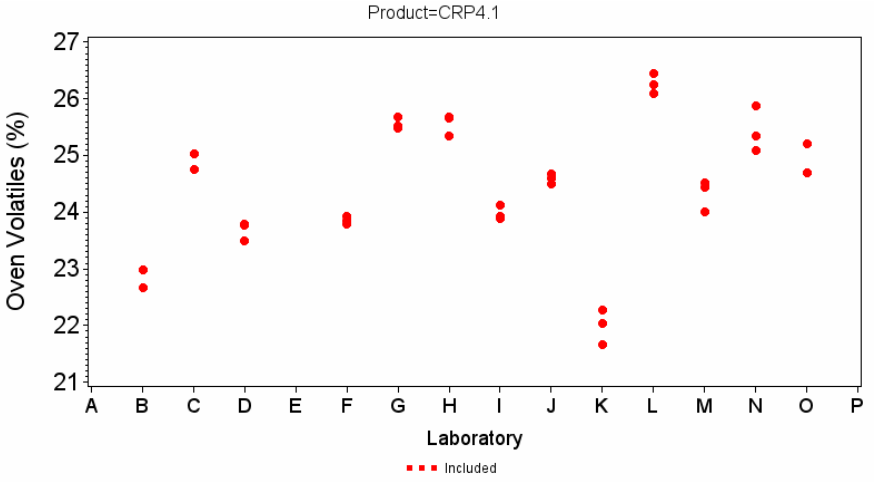

Product=CRP3.1
NAB (ug/g) as-is
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded

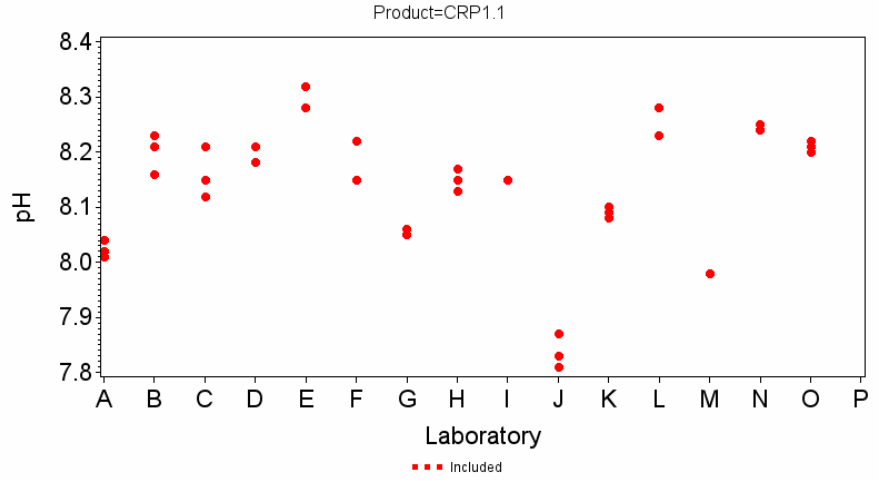

Product=CRP4.1
NAB (ug/g) as-is
0.14
0.13
0.12
0.11
0.10
0.09
0.08
A B C D E F G H I J K L M N O P
Laboratory
Included
Excluded

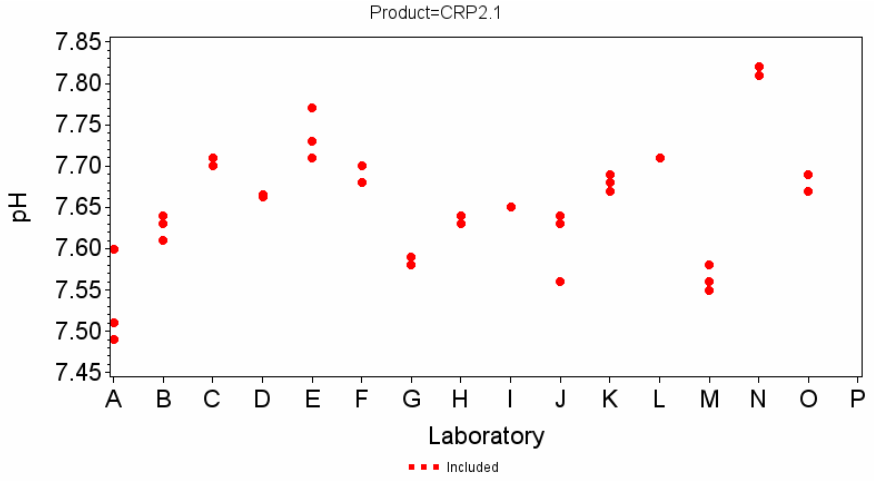

Product=CRP1.1
Oven Volatiles (%)
56
54
52
50
48
46
44
42
A B C D E F G H I J K L M N O P
Laboratory
Included

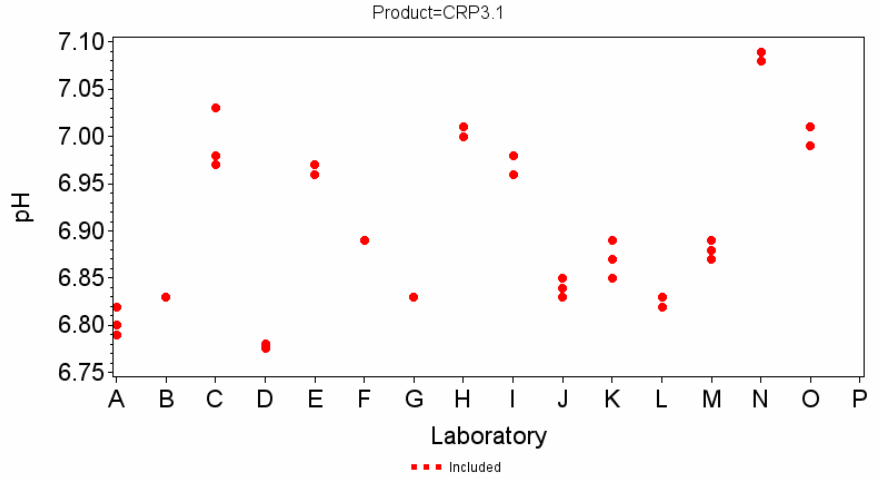

Product=CRP2.1
Oven Volatiles (%)
53
52
51
50
49
48
47
46
A B C D E F G H I J K L M N O P
Laboratory
Included

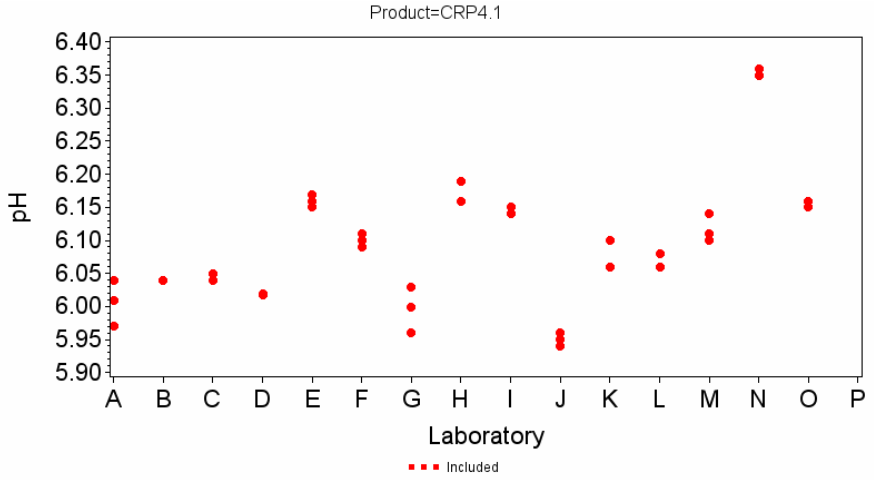

Product=CRP3.1
Oven Volatiles (%)
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
A B C D E F G H I J K L M N O P
Laboratory
Included


Product=CRP4.1
Oven Volatiles (%)
27
26
25
24
23
22
21
A B C D E F G H I J K L M N O P
Laboratory
Included

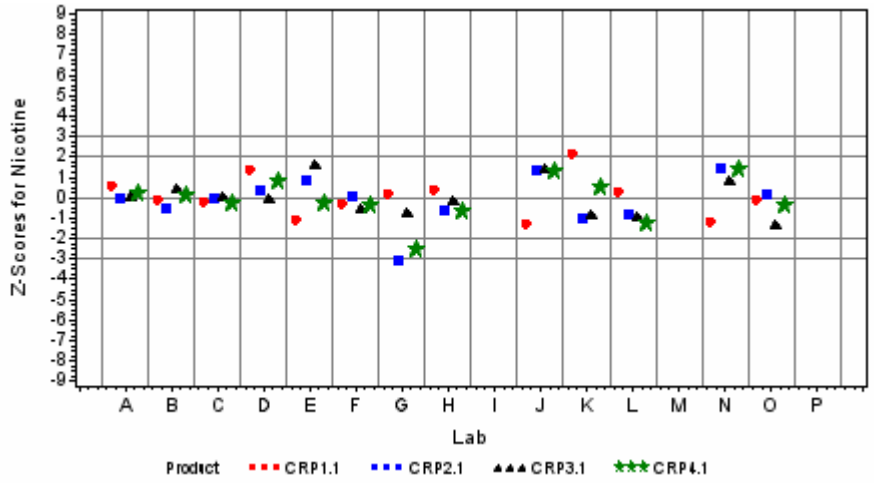

Product=CRP1.1
pH
8.4
8.3
8.2
8.1
8.0
7.9
7.8
A B C D E F G H I J K L M N O P
Laboratory
Included

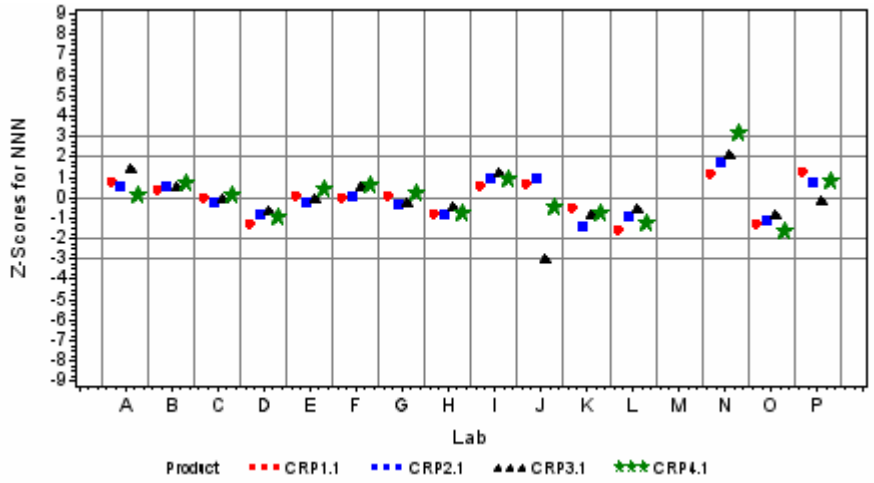

Product=CRP2.1
pH
7.85
7.80
7.75
7.70
7.65
7.60
7.55
7.50
7.45
A B C D E F G H I J K L M N O P
Laboratory
Included

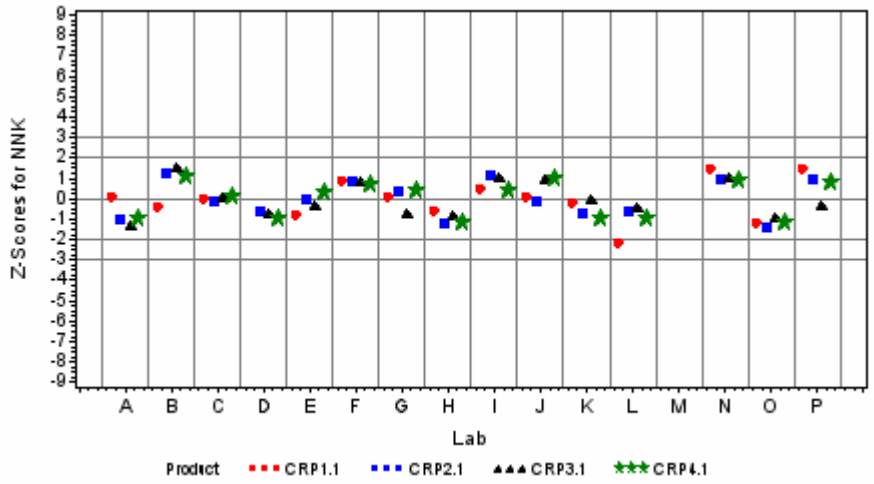

Product=CRP3.1
pH
7.10
7.05
7.00
6.95
6.90
6.85
6.80
6.75
A B C D E F G H I J K L M N O P
Laboratory
Included

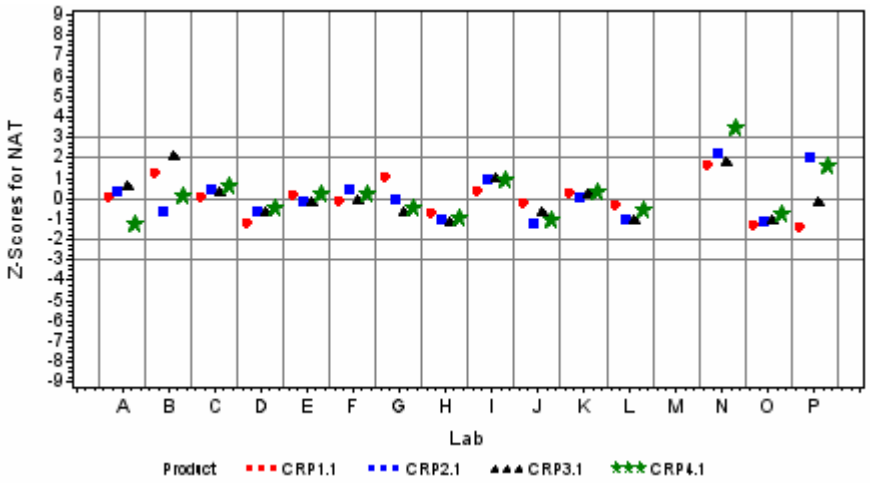

Product=CRP4.1
pH
6.40
6.35
6.30
6.25
6.20
6.15
6.10
6.05
6.00
5.95
5.90
A B C D E F G H I J K L M N O P
Laboratory
Included

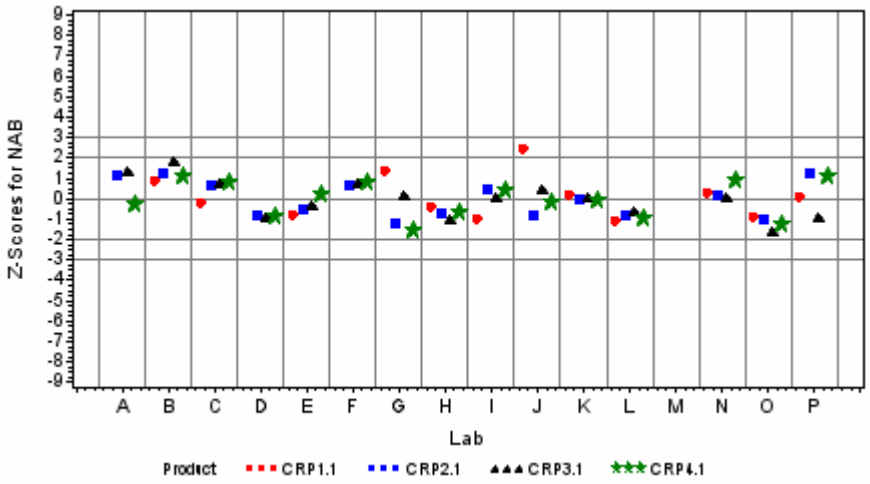
APPENDIX D: Z-scores Plots

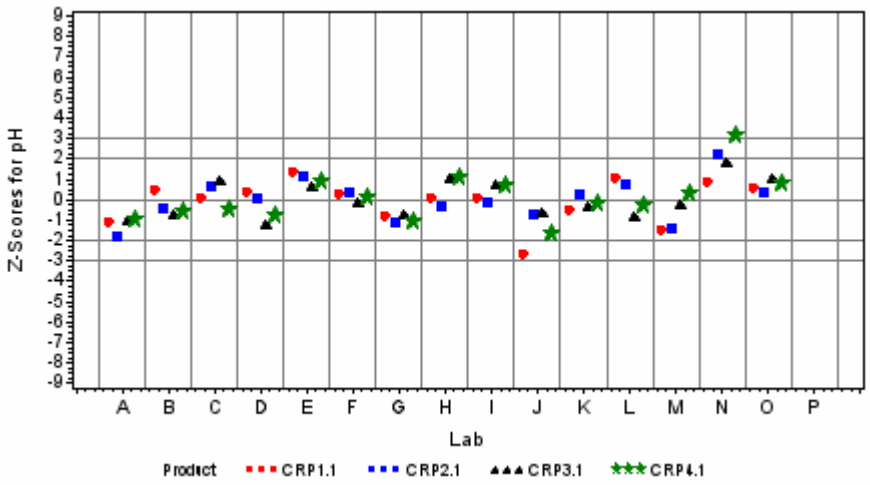


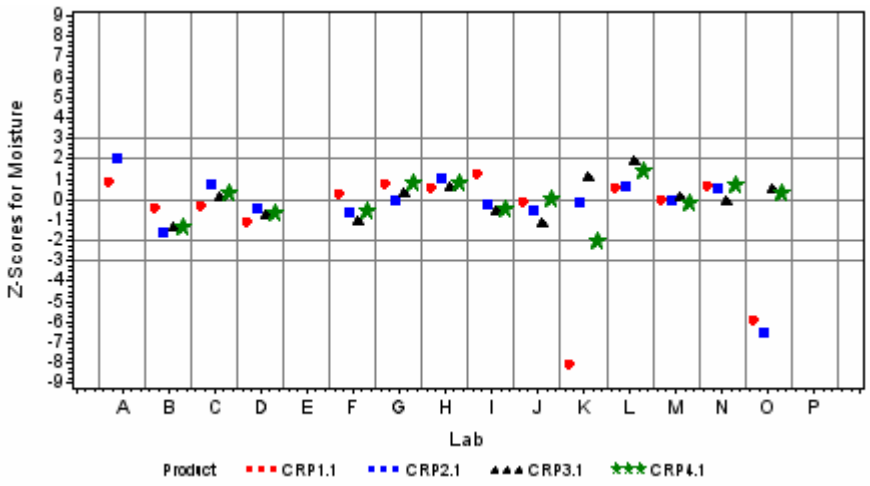





Z-Scores for NAT
Lab
Product CRP1.1 CRP2.1 CRP3.1 CRP4.1


Z-Scores for NAB
Lab
Product CRP1.1 CRP2.1 CRP3.1 CRP4.1


Z-Scores for pH
Lab
Product CRP1.1 CRP2.1 CRP3.1 CRP4.1


Z-Scores for Moisture
Lab
Product CRP1.1 CRP2.1 CRP3.1 CRP4.1