Tobacco and Tobacco Products Analytes Sub-Group

Technical Report

CORESTA Reference Products 2021 Analysis

September 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 the third stability 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, r and R estimates, and z-scores.

The results from this 2021 stability analysis generally compared well to the 2016 and 2019 analyses[1,2]. The only statistically significant differences from the 2016 and 2019 results were slight changes in moisture for CRP3.1 and CRP 2.1. The TTPA recommends that the stability of the 2016 CRPs continued 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, 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[3] and demonstrated that the CRPs were stable for a minimum of 10 years when held at the recommended storage conditions of −20 °C.

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. Even though the 2016 CRPs included the same 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 completed and nicotine, pH, moisture, TSNAs, ammonia and B[a]P were reported using the applicable CRMs[1]. The collaborative study conducted in 2019 was the first stability timepoint.[2]. This report serves as the second stability assessment of the 2016 CRPs by reporting the levels of nicotine, pH, moisture, and TSNAs using the applicable CRMs.

The CRPs produced in 2016 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 2009 Reference Products 2019 Analysis.

2.1 Objective

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)) for the purpose of examining the stability of the CRPs. Results were reported on an as-is basis and not corrected for moisture. This work was conducted using the applicable CRMs referenced in Section 3.

This study was conducted to provide an assessment of inter-laboratory variability, and to examine the stability of the CRPs. 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. The results from this year's evaluation were compared to the results from 2016 and 2019 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 the table below. 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, UK
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
Scandinavian Tobacco Group, Eersel, The Netherlands
Swedish Match North America, United States
Swedish Match Northern Europe, Sweden
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 procuring each of 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 initiate the study by December 2020 and report data by March 1, 2021. The samples are identified in the table below.

Table 2: Sample Identification

Product 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 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

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 E: Minor deviations. 0.2 g sample was extracted by 0.5 ml NaOH and 5 ml MTBE, instead of 1.0 g sample extracted by 7 ml NaOH and 50 ml MTBE, as stated in CRM N° 62. These results were included in the study.
- Lab J: Major deviations. TSNA was performed with GC-TEA alkaline dichloromethane extraction ISO/TS 22304: 2008 and deviated from the extraction described in CRM N° 72. These results were excluded from the study.
- Lab K: Minor deviations. 2M NaOH instead of 5M 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 °C ± 1 °C instead of 100 °C ± 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 N° 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 L: Minor deviations. 5 mL of a 2N NaOH solution was used instead of 7 mL of 5N NaOH solution as described in CRM N° 62. These results were included in the study.
- Lab N: Major deviations. The samples were extracted with methanol/water (50/50, v/v) instead of ammonium acetate as described in CRM N° 72 and the calibration standards are prepared with methanol/water (50/50, v/v) instead of acetonitrile/water and ammonium acetate. These results 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. Raw Data

The full data set for the study, including outliers, 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, without removal of outliers, are given in Appendix C.

5. Statistical Analysis

A 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 it was not necessary for the objective of evaluating stability of the reference products, 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 and to support their laboratory accreditation. Raw data plots that include all replicates, without removal of outliers, are shown in Appendix C.

5.1 Exclusion of Outliers

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. 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[4]. The outliers are presented in the table below.

Table 3: Outliers

Product	Analyte	Levene's Outlier Lab	Grubbs' Outlier Lab
CRP1.1	NNN	E	–
CRP2.1	Nicotine	E	–
CRP2.1	Moisture	G	–
CRP2.1	NNK	–	M
CRP3.1	NAT	L	–
CRP4.1	NNN	–	M

The (–) symbol indicates an outlier of that type 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 limits (r & R) were calculated. The r & R results are shown in the table below and reflect both laboratory variability and product homogeneity.

[4] 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.

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

Analyte	Product	Mean	N° of Labs*	r	% r	R	% R
Nicotine (mg/g)	CRP1.1	7,53	13	0,49	6,5%	1,02	13,6%
Nicotine (mg/g)	CRP2.1	10,61	12	0,17	1,6%	1,55	14,7%
Nicotine (mg/g)	CRP3.1	16,82	13	0,33	2,0%	1,36	8,1%
Nicotine (mg/g)	CRP4.1	8,94	13	0,23	2,5%	1,10	12,3%
pH	CRP1.1	8,20	16	0,079	NA	0,34	NA
pH	CRP2.1	7,71	16	0,038	NA	0,20	NA
pH	CRP3.1	6,90	16	0,064	NA	0,15	NA
pH	CRP4.1	6,09	16	0,053	NA	0,19	NA
Moisture (%)	CRP1.1	53,72	13	2,04	3,8%	2,52	4,7%
Moisture (%)	CRP2.1	51,06	12	0,83	1,6%	1,34	2,6%
Moisture (%)	CRP3.1	8,31	13	0,32	3,8%	1,25	15,0%
Moisture (%)	CRP4.1	24,14	13	0,67	2,8%	2,31	9,6%
NNN (µg/g)	CRP1.1	0,197	12	0,024	12,1%	0,060	30,7%
NNN (µg/g)	CRP2.1	3,46	13	0,22	6,5%	0,87	25,1%
NNN (µg/g)	CRP3.1	5,61	13	0,53	9,5%	1,77	31,5%
NNN (µg/g)	CRP4.1	3,58	12	0,38	10,6%	0,61	17,1%
NNK (µg/g)	CRP1.1	0,050	13	0,0073	14,5%	0,016	31,7%
NNK (µg/g)	CRP2.1	2,09	12	0,18	8,8%	0,30	14,3%
NNK (µg/g)	CRP3.1	2,55	13	0,15	6,0%	0,45	17,5%
NNK (µg/g)	CRP4.1	0,81	13	0,10	11,9%	0,18	22,7%
NAT (µg/g)	CRP1.1	0,14	13	0,025	17,7%	0,033	23,9%
NAT (µg/g)	CRP2.1	3,97	13	0,29	7,2%	1,10	27,8%
NAT (µg/g)	CRP3.1	4,04	12	0,18	4,4%	0,98	24,3%
NAT (µg/g)	CRP4.1	1,52	13	0,11	7,4%	0,26	17,4%
NAB (µg/g)	CRP1.1	0,0086	13	0,0023	26,7%	0,0045	52,5%
NAB (µg/g)	CRP2.1	0,267	13	0,0364	13,6%	0,0699	26,2%
NAB (µg/g)	CRP3.1	0,298	13	0,0346	11,6%	0,0650	21,8%
NAB (µg/g)	CRP4.1	0,115	13	0,0094	8,2%	0,0287	24,9%

* This is the number of laboratory data sets reporting numerical values and after removal of outliers.
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 and 2019 studies in Table 5. The results from this study are generally comparable to or a little less variable than the 2016 and 2019 results.

Table 5. Comparison of Repeatability and Reproducibility

Parameter	Product	2016	2019	2021	2016	2019	2021
		% r	% r	% r	% R	% R	% R
Nicotine (mg/g)	CRP1.1	11,0%	10,6%	6,54%	16,8%	17,7%	13,6%
Nicotine (mg/g)	CRP2.1	5,00%	2,80%	1,64%	24,8%	15,6%	14,7%
Nicotine (mg/g)	CRP3.1	1,90%	2,70%	1,98%	12,2%	11,5%	8,1%
Nicotine (mg/g)	CRP4.1	7,90%	1,50%	2,55%	13,1%	10,1%	12,3%
Moisture (%)	CRP1.1	2,10%	2,70%	3,79%	3,40%	3,00%	4,70%
Moisture (%)	CRP2.1	0,60%	1,40%	1,62%	1,40%	2,70%	2,62%
Moisture (%)	CRP3.1	2,60%	3,00%	3,83%	10,1%	20,1%	15,0%
Moisture (%)	CRP4.1	2,50%	3,10%	2,76%	8,90%	12,60%	9,57%
NNN (µg/g)	CRP1.1	22,1%	17,7%	12,1%	35,8%	30,4%	30,7%
NNN (µg/g)	CRP2.1	6,30%	6,30%	6,47%	25,8%	24,8%	25,1%
NNN (µg/g)	CRP3.1	5,70%	4,70%	9,47%	27,3%	31,2%	31,5%
NNN (µg/g)	CRP4.1	5,8%	8,5%	10,6%	21,8%	19,8%	17,1%
NNK (µg/g)	CRP1.1	25,2%	29,0%	14,5%	56,6%	71,2%	31,7%
NNK (µg/g)	CRP2.1	10,20%	5,20%	8,75%	25,5%	16,6%	14,3%
NNK (µg/g)	CRP3.1	5,50%	7,90%	6,04%	22,6%	16,9%	17,5%
NNK (µg/g)	CRP4.1	11,4%	12,4%	11,9%	33,1%	16,6%	22,7%
NAT (µg/g)	CRP1.1	22,9%	19,6%	17,7%	50,7%	25,9%	23,9%
NAT (µg/g)	CRP2.1	8,80%	6,80%	7,20%	21,0%	20,2%	27,8%
NAT (µg/g)	CRP3.1	2,70%	6,20%	4,36%	20,2%	17,0%	24,3%
NAT (µg/g)	CRP4.1	6,80%	10,90%	7,43%	23,8%	19,4%	17,4%
NAB (µg/g)	CRP1.1	35,9%	30,6%	26,7%	63,2%	59,6%	52,5%
NAB (µg/g)	CRP2.1	10,2%	10,2%	13,6%	25,4%	17,4%	26,2%
NAB (µg/g)	CRP3.1	10,8%	12,8%	11,6%	28,0%	18,4%	21,8%
NAB (µg/g)	CRP4.1	9,40%	7,90%	8,18%	37,9%	11,3%	24,9%

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

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. The assigned value and standard deviation for proficiency assessment were calculated after removing outliers; however, z-scores were calculated for the outliers. 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	pH	Moisture	NAB	NAT	NNK	NNN
CRP1.1	A	–	0,36	–	0,47	0,91	0,24	0,98
CRP1.1	B	–	0,33	–	0,03	1,23	0,59	0,64
CRP1.1	C	-0,81	-0,80	0,45	-0,14	-0,27	-0,19	-0,32
CRP1.1	D	1,19	0,36	0,04	-0,36	-0,36	-0,75	0,19
CRP1.1	E	0,87	0,14	2,96	0,09	-0,05	-1,32	1,26
CRP1.1	F	-0,12	-0,67	0,53	1,87	0,47	-0,02	0,27
CRP1.1	G	1,28	1,05	-0,72	0,73	0,66	0,82	-0,14
CRP1.1	H	0,44	-0,36	1,40	0,11	-0,07	0,00	-0,85
CRP1.1	I	-0,94	0,83	-0,20	-0,40	0,45	0,26	-0,02
CRP1.1	J	0,15	-1,42	-1,30	-1,02	-0,61	1,43	0,12
CRP1.1	K	0,23	0,73	-0,33	0,11	0,33	0,20	-0,47
CRP1.1	L	-0,43	0,77	0,25	-1,40	1,27	2,26	1,74
CRP1.1	M	–	1,43	-0,99	1,88	-1,60	-0,44	-2,85
CRP1.1	N	-1,32	-0,70	–	-1,68	-2,89	-2,77	-2,24
CRP1.1	O	-1,18	-0,58	-1,03	–	–	–	–
CRP1.1	P	0,64	-3,08	0,40	0,29	-0,95	-0,80	-0,16
CRP2.1	A	–	0,72	–	1,68	-1,13	-0,28	0,39
CRP2.1	B	–	0,59	–	-1,37	-0,70	-0,76	-0,48
CRP2.1	C	-1,42	-1,34	0,83	0,03	0,38	1,29	1,19
CRP2.1	D	-0,18	-0,30	0,51	-0,90	-0,60	-0,37	0,17
CRP2.1	E	0,52	-0,17	-0,17	1,08	0,71	1,29	-0,11
CRP2.1	F	-0,57	-0,39	-0,40	0,27	0,24	0,35	-0,33
CRP2.1	G	0,61	0,05	1,49	0,37	0,93	0,15	-0,01
CRP2.1	H	0,33	0,14	0,83	-1,14	-0,02	-0,59	-0,80
CRP2.1	I	-0,91	1,89	0,22	0,60	0,95	0,40	1,24
CRP2.1	J	1,27	-1,21	-2,45	-0,73	0,05	-0,03	0,15
CRP2.1	K	0,73	0,25	0,09	0,43	0,81	-0,07	-0,01
CRP2.1	L	-0,26	0,59	-2,60	0,84	1,30	1,21	1,58
CRP2.1	M	–	1,53	-0,80	-0,17	-2,90	-3,52	-2,88
CRP2.1	N	-1,84	-0,38	–	-1,16	-1,30	-1,86	-1,69
CRP2.1	O	0,57	-0,08	0,08	–	–	–	–
CRP2.1	P	0,78	-1,48	0,33	0,35	-0,11	0,42	0,08
CRP3.1	A	–	-0,22	–	-0,26	-2,51	-1,53	-1,13
CRP3.1	B	–	0,83	–	-0,74	-0,23	0,25	0,12
CRP3.1	C	-1,73	0,24	1,05	0,12	0,22	1,49	1,20
CRP3.1	D	0,16	-1,55	-0,23	-1,31	-0,70	-0,47	0,41

Product	Lab	Nicotine	pH	Moisture	NAB	NAT	NNK	NNN
CRP3.1	E	0,16	0,12	-0,39	1,14	0,10	0,94	0,94
CRP3.1	F	-0,28	-1,25	0,30	-0,01	-0,30	0,94	-0,16
CRP3.1	G	1,04	-1,09	1,72	0,24	1,06	0,39	-0,13
CRP3.1	H	0,17	-0,11	-0,33	-1,14	-0,44	-0,43	-0,66
CRP3.1	I	-1,11	1,45	-0,57	0,68	1,62	0,61	1,26
CRP3.1	J	0,74	-1,09	-0,49	1,06	1,19	-1,43	-0,12
CRP3.1	K	1,32	0,80	-0,62	0,30	0,63	-0,38	-0,05
CRP3.1	L	-0,53	0,53	1,43	1,48	0,27	0,70	1,05
CRP3.1	M	–	0,87	-1,21	-1,32	-3,75	-0,58	-3,29
CRP3.1	N	-1,20	-0,58	–	-0,48	-0,46	-0,76	-1,30
CRP3.1	O	0,32	0,70	-0,94	–	–	–	–
CRP3.1	P	0,72	0,30	0,49	0,24	0,16	0,22	0,08
CRP4.1	A	–	0,08	–	2,01	-0,13	0,80	0,48
CRP4.1	B	–	0,50	–	-0,65	0,20	0,15	0,35
CRP4.1	C	-0,96	1,00	0,19	0,39	0,80	1,35	1,05
CRP4.1	D	0,11	-0,27	-1,20	-0,91	-0,44	-0,50	0,32
CRP4.1	E	0,33	-0,60	0,10	0,24	0,75	0,14	1,29
CRP4.1	F	0,23	-0,44	-0,68	0,98	0,85	0,92	0,38
CRP4.1	G	0,29	-2,14	0,95	0,05	0,69	-0,16	-0,08
CRP4.1	H	-0,59	0,48	1,38	-1,05	-0,08	-1,01	-1,19
CRP4.1	I	-0,97	0,48	-0,49	-0,30	0,90	-0,03	0,76
CRP4.1	J	-0,29	-2,94	0,05	0,90	-2,36	-0,16	-0,62
CRP4.1	K	1,21	-0,94	-0,22	0,11	0,69	-0,06	-0,41
CRP4.1	L	-0,25	0,97	0,37	1,18	0,42	1,28	0,71
CRP4.1	M	–	0,54	-1,06	-0,72	-1,81	-1,78	-3,27
CRP4.1	N	-1,70	0,40	–	-4,10	-4,07	-4,08	-4,22
CRP4.1	O	1,45	1,45	-0,90	–	–	–	–
CRP4.1	P	0,96	-0,66	1,57	-0,21	-0,15	0,28	-0,04

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, and 2021.

Table 7: Summary of Stability Analysis

Variable	Product	2016 Avg.	2019 Avg.	2021 Avg.	Slope	p-value
Nicotine (mg/g)	CRP1.1	7,62	7,30	7,53	-0,024	0,440
Nicotine (mg/g)	CRP2.1	10,69	10,16	10,61	-0,032	0,588
Nicotine (mg/g)	CRP3.1	17,22	16,44	16,82	-0,094	0,083
Nicotine (mg/g)	CRP4.1	8,86	8,77	8,94	0,014	0,630
pH	CRP1.1	8,30	8,19	8,20	-0,021	0,040
pH	CRP2.1	7,74	7,68	7,71	-0,006	0,278
pH	CRP3.1	6,96	6,89	6,90	-0,012	0,079
pH	CRP4.1	6,08	6,06	6,09	0,001	0,927
Moisture (%)	CRP1.1	53,95	53,56	53,72	-0,056	0,177
Moisture (%)	CRP2.1	51,41	51,22	51,06	-0,069	0,010
Moisture (%)	CRP3.1	7,33	7,87	8,31	0,194	0,000
Moisture (%)	CRP4.1	24,19	24,12	24,14	-0,012	0,846
NNN (µg/g)	CRP1.1	0,190	0,199	0,197	0,002	0,352
NNN (µg/g)	CRP2.1	3,39	3,49	3,46	0,015	0,552
NNN (µg/g)	CRP3.1	5,63	5,74	5,61	-0,003	0,957
NNN (µg/g)	CRP4.1	3,39	3,57	3,58	0,038	0,053
NNK (µg/g)	CRP1.1	0,052	0,050	0,050	0,000	0,535
NNK (µg/g)	CRP2.1	2,06	2,07	2,09	0,008	0,532
NNK (µg/g)	CRP3.1	2,51	2,59	2,55	0,004	0,484
NNK (µg/g)	CRP4.1	0,790	0,816	0,807	0,006	0,594
NAT (µg/g)	CRP1.1	0,140	0,141	0,139	0,000	0,827
NAT (µg/g)	CRP2.1	4,24	4,12	3,97	-0,013	0,138
NAT (µg/g)	CRP3.1	4,33	4,25	4,04	-0,053	0,049
NAT (µg/g)	CRP4.1	1,59	1,59	1,52	-0,056	0,035
NAB (µg/g)	CRP1.1	0,0090	0,0090	0,0086	0,001	0,765
NAB (µg/g)	CRP2.1	0,265	0,268	0,267	-0,001	0,615
NAB (µg/g)	CRP3.1	0,303	0,309	0,298	0,000	0,833
NAB (µg/g)	CRP4.1	0,115	0,115	0,115	0,000	0,965

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.

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 footnote 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 moisture for CRP2.1 and 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]. The loss in moisture with CRP2.1, though statistically significant, is quite small. Graphs of the two statistically significant moisture trends are shown in Figures 1 and 2. Each data point in the two graphs represents a lab average.

Figure 1. Moisture Results for CRP2.1 for 2016, 2019, and 2021

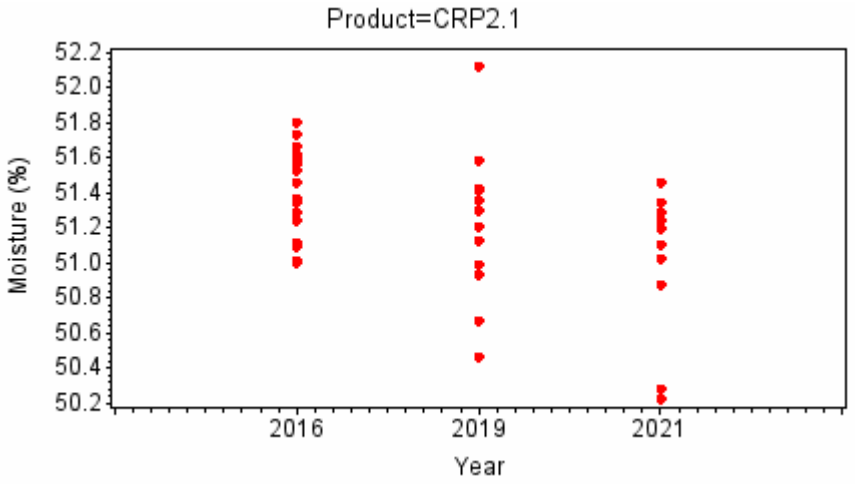
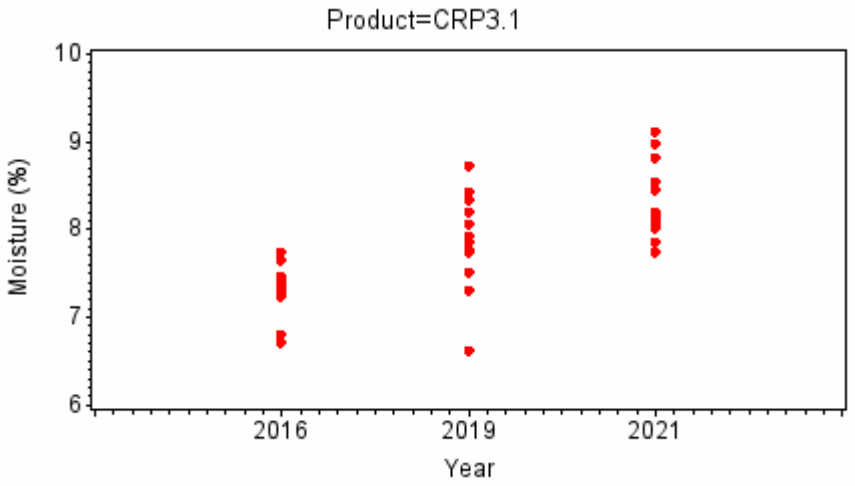


Figure 2. Moisture Results for CRP3.1 for 2016, 2019, and 2021



6. Data Interpretation

In terms of stability, moisture for CRP3.1 showed a statistically significant difference in comparison to the testing conducted in 2016 and 2019. That increase was consistent with the pattern seen for CRP3 from the 2009 production. The CRP3.1 moisture increase from the time of manufacture is approximately 0.5 %. Moisture for CRP 2.1 also showed a statistically significant trend, although the year-to-year differences were small. As mentioned earlier, results are presented on an as-is basis, however, this negligible shift in moisture for CRP3.1 and CRP 2.1 would not be evident in the other chemical analyses included in this study considering lab-to-lab method variability.

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

A comparison of the r & R results was presented in Table 5. Generally speaking, both the repeatability and reproducibility in this study were comparable or slightly less than the corresponding values seen in the 2016 and 2019 studies.

7. Recommendations

The results of this study were consistent with those in studies of the 2009 CRPs. Most analytes and measures were not statistically significantly different from the results from the initial characterization study conducted in 2016, after manufacturing. The exception was moisture for CRP3.1 and CRP2.1. 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: Analysis of 2016 CORESTA Reference Products - 2021 Analysis

Type of Document: Collaborative Study Protocol

Date: November 20, 2020

Written by: Johan Patring

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, Sub-Group 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), NNitrosoanabasine (NAB) and 4-(N-nitrosomethylamino)-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 2016 and 2009 CRPs. The results of the 2016 study established a baseline for future biennial stability studies.

At the October 2020 virtual Tobacco and Tobacco Products Analytes (TTPA) Sub-Group meeting, the group agreed to initiate the third stability study for the four 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 the 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 third stability 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 5725-2:1994. 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 do not impact the study timelines.

Table 1: Study Timeline

Date	Activity
November 2020	Participants order CRPs
November-February	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	TSNA CRM 72	PH CRM 69	OV CRM 76
Altria	Yes	Yes	Yes	Yes
American Snuff Company	Yes	Yes	Yes	Yes
British American Tobacco, Brazil	Yes	Yes	Yes	Yes
C.I.T. Montepaz S.A., Uruguay	Yes	Yes	Yes	Yes
Enthalpy Analytical, LLC	No	Yes	No	Yes
Essentra, United Kingdom	No	Yes	Yes	Yes
Eurofins Food & Feed Testing Sweden AB	No	Yes	Yes	No
Global Laboratory Services, Inc.	Yes	Yes	Yes	Yes
Imperial Brands, Reemtsma, Germany	Yes	Yes	Yes	No
Japan Tobacco, Leaf Tobacco Research Center	Yes	Yes	Yes	Yes
JTI Ökolab; Vienna, Austria	No	Yes	Yes	No
KT&G	Yes	Yes	Yes	Yes
Labstat ULC	Yes	Yes	Yes	Yes
National Institute of Standards and Technology	Yes	Yes	Yes	No
RJ Reynolds Tobacco Company	Yes	Yes	Yes	Yes
Scandinavian Tobacco Group, The Netherlands	Yes	No	Yes	Yes
Swedish Match NE, Sweden	Yes	Yes	Yes	Yes
Swedish Match North America	Yes	Yes	Yes	Yes
Swisher International	Yes	Yes	Yes	Yes
University of Kentucky	Yes	Yes	Yes	Yes
Zhengzhou Tobacco Research Institute of CNTC	No	No	Yes	Yes

5. Samples

5.1 Samples

Each participating laboratory should request sufficient quantities of the 2016 CRPs from The North Carolina State University Tobacco Analytical Services Laboratory (TASL). Laboratories may use CRPs that they have onsite if the CRPs are unopened and have been maintained at the recommended storage conditions of −20 °C. Samples not stored at −20 °C should not be used for this study. This study does not involve the analysis of the 2009 CRPs. The 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 the 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. 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.

5.2 Receipt

Upon receipt, the samples must immediately be stored in the refrigerator at approximately 4 °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.

5.3 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 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 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.
- 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 analysed as-is, without grinding.

6. Analysis

6.1 Replicates

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

6.2 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 N° 69, Determination of pH in Tobacco and Tobacco Products
- CRM N° 76, Determination of Moisture Content (Oven Volatiles) of Tobacco and Tobacco Products
- CRM N° 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 N° 72, Determination of Tobacco Specific Nitrosamines in Tobacco and Tobacco Products by Liquid Chromatography - Tandem Mass Spectrometry

7. 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 5725-2:1994. Additionally, z-scores will be calculated in order to allow laboratories to compare their results to those of other laboratories.

8. 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 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.
- All test results shall be reported as is (with no correction for moisture content).
- Specify the method used for the analysis of nicotine (CRM N° 87, CRM N° 62 [MTBE], or CRM N° 62 [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 a technical report will be drafted.

APPENDIX B: Raw Data

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

Lab Code	Product	Replicate	Nicotine	NNK	NNN	NAT	NAB	Oven volatiles	pH
			mg/g	µg/g	µg/g	µg/g	µg/g	%	
A	CRP1.1	1	–	0,0509	0,2210	0,1523	0,009049*	–	8,19
A	CRP1.1	2	–	0,0496	0,2149	0,1481	0,00895*	–	8,27
A	CRP1.1	3	–	0,0522	0,2134	0,1423	0,0089*	–	8,28
A	CRP2.1	1	–	2,014	3,652	3,692	0,3122	–	7,77
A	CRP2.1	2	–	2,056	3,591	3,710	0,3041	–	7,76
A	CRP2.1	3	–	1,992	3,456	3,356	0,3019	–	7,76
A	CRP3.1	1	–	2,181	5,176	3,342	0,3045	–	6,89
A	CRP3.1	2	–	2,201	5,206	3,594	0,3013	–	6,89
A	CRP3.1	3	–	2,313	4,775	3,654	0,2715	–	6,89
A	CRP4.1	1	–	0,8319	3,583	1,416	0,1345	–	6,13
A	CRP4.1	2	–	0,8690	3,745	1,512	0,1381	–	6,08
A	CRP4.1	3	–	0,8595	3,556	1,507	0,1362	–	6,09
B	CRP1.1	1	–	0,0534	0,2176	0,1590	0,0082	–	8,243
B	CRP1.1	2	–	0,0551	0,2158	0,1551	0,0086	–	8,240
B	CRP1.1	3	–	0,0503	0,1972	0,1394	0,0079	–	8,245
B	CRP2.1	1	–	1,984	3,374	3,713	0,2232	–	7,757
B	CRP2.1	2	–	1,910	3,297	3,772	0,2264	–	7,755
B	CRP2.1	3	–	1,981	3,321	3,715	0,2334	–	7,749
B	CRP3.1	1	–	2,542	5,603	4,080	0,2797	–	6,951
B	CRP3.1	2	–	2,548	5,798	4,071	0,2798	–	6,949
B	CRP3.1	3	–	2,609	5,644	4,042	0,2846	–	6,953
B	CRP4.1	1	–	0,8045	3,602	1,499	0,1096	–	6,116
B	CRP4.1	2	–	0,8281	3,619	1,535	0,1064	–	6,128
B	CRP4.1	3	–	0,7984	3,551	1,519	0,1044	–	6,129
C	CRP1.1	1	7,266	0,0426	0,1697	0,1151	0,0065	54,22	8,12
C	CRP1.1	2	7,208	0,0521	0,2069	0,1468	0,0086	53,75	8,12
C	CRP1.1	3	7,199	0,0504	0,2015	0,1405	0,0088	53,79	8,13
C	CRP2.1	1	9,860	2,232	3,780	4,070	0,2640	51,29	7,61
C	CRP2.1	2	9,834	2,212	3,803	4,039	0,2650	51,58	7,61
C	CRP2.1	3	9,814	2,223	3,767	4,189	0,2620	51,52	7,61
C	CRP3.1	1	15,96	2,808	6,249	4,147	0,2980	8,740	6,91
C	CRP3.1	2	15,92	2,791	6,250	4,170	0,3040	8,840	6,92
C	CRP3.1	3	15,91	2,796	6,179	4,194	0,3020	8,830	6,92

Lab Code	Product	Replicate	Nicotine	NNK	NNN	NAT	NAB	Oven volatiles	pH
			mg/g	µg/g	µg/g	µg/g	µg/g	%	
C	CRP4.1	1	8,565	0,8660	3,832	1,578	0,1170	24,41	6,15
C	CRP4.1	2	8,523	0,9000	3,745	1,611	0,1190	24,31	6,15
C	CRP4.1	3	8,533	0,9050	3,787	1,575	0,1190	24,18	6,16
D	CRP1.1	1	8,033	0,04479*	0,2028	0,1370	0,007837*	52,20	8,221
D	CRP1.1	2	8,099	0,04335*	0,1976	0,1285	0,007159*	54,28	8,255
D	CRP1.1	3	7,811	0,04689*	0,2055	0,1337	0,007814*	54,56	8,263
D	CRP2.1	1	10,51	1,984	3,553	3,766	0,2382	51,35	7,692
D	CRP2.1	2	10,57	2,002	3,462	3,756	0,2426	51,47	7,687
D	CRP2.1	3	10,57	2,041	3,503	3,776	0,2384	51,22	7,684
D	CRP3.1	1	16,82	2,377	5,771	3,977	0,2677	8,148	6,813
D	CRP3.1	2	16,95	2,446	5,948	3,973	0,2673	8,275	6,803
D	CRP3.1	3	16,95	2,470	5,767	3,910	0,2687	8,153	6,824
D	CRP4.1	1	8,990	0,7612	3,499	1,439	0,1025	22,70	6,072
D	CRP4.1	2	9,010	0,7905	3,665	1,438	0,1045	23,14	6,081
D	CRP4.1	3	8,990	0,7486	3,582	1,449	0,1048	23,33	6,085
E	CRP1.1	1	7,718	0,037	0,172	0,113	0,008	55,440	8,240
E	CRP1.1	2	7,783	0,048	0,268	0,146	0,009	55,431	8,230
E	CRP1.1	3	8,081	0,040	0,225	0,151	0,008	55,358	8,200
E	CRP2.1	1	10,895	2,102	3,236	4,178	0,296	51,224	7,710
E	CRP2.1	2	10,727	2,430	3,594	4,420	0,314	50,835	7,700
E	CRP2.1	3	11,235	2,134	3,460	4,040	0,262	51,251	7,680
E	CRP3.1	1	16,945	2,684	6,598	4,164	0,294	8,223	6,940
E	CRP3.1	2	16,813	2,668	6,106	4,148	0,328	8,289	6,900
E	CRP3.1	3	16,963	2,732	5,574	4,112	0,354	7,837	6,890
E	CRP4.1	1	9,217	0,866	3,888	1,556	0,122	24,359	6,050
E	CRP4.1	2	9,092	0,738	3,998	1,646	0,118	24,022	6,060
E	CRP4.1	3	8,958	0,824	3,684	1,542	0,110	24,271	6,070
F	CRP1.1	1	7,615	0,04963	0,2093	0,1459	0,01179	54,51	8,143
F	CRP1.1	2	7,471	0,05159	0,2034	0,1414	0,00899	53,67	8,127
F	CRP1.1	3	7,366	0,04687	0,1980	0,1402	0,01313	53,73	8,139
F	CRP2.1	1	10,27	2,052	3,387	4,015	0,2614	51,15	7,689
F	CRP2.1	2	10,30	2,093	3,348	4,121	0,2817	50,96	7,680
F	CRP2.1	3	10,40	2,158	3,380	4,021	0,2666	50,95	7,674
F	CRP3.1	1	16,63	2,734	5,398	4,065	0,2935	8,453	6,819
F	CRP3.1	2	16,74	2,746	5,590	4,024	0,3062	8,476	6,833
F	CRP3.1	3	16,67	2,607	5,640	4,053	0,2957	8,401	6,840

Lab Code	Product	Replicate	Nicotine	NNK	NNN	NAT	NAB	Oven volatiles	pH
			mg/g	µg/g	µg/g	µg/g	µg/g	%	
F	CRP4.1	1	9,051	0,9136	3,632	1,634	0,1259	23,52	6,071
F	CRP4.1	2	9,026	0,8206	3,496	1,597	0,1230	23,46	6,069
F	CRP4.1	3	9,065	0,8501	3,673	1,548	0,1256	23,59	6,068
G	CRP1.1	1	8,2231	0,0539	0,1978	0,1435	0,0094	53,39	8,340
G	CRP1.1	2	8,0525	0,0544	0,1946	0,1457	0,0095	53,00	8,300
G	CRP1.1	3	7,7777	0,0546	0,1954	0,1449	0,0093	53,30	8,320
G	CRP2.1	1	10,9361	2,0756	3,4499	4,3693	0,2784	49,84	7,730
G	CRP2.1	2	11,0784	2,0841	3,4545	4,2869	0,2725	53,30	7,710
G	CRP2.1	3	10,9872	2,0677	3,4716	4,2019	0,2664	51,96	7,700
G	CRP3.1	1	17,3501	2,5835	5,5756	4,3606	0,3040	9,124	6,850
G	CRP3.1	2	17,3729	2,5911	5,5856	4,3658	0,3086	9,181	6,870
G	CRP3.1	3	17,3711	2,5998	5,5109	4,3768	0,3001	9,060	6,800
G	CRP4.1	1	9,1182	0,7867	3,4394	1,5647	0,1146	24,89	5,970
G	CRP4.1	2	9,0869	0,8029	3,5569	1,5708	0,1164	25,17	5,980
G	CRP4.1	3	9,0162	0,7791	3,4179	1,5899	0,1128	24,88	5,960
H	CRP1.1	1	7,482	0,0508	0,1900	0,1360	0,0080	53,76	8,16
H	CRP1.1	2	7,861	0,0479	0,1750	0,1350	0,0079	54,89	8,18
H	CRP1.1	3	7,754	0,0498	0,1840	0,1380	0,0092	54,81	8,17
H	CRP2.1	1	10,96	1,944	3,212	3,963	0,2390	51,39	7,72
H	CRP2.1	2	10,74	2,014	3,316	4,061	0,2400	51,24	7,72
H	CRP2.1	3	10,83	1,985	3,202	3,869	0,2220	51,76	7,72
H	CRP3.1	1	16,85	2,417	5,327	4,058	0,2660	8,248	6,90
H	CRP3.1	2	16,89	2,479	5,312	3,897	0,2780	8,280	6,89
H	CRP3.1	3	17,00	2,421	5,229	4,087	0,2720	7,913	6,90
H	CRP4.1	1	8,812	0,7610	3,118	1,506	0,1030	26,01	6,12
H	CRP4.1	2	8,538	0,7080	3,137	1,480	0,1010	25,06	6,13
H	CRP4.1	3	8,746	0,7290	3,224	1,468	0,1030	25,05	6,12
I	CRP1.1	1	7,189	0,0508	0,1995	0,1400	0,0074	53,75	8,25
I	CRP1.1	2	7,137	0,0499	0,1943	0,1408	0,0076	53,47	8,27
I	CRP1.1	3	7,192	0,0523	0,2008	0,1460	0,0076	53,39	8,37
I	CRP2.1	1	10,10	2,1060	3,889	4,264	0,2787	51,23	7,85
I	CRP2.1	2	10,17	2,110	3,790	4,285	0,2736	51,30	7,85
I	CRP2.1	3	10,12	2,108	3,716	4,332	0,2829	51,20	7,85
I	CRP3.1	1	16,27	2,633	6,268	4,507	0,3074	8,069	7,00
I	CRP3.1	2	16,21	2,641	6,284	4,506	0,3183	7,927	6,97
I	CRP3.1	3	16,27	2,625	6,208	4,478	0,3180	8,092	6,99

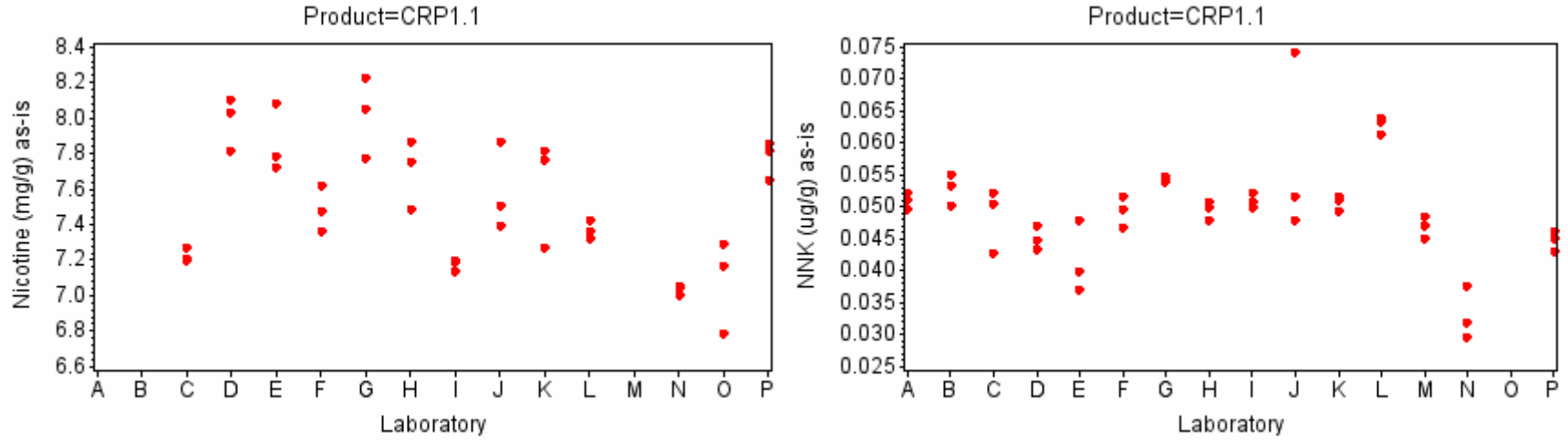
Lab Code	Product	Replicate	Nicotine	NNK	NNN	NAT	NAB	Oven volatiles	pH
			mg/g	µg/g	µg/g	µg/g	µg/g	%	
I	CRP4.1	1	8,443	0,7901	3,666	1,570	0,1093	23,77	6,12
I	CRP4.1	2	8,576	0,8016	3,721	1,604	0,1089	23,72	6,13
I	CRP4.1	3	8,590	0,8029	3,730	1,626	0,1139	23,58	6,12
J	CRP1.1	1	7,393	0,0516	0,2028	0,1464	0,0048	51,86	8,08
J	CRP1.1	2	7,867	0,0743	0,2103	0,1246	0,0057	51,75	8,09
J	CRP1.1	3	7,501	0,0478	0,1889	0,1199	0,0090	55,04	8,00
J	CRP2.1	1	11,34	2,073	3,545	3,908	0,2328	49,73	7,60
J	CRP2.1	2	11,43	2,119	3,498	4,106	0,2303	50,64	7,62
J	CRP2.1	3	11,38	1,965	3,462	3,952	0,2696	50,48	7,64
J	CRP3.1	1	17,38	2,224	5,698	4,200	0,3075	8,158	6,91
J	CRP3.1	2	17,21	2,287	5,629	4,397	0,3267	8,087	6,79
J	CRP3.1	3	17,04	2,240	5,364	4,594	0,3363	7,967	6,82
J	CRP4.1	1	8,810	0,7374	3,170	1,250	0,1126	24,63	5,89
J	CRP4.1	2	8,841	0,8346	3,386	1,262	0,1565	24,03	5,93
J	CRP4.1	3	8,832	0,7969	3,402	1,141	0,1028	23,86	5,95
K	CRP1.1	1	7,813	0,0515	0,1924	0,1426	0,0085	52,91	8,270
K	CRP1.1	2	7,764	0,0511	0,1913	0,1432	0,0087	53,41	8,287
K	CRP1.1	3	7,272	0,0493	0,1859	0,1369	0,0079	54,05	8,301
K	CRP2.1	1	11,08	2,0360	3,507	4,257	0,2737	50,93	7,731
K	CRP2.1	2	11,05	2,0430	3,401	4,292	0,2677	51,16	7,728
K	CRP2.1	3	11,09	2,0620	3,465	4,187	0,2802	51,50	7,725
K	CRP3.1	1	17,57	2,4200	5,589	4,247	0,3048	8,010	6,925
K	CRP3.1	2	17,45	2,4620	5,669	4,315	0,3081	8,020	6,965
K	CRP3.1	3	17,50	2,4580	5,538	4,234	0,3040	7,992	6,957
K	CRP4.1	1	9,416	0,8487	3,384	1,614	0,1169	24,06	6,037
K	CRP4.1	2	9,485	0,7697	3,423	1,570	0,1163	23,68	6,036
K	CRP4.1	3	9,493	0,7698	3,328	1,541	0,1125	24,07	6,047
L	CRP1.1	1	7,321	0,0612	0,2455	0,1600	0,0066	53,71	8,280
L	CRP1.1	2	7,358	0,0640	0,2249	0,1462	0,0054	53,74	8,300
L	CRP1.1	3	7,428	0,0632	0,2209	0,1487	0,0056	53,96	8,290
L	CRP2.1	1	10,49	2,253	3,999	4,277	0,3002	49,43	7,750
L	CRP2.1	2	10,46	2,287	3,751	4,375	0,2470	50,45	7,755
L	CRP2.1	3	10,56	2,096	3,916	4,582	0,3064	50,80	7,755
L	CRP3.1	1	16,53	2,521	5,832	4,434	0,3140	8,985	6,925
L	CRP3.1	2	16,57	2,625	6,313	3,928	0,3596	8,967	6,935
L	CRP3.1	3	16,55	2,806	6,297	4,185	0,3268	9,000	6,940

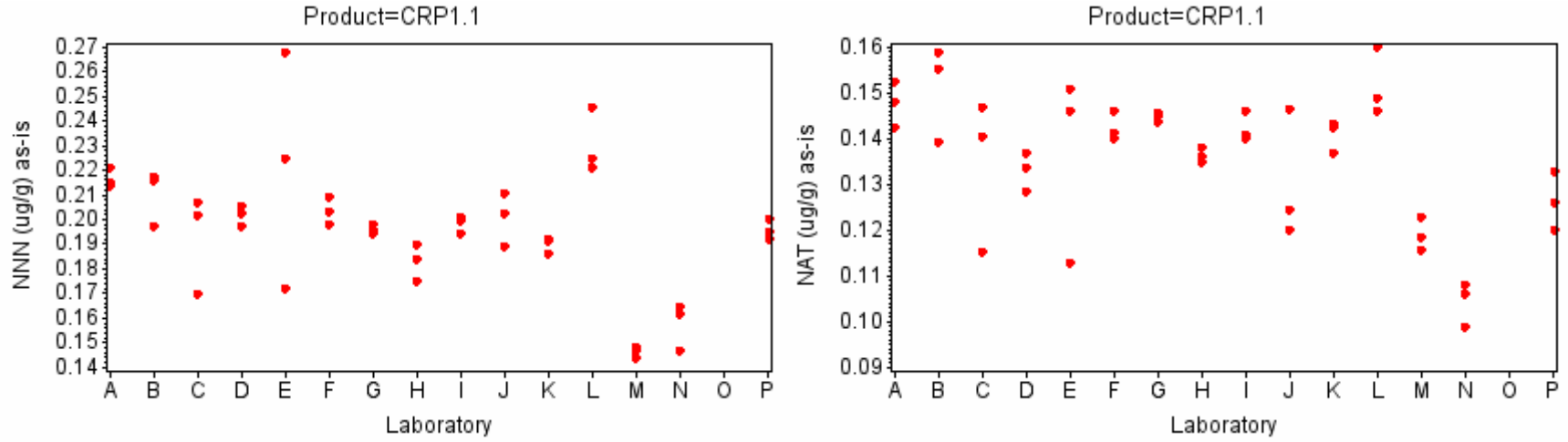
Lab Code	Product	Replicate	Nicotine	NNK	NNN	NAT	NAB	Oven volatiles	pH
			mg/g	µg/g	µg/g	µg/g	µg/g	%	
L	CRP4.1	1	8,879	0,9039	3,823	1,465	0,1278	24,43	6,150
L	CRP4.1	2	8,851	0,9341	3,257	1,637	0,1186	24,50	6,145
L	CRP4.1	3	8,795	0,8193	3,997	1,527	0,1347	24,46	6,160
M	CRP1.1	1	–	0,04515	0,1440	0,1155	0,01155	52,93	8,34
M	CRP1.1	2	–	0,04690	0,1465	0,1185	0,01025	53,77	8,35
M	CRP1.1	3	–	0,04850	0,1480	0,1230	0,01215	52,50	8,39
M	CRP2.1	1	–	1,570	2,640	2,960	0,2530	50,78	7,82
M	CRP2.1	2	–	1,590	2,650	2,980	0,2645	51,02	7,83
M	CRP2.1	3	–	1,650	2,740	3,010	0,2585	50,83	7,82
M	CRP3.1	1	–	2,440	4,000	3,290	0,2700	7,713	6,97
M	CRP3.1	2	–	2,400	3,930	3,240	0,2680	7,737	6,95
M	CRP3.1	3	–	2,390	3,970	3,190	0,2650	7,737	6,94
M	CRP4.1	1	–	0,6850	2,575	1,305	0,1060	23,18	6,13
M	CRP4.1	2	–	0,6800	2,570	1,265	0,1085	23,20	6,13
M	CRP4.1	3	–	0,6800	2,580	1,275	0,1035	23,17	6,12
N	CRP1.1	1	7,054	0,03773*	0,1614	0,106	0,004565*	–	8,145
N	CRP1.1	2	7,039	0,03202*	0,1642	0,108	0,006102*	–	8,116
N	CRP1.1	3	7,000	0,02963*	0,1467	0,099	0,005502*	–	8,141
N	CRP2.1	1	9,557	1,861	3,015	3,551	0,2428	–	7,686
N	CRP2.1	2	9,570	1,817	2,952	3,625	0,2346	–	7,680
N	CRP2.1	3	9,644	1,773	3,033	3,407	0,2216	–	7,678
N	CRP3.1	1	16,31	2,378	5,146	3,988	0,2881	–	6,858
N	CRP3.1	2	16,18	2,430	4,879	4,010	0,3042	–	6,859
N	CRP3.1	3	16,13	2,322	4,880	4,033	0,2696	–	6,891
N	CRP4.1	1	8,245	0,480	2,024	0,8802	0,0604	–	6,106
N	CRP4.1	2	8,155	0,545	2,468	1,093	0,0703	–	6,140
N	CRP4.1	3	8,280	0,558	2,428	1,076	0,0752	–	6,110
O	CRP1.1	1	7,169	–	–	–	–	53,49	8,15
O	CRP1.1	2	6,790	–	–	–	–	52,80	8,17
O	CRP1.1	3	7,293	–	–	–	–	52,84	8,12
O	CRP2.1	1	11,03	–	–	–	–	51,23	7,71
O	CRP2.1	2	11,00	–	–	–	–	51,20	7,70
O	CRP2.1	3	10,91	–	–	–	–	51,15	7,70
O	CRP3.1	1	17,37	–	–	–	–	7,780	6,95
O	CRP3.1	2	16,82	–	–	–	–	8,070	6,94
O	CRP3.1	3	16,78	–	–	–	–	7,720	6,94

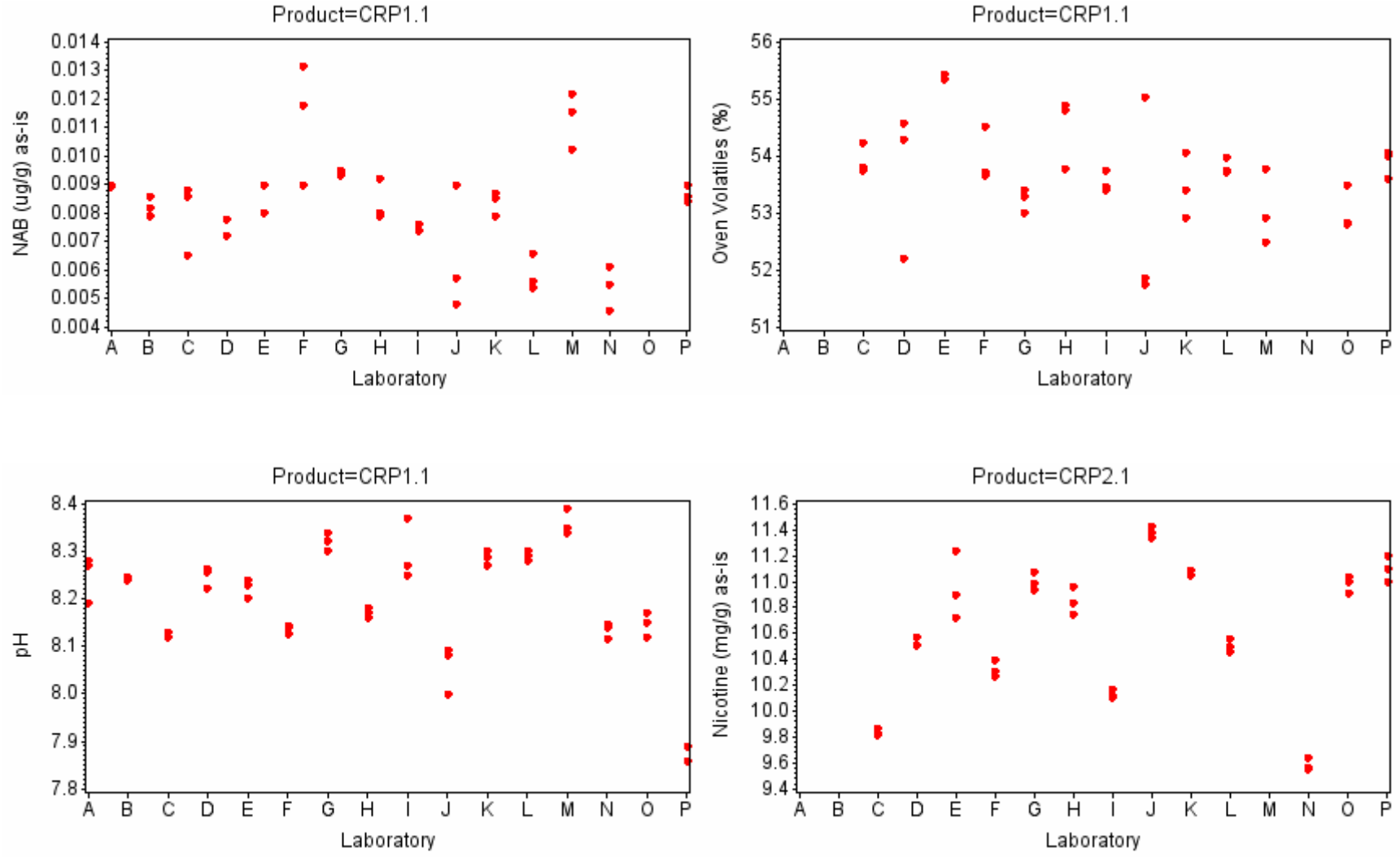
Lab Code	Product	Replicate	Nicotine	NNK	NNN	NAT	NAB	Oven volatiles	pH
			mg/g	µg/g	µg/g	µg/g	µg/g	%	
O	CRP4.1	1	9,766	–	–	–	–	23,17	6,24
O	CRP4.1	2	9,454	–	–	–	–	23,35	6,15
O	CRP4.1	3	9,481	–	–	–	–	23,46	6,15
P	CRP1.1	1	7,650	0,0462	0,2000	0,1330	0,0084	54,01	7,89
P	CRP1.1	2	7,810	0,0451	0,1950	0,1260	0,0090	54,05	7,86
P	CRP1.1	3	7,860	0,0429	0,1920	0,1200	0,0086	53,61	7,89
P	CRP2.1	1	11,00	2,110	3,450	3,910	0,2780	51,17	7,57
P	CRP2.1	2	11,20	2,110	3,460	3,990	0,2680	51,22	7,65
P	CRP2.1	3	11,10	2,110	3,540	3,900	0,2700	51,46	7,58
P	CRP3.1	1	17,20	2,560	5,760	4,190	0,2980	8,540	6,04
P	CRP3.1	2	17,10	2,570	5,730	4,160	0,3090	8,560	6,08
P	CRP3.1	3	17,30	2,550	5,490	4,120	0,3060	8,500	6,05
P	CRP4.1	1	9,390	0,8400	3,440	1,500	0,1110	25,53	6,90
P	CRP4.1	2	9,350	0,8300	3,570	1,500	0,1140	25,62	6,95
P	CRP4.1	3	9,340	0,7870	3,440	1,430	0,1100	25,48	6,91

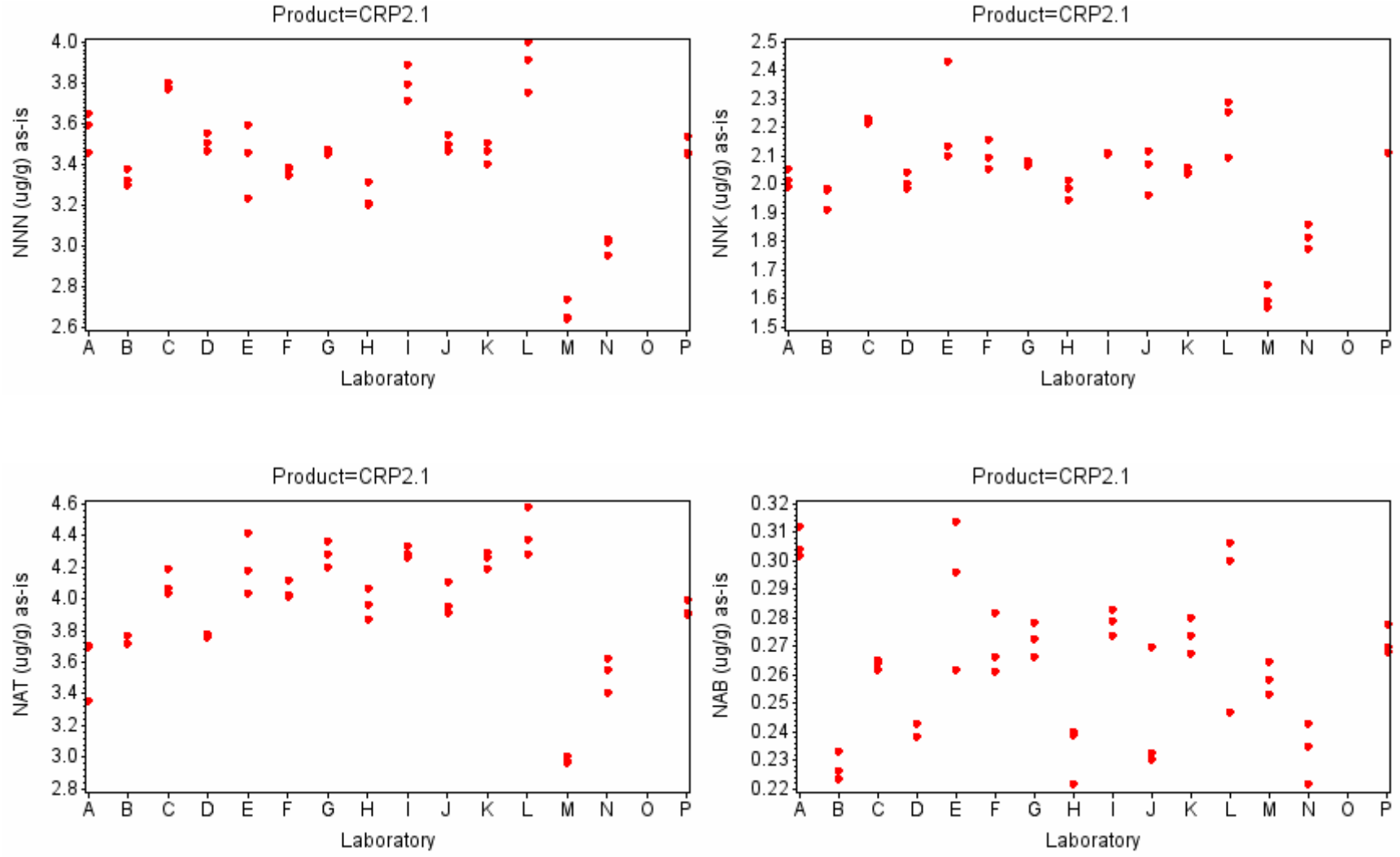
The (–) symbol indicates the laboratory did not submit data for that sample analysis.
The * symbol indicates that the value was below their reporting limit.

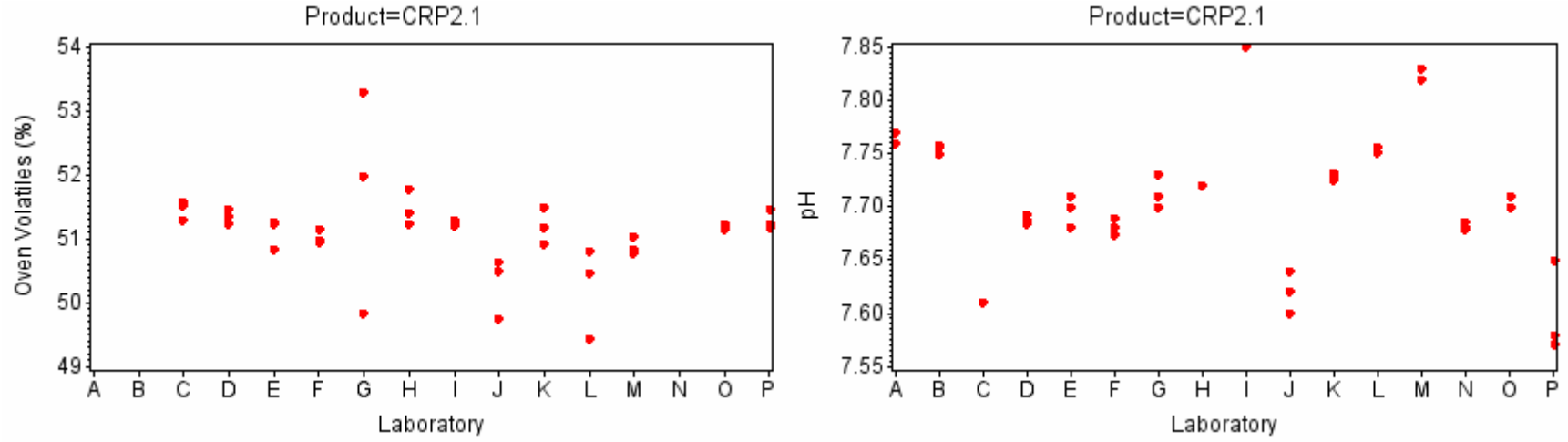
APPENDIX C: Raw Data Plots for 2021 Study



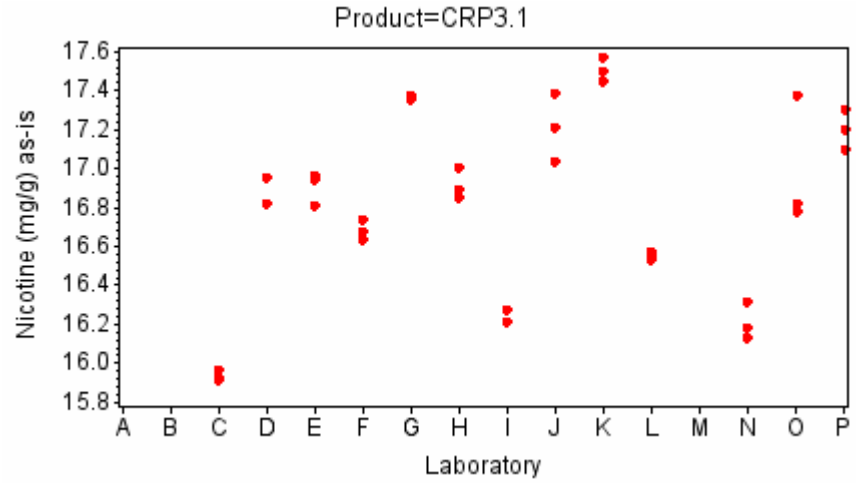



Product=CRP1.1
NAB (ug/g) as-is
0.014
0.013
0.012
0.011
0.010
0.009
0.008
0.007
0.006
0.005
0.004
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP1.1
Oven Volatiles (%)
56
55
54
53
52
51
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP1.1
pH
8.4
8.3
8.2
8.1
8.0
7.9
7.8
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP2.1
Nicotine (mg/g) as-is
11.6
11.4
11.2
11.0
10.8
10.6
10.4
10.2
10.0
9.8
9.6
9.4
A B C D E F G H I J K L M N O P
Laboratory


Product=CRP2.1
NNN (ug/g) as-is
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP2.1
NNK (ug/g) as-is
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP2.1
NAT (ug/g) as-is
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP2.1
NAB (ug/g) as-is
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


Product=CRP2.1
Oven Volatiles (%)
54
53
52
51
50
49
A B C D E F G H I J K L M N O P
Laboratory

Product=CRP2.1
pH
7.85
7.80
7.75
7.70
7.65
7.60
7.55
A B C D E F G H I J K L M N O P
Laboratory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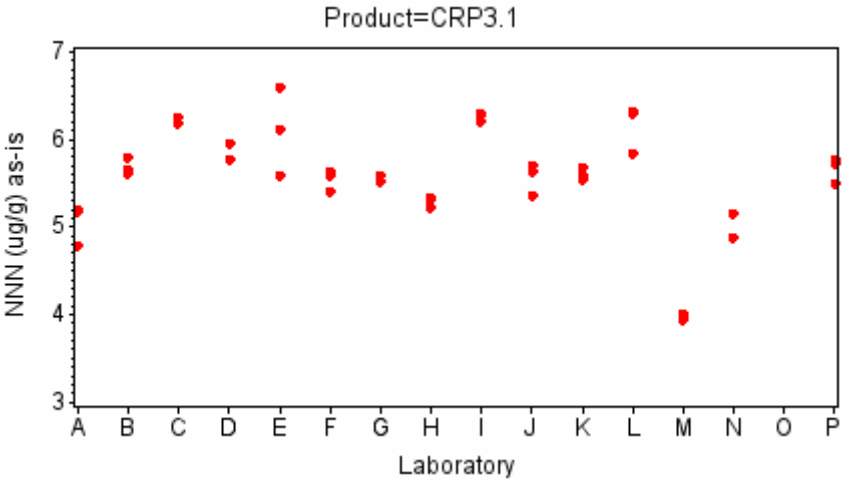
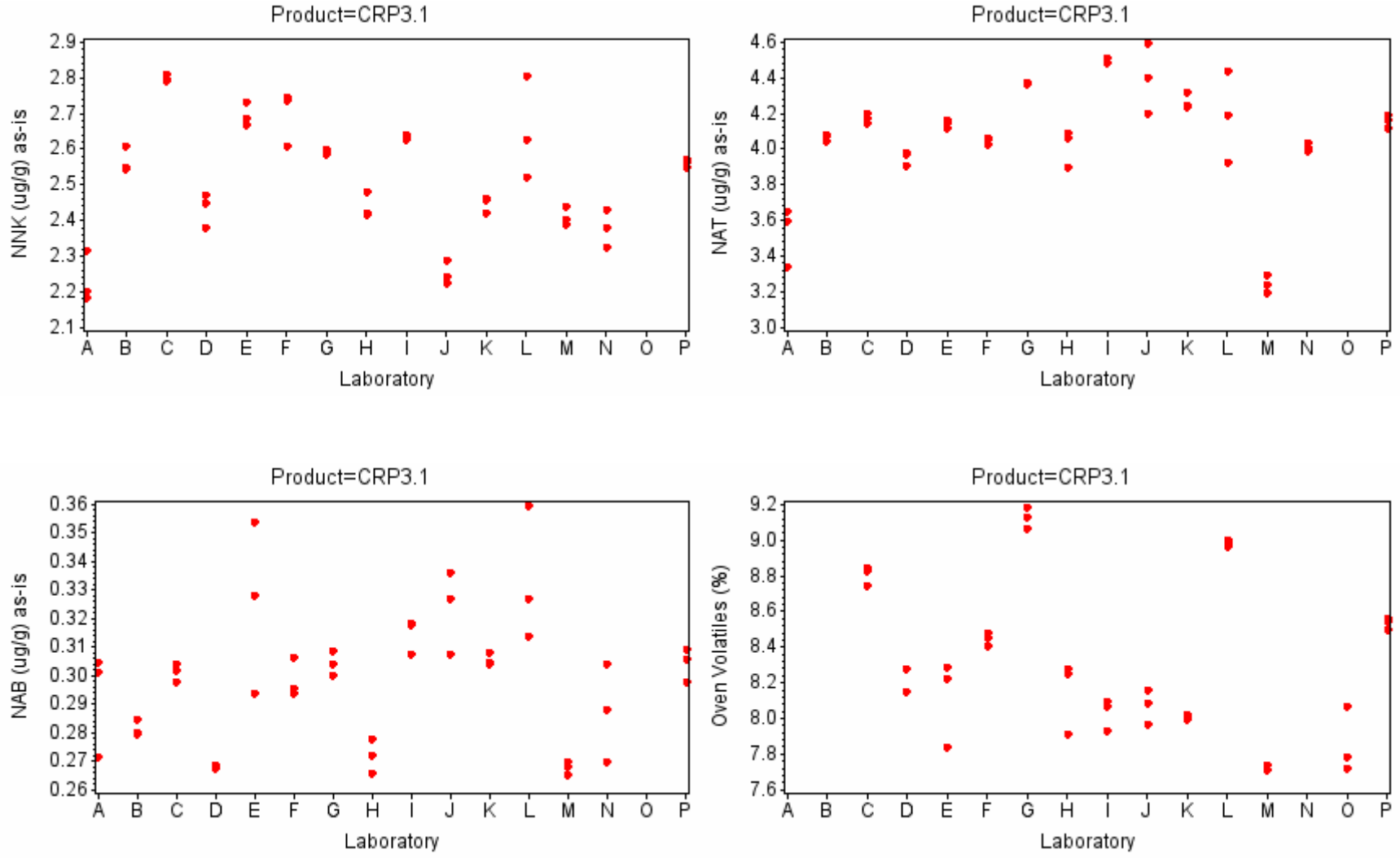
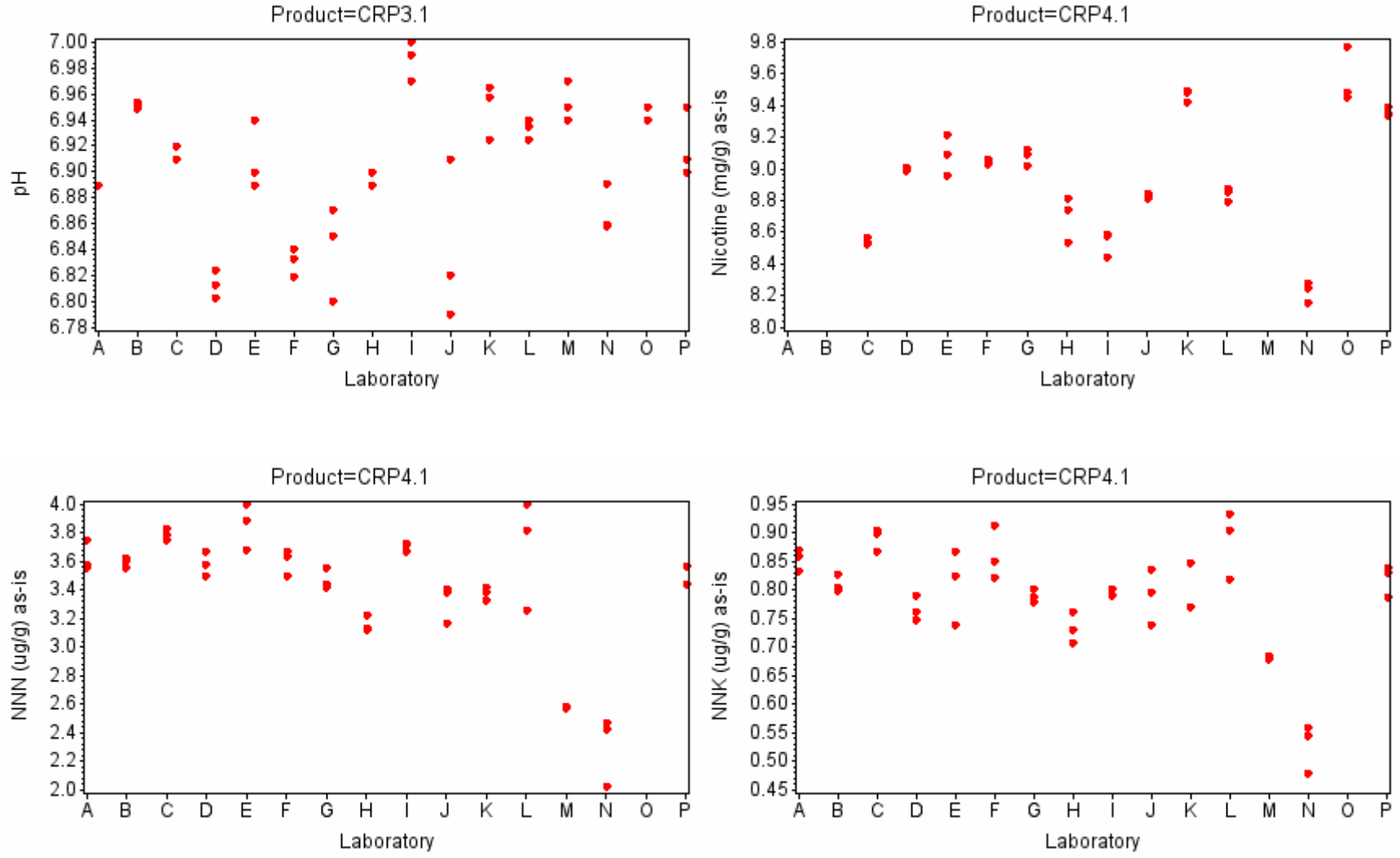
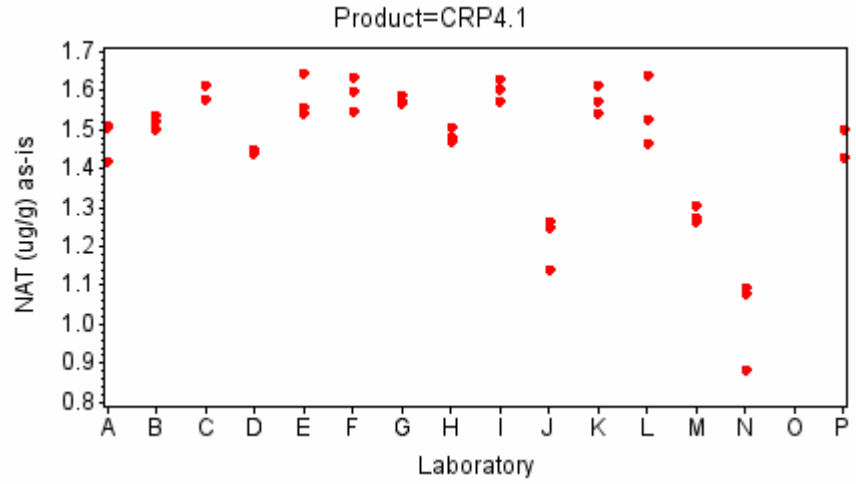
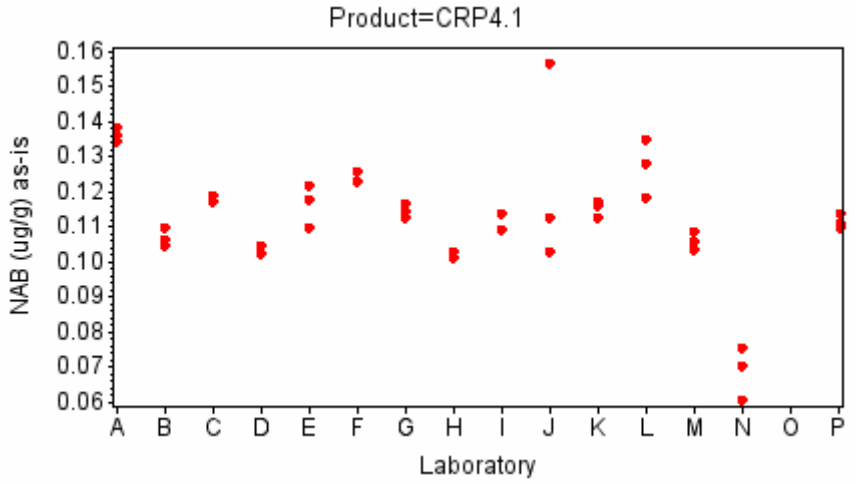
Nicotine (mg/g) as-is
17.6
17.4
17.2
17.0
16.8
16.6
16.4
16.2
16.0
15.8
A B C D E F G H I J K L M N O P
Laboratory

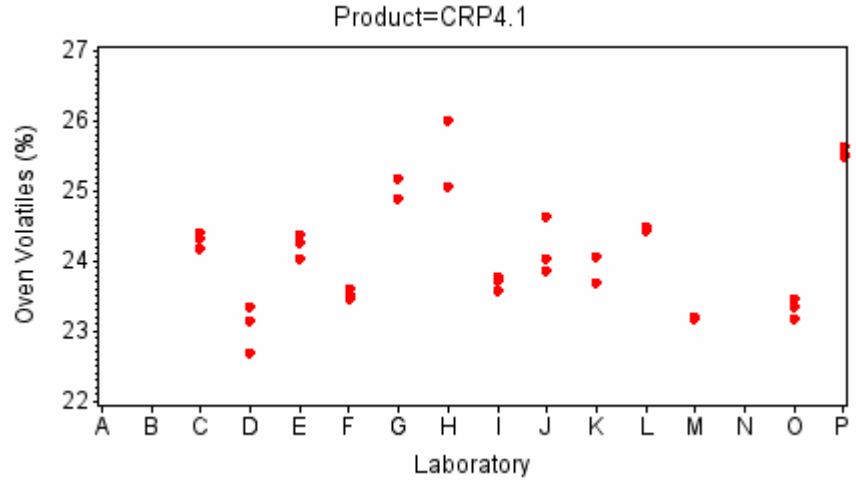

Product=CRP3.1
NNN (ug/g) as-is
7
6
5
4
3
A B C D E F G H I J K L M N O P
Laboratory

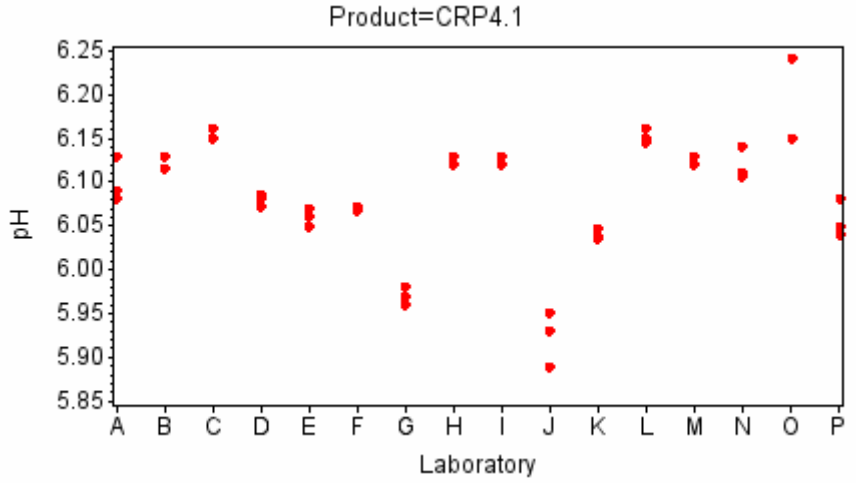

Product=CRP3.1
NNK (ug/g) as-is
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP3.1
NAT (ug/g) as-is
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP3.1
NAB (ug/g) as-is
0.36
0.35
0.34
0.33
0.32
0.31
0.30
0.29
0.28
0.27
0.26
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP3.1
Oven Volatiles (%)
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
A B C D E F G H I J K L M N O P
Laboratory


Product=CRP3.1
pH
7.00
6.98
6.96
6.94
6.92
6.90
6.88
6.86
6.84
6.82
6.80
6.78
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP4.1
Nicotine (mg/g) as-is
9.8
9.6
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP4.1
NNN (ug/g) as-is
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
A B C D E F G H I J K L M N O P
Laboratory
Product=CRP4.1
NNK (ug/g) as-is
0.95
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
Laboratory

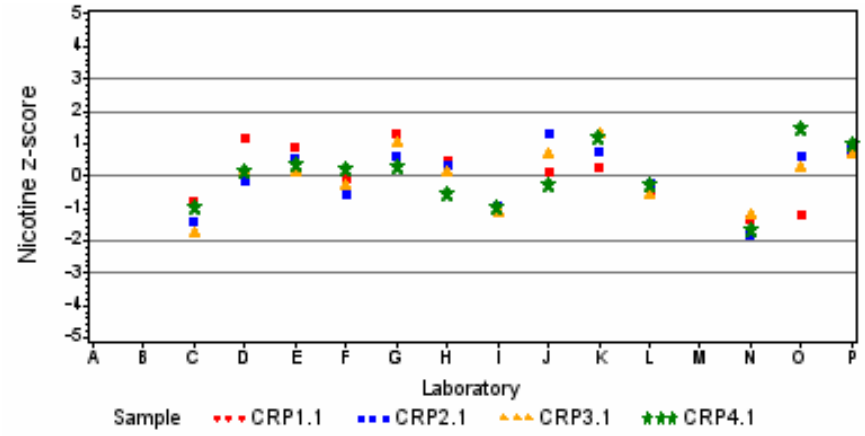

Product=CRP4.1
NAT (ug/g) as-is
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
A B C D E F G H I J K L M N O P
Laboratory

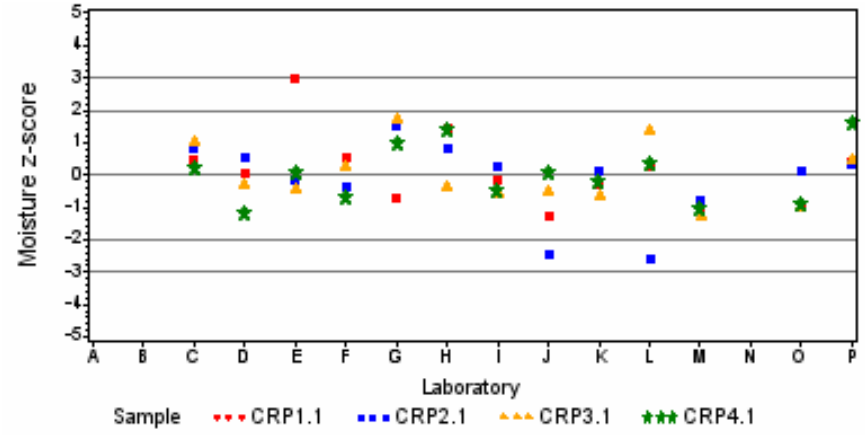

Product=CRP4.1
NAB (ug/g) as-is
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
A B C D E F G H I J K L M N O P
Laboratory

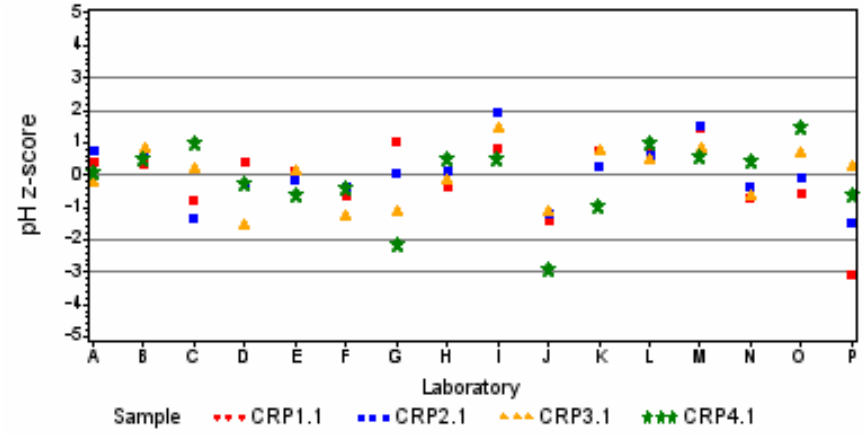

Product=CRP4.1
Oven Volatiles (%)
27
26
25
24
23
22
A B C D E F G H I J K L M N O P
Laboratory

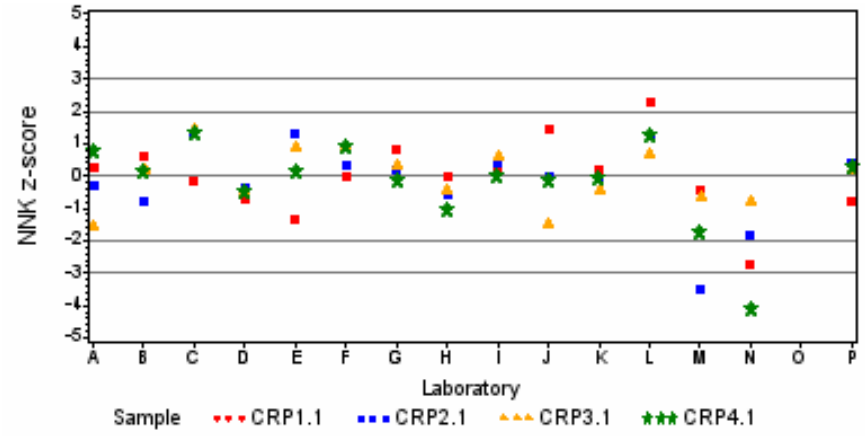

Product=CRP4.1
pH
6.25
6.20
6.15
6.10
6.05
6.00
5.95
5.90
5.85
A B C D E F G H I J K L M N O P
Laboratory

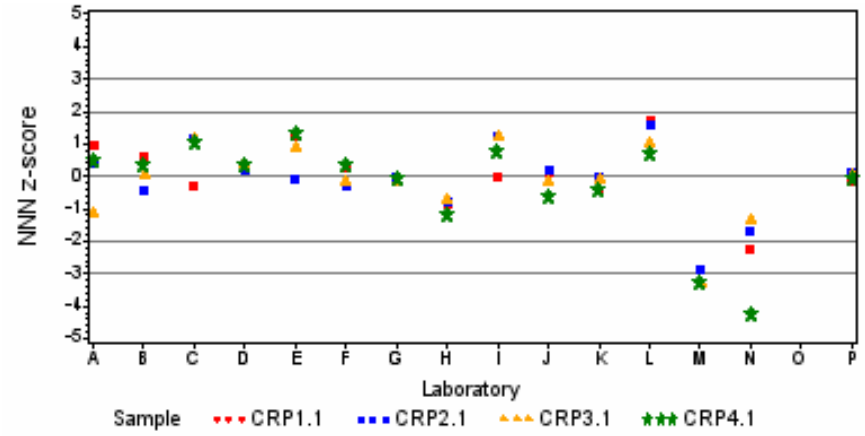
APPENDIX D: Graphs of the Z-Scores

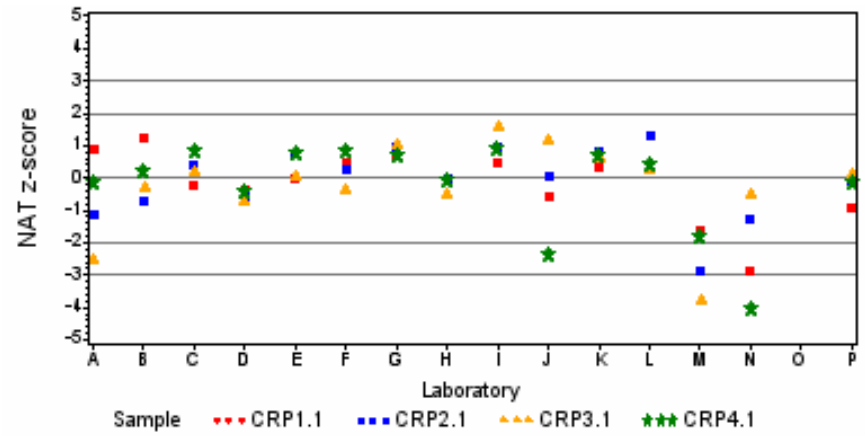


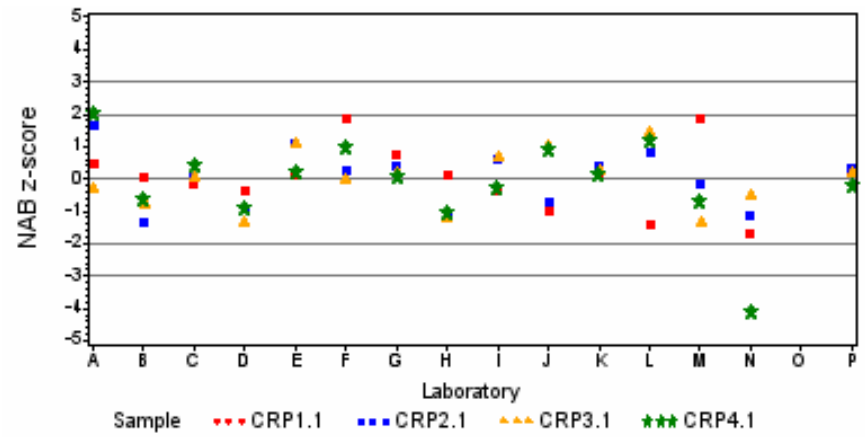





NNK z-score
5
4
3
2
1
0
-1
-2
-3
-4
-5
A B C D E F G H I J K L M N O P
Laboratory
Sample CRP1.1 CRP2.1 CRP3.1 CRP4.1


NNN z-score
5
4
3
2
1
0
-1
-2
-3
-4
-5
A B C D E F G H I J K L M N O P
Laboratory
Sample CRP1.1 CRP2.1 CRP3.1 CRP4.1


NAT z-score
5
4
3
2
1
0
-1
-2
-3
-4
-5
A B C D E F G H I J K L M N O P
Laboratory
Sample CRP1.1 CRP2.1 CRP3.1 CRP4.1


NAB z-score
5
4
3
2
1
0
-1
-2
-3
-4
-5
A B C D E F G H I J K L M N O P
Laboratory
Sample CRP1.1 CRP2.1 CRP3.1 CRP4.1