Smokeless Tobacco Sub-Group

Technical Report

CORESTA Reference Products

2012 and 2013 Analysis

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1. Summary

In 2012 and 2013 the CORESTA Smokeless Tobacco Sub-Group (STS) conducted the second and third consecutive Working Group 4 (WG4) interlaboratory studies designed to assess the stability of the four CORESTA Reference Products (CRPs). Eight laboratories participated in the 2012 study and ten laboratories participated in the 2013 study. The participating laboratories reported the levels of nicotine, pH, moisture (oven volatiles) and tobacco specific nitrosamines (TSNAs) using CORESTA Recommended Methods (CRMs). These analytes were recommended by the STS as appropriate markers for monitoring product stability in an initial study conducted in 2010. Tabulated data are presented along with repeatability (r) and reproducibility (R) and z-scores for these two study years.

The results from the stability analysis over all study years showed only a few trends. Specifically:

- There was a statistically significant increase in moisture for CRP3 over the study years.
- There was an increase in interlaboratory variability for pH for CRP3 over the study years.
- There was an increase in interlaboratory variability for moisture for CRP4, with a notable increase in 2012.

Based on the results taken in their entirety, the conclusion of this study is that all four CRPs are suitable for continued use as reference products. Lack of any clear and significant trends in the stability results indicate that storage at −20 °C is an appropriate storage condition for the four reference products. The recommendations for 2014 are to continue to monitor the stability of the CRPs and to require all participating laboratories to procure the CRPs immediately prior to the laboratory phase of the study to mitigate differences in long term storage conditions.

2. Introduction

In November 2008, the Smokeless Tobacco Sub-Group (STS) 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 greater than ten years old.

At the Amelia Island, Florida STS meeting (October 2009), Working Group Four (WG4) was organized to proceed with the chemical characterization of the four CRPs. The protocol for the first WG4 study[1] was distributed in December 2009 and the study was conducted in 2010. This study included 43 analytes and did not specify methods of analysis. Many of the results from this study showed a wide range in analyte yields, which is not unexpected since the methods were not harmonized. The recommendations from the 2010 study were to monitor the stability of the CRPs on an annual basis, by determining the levels of nicotine, pH, moisture (oven volatiles) and TSNAs using CORESTA Recommended Methods (CRMs) or draft CRMs. This annual analysis would allow the STS to determine when the CRPs should be remanufactured.

At the STS Meeting in Hamburg, Germany (May 2011), it was decided to adopt the recommendations from the 2010 WG4 study and initiate the first annual analysis of the four CRPs using CRMs or draft CRMs. The first WG4 study using CRMs or draft CRMs was

[1] Smokeless Tobacco Sub-Group Technical Report: CORESTA Reference Products 2010 Analysis, February, 2014

conducted in 2011, and a similar study has been carried out annually since that time. The focus of this report is the 2012 and 2013 studies. Repeatability and reproducibility (r & R) and z-scores were calculated for the 2012 and 2013 studies while the stability analysis includes data from 2010-2013. As mentioned earlier, methods of analysis were not specified for the 2010 WG4; however, the data were considered to be similar enough for inclusion in the analysis.

The CRPs continue to be stored at -20 °C and distributed by the North Carolina State University (NCSU) Tobacco Analytical Services Lab under the direction of Dr. Ramsey Lewis[2] and Karen Andres[3].

2.1 Objective

The participating laboratories, in the 2012 and 2013 WG4 analysis of the CRPs, were to provide analytical results for pH, moisture (oven volatiles), nicotine, and the four TSNAs (N-nitrosonornicotine (NNN), N-nitrosoanatabine (NAT), N-nitrosoanabasine (NAB) and 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)). This work was conducted using the applicable CRMs referenced in Section 3.

These studies were conducted to support the assessment of stability of the CRPs and to provide an assessment of inter-laboratory variability. Data were collected from the participating laboratories and statistically evaluated in basic conformance with the recommendations of ISO 5725(2) and ISO/TR 22971. The stability analysis was conducted using the study data from 2010 through 2013. Additionally, z-scores were calculated for the 2012 and 2013 data as an additional measure of a laboratory's performance as compared to the results of other laboratories.

3. Organisation

3.1 Participants

A list of the participating laboratories is provided in Table 1. 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. Since the statistical analysis covers several years, the laboratory letter codes were maintained between the tables for all years. Furthermore, not all laboratories participated in all time points or submitted data for all analyses.

Table 1: List of Participating Laboratories in 2012 and 2013 WG4 Studies

2012 WG4 Participants	2013 WG4 Participants
American Snuff Company	Altria Client Services
Arista Laboratories	American Snuff Company
Global Laboratory Services	ITC Limited
ITC Limited	Imperial Tobacco Group, Reemtsma
Japan Tobacco, Inc.	Japan Tobacco, Inc.
Labstat International	R.J. Reynolds Tobacco Company
R.J. Reynolds Tobacco Company	Swedish Match Northern Europe
Swedish Match NE	Swisher International, Inc.
	Technical Center of Shanghai Tobacco Group Co., Ltd
	University of Kentucky

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3.2 Protocol

Specific details from the protocol are described below:

3.2.1 Sample Shipment

Laboratories were responsible for procuring approximately 150g of each of the CRPs for each of the studies. Therefore, the participants may have either procured samples from NCSU immediately before starting a study or they may have chosen to use sample retains, maintained at their facility. The 2012 study start date was August 1, 2012, while the 2013 study start date was July 14, 2013. Laboratories were requested to submit data within 4-6 weeks after initiating each of the studies. The samples are identified in Table 2.

Table 2: Sample Identification

Product Type
CRP1 - Swedish style snus pouch
CRP2 - American-style loose moist snuff
CRP3 - American-style loose dry snuff powder
CRP4 - American-style loose-leaf chewing tobacco

3.2.2 Within Laboratory Sample Preparation

The laboratories were directed to thaw the samples at room temperature for at least 2 hours before use. After this initial thawing, the samples were to be stored at approximately 4 °C in between use. Additionally:

- The snus pouch (CRP1) was to be cut into 2 halves and placed directly into the extraction vessel. Both the tobacco and pouch material were included in the analysis.
- The moist snuff reference product (CRP2) and the dry snuff reference product (CRP3) did not require sample grinding and were analysed as received.
- The loose leaf reference product (CRP4) was to be ground according the participating laboratory's standard procedure.

3.2.3 Sample Analysis and Data Reporting

The WG4 participating laboratories were instructed to conduct triplicate analyses of the following analytes: pH, moisture (oven volatiles), nicotine, and TSNAs. The laboratories were requested to use the following CRMs:

- pH: CRM N° 69, Determination of pH in Smokeless Tobacco Products
- Moisture (oven volatiles): Draft CRM N° 76, Determination of Moisture Content (Oven Volatiles) of Smokeless Tobacco Products
- Nicotine: CRM N° 62, Determination of Nicotine in Tobacco and Tobacco Products by Gas Chromatographic Analysis
- TSNAs: Draft CRM N° 72, Determination of Tobacco Specific Nitrosamines in Smokeless Tobacco Products by Liquid Chromatography - Tandem Mass Spectrometry

Participating laboratories were requested to document any deviations from the CRMs and submit the deviations with their results. All test results were to be reported on an *as-is* basis with no correction for moisture content. The results were not to be rounded and ideally reported to at least one more digit than typically required.

The spread sheet with the results of the analysis and the comments were to be sent by e-mail to John E. Bunch, Study Coordinator.

4. Data - Raw

The full data sets for the 2012 and 2013 WG4 studies are listed in Tables 3 and 4, respectively. Each analysis includes three replicates. Raw data plots that include all replicates, without removal of outliers, are given in Appendices A and B for the two study years.

Table 3: Full Data Set for 2012 WG4 Study

Lab Code	Product	Nicotine	pH	Moisture	NNN	NNK	NAT	NAB
		%		%	µg/g	µg/g	µg/g	µg/g
A	CRP1	1.0036	7.97	51.83	0.591	0.213	0.587	0.040
A	CRP1	0.9920	7.99	51.67	0.661	0.219	0.527	0.040
A	CRP1	1.0050	7.99	51.33	0.593	0.226	0.530	0.034
A	CRP2	1.2662	7.72	55.14	1.713	0.474	1.764	0.169
A	CRP2	1.2553	7.71	54.51	1.884	0.499	2.032	0.162
A	CRP2	1.2502	7.70	54.79	1.805	0.436	2.171	0.151
A	CRP3	2.2681	6.77	9.84	8.374	4.226	5.951	0.423
A	CRP3	2.2871	6.73	10.03	8.799	4.011	5.869	0.410
A	CRP3	2.2750	6.74	9.98	8.377	4.648	6.064	0.400
A	CRP4	1.0619	5.88	22.51	1.839	0.472	1.254	0.058
A	CRP4	1.0669	5.87	22.52	1.780	0.488	1.293	0.065
A	CRP4	1.0812	5.88	22.47	2.207	0.425	1.362	0.066
D	CRP1	0.9040	7.98	51.10	0.732	0.188	0.454	0.047
D	CRP1	0.9060	7.98	51.00	0.744	0.190	0.457	0.044
D	CRP1	0.9050	7.99	50.80	0.763	0.196	0.473	0.048
D	CRP2	1.1900	7.75	54.60	1.918	0.382	1.479	0.175
D	CRP2	1.1900	7.73	54.50	1.929	0.393	1.485	0.171
D	CRP2	1.1900	7.75	54.50	1.856	0.370	1.433	0.162
D	CRP3	2.0900	6.82	9.29	9.236	4.118	5.078	0.448
D	CRP3	2.1100	6.83	9.28	9.624	4.295	5.170	0.437
D	CRP3	2.0900	6.82	9.24	9.973	4.428	5.266	0.487
D	CRP4	1.0600	5.99	17.50	2.195	0.395	1.058	0.078
D	CRP4	1.0700	5.95	17.50	2.213	0.369	1.059	0.078
D	CRP4	1.0800	5.97	17.50	2.183	0.396	1.022	0.077
F	CRP1	0.7690	7.80	50.56	0.239	0.278	0.619	0.029
F	CRP1	0.8340	7.79	50.98	0.230	0.216	0.591	0.031
F	CRP1	0.7670	7.74	51.28	0.244	0.205	0.585	0.026
F	CRP2	1.1000	7.55	53.02	1.513	0.427	2.021	0.181
F	CRP2	1.1100	7.52	53.54	1.595	0.385	2.056	0.181
F	CRP2	1.1000	7.55	53.48	1.606	0.434	2.164	0.195
F	CRP3	1.9500	6.72	8.96	7.942	4.592	7.718	0.473
F	CRP3	1.9800	6.66	9.05	7.851	4.944	7.103	0.451
F	CRP3	1.9900	6.67	9.09	7.605	4.246	7.372	0.449

Lab Code	Product	Nicotine	pH	Moisture	NNN	NNK	NAT	NAB
		%		%	µg/g	µg/g	µg/g	µg/g
F	CRP4	0.8800	5.73	13.34	1.527	0.436	1.249	0.063
F	CRP4	0.9500	5.68	13.26	1.689	0.488	1.426	0.055
F	CRP4	0.9300	5.66	13.24	1.688	0.502	1.425	0.064
G	CRP1	0.9200	7.86	52.05	0.703	0.197	0.543	0.032
G	CRP1	0.9000	7.89	52.07	0.694	0.213	0.543	0.036
G	CRP1	0.9200	7.86	51.80	0.720	0.204	0.561	0.030
G	CRP2	1.2300	7.69	54.74	1.892	0.418	1.747	0.167
G	CRP2	1.2300	7.70	54.76	1.898	0.448	1.784	0.169
G	CRP2	1.2200	7.70	54.66	1.878	0.464	1.787	0.171
G	CRP3	2.0800	6.73	8.95	8.900	4.428	6.080	0.474
G	CRP3	2.1200	6.72	9.08	8.948	4.576	6.412	0.492
G	CRP3	2.1000	6.73	8.94	9.203	4.452	6.304	0.450
G	CRP4	1.1332	5.90	22.13	2.155	0.444	1.288	0.064
G	CRP4	1.1300	5.93	22.31	2.103	0.421	1.257	0.058
G	CRP4	1.1200	5.89	22.29	2.189	0.429	1.265	0.067
H	CRP1	0.9324	8.00	52.18	0.606	0.192	0.518	0.031
H	CRP1	0.9286	8.00	51.14	0.591	0.191	0.505	0.031
H	CRP1	0.9666	8.02	50.61	0.582	0.178	0.483	0.028
H	CRP2	1.2943	7.73	55.63	1.787	0.426	1.809	0.150
H	CRP2	1.2977	7.74	54.94	1.795	0.415	1.829	0.148
H	CRP2	1.2821	7.76	54.67	1.786	0.411	1.816	0.147
H	CRP3	2.2134	6.83	7.96	7.263	3.802	5.417	0.378
H	CRP3	2.1980	6.84	8.61	7.967	3.904	5.778	0.382
H	CRP3	2.2053	6.81	8.40	8.021	3.939	5.794	0.381
H	CRP4	1.0641	6.01	23.85	1.776	0.383	1.161	0.057
H	CRP4	1.0467	6.00	24.00	1.731	0.390	1.097	0.058
H	CRP4	1.0582	5.96	24.25	1.769	0.359	1.151	0.054
I	CRP1	0.9800	7.91	50.80	0.655	0.213	0.568	0.032
I	CRP1	0.9700	NA	NA	0.685	0.208	0.554	0.030
I	CRP1	0.9700	NA	NA	0.686	0.207	0.555	0.030
I	CRP2	1.3300	7.66	54.23	1.940	0.470	1.960	0.152
I	CRP2	1.3300	7.66	54.08	1.690	0.394	1.710	0.136
I	CRP2	1.3200	7.61	54.07	1.920	0.456	1.950	0.157
I	CRP3	2.2600	6.86	9.28	8.560	4.000	5.880	0.390
I	CRP3	2.2700	6.95	9.35	8.790	3.970	5.960	0.379
I	CRP3	2.2600	6.86	9.22	8.550	3.860	6.030	0.386
I	CRP4	1.1200	5.92	19.93	2.190	0.475	1.300	0.058
I	CRP4	1.1400	5.92	19.89	2.240	0.489	1.330	0.060
I	CRP4	1.1500	5.85	19.86	2.110	0.446	1.280	0.059
K	CRP1	0.9500	7.76	51.24	0.520	0.230	0.460	0.140
K	CRP1	0.9400	7.73	51.08	0.520	0.250	0.430	0.130
K	CRP1	0.9300	7.77	51.14	0.490	0.240	0.450	0.130
K	CRP2	1.3000	7.62	53.88	1.160	0.350	1.190	0.200
K	CRP2	1.2900	7.59	53.98	1.270	0.360	1.200	0.200
K	CRP2	1.2900	7.59	53.74	1.160	0.390	1.180	0.190
K	CRP3	2.1800	6.74	7.96	4.450	2.390	3.310	0.320
K	CRP3	2.1700	6.75	8.08	4.530	2.340	3.400	0.310
K	CRP3	2.1400	6.77	8.16	4.660	2.520	3.360	0.310
K	CRP4	1.0700	5.92	20.90	1.220	0.350	0.850	0.160

Lab Code	Product	Nicotine	pH	Moisture	NNN	NNK	NAT	NAB
		%		%	µg/g	µg/g	µg/g	µg/g
K	CRP4	1.0800	5.81	21.06	1.300	0.370	0.880	0.150
K	CRP4	1.0500	5.79	21.08	1.180	0.360	0.880	0.160
M	CRP1	0.9186	7.97	51.54	0.579	0.184	0.554	0.030
M	CRP1	0.9052	7.97	51.52	0.633	0.201	0.547	0.030
M	CRP1	0.9041	7.99	50.62	0.598	0.184	0.588	0.029
M	CRP2	1.1771	7.74	54.83	1.876	0.434	1.696	0.160
M	CRP2	1.1780	7.75	55.02	1.818	0.445	1.827	0.140
M	CRP2	1.1888	7.75	55.04	1.755	0.460	2.138	0.154
M	CRP3	1.9388	6.88	10.05	8.127	3.946	6.523	0.392
M	CRP3	1.9127	6.89	10.24	8.471	4.119	5.808	0.391
M	CRP3	1.9044	6.89	10.09	8.257	4.095	5.923	0.368
M	CRP4	0.9988	5.98	26.34	1.873	0.404	1.267	0.058
M	CRP4	1.0107	5.99	27.58	1.873	0.353	1.251	0.053
M	CRP4	0.9984	6.00	26.86	1.852	0.368	1.143	0.058

Table 4: Full Data Set for 2013 WG4 Study

Lab Code	Product	Nicotine	pH	Moisture	NNN	NNK	NAT	NAB
		%		%	µg/g	µg/g	µg/g	µg/g
A	CRP1	1.0430	8.02	52.18	0.660	0.217	0.574	0.039
A	CRP1	0.9440	7.98	52.94	0.621	0.211	0.521	0.039
A	CRP1	0.9440	7.98	52.57	0.638	0.209	0.599	0.034
A	CRP2	1.1940	7.71	54.60	1.908	0.487	1.989	0.157
A	CRP2	1.2170	7.71	54.57	1.929	0.504	1.867	0.165
A	CRP2	1.2220	7.70	54.66	1.814	0.468	1.925	0.160
A	CRP3	2.1370	6.75	9.97	8.334	4.434	5.826	0.433
A	CRP3	2.1340	6.79	9.95	8.712	4.532	5.700	0.447
A	CRP3	2.1360	6.75	9.93	8.713	4.641	6.407	0.428
A	CRP4	1.0880	5.92	22.17	2.171	0.523	1.426	0.079
A	CRP4	1.0880	5.92	22.18	2.220	0.506	1.366	0.075
A	CRP4	1.0850	5.91	22.18	2.164	0.469	1.422	0.067
G	CRP1	0.8710	7.84	52.67	0.735	0.211	0.575	0.041
G	CRP1	0.8730	7.80	52.72	0.746	0.220	0.557	0.042
G	CRP1	0.8710	7.87	52.82	0.757	0.222	0.559	0.042
G	CRP2	1.2160	7.56	54.19	2.018	0.440	1.834	0.163
G	CRP2	1.2130	7.57	54.07	1.995	0.443	1.828	0.173
G	CRP2	1.2110	7.61	54.07	1.944	0.440	1.827	0.174
G	CRP3	2.1080	6.67	9.92	9.237	3.823	6.022	0.453
G	CRP3	2.1040	6.63	9.96	9.211	3.854	6.070	0.462
G	CRP3	2.1120	6.67	9.93	9.264	3.806	5.961	0.449
G	CRP4	1.1230	5.85	21.31	2.202	0.431	1.256	0.061
G	CRP4	1.1180	5.84	21.33	2.190	0.430	1.252	0.063
G	CRP4	1.1090	5.83	21.33	2.272	0.438	1.272	0.062
I	CRP1	0.9200	8.00	48.08	0.630	0.194	0.506	0.032
I	CRP1	0.9600	8.00	49.65	0.660	0.188	0.503	0.033
I	CRP1	0.9300	8.00	48.86	0.696	0.198	0.520	0.034
I	CRP2	1.2400	7.72	54.09	1.860	0.426	1.770	0.160
I	CRP2	1.2300	7.72	53.93	1.780	0.406	1.710	0.161
I	CRP2	1.2100	7.73	54.05	1.820	0.416	1.700	0.150
I	CRP3	2.0700	6.91	8.25	8.420	3.820	5.730	0.401

Lab Code	Product	Nicotine	pH	Moisture	NNN	NNK	NAT	NAB
		%		%	µg/g	µg/g	µg/g	µg/g
I	CRP3	2.1000	6.89	8.26	8.260	3.780	5.500	0.382
I	CRP3	2.0900	6.89	8.19	8.420	3.900	5.430	0.405
I	CRP4	1.0600	5.91	17.06	1.920	0.417	1.150	0.056
I	CRP4	1.0500	6.17	17.51	1.900	0.415	1.200	0.059
I	CRP4	1.0500	5.93	17.46	1.910	0.403	1.200	0.060
K	CRP1	0.9900	7.72	50.66	0.587	0.199	0.369	0.051
K	CRP1	0.9800	7.73	50.74	0.583	0.180	0.315	0.044
K	CRP1	0.9900	7.73	51.58	0.593	0.177	0.373	0.051
K	CRP2	1.3100	7.55	54.12	1.463	0.392	1.142	0.136
K	CRP2	1.3100	7.54	54.11	1.452	0.377	1.169	0.143
K	CRP2	1.3200	7.55	53.75	1.359	0.413	1.219	0.152
K	CRP3	2.3000	6.75	9.14	6.361	3.314	3.595	0.338
K	CRP3	2.2800	6.75	9.07	6.261	3.333	3.754	0.301
K	CRP3	2.2900	6.76	8.94	6.153	3.125	3.773	0.296
K	CRP4	1.2100	6.09	18.49	1.391	0.276	0.732	0.054
K	CRP4	1.2400	5.99	18.64	1.332	0.241	0.714	0.053
K	CRP4	1.1800	5.93	18.16	1.441	0.259	0.754	0.051
L	CRP1	0.9530	7.99	49.67	0.688	0.213	0.519	0.032
L	CRP1	0.9770	7.95	49.72	0.683	0.218	0.531	0.032
L	CRP1	0.9790	7.93	49.18	0.643	0.224	0.525	0.032
L	CRP2	1.1900	7.70	54.62	1.744	0.414	1.654	0.140
L	CRP2	1.1920	7.66	54.74	1.730	0.422	1.653	0.139
L	CRP2	1.1960	7.66	54.61	1.700	0.416	1.633	0.138
L	CRP3	2.0770	6.62	9.82	8.632	4.494	5.849	0.368
L	CRP3	2.0810	6.61	9.51	8.548	4.695	5.884	0.372
L	CRP3	2.0780	6.61	9.80	8.601	4.478	5.880	0.374
L	CRP4	0.9920	5.80	22.58	1.889	0.423	1.085	0.057
L	CRP4	0.9880	5.78	22.64	1.846	0.407	1.105	0.061
L	CRP4	0.9870	5.75	22.39	1.894	0.394	1.118	0.058
M	CRP1	0.9360	7.98	49.62	0.736	0.224	0.572	0.034
M	CRP1	0.9060	7.94	49.33	0.631	0.204	0.496	0.031
M	CRP1	0.9240	7.92	49.96	0.666	0.206	0.513	0.037
M	CRP2	1.2440	7.68	53.46	1.957	0.427	1.838	0.139
M	CRP2	1.2650	7.67	53.70	1.728	0.468	1.773	0.148
M	CRP2	1.2640	7.67	54.30	1.879	0.445	1.800	0.154
M	CRP3	2.1250	6.85	10.27	8.620	4.079	5.872	0.374
M	CRP3	2.1230	6.84	10.27	7.624	3.917	6.250	0.383
M	CRP3	2.1260	6.84	10.50	8.090	4.102	5.754	0.372
M	CRP4	0.9840	5.97	21.77	1.892	0.470	1.157	0.062
M	CRP4	0.9990	5.96	21.28	1.866	0.471	1.177	0.059
M	CRP4	0.9840	5.97	21.94	1.999	0.470	1.215	0.065
N	CRP1	0.9870	7.59	46.97	0.810	0.180	0.580	0.040
N	CRP1	1.0140	7.64	47.91	0.790	0.190	0.580	0.040
N	CRP1	0.8870	7.61	47.16	0.860	0.200	0.620	0.040
N	CRP2	1.2050	7.70	54.89	2.160	0.440	2.040	0.190
N	CRP2	1.2210	7.70	54.81	2.070	0.490	2.150	0.170
N	CRP2	1.2110	7.71	54.49	2.090	0.470	2.150	0.180
N	CRP3	2.1710	6.91	8.83	9.680	5.160	7.870	0.550
N	CRP3	2.1700	6.92	8.72	9.390	4.650	7.330	0.500

Lab Code	Product	Nicotine	pH	Moisture	NNN	NNK	NAT	NAB
		%		%	µg/g	µg/g	µg/g	µg/g
N	CRP3	2.1780	6.92	8.73	9.470	4.960	7.380	0.500
N	CRP4	1.0330	6.03	22.48	1.570	0.300	0.990	0.040
N	CRP4	1.0200	6.02	22.47	1.660	0.370	1.050	0.050
N	CRP4	1.0200	6.02	22.54	1.680	0.330	0.970	0.050
O	CRP1	0.9200	7.78	49.81	0.672	0.200	0.545	0.044
O	CRP1	0.9300	7.78	49.75	0.648	0.186	0.489	0.040
O	CRP1	0.9200	7.79	49.72	0.651	0.189	0.524	0.043
O	CRP2	1.2100	7.58	54.68	1.767	0.436	1.885	0.160
O	CRP2	1.2300	7.59	54.69	1.739	0.427	1.848	0.150
O	CRP2	1.2000	7.58	54.74	1.840	0.447	1.830	0.157
O	CRP3	2.1200	6.71	8.96	7.150	4.520	6.090	0.404
O	CRP3	2.1600	6.71	9.01	6.620	4.242	5.897	0.379
O	CRP3	2.1500	6.69	8.93	6.856	4.261	5.379	0.392
O	CRP4	1.0200	5.97	22.26	1.878	0.389	1.185	0.067
O	CRP4	1.0300	5.95	22.24	1.918	0.362	1.034	0.059
O	CRP4	1.0200	5.97	22.29	1.882	0.374	1.183	0.063
P	CRP1	0.9200	7.91	50.01	0.598	0.272	0.399	0.031
P	CRP1	0.8900	7.91	49.59	0.618	0.368	0.417	0.040
P	CRP1	0.9000	7.91	50.58	0.558	0.420	0.466	0.039
P	CRP2	1.2000	7.65	54.37	1.406	0.436	1.254	0.143
P	CRP2	1.2100	7.68	54.48	1.409	0.446	1.170	0.138
P	CRP2	1.2200	7.68	54.68	1.368	0.462	1.141	0.129
P	CRP3	2.1000	6.81	8.72	6.497	4.193	5.343	0.459
P	CRP3	2.0600	6.83	8.76	6.515	4.410	5.253	0.479
P	CRP3	2.0500	6.83	9.05	6.711	4.393	4.851	0.468
P	CRP4	0.9800	5.96	21.77	1.691	0.527	0.885	0.070
P	CRP4	0.9900	5.95	22.16	1.625	0.520	0.974	0.062
P	CRP4	0.9900	5.95	22.56	1.713	0.535	0.859	0.053

5. Data – 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 ISO 5725-2 does not suggest calculation of z-scores, z-scores are presented in section 5.3 so that the participating laboratories would have an additional measure of their performance compared to their peers. Section 5.4 describes the stability analysis conducted using data from the 2010-2013 studies. Raw data plots that include all replicates, without removal of outliers, are given in Appendices A and B for the two study years.

5.1 Exclusion of Outliers

Procedures outlined in ISO 5725(2) and ISO/TR 22971 were generally used for the exclusion of outliers. ISO 5725(2) specifies the use of Cochran's test for eliminating laboratories with overly large repeatability standard deviations and Grubbs' test for eliminating laboratories with outlying mean values.

The intent of ISO 5725(2) is to eliminate outliers that exceed a 1% critical value. However, Cochran's test is very sensitive to deviations from normality and, as demonstrated by Conover et al. (1981)[4], the test is prone to falsely identify laboratories as outliers much more frequently than the nominal rate of 1%. From a practical perspective, this means that data are eliminated too easily which may lead to the calculation of an unrealistically low level of method variation. The repeated application of Cochran's test is likely to exacerbate the effect. ISO 5725(2) also recognizes this potential difficulty (see 7.3.3.6). For this reason, a single application of Cochran's test was employed for this analysis.

Grubbs' and a single iteration of Cochran's tests were applied at the standard nominal 1% significance level to determine outliers and the results are shown in Tables 5 and 6 for the 2012 and 2013 data, respectively. As noted above, it is likely that the Cochran outliers are identified with a much higher probability than the nominally stated rate.

Table 5: Outliers among 2012 data

Analyte	Product	Cochran's Outliers - Lab	Grubbs' Outliers - Lab
Nicotine	CRP1	F	–
Moisture	CRP3	H	–
Moisture	CRP4	M	–
NNN	CRP4	A	–
NNK	CRP1	F	–
NNK	CRP3	–	K
NAB	CRP1	–	K
NAB	CRP4	–	K

Table 6: Outliers among 2013 Data.

Analyte	Product	Cochran's Outliers - Lab	Grubbs' Outliers - Lab
Nicotine	CRP3	Q	–
Nicotine	CRP4	K	–
Moisture	CRP2	Q	–
Moisture	CRP3	Q	–
Moisture	CRP4	Q	–
pH	CRP4	I	–
NNK	CRP1	P	–

[4] Conover, W.J., Johnson, M.E., & Johnson, M.M. (1981). A comparative study of tests for homogeneity of variances, with applications to the outer continental shelf bidding data. Technometrics, 23, 351-361

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

After removal of outlying data based on numerical data consistency methods (GRUBBS' test, COCHRAN's test), the final repeatability and reproducibility (r & R) results were calculated. The r & R results are shown in Tables 7 and 8 for the 2012 and 2013 WG4 studies, respectively.

Table 7: Repeatability (r) and Reproducibility for 2012 WG4 Study

Parameter	Product	No of Labs*	Mean	Repeatability		Reproducibility	
				r	% r	R	% R
Nicotine (%)	CRP1	7	0.94	0.030	3.2%	0.103	10.9%
Nicotine (%)	CRP2	8	1.23	0.017	1.4%	0.206	16.7%
Nicotine (%)	CRP3	8	2.12	0.043	2.0%	0.364	17.1%
Nicotine (%)	CRP4	8	1.06	0.046	4.3%	0.197	18.7%
pH	CRP1	8	7.91	0.050	NA	0.293	NA
pH	CRP2	8	7.68	0.044	NA	0.217	NA
pH	CRP3	8	6.79	0.068	NA	0.220	NA
pH	CRP4	8	5.90	0.096	NA	0.289	NA
Moisture (%)	CRP1	8	51.2	1.14	2.2%	1.39	2.7%
Moisture (%)	CRP2	8	54.4	0.68	1.2%	1.77	3.3%
Moisture (%)	CRP3	7	9.20	0.23	2.4%	1.91	20.7%
Moisture (%)	CRP4	7	20.1	0.27	1.3%	10.2	51.0%
NNN (µg/g)	CRP1	8	0.59	0.059	10.1%	0.447	76.3%
NNN (µg/g)	CRP2	8	1.73	0.194	11.3%	0.683	39.6%
NNN (µg/g)	CRP3	8	8.02	0.694	8.7%	4.324	53.9%
NNN (µg/g)	CRP4	7	1.86	0.148	8.0%	0.999	53.7%
NNK (µg/g)	CRP1	7	0.21	0.021	10.2%	0.056	27.3%
NNK (µg/g)	CRP2	8	0.42	0.068	16.0%	0.112	26.4%
NNK (µg/g)	CRP3	7	4.22	0.557	13.2%	0.883	20.9%
NNK (µg/g)	CRP4	8	0.42	0.064	15.4%	0.143	34.4%
NAT (µg/g)	CRP1	8	0.53	0.052	9.9%	0.155	29.2%
NAT (µg/g)	CRP2	8	1.76	0.345	19.6%	0.866	49.2%
NAT (µg/g)	CRP3	8	5.73	0.580	10.1%	3.253	56.7%
NAT (µg/g)	CRP4	8	1.19	0.143	12.0%	0.476	40.0%
NAB (µg/g)	CRP1	7	0.034	0.0064	19.0%	0.0186	55.2%
NAB (µg/g)	CRP2	8	0.166	0.0212	12.7%	0.0527	31.7%
NAB (µg/g)	CRP3	8	0.408	0.0408	10.0%	0.1528	37.5%
NAB (µg/g)	CRP4	7	0.062	0.0094	15.0%	0.0219	35.1%

*This includes the number of laboratory data sets included after removal of outliers. NA = Since pH is not a proportional scale, it is not appropriate to calculate %r or %R.

Table 8: Repeatability (r) and Reproducibility for 2013 WG4 Study

Parameter	Product	No of Labs*	Mean	Repeatability		Reproducibility	
				r	% r	R	% R
Nicotine (%)	CRP1	10	0.94	0.084	8.9%	0.122	13.0%
Nicotine (%)	CRP2	10	1.23	0.030	2.4%	0.108	8.7%
Nicotine (%)	CRP3	9	2.13	0.036	1.7%	0.190	8.9%
Nicotine (%)	CRP4	9	1.05	0.026	2.4%	0.174	16.6%
pH	CRP1	10	7.85	0.062	NA	0.382	NA

Parameter	Product	No of Labs*	Mean	Repeatability		Reproducibility	
				r	% r	R	% R
pH	CRP2	10	7.66	0.048	NA	0.178	NA
pH	CRP3	10	6.79	0.042	NA	0.296	NA
pH	CRP4	9	5.94	0.092	NA	0.249	NA
Moisture (%)	CRP1	10	50.3	1.23	2.4%	4.77	9.5%
Moisture (%)	CRP2	9	54.4	0.53	1.0%	1.09	2.0%
Moisture (%)	CRP3	9	9.3	0.29	3.1%	1.97	21.2%
Moisture (%)	CRP4	9	21.2	0.60	2.8%	5.38	25.4%
NNN (µg/g)	CRP1	10	0.67	0.081	12.2%	0.207	31.1%
NNN (µg/g)	CRP2	10	1.77	0.159	9.0%	0.636	35.8%
NNN (µg/g)	CRP3	10	8.03	0.607	7.6%	3.138	39.1%
NNN (µg/g)	CRP4	10	1.87	0.118	6.3%	0.714	38.1%
NNK (µg/g)	CRP1	9	0.20	0.023	11.5%	0.042	20.4%
NNK (µg/g)	CRP2	10	0.44	0.043	9.6%	0.082	18.7%
NNK (µg/g)	CRP3	10	4.16	0.380	9.1%	1.365	32.8%
NNK (µg/g)	CRP4	10	0.42	0.056	13.4%	0.236	56.1%
NAT (µg/g)	CRP1	10	0.51	0.076	15.0%	0.208	40.8%
NAT (µg/g)	CRP2	10	1.70	0.115	6.7%	0.848	49.8%
NAT (µg/g)	CRP3	10	5.72	0.652	11.4%	2.678	46.8%
NAT (µg/g)	CRP4	10	1.12	0.114	10.2%	0.550	49.2%
NAB (µg/g)	CRP1	10	0.038	0.0071	18.9%	0.0172	45.9%
NAB (µg/g)	CRP2	10	0.153	0.0179	11.7%	0.0422	27.5%
NAB (µg/g)	CRP3	10	0.410	0.0413	10.1%	0.1690	41.2%
NAB (µg/g)	CRP4	10	0.060	0.0120	20.0%	0.0220	36.7%

*This includes the number of laboratory data sets included after removal of outliers. NA = Since pH is not a proportional scale, it is not appropriate to calculate %r or %R.

5.3 Calculation of Z-Scores

Although calculation of z-scores is not suggested in ISO 5725-2, z-scores were calculated so that the participating laboratories could compare their results to those of their peers. It is expected that most of the data should fall within the range of ±2. A final summary table of z-scores is presented in Tables 9 and 10 for the 2012 and 2013 WG4 studies, respectively. Outliers detected with the GRUBBS' test and COCHRAN'S test, were removed prior to the calculation of the z-scores and are given as "outlier" in the tables.

Table 9: Z-Scores for 2012 WG4 Study

Product	Lab	Nicotine	pH	Moisture	NAB	NAT	NNK	NNN
CRP1	A	1.67	0.77	0.94	0.67	0.37	0.70	0.18
CRP1	D	-1.00	0.77	-0.73	1.98	-1.26	-0.76	1.01
CRP1	F	outlier	-1.32	-0.80	-0.79	1.32	outlier	-2.19
CRP1	G	-0.76	-0.38	1.88	-0.16	0.39	-0.06	0.76
CRP1	H	0.06	1.00	0.16	-0.58	-0.50	-0.99	0.05
CRP1	I	0.92	0.03	-1.16	-0.48	0.58	0.18	0.56
CRP1	K	-0.01	-1.56	-0.24	outlier	-1.54	1.78	-0.48
CRP1	M	-0.88	0.70	-0.05	-0.64	0.65	-0.85	0.11
CRP2	A	0.32	0.42	0.64	-0.31	0.78	1.36	0.31
CRP2	D	-0.60	0.86	0.17	0.18	-1.00	-1.18	0.73

Product	Lab	Nicotine	pH	Moisture	NAB	NAT	NNK	NNN
CRP2	F	-1.78	-1.80	-1.80	1.10	1.10	-0.21	-0.66
CRP2	G	-0.10	0.25	0.48	0.16	0.05	0.60	0.68
CRP2	H	0.78	0.86	1.08	-1.00	0.20	-0.15	0.26
CRP2	I	1.26	-0.45	-0.51	-1.00	0.39	0.50	0.52
CRP2	K	0.81	-1.02	-0.94	1.71	-1.95	-1.61	-2.23
CRP2	M	-0.71	0.90	0.88	-0.83	0.44	0.69	0.38
CRP3	A	1.18	-0.60	1.04	0.06	0.20	0.28	0.32
CRP3	D	-0.22	0.41	0.04	0.93	-0.49	0.23	1.04
CRP3	F	-1.17	-1.43	-0.31	0.94	1.45	1.39	-0.14
CRP3	G	-0.19	-0.86	-0.38	1.21	0.46	0.99	0.65
CRP3	H	0.63	0.46	outlier	-0.51	-0.06	-1.25	-0.18
CRP3	I	1.07	1.29	0.06	-0.42	0.20	-1.02	0.40
CRP3	K	0.30	-0.51	-1.73	-1.77	-2.07	outlier	-2.27
CRP3	M	-1.59	1.25	1.30	-0.45	0.31	-0.61	0.17
CRP4	A	0.20	-0.18	0.67	0.10	0.69	0.93	outlier
CRP4	D	0.20	0.75	-0.70	2.10	-0.87	-0.64	0.95
CRP4	F	-1.97	-2.06	-1.86	-0.22	1.08	1.22	-0.64
CRP4	G	1.03	0.12	0.60	0.10	0.49	0.30	0.82
CRP4	H	0.00	0.96	1.09	-0.81	-0.32	-0.84	-0.29
CRP4	I	1.16	0.02	-0.05	-0.45	0.69	1.11	0.90
CRP4	K	0.15	-0.55	0.26	outlier	-1.94	-1.20	-1.77
CRP4	M	-0.78	0.96	outlier	-0.81	0.19	-0.88	0.02

Outliers detected with the GRUBBS' test and COCHRAN'S test were removed prior to the calculation of the z-scores.

Table 10: Z-Scores for 2013 WG4 Study

Product	Lab	Nicotine	pH	Moisture	NAB	NAT	NNK	NNN
CRP1	A	1.01	1.08	1.37	-0.03	0.77	0.67	-0.40
CRP1	G	-1.91	-0.08	1.47	0.72	0.76	1.07	1.11
CRP1	I	-0.11	1.13	-0.86	-0.78	0.00	-0.78	-0.08
CRP1	K	1.28	-0.89	0.42	1.93	-2.22	-1.38	-1.14
CRP1	L	0.81	0.81	-0.46	-0.95	0.21	1.12	0.05
CRP1	M	-0.52	0.74	-0.39	-0.60	0.24	0.59	0.14
CRP1	N	0.61	-1.73	-1.77	0.43	1.18	-1.03	2.17
CRP1	O	-0.48	-0.47	-0.32	0.83	0.13	-0.90	-0.15
CRP1	P	-1.03	0.47	-0.14	-0.14	-1.17	outlier	-1.09
CRP1	Q	0.34	-1.06	0.66	-1.41	0.10	0.64	-0.60
CRP2	A	-0.57	0.83	0.72	0.53	0.75	1.71	0.49
CRP2	G	-0.51	-1.22	-0.67	1.19	0.42	0.01	0.95
CRP2	I	-0.15	1.10	-0.91	0.27	0.08	-0.93	0.20
CRP2	K	2.16	-1.75	-1.00	-0.67	-1.75	-1.75	-1.57
CRP2	L	-1.06	0.29	0.85	-1.00	-0.19	-0.88	-0.23
CRP2	M	0.68	0.29	-1.48	-0.44	0.34	0.22	0.36
CRP2	N	-0.54	0.77	1.06	1.90	1.36	0.97	1.49
CRP2	O	-0.51	-1.16	0.98	0.17	0.50	-0.15	0.03
CRP2	P	-0.60	0.24	0.44	-1.17	-1.71	0.27	-1.71
CRP2	Q	1.10	0.61	outlier	-0.77	0.19	0.53	-0.02
CRP3	A	0.02	-0.21	0.92	0.44	0.27	0.79	0.50
CRP3	G	-0.39	-1.23	0.90	0.76	0.31	-0.70	1.09
CRP3	I	-0.71	1.06	-1.54	-0.23	-0.18	-0.69	0.30

Product	Lab	Nicotine	pH	Moisture	NAB	NAT	NNK	NNN
CRP3	K	2.32	-0.31	-0.37	-1.66	-2.15	-1.90	-1.60
CRP3	L	-0.83	-1.64	0.57	-0.65	0.16	0.83	0.51
CRP3	M	-0.15	0.55	1.48	-0.57	0.25	-0.27	0.07
CRP3	N	0.57	1.25	-0.79	1.81	1.92	1.61	1.34
CRP3	O	0.13	-0.78	-0.49	-0.31	0.07	0.38	-1.04
CRP3	P	-0.96	0.36	-0.67	0.99	-0.61	0.36	-1.32
CRP3	Q	outlier	0.96	outlier	-0.58	-0.05	-0.41	0.14
CRP4	A	0.64	-0.29	0.53	1.93	1.49	0.95	1.24
CRP4	G	1.12	-1.20	0.09	0.27	0.74	0.15	1.38
CRP4	I	0.09	outlier	-1.99	-0.25	0.34	-0.11	0.15
CRP4	K	outlier	0.73	-1.43	-1.05	-1.98	-1.96	-1.92
CRP4	L	-0.95	-1.94	0.72	-0.20	-0.07	-0.16	0.01
CRP4	M	-0.95	0.30	0.27	0.27	0.34	0.60	0.18
CRP4	N	-0.38	0.96	0.70	-1.90	-0.59	-1.06	-0.93
CRP4	O	-0.39	0.26	0.58	0.42	0.09	-0.56	0.08
CRP4	P	-0.99	0.14	0.53	0.23	-1.09	1.29	-0.78
CRP4	Q	1.81	1.04	outlier	0.27	0.73	0.87	0.59

Outliers detected with the GRUBBS' test and COCHRAN'S test were removed prior to the calculation of the z-scores.

5.4 CRP Stability Assessment

The data were evaluated for stability by examining the results from the 2010-2013 WG4 studies for trends over time, for all analytes. Even though the stability trend analysis, described below, was the primary evaluation of stability, the data were also graphically evaluated for changes in between-lab variability over the study years. As mentioned in the introduction, the 2010 study did not specify methods of analysis and the 2011 study was the first study year initiated to assess stability of the CRPs where methods were specified. However, after evaluating the data for all four years, it was determined that the 2010 data were consistent with the data from later years and were included in the stability analysis.

The stability trend analysis included two factors: year of analysis and laboratory. To account for potential year-to-year correlation in a laboratory's results, the laboratory variable was treated as a random factor.

There is also a potential need to account for multiplicity of testing. That is, if four statistical tests are each carried out with a 5% chance to falsely declare a time trend, then there is just under a 20% chance that at least one of them will give a false indication of a time trend. In this instance there are four reference products and seven separate analytes, thus twenty-eight comparisons in all. The approach taken was to correct for testing multiplicity for each analyte and not across all analytes. The correction employed was to allow for four tests being conducted, not all twenty-eight. That meant that each test was judged statistically significant if the associated p-value was less than 5%/4 = 1.25%. Therefore, there is roughly a 5% chance that each analyte will be declared to show a time trend on one or more of the CRPs by chance alone.

Graphs of the mean data plots for all years and all analytes are shown in Appendix C (outliers were not removed). Table 11 summarizes the trend analysis of each analyte and each product. Although there are apparent outliers in the mean data plots, the only item showing statistical significance is moisture level for CRP3 in which there was a trend in which moisture increased over the four years.

Table 11: Summary of Stability Analysis

Product	Variable	Estimated Slope	p-value[1]
CRP1	Nicotine	-0.011	17.3%
CRP2	Nicotine	-0.015	12.8%
CRP3	Nicotine	-0.002	89.4%
CRP4	Nicotine	-0.027	6.8%
CRP1	pH	-0.027	11.8%
CRP2	pH	-0.014	22.9%
CRP3	pH	-0.002	83.6%
CRP4	pH	0.008	59.7%
CRP1	Moisture	-0.407	1.5%
CRP2	Moisture	-0.037	57.1%
CRP3	Moisture	0.374	0.1%
CRP4	Moisture	-0.217	56.7%
CRP1	NNN	-0.009	62.5%
CRP2	NNN	-0.034	29.4%
CRP3	NNN	-0.007	95.4%
CRP4	NNN	-0.042	30.3%
CRP1	NNK	-0.001	89.9%
CRP2	NNK	-0.008	17.2%
CRP3	NNK	0.070	28.5%
CRP4	NNK	-0.003	77.7%
CRP1	NAT	-0.011	30.7%
CRP2	NAT	-0.053	16.8%
CRP3	NAT	-0.014	90.9%
CRP4	NAT	-0.039	18.4%
CRP1	NAB	0.003	34.2%
CRP2	NAB	0.000	90.4%
CRP3	NAB	0.003	70.5%
CRP4	NAB	0.001	82.6%

1. P-values are declared statistically significant if $p < 1.25\%$. This is after dividing the nominal 5% level by 4 to account for testing multiplicity.
2. Calculated omitting the Lab E result in 2010.

6. Data Interpretation

The only parameter showing a statistically significant trend over time was an increase in moisture for CRP3 over the four study years. However, this increase was marginal and equated to an increase of approximately 1% when calculated as an average for all labs. CRP3 has the lowest moisture content so this product would be most likely to absorb water over time as compared to the other CRPs. As mentioned earlier, results are presented on an as-is basis; however, this negligible shift in moisture for CRP3 would not be evident in the other analyses due to method variability.

In examining the mean data plots (Appendix C), a number of potential outliers are evident; therefore, the stability analyses were conducted both with and without the outliers included. The only instance in which the exclusion of outliers affected the conclusion was moisture for CRP1. If the 2010 Lab E outlying value was included, the estimated slope was statistically significant; however, this was not the case with the outlier omitted. In other words, there was a statistically significant increase in moisture over time with the outlier included, but not with the outlier excluded. The adjusted p-value given in Table 11 was calculated excluding the 2010 Lab E moisture outlying result for CRP1.

As mentioned above, the mean data plots for all years (Appendix C) were examined and there were some indications of changes in between-lab variability over the study years. Two notable examples of increased variability between labs are pH for CRP3 and moisture for CRP4. The variability for pH for CRP3 increased across all study years while the variability in moisture was notably worse in 2012 as compared to the other years. In 2012, the measured moisture values for CRP4 ranged from 13.3% to 26.9%. The reason for the increased variability in the pH analysis is not evident. Regarding the increased variability for moisture in 2012, these results do not seem to correlate with nicotine suggesting that sample instability is not root cause.

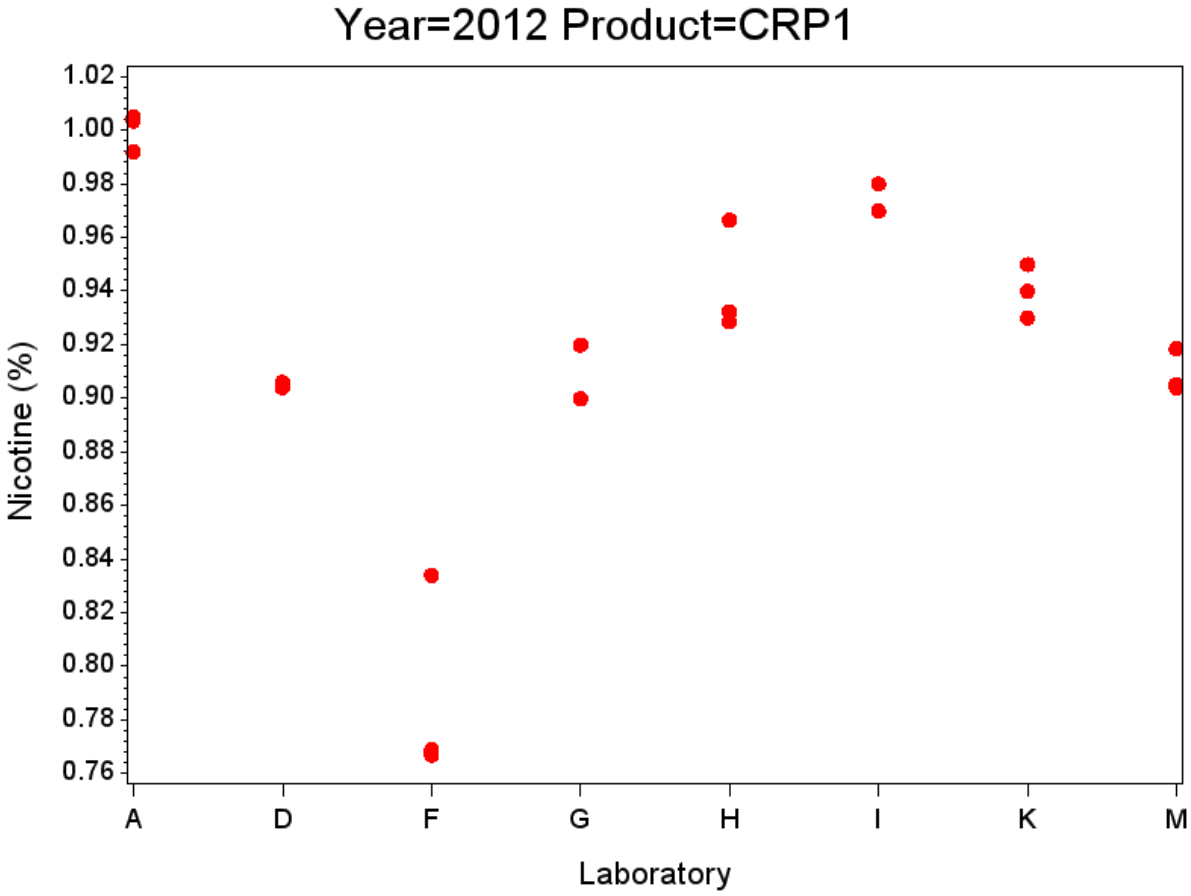
Overall, both nicotine and TSNAs are shown to be stable over the four study years, for all CRPs. This is not unexpected as both nicotine and TSNAs should be stable in these products when the products are maintained at −20 °C.

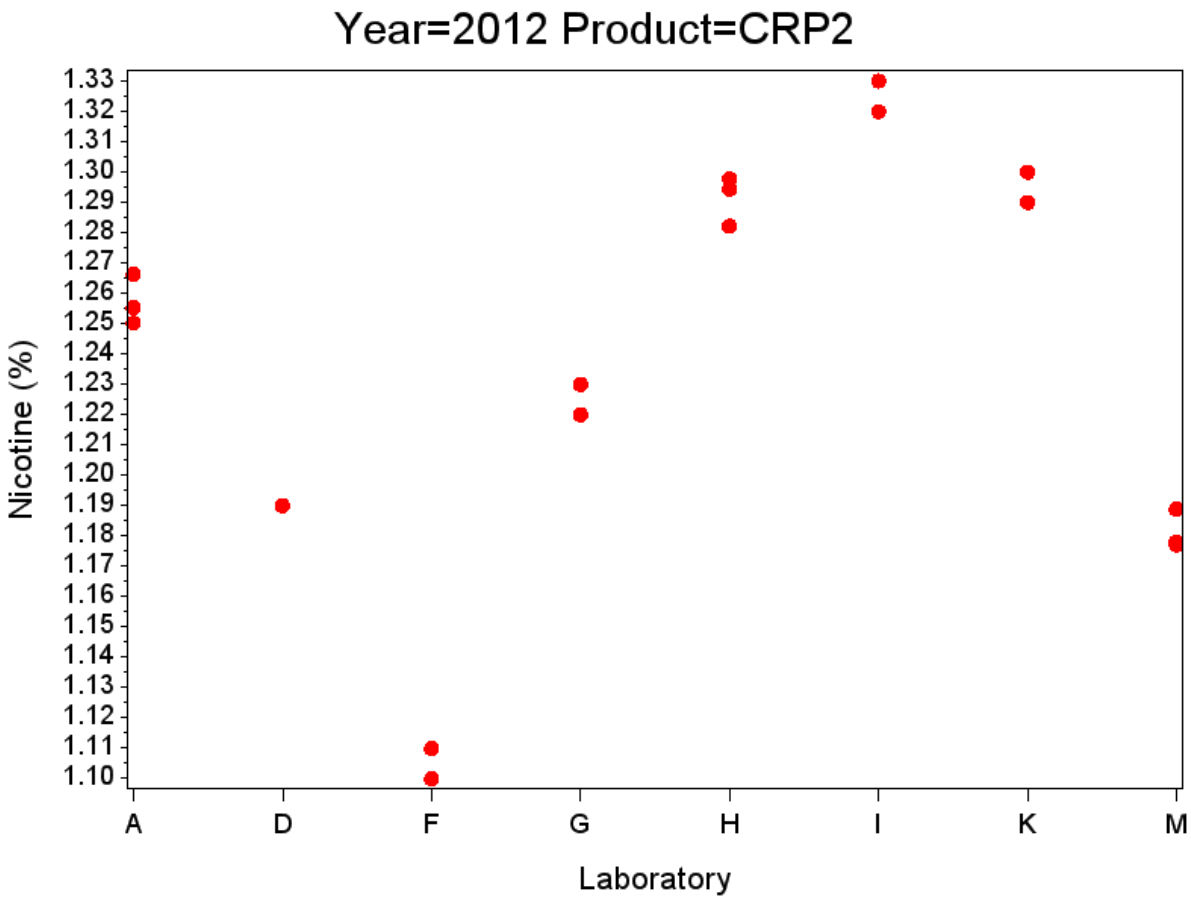
Based on the results taken in their entirety, the conclusion of this study is that all four CRPs are suitable for continued use as reference products. Lack of any clear and significant trends in the stability results indicate that storage at −20C is an appropriate storage condition for the four reference products. Furthermore, given that changes in moisture content should also affect other analytes on a wet weight basis the apparent changes in moisture content observed in CRP3 may be related to factors other than product instability.

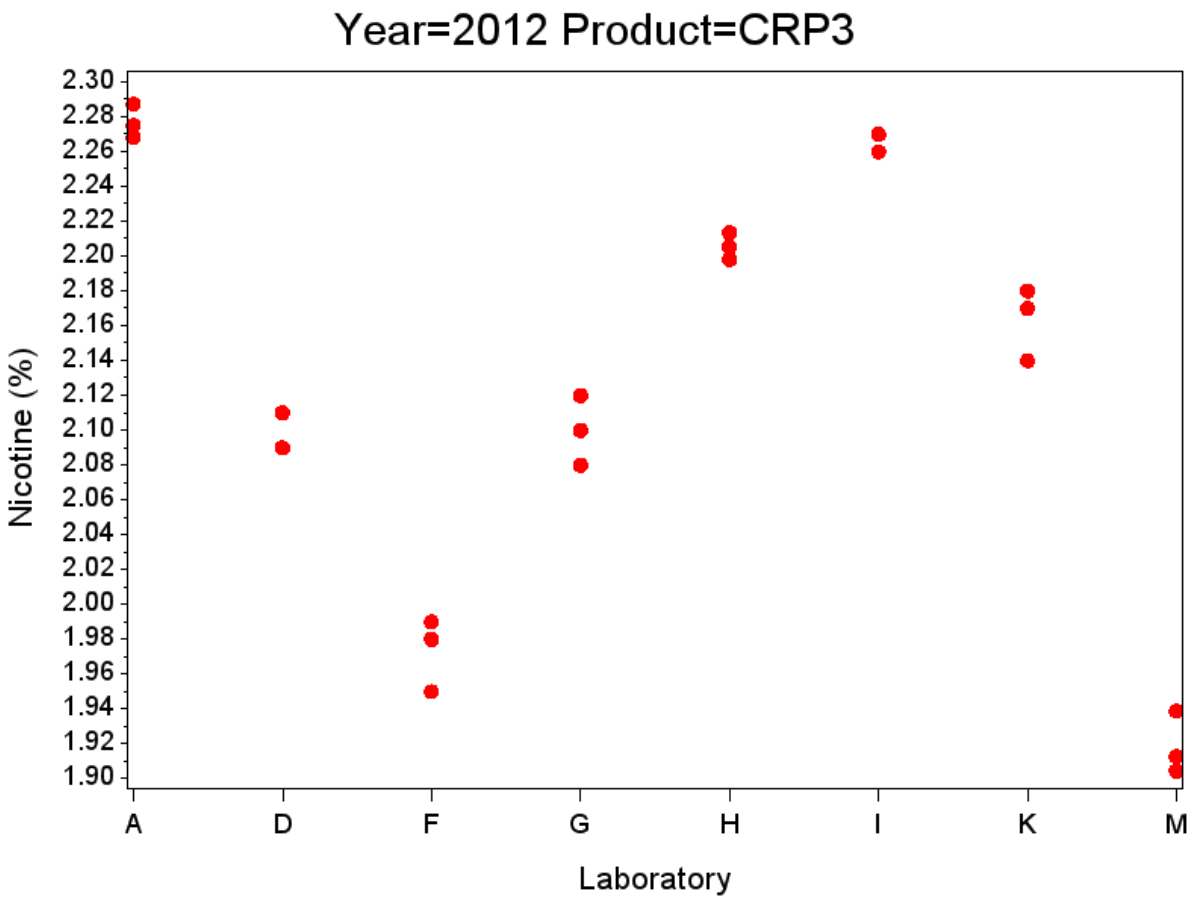
7. Recommendations

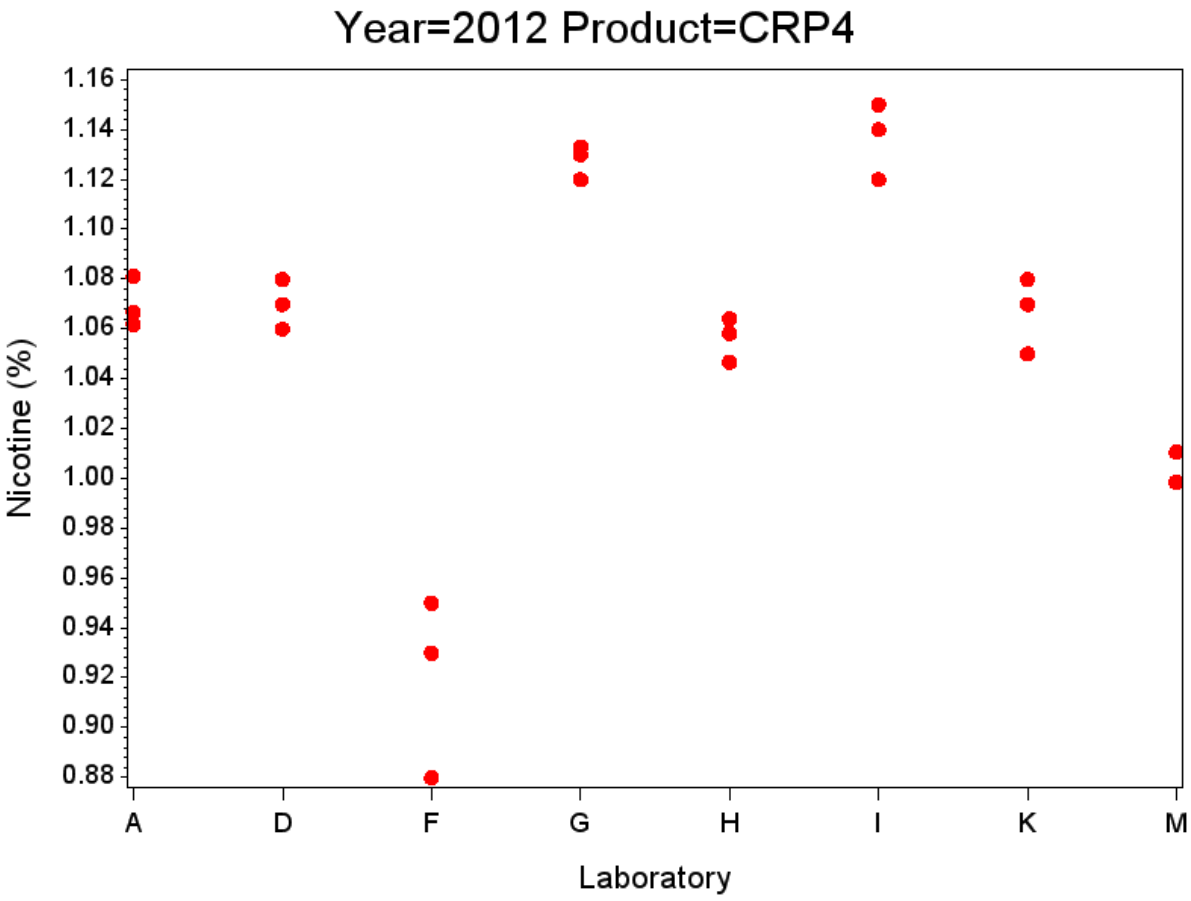
The STS recommends that WG4 continue to monitor stability of the CRPs on an annual basis. As stated earlier, data generated in 2010 will serve as the baseline for future WG4 stability analyses. A formal analysis of product stability should be conducted with data obtained from the next WG4 study scheduled for 2014. For future analyses, we are recommending that all CRPs be obtained from NC State just prior to the study to mitigate differences in long term storage conditions by the participating laboratories.

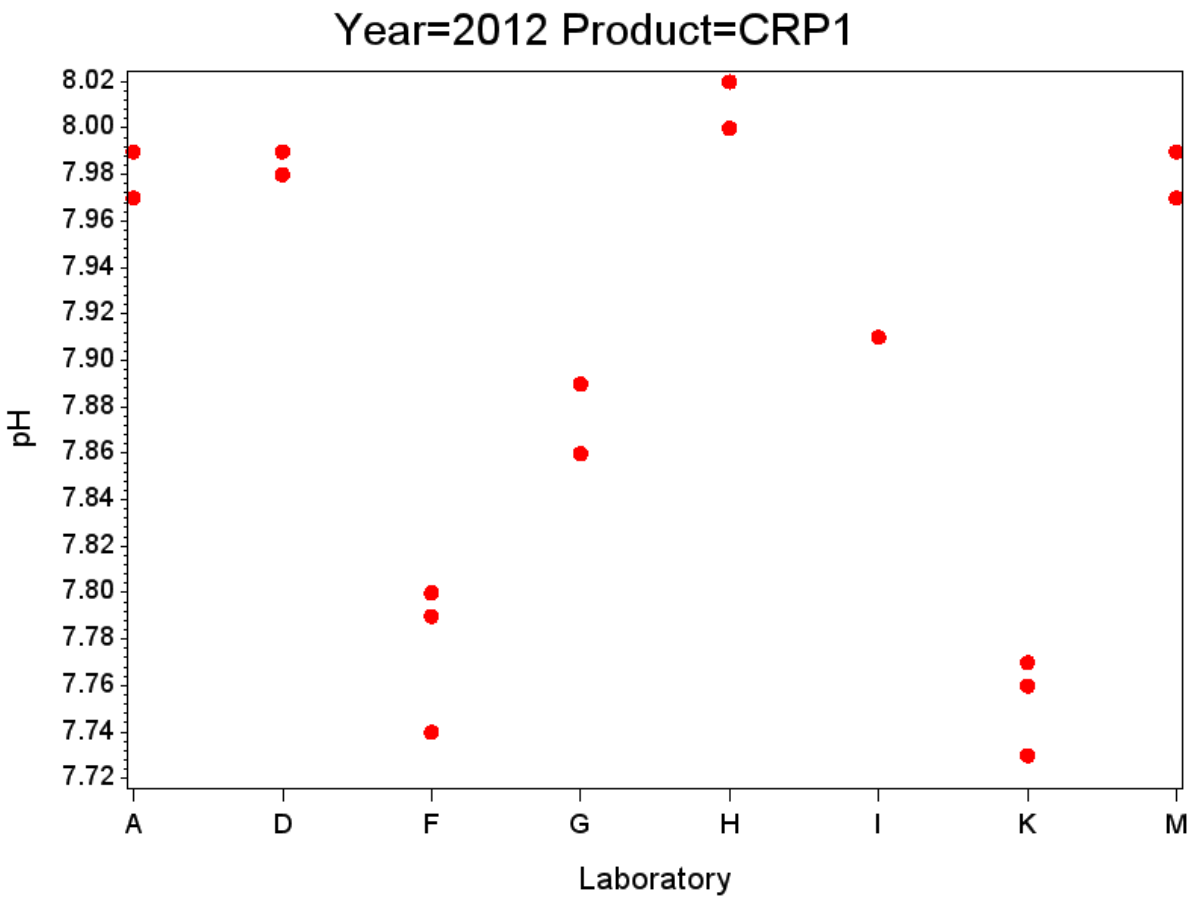
APPENDIX A: Raw Data Plots for 2012 WG4 Study

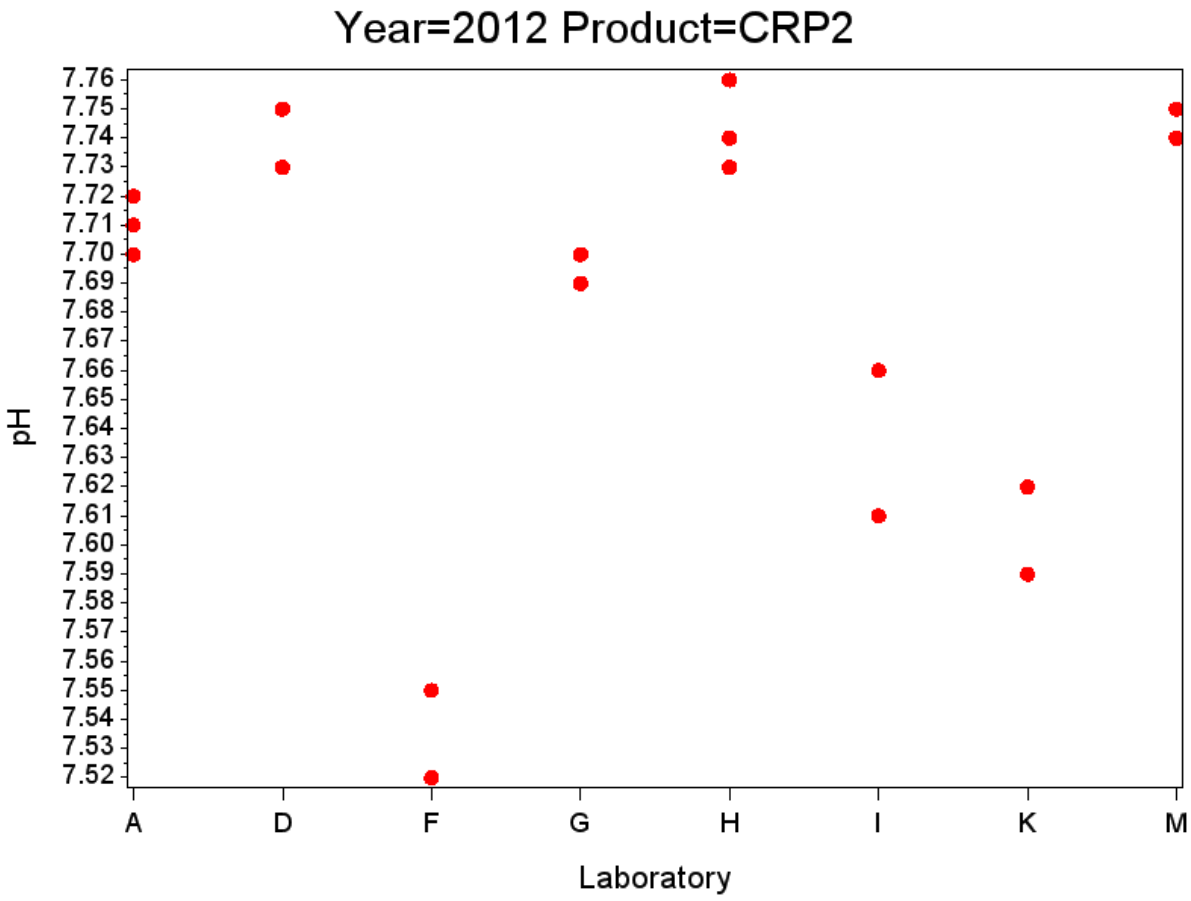


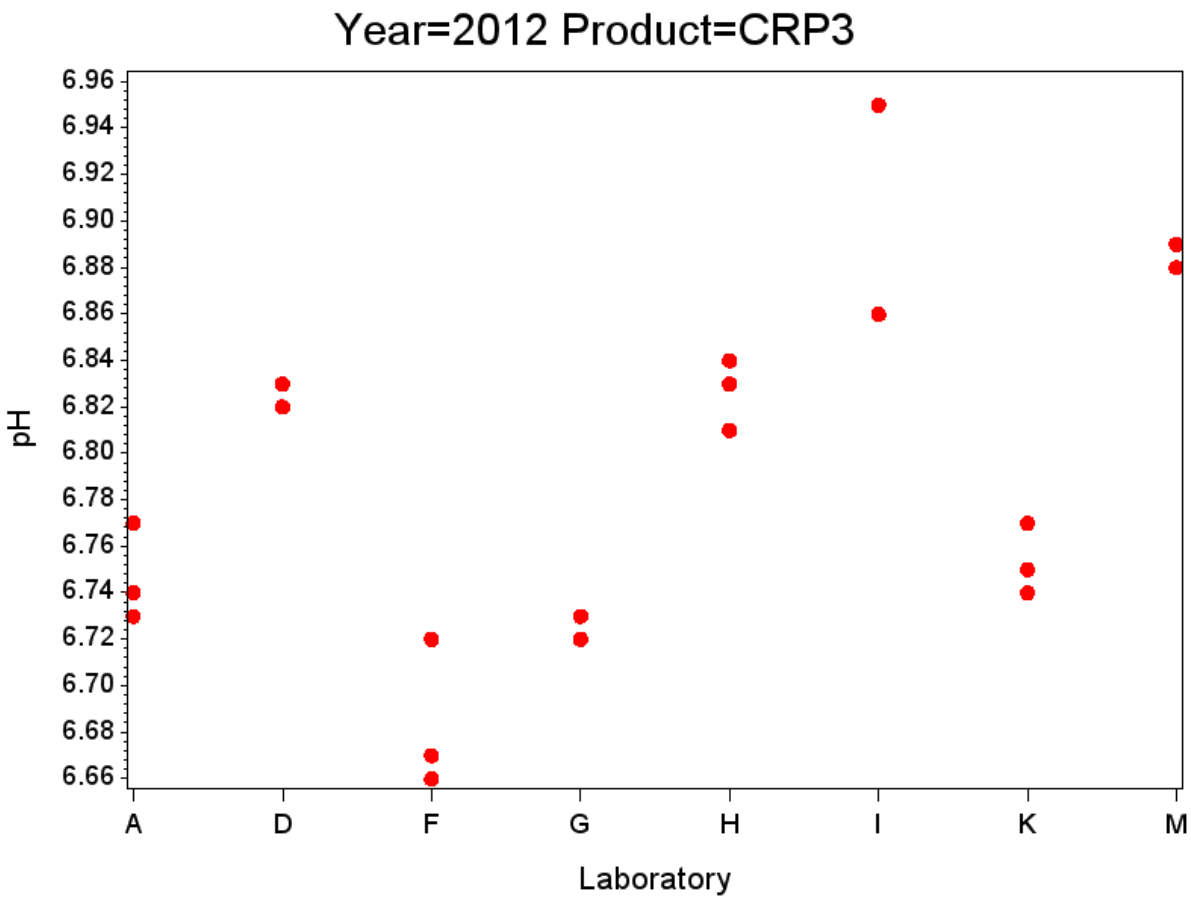


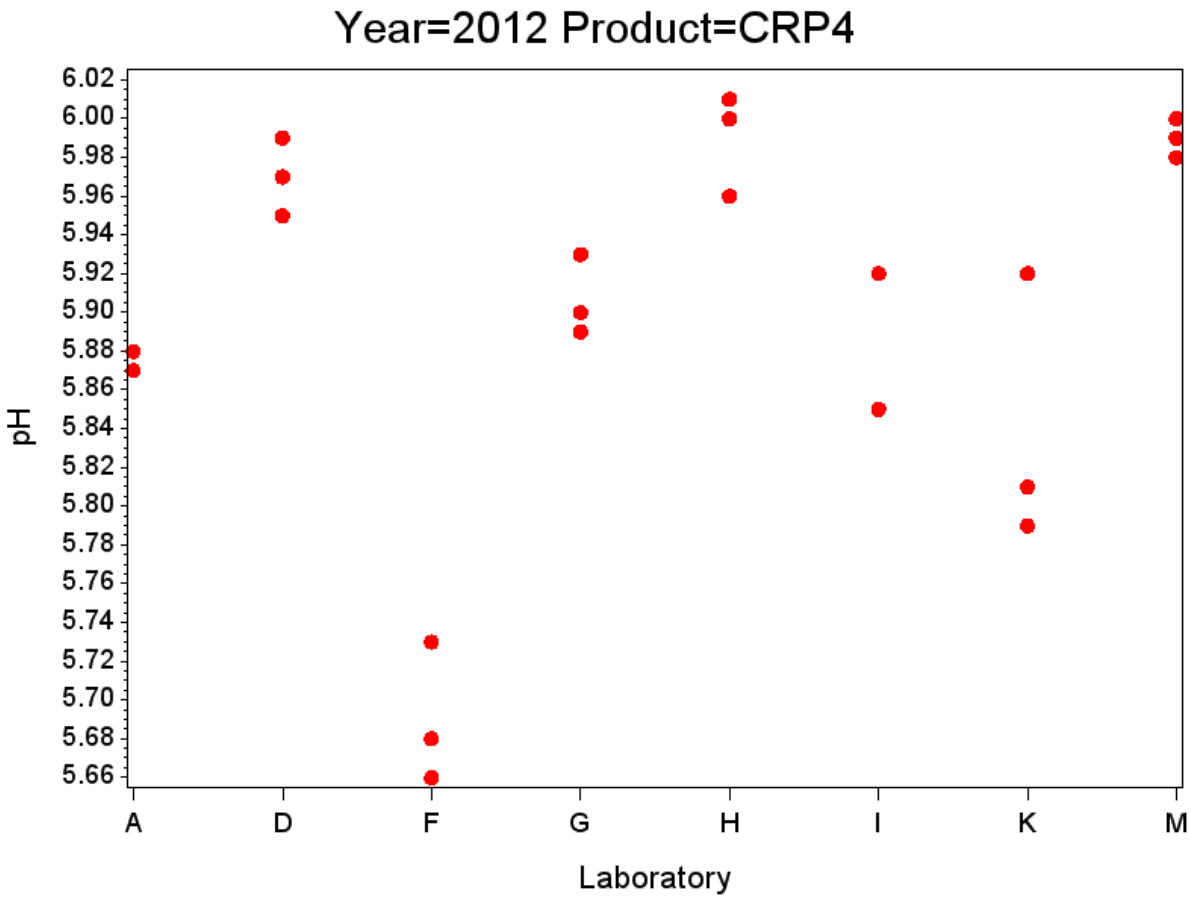

Year=2012 Product=CRP3
Nicotine (%)
2.30
2.28
2.26
2.24
2.22
2.20
2.18
2.16
2.14
2.12
2.10
2.08
2.06
2.04
2.02
2.00
1.98
1.96
1.94
1.92
1.90
A
D
F
G
H
I
K
M
Laboratory

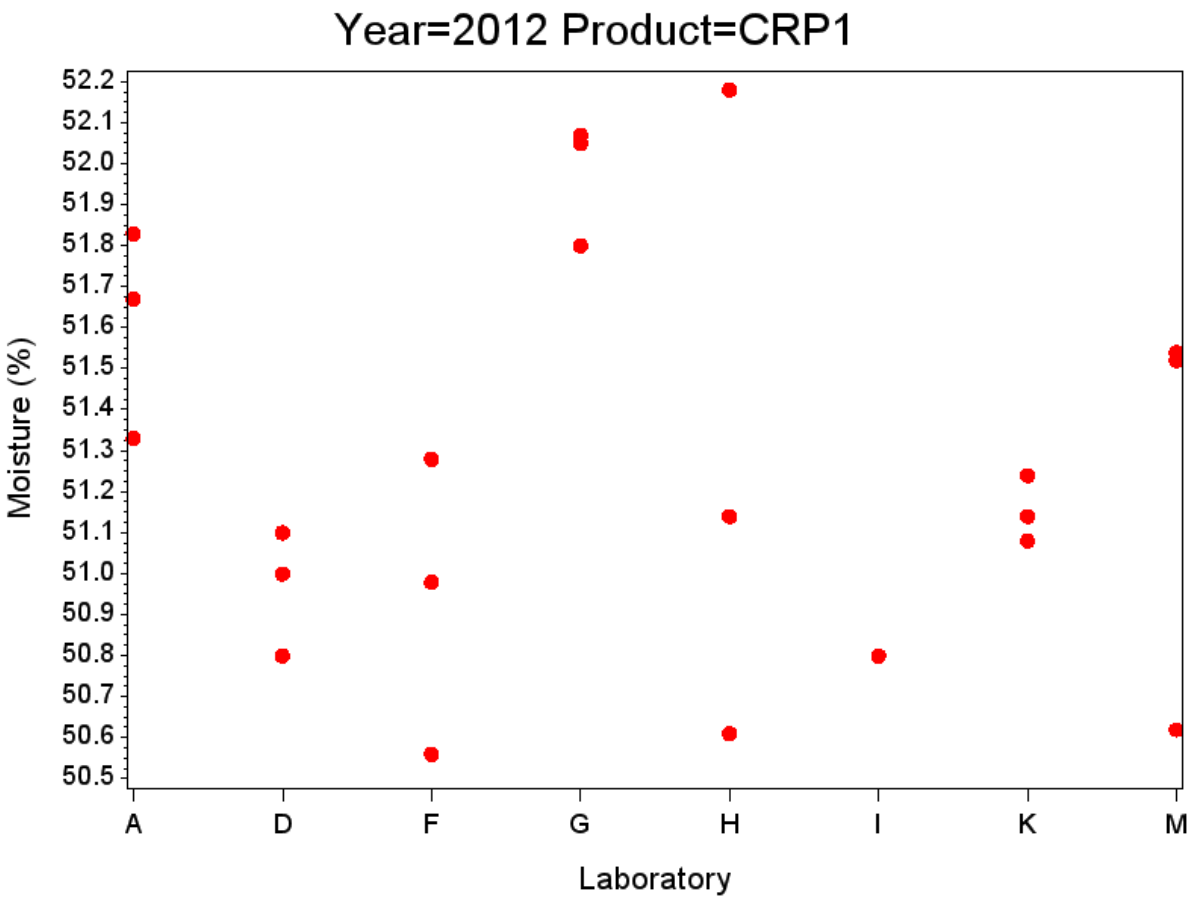

Year=2012 Product=CRP4
Nicotine (%)
1.16
1.14
1.12
1.10
1.08
1.06
1.04
1.02
1.00
0.98
0.96
0.94
0.92
0.90
0.88
A
D
F
G
H
I
K
M
Laboratory

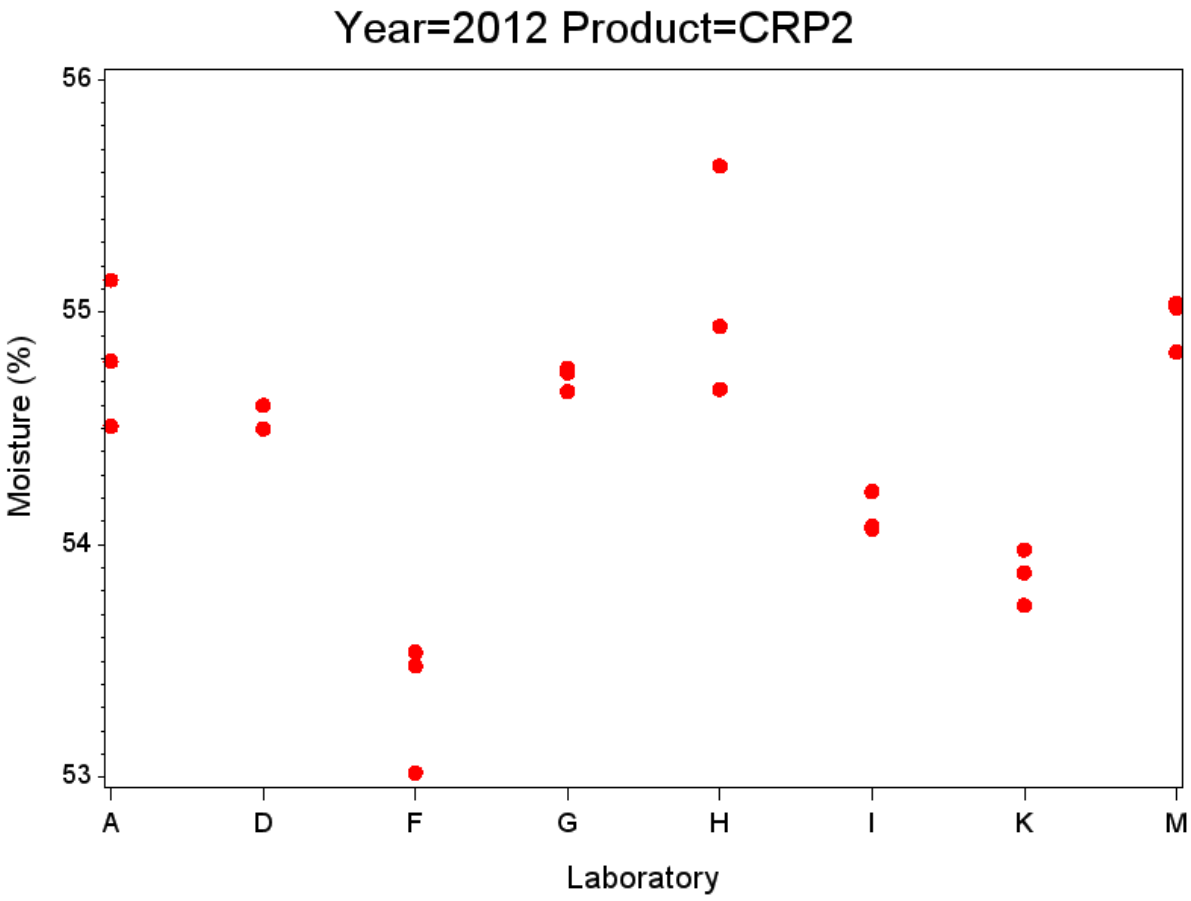

Year=2012 Product=CRP1
pH
8.02
8.00
7.98
7.96
7.94
7.92
7.90
7.88
7.86
7.84
7.82
7.80
7.78
7.76
7.74
7.72
A
D
F
G
H
I
K
M
Laboratory

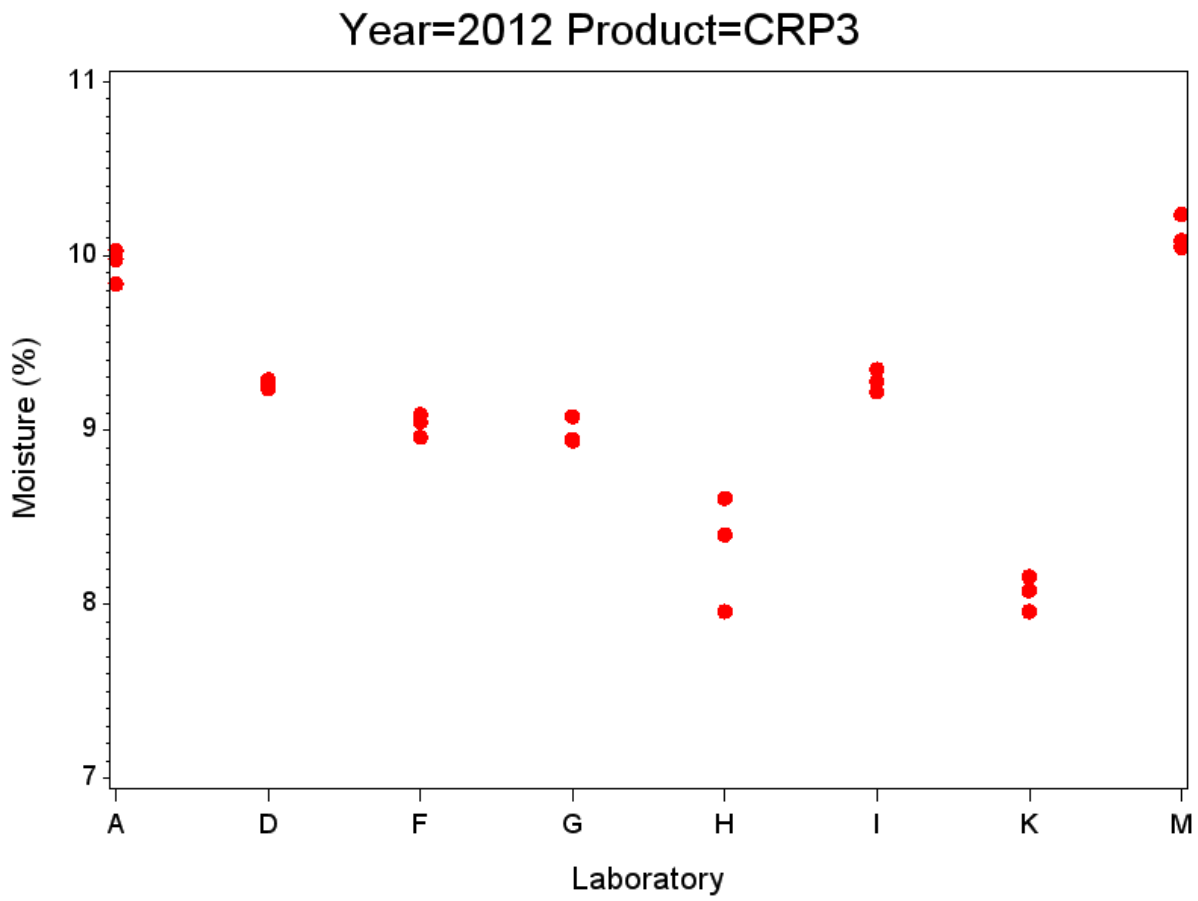

Year=2012 Product=CRP2
pH
7.76
7.75
7.74
7.73
7.72
7.71
7.70
7.69
7.68
7.67
7.66
7.65
7.64
7.63
7.62
7.61
7.60
7.59
7.58
7.57
7.56
7.55
7.54
7.53
7.52
A
D
F
G
H
I
K
M
Laboratory

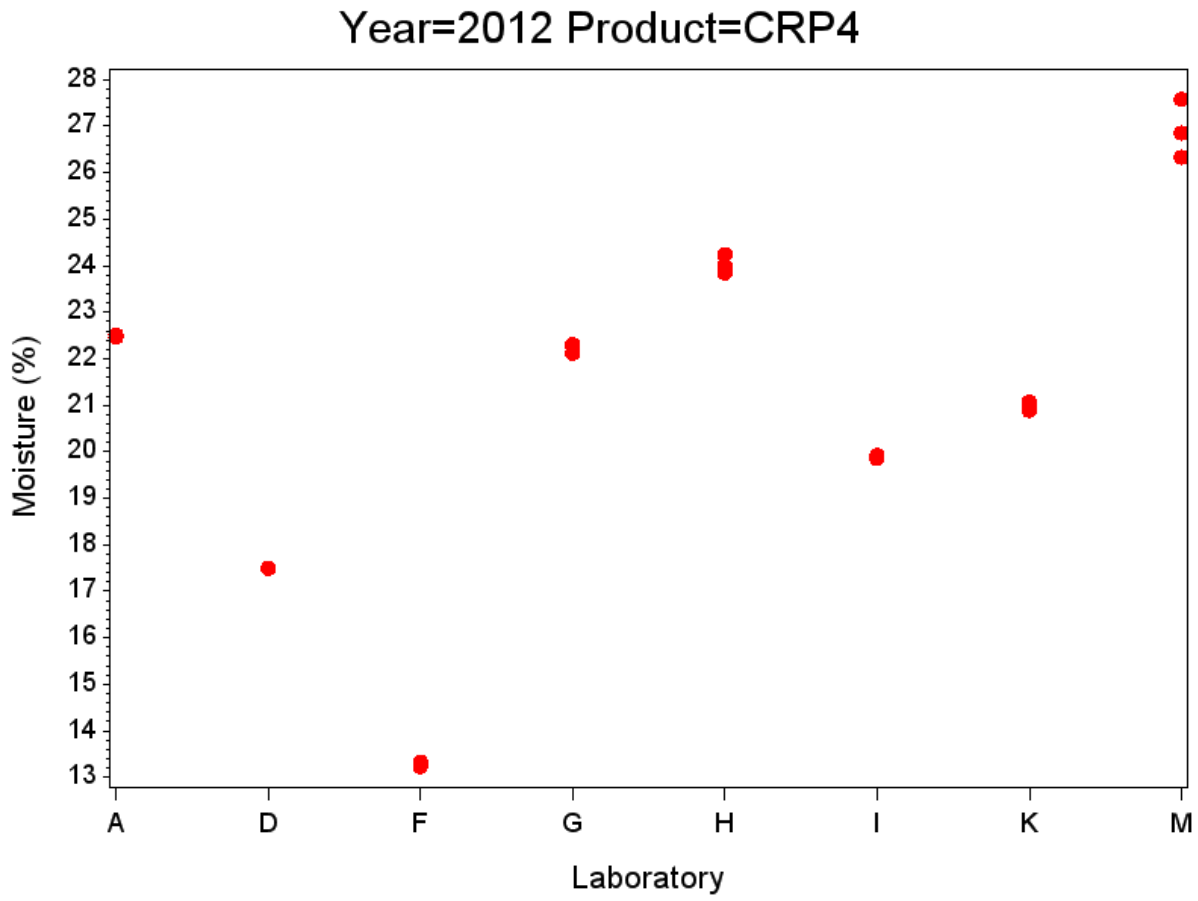

Year=2012 Product=CRP3
pH
6.96
6.94
6.92
6.90
6.88
6.86
6.84
6.82
6.80
6.78
6.76
6.74
6.72
6.70
6.68
6.66
A
D
F
G
H
I
K
M
Laboratory

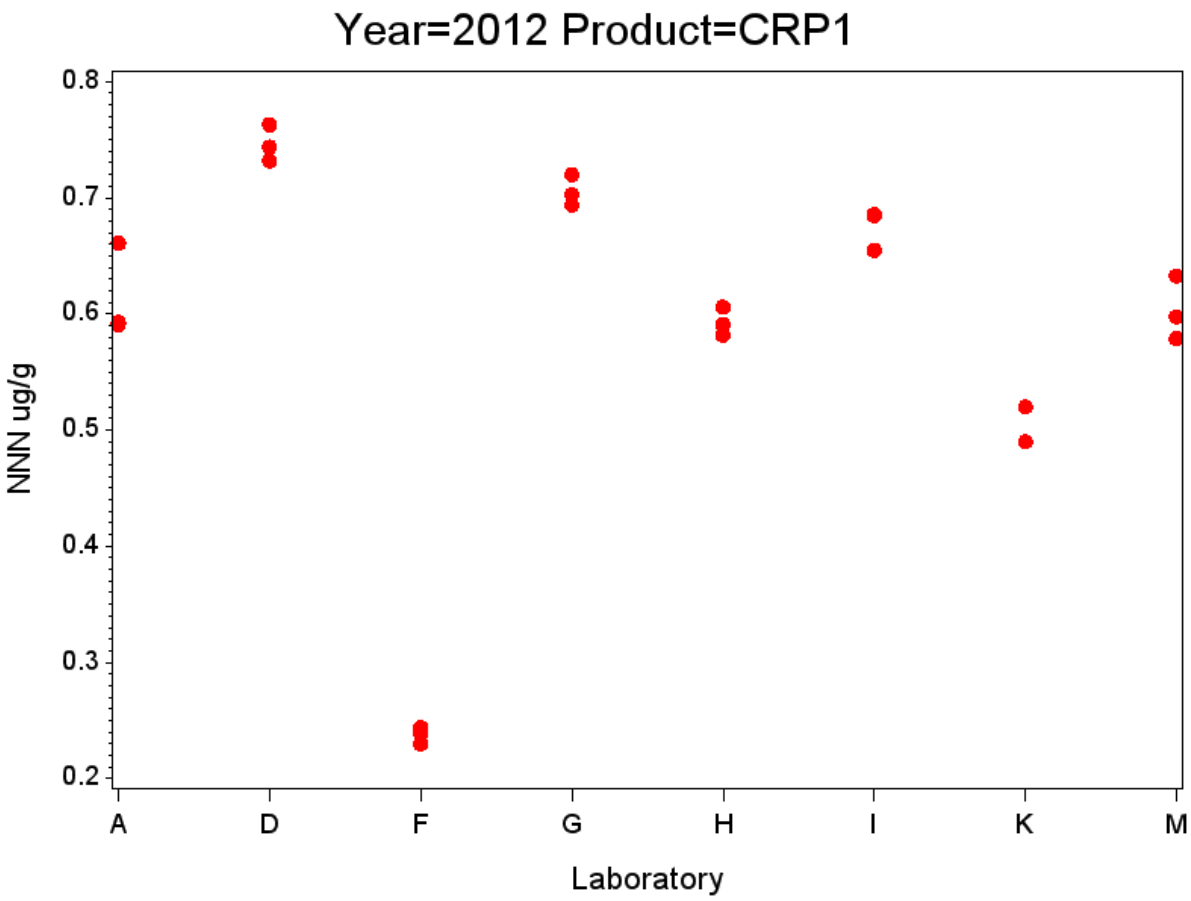

Year=2012 Product=CRP4
pH
6.02
6.00
5.98
5.96
5.94
5.92
5.90
5.88
5.86
5.84
5.82
5.80
5.78
5.76
5.74
5.72
5.70
5.68
5.66
A
D
F
G
H
I
K
M
Laboratory

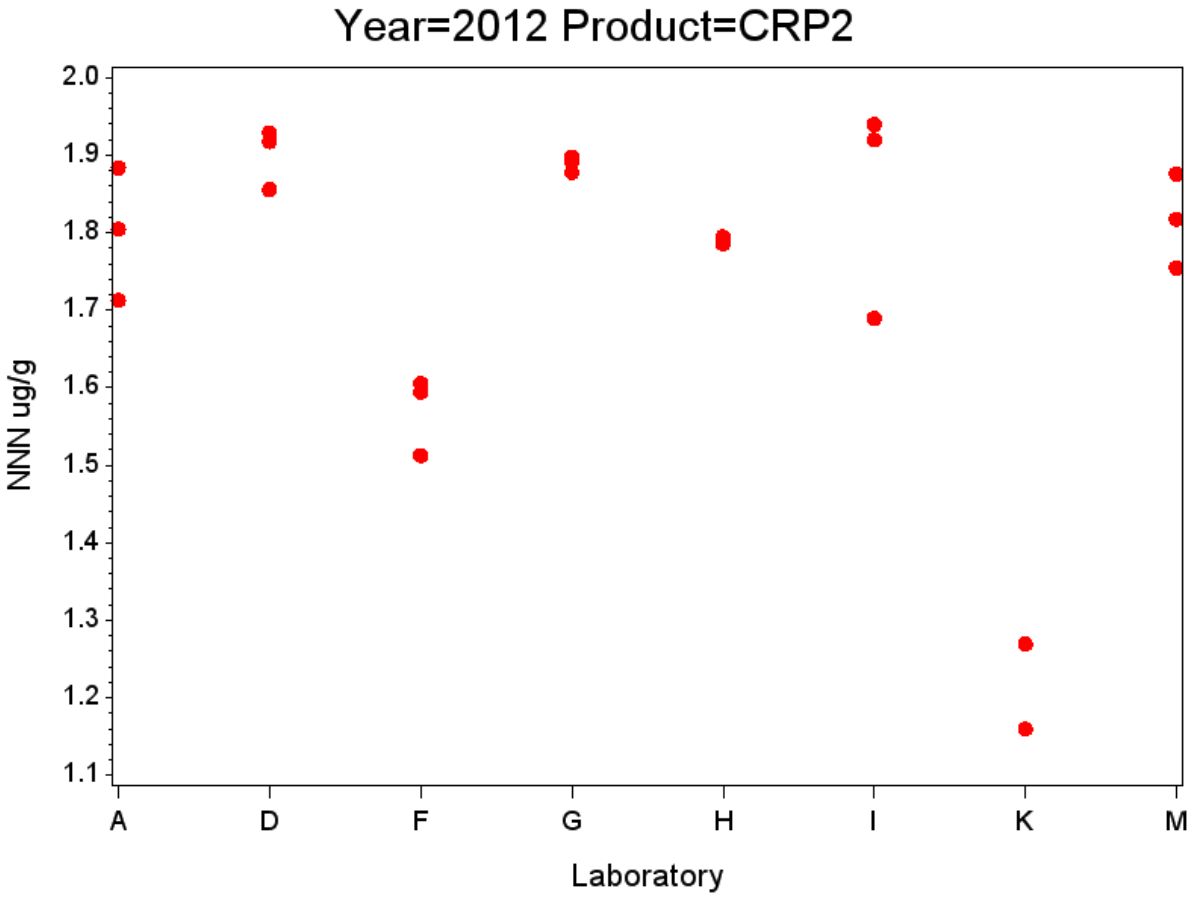

Year=2012 Product=CRP1
Moisture (%)
52.2
52.1
52.0
51.9
51.8
51.7
51.6
51.5
51.4
51.3
51.2
51.1
51.0
50.9
50.8
50.7
50.6
50.5
A
D
F
G
H
I
K
M
Laboratory

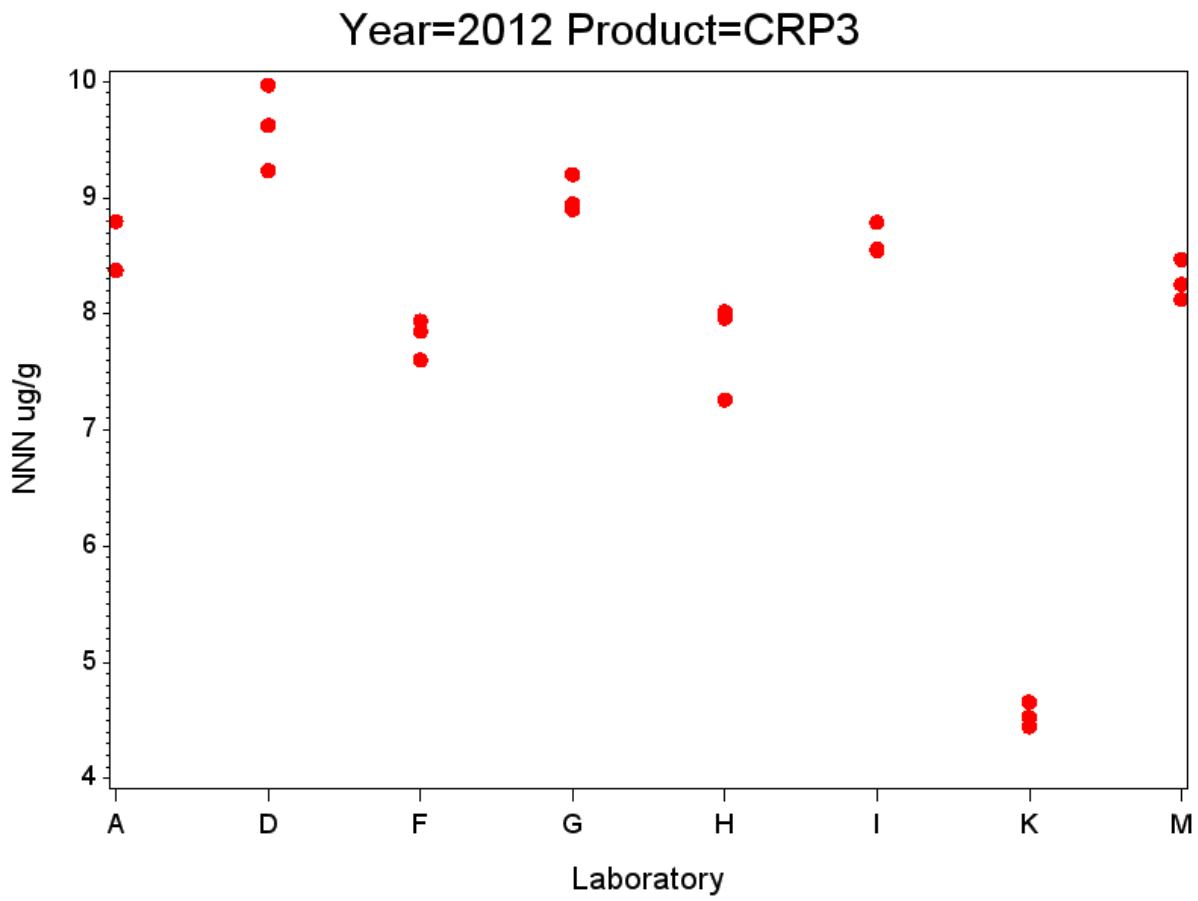

Year=2012 Product=CRP2
Moisture (%)
56
55
54
53
A
D
F
G
H
I
K
M
Laboratory

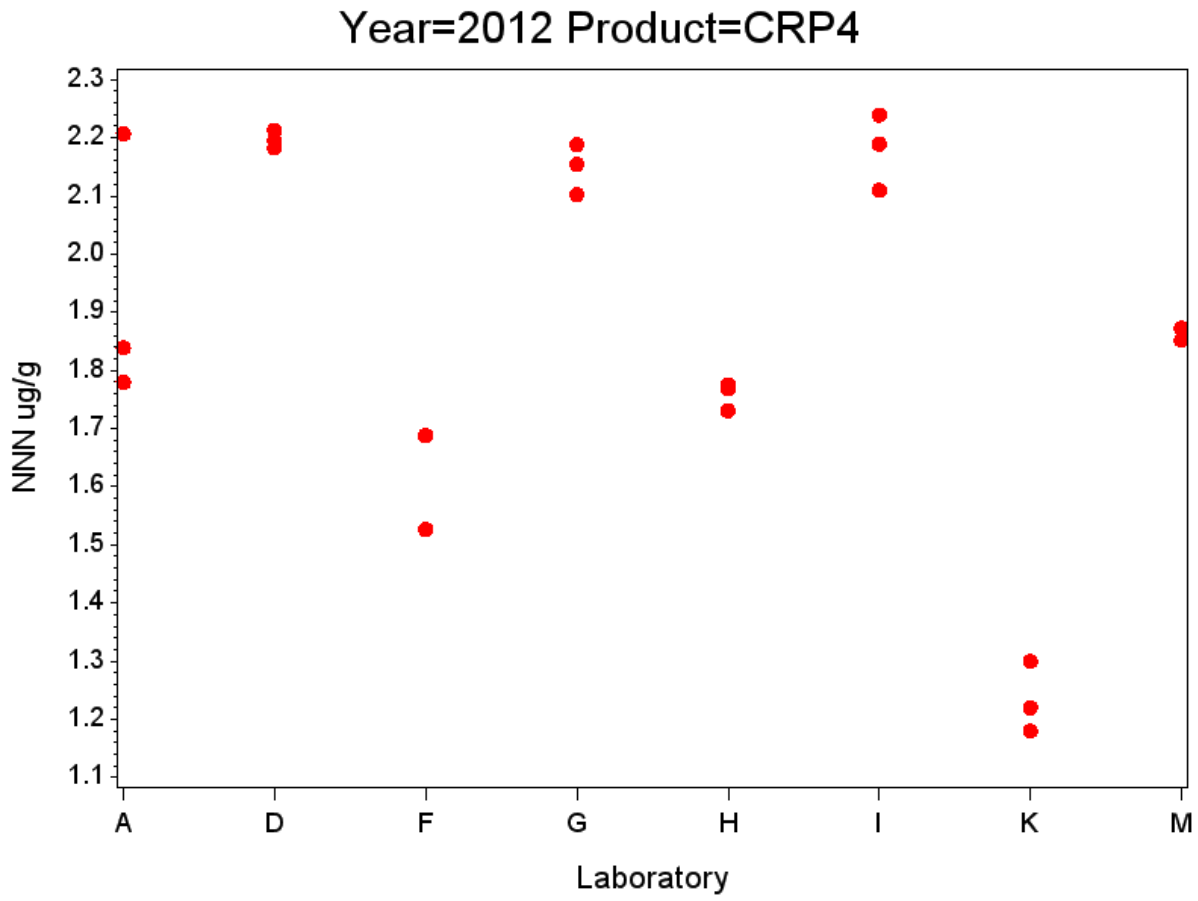

Year=2012 Product=CRP3
Moisture (%)
11
10
9
8
7
A
D
F
G
H
I
K
M
Laboratory

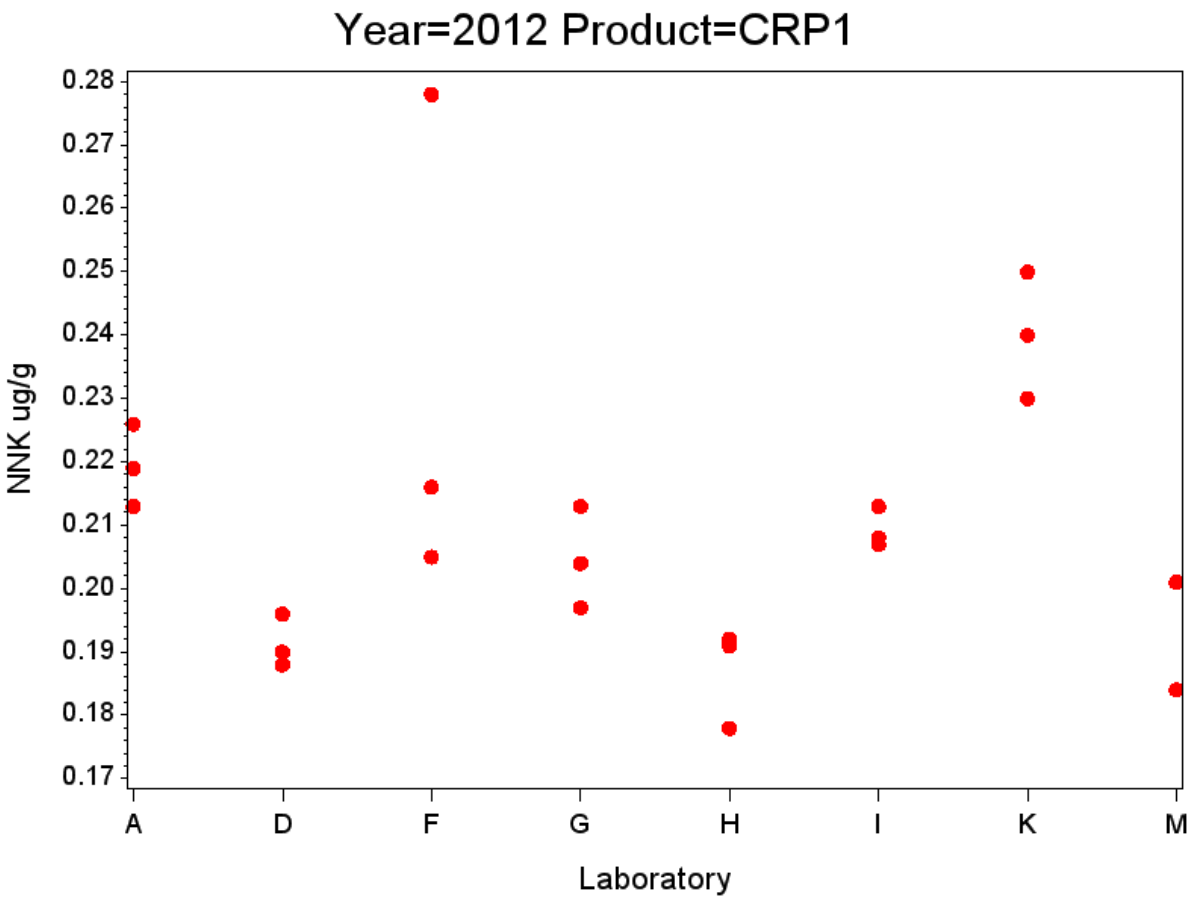

Year=2012 Product=CRP4
Moisture (%)
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
A
D
F
G
H
I
K
M
Laboratory

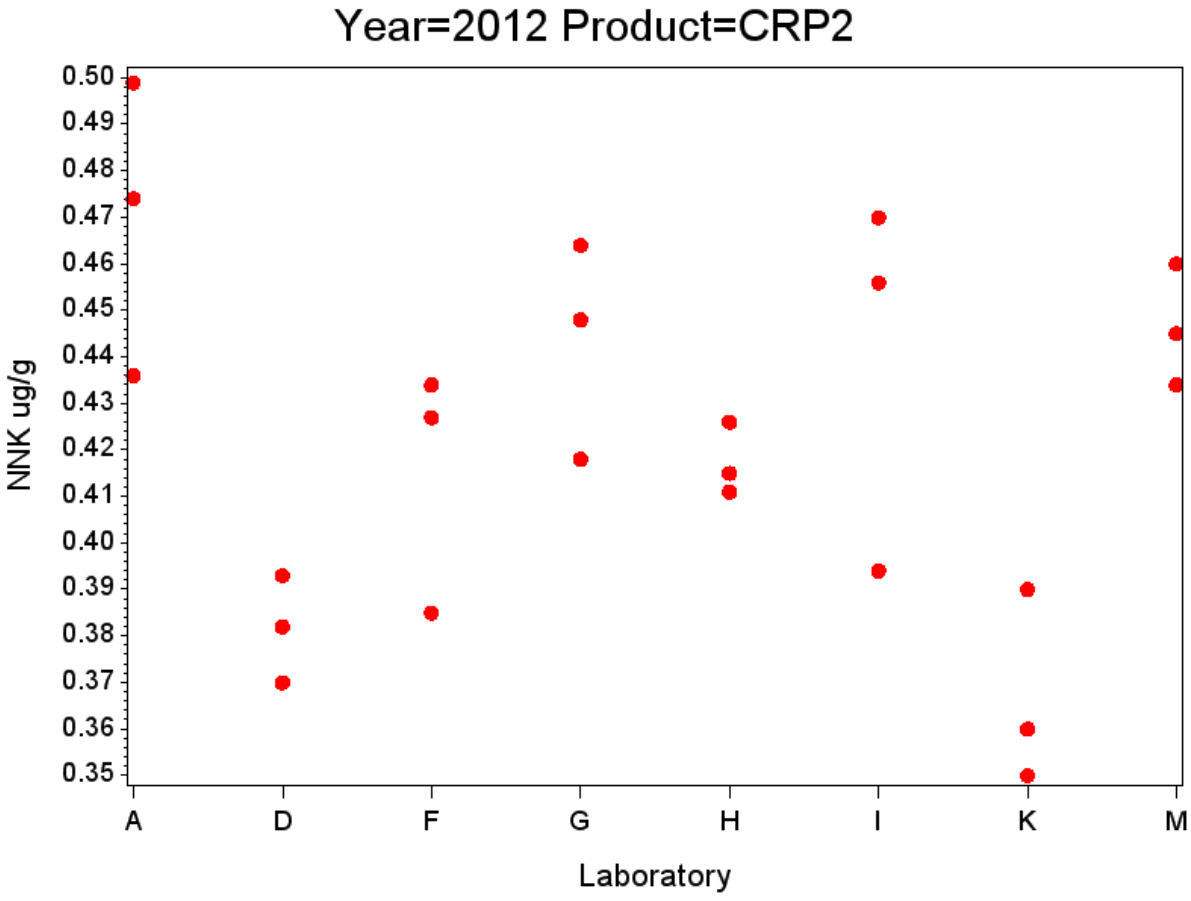

Year=2012 Product=CRP1
NNN ug/g
0.8
0.7
0.6
0.5
0.4
0.3
0.2
A
D
F
G
H
I
K
M
Laboratory

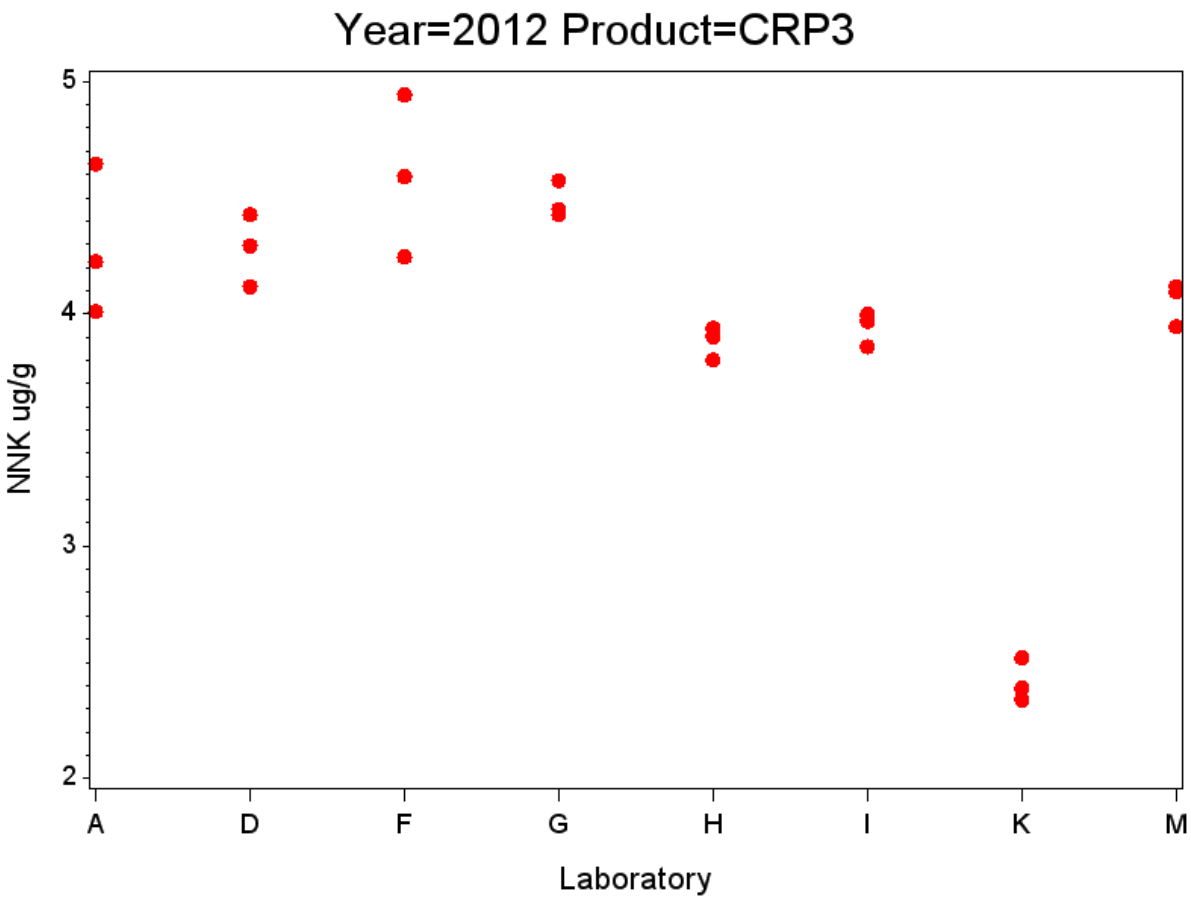

Year=2012 Product=CRP2
NNN ug/g
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
A
D
F
G
H
I
K
M
Laboratory

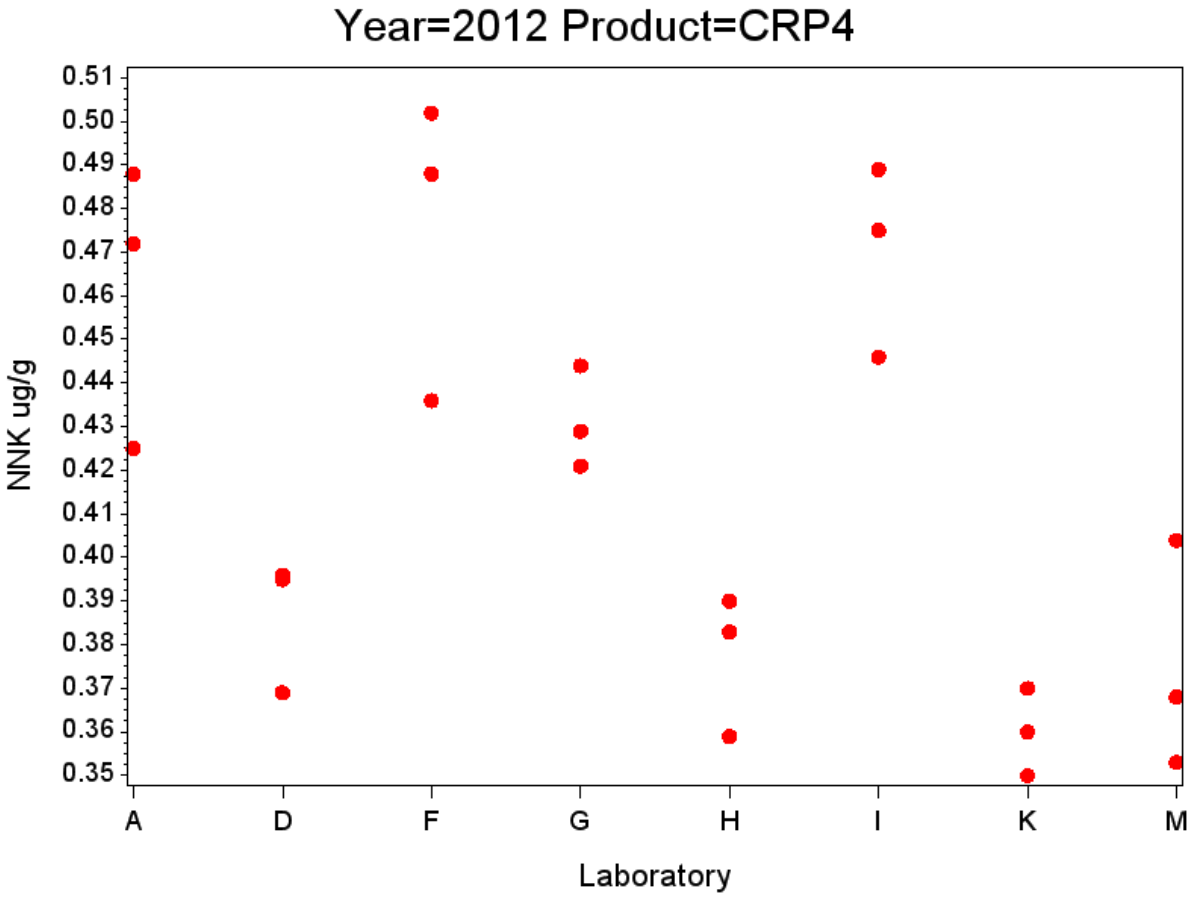

Year=2012 Product=CRP3
NNN ug/g
10
9
8
7
6
5
4
A
D
F
G
H
I
K
M
Laboratory

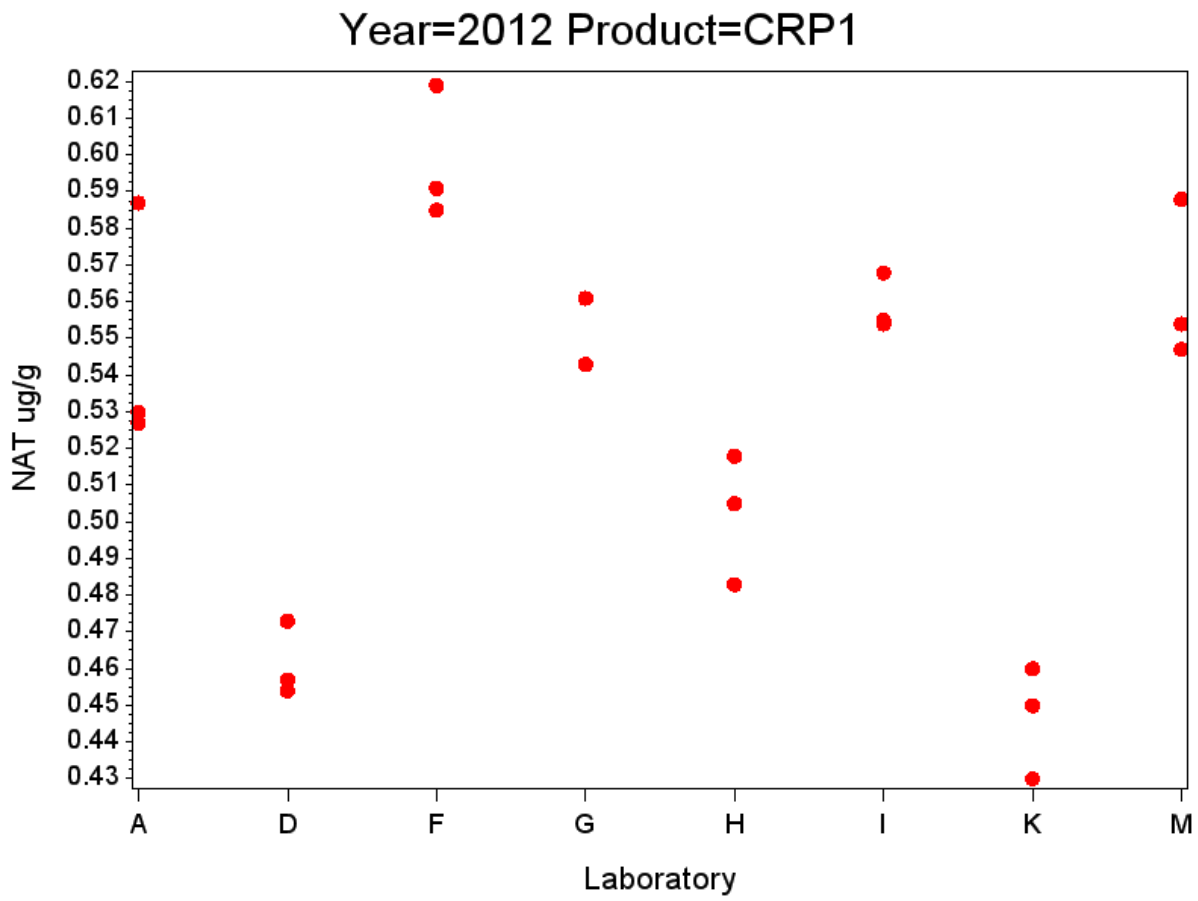

Year=2012 Product=CRP4
NNN ug/g
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
A
D
F
G
H
I
K
M
Laboratory

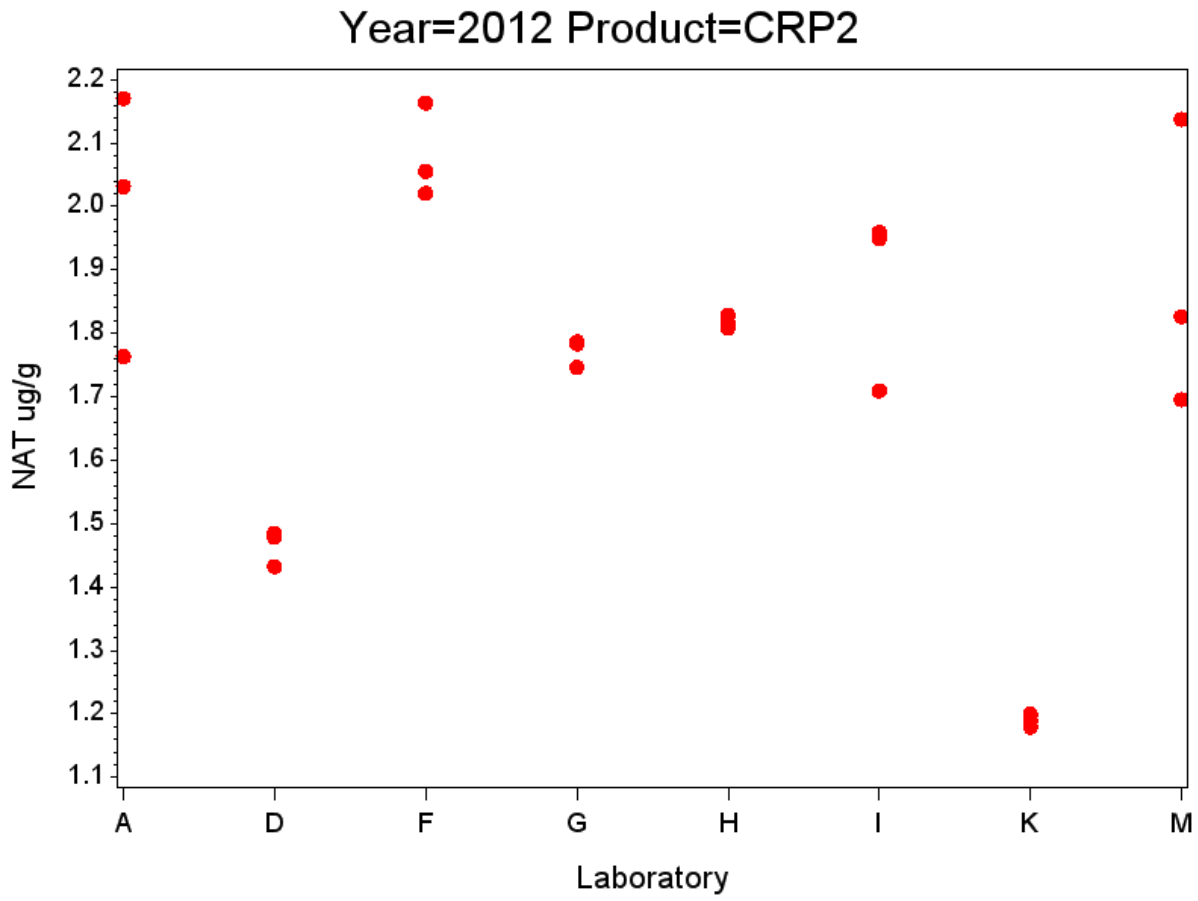

Year=2012 Product=CRP1
NNK ug/g
0.28
0.27
0.26
0.25
0.24
0.23
0.22
0.21
0.20
0.19
0.18
0.17
A
D
F
G
H
I
K
M
Laboratory

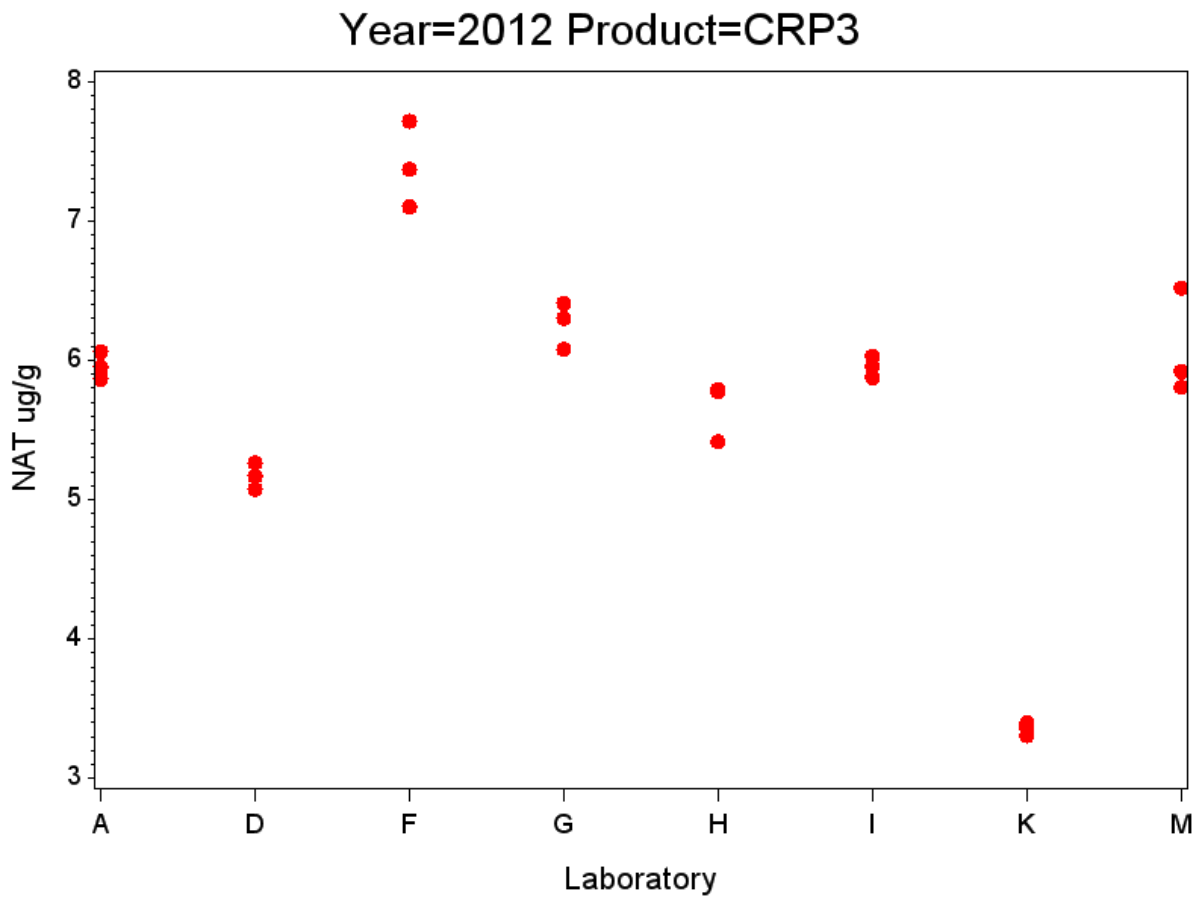

Year=2012 Product=CRP2
NNK ug/g
0.50
0.49
0.48
0.47
0.46
0.45
0.44
0.43
0.42
0.41
0.40
0.39
0.38
0.37
0.36
0.35
A
D
F
G
H
I
K
M
Laboratory

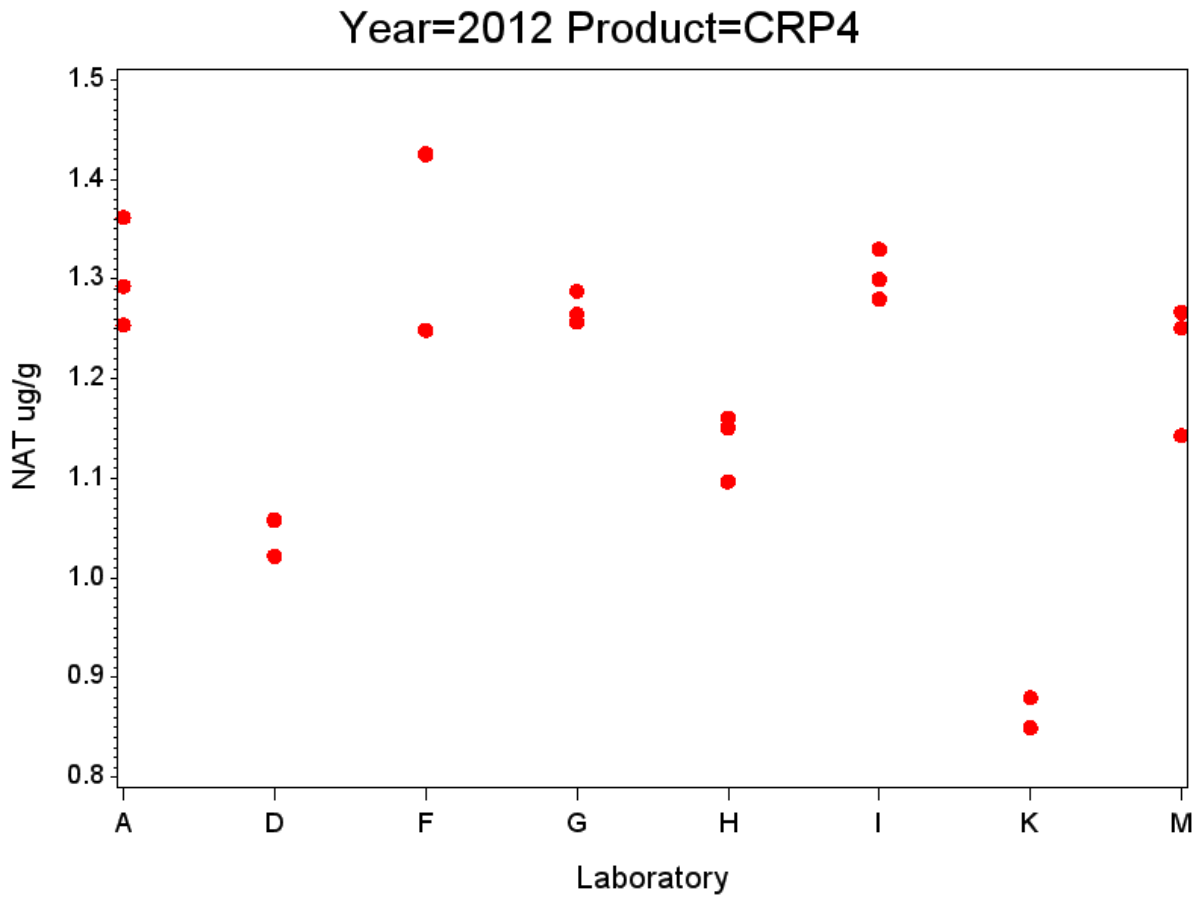

Year=2012 Product=CRP3
NNK ug/g
5
4
3
2
A
D
F
G
H
I
K
M
Laboratory

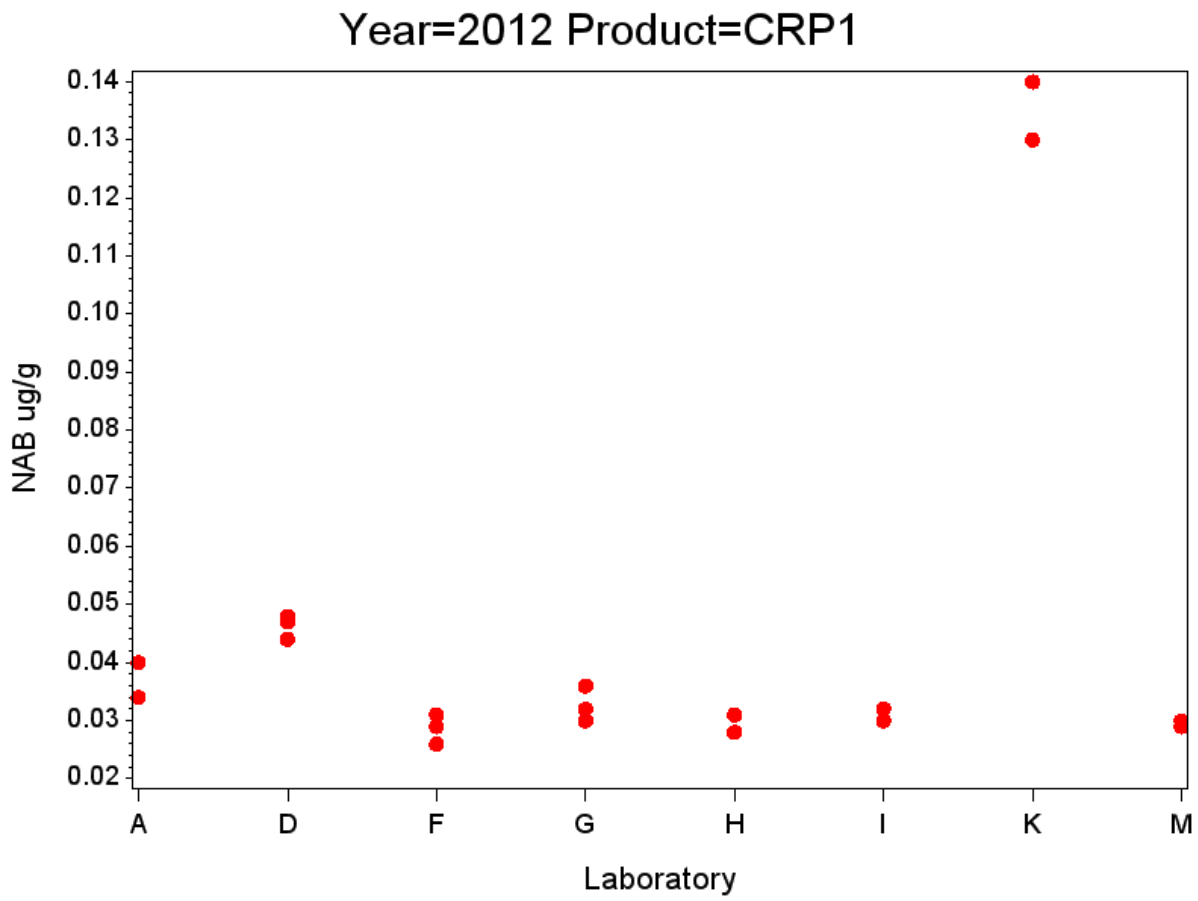

Year=2012 Product=CRP4
NNK ug/g
0.51
0.50
0.49
0.48
0.47
0.46
0.45
0.44
0.43
0.42
0.41
0.40
0.39
0.38
0.37
0.36
0.35
A
D
F
G
H
I
K
M
Laboratory

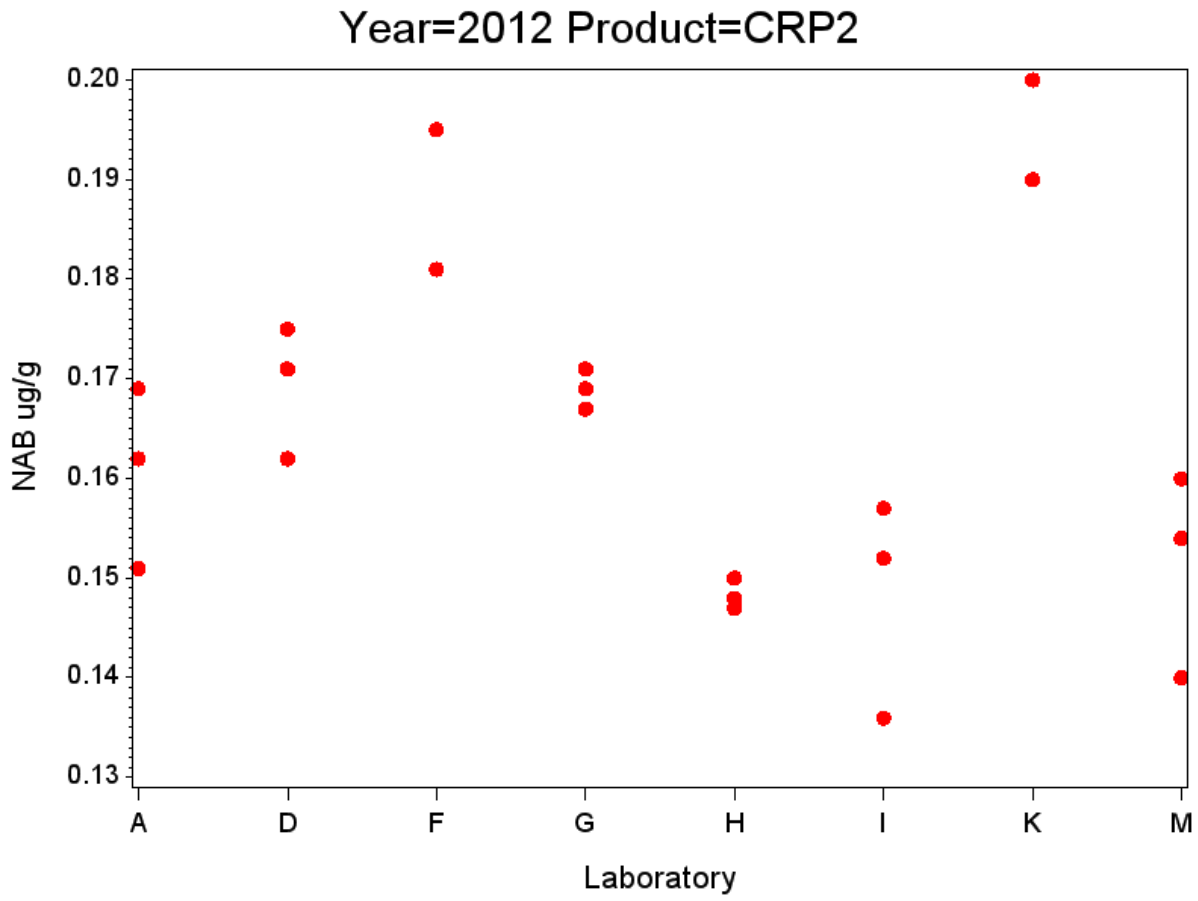

Year=2012 Product=CRP1
NAT ug/g
0.62
0.61
0.60
0.59
0.58
0.57
0.56
0.55
0.54
0.53
0.52
0.51
0.50
0.49
0.48
0.47
0.46
0.45
0.44
0.43
A
D
F
G
H
I
K
M
Laboratory

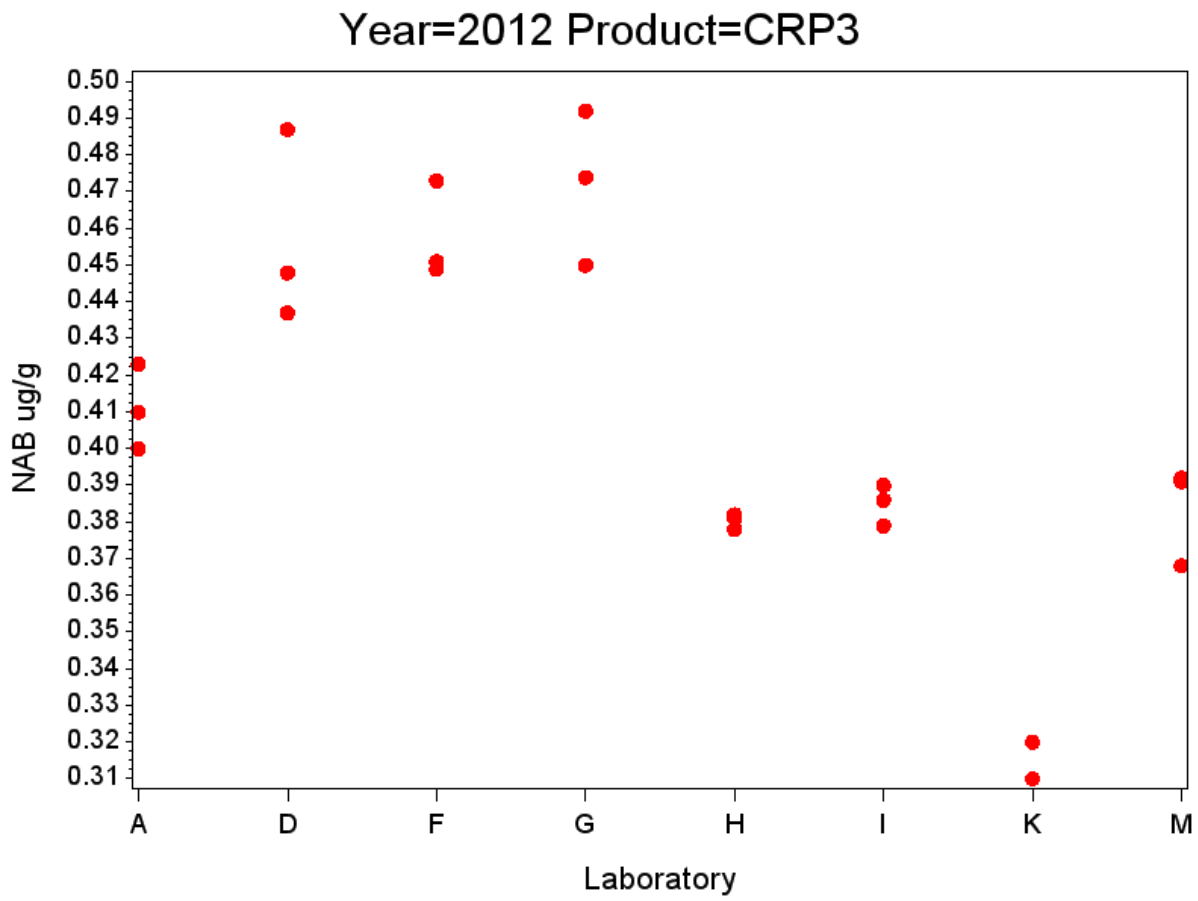

Year=2012 Product=CRP2
NAT ug/g
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
A
D
F
G
H
I
K
M
Laboratory

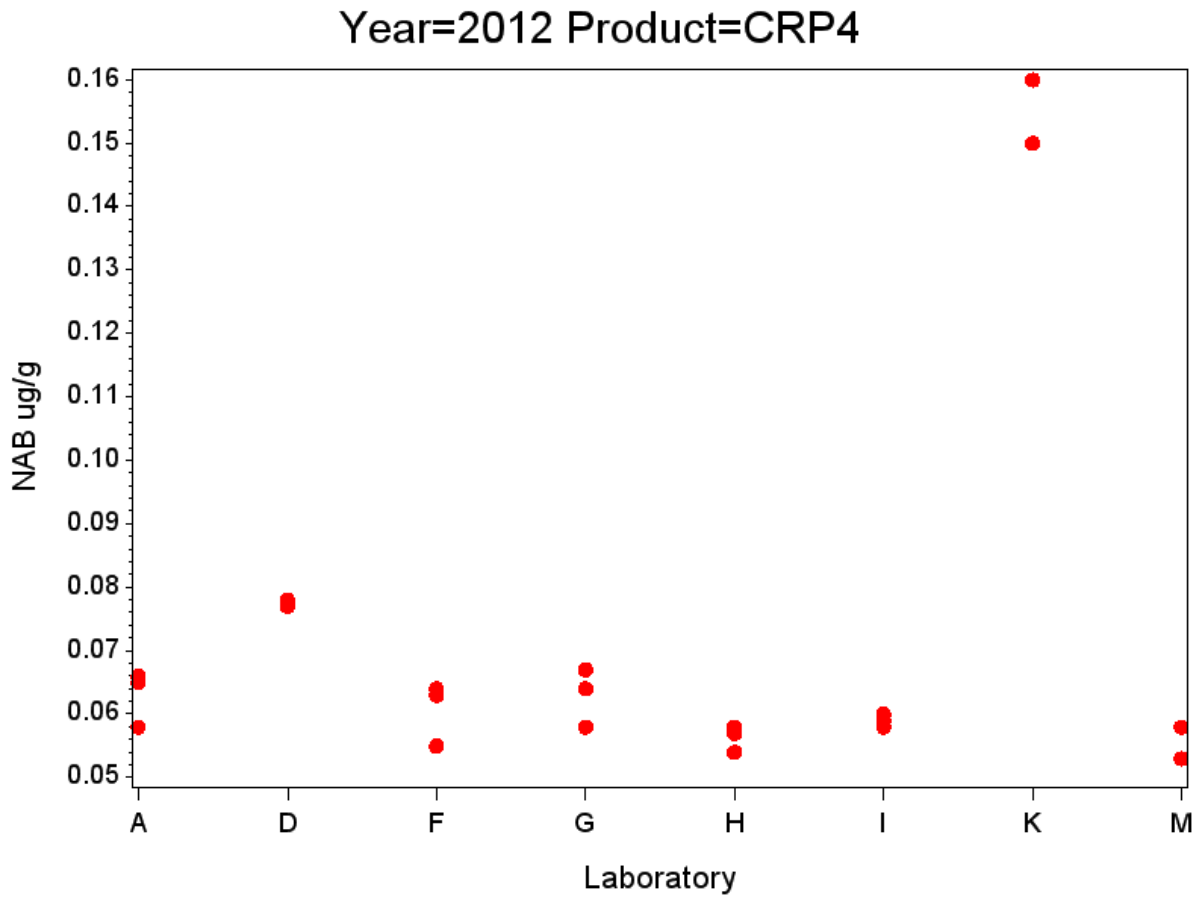

Year=2012 Product=CRP3
NAT ug/g
8
7
6
5
4
3
A
D
F
G
H
I
K
M
Laboratory


Year=2012 Product=CRP4
NAT ug/g
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
A
D
F
G
H
I
K
M
Laboratory

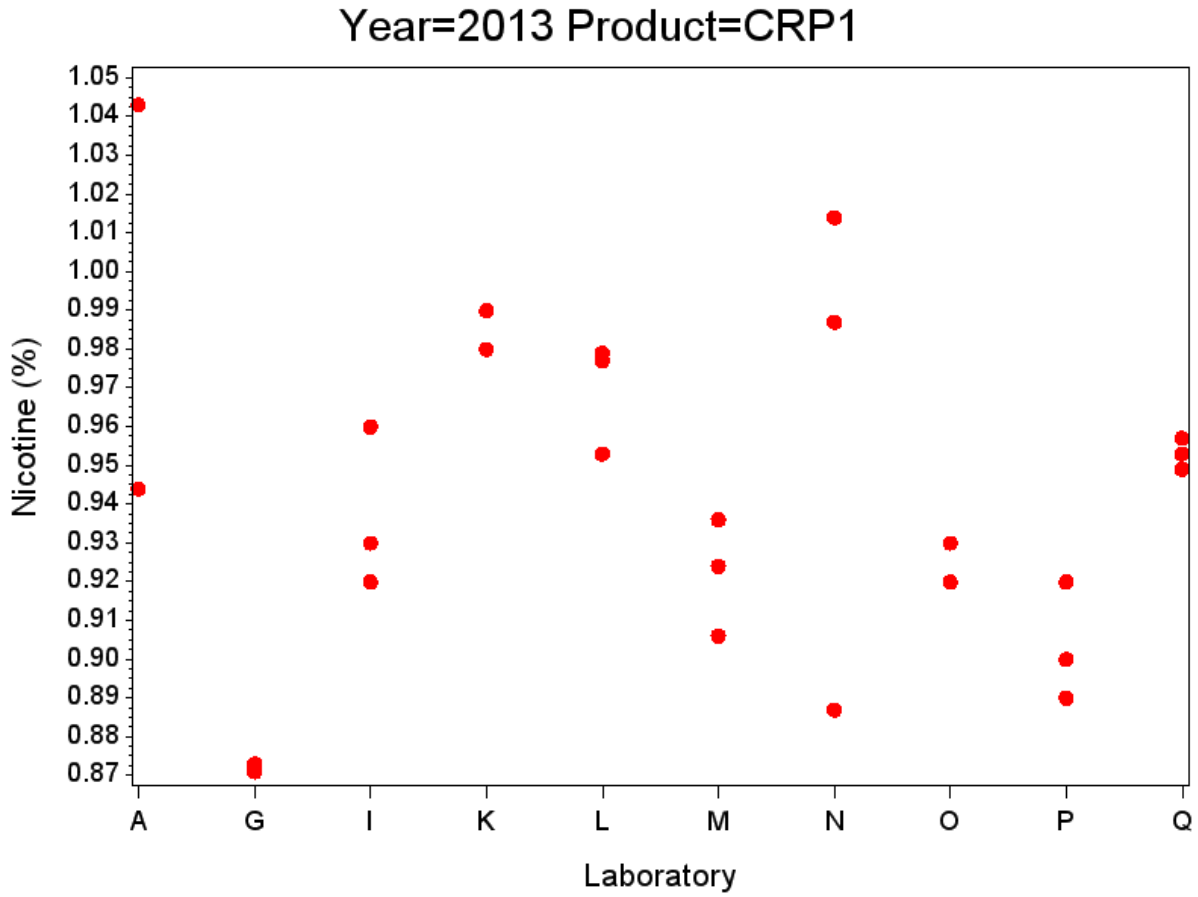

Year=2012 Product=CRP1
NAB ug/g
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
A
D
F
G
H
I
K
M
Laboratory

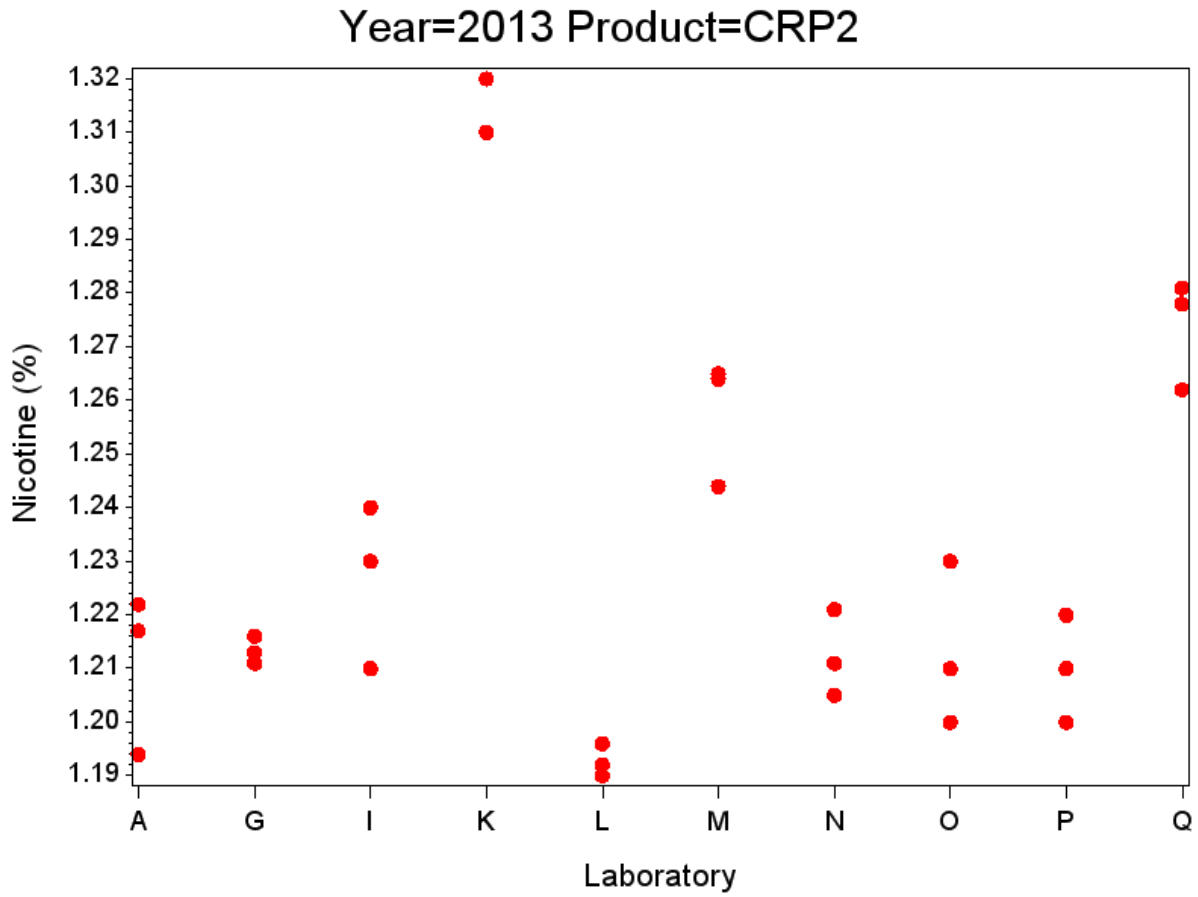

Year=2012 Product=CRP2
NAB ug/g
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
A
D
F
G
H
I
K
M
Laboratory

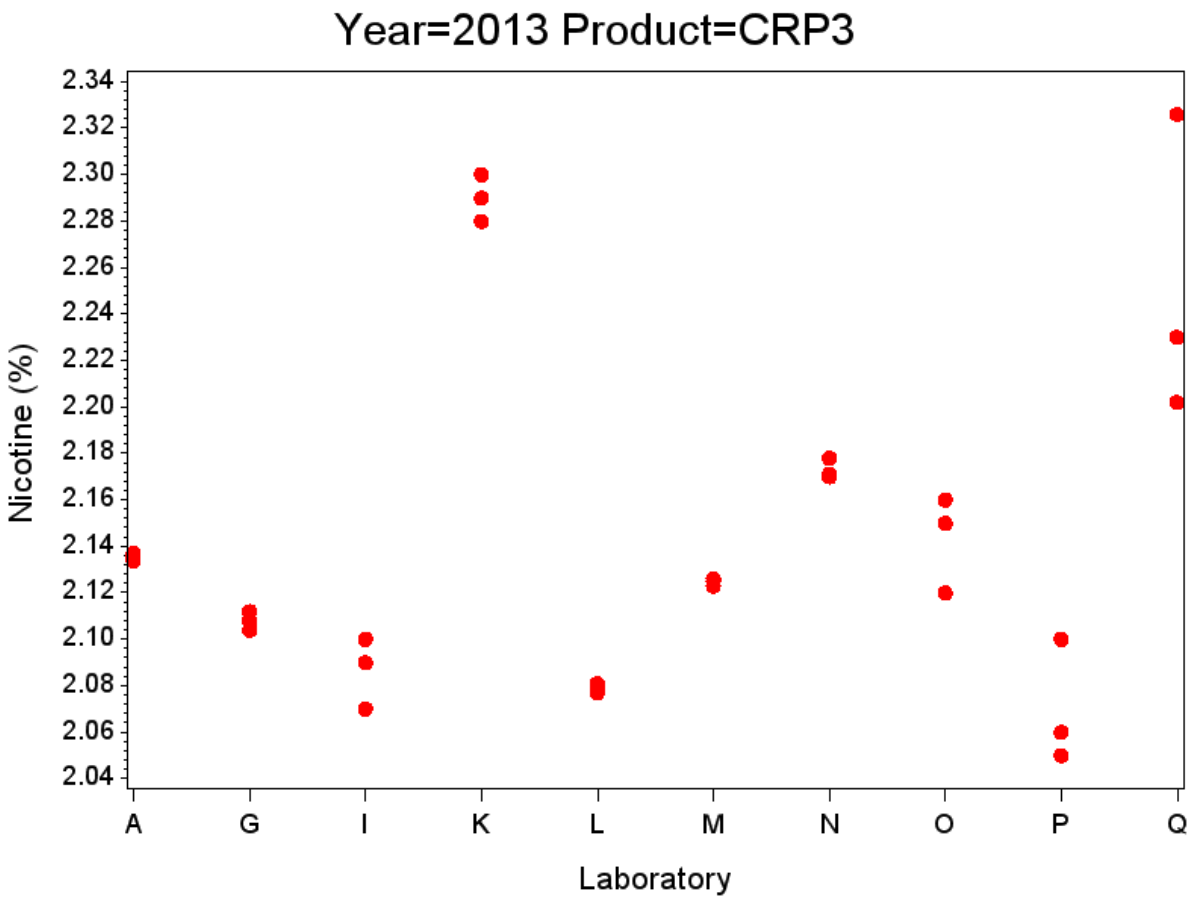

Year=2012 Product=CRP3
NAB ug/g
0.50
0.49
0.48
0.47
0.46
0.45
0.44
0.43
0.42
0.41
0.40
0.39
0.38
0.37
0.36
0.35
0.34
0.33
0.32
0.31
A
D
F
G
H
I
K
M
Laboratory

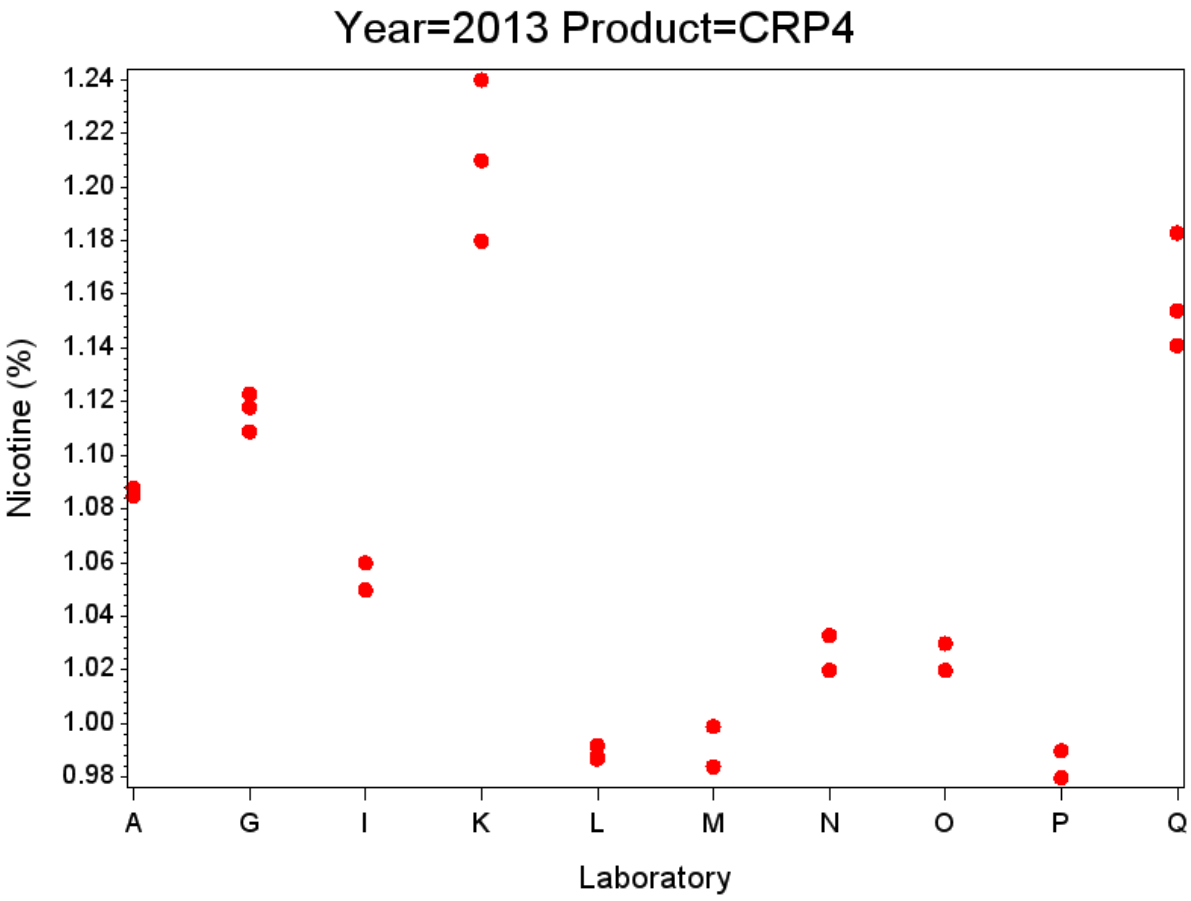

Year=2012 Product=CRP4
NAB ug/g
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
A
D
F
G
H
I
K
M
Laboratory

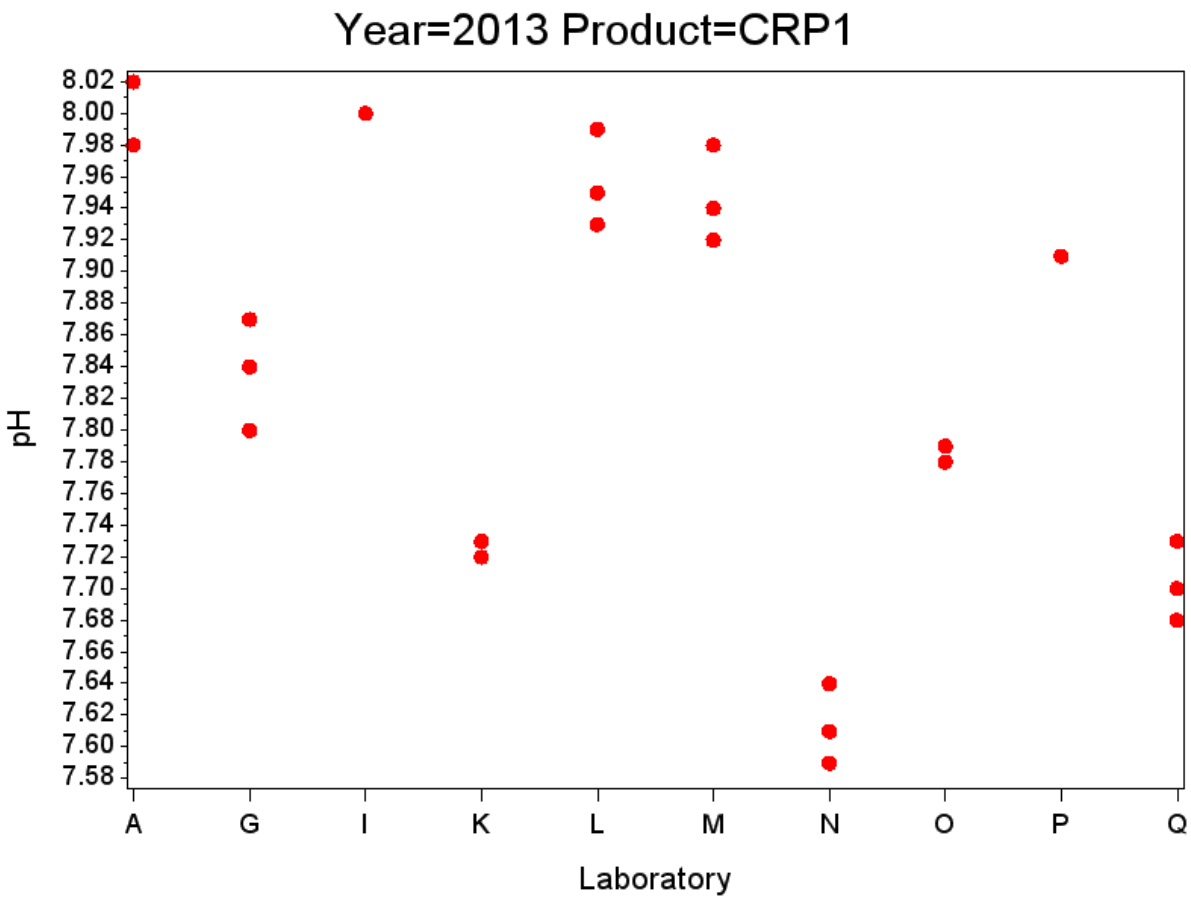
APPENDIX B: Raw Data Plots for 2013 WG4 Study

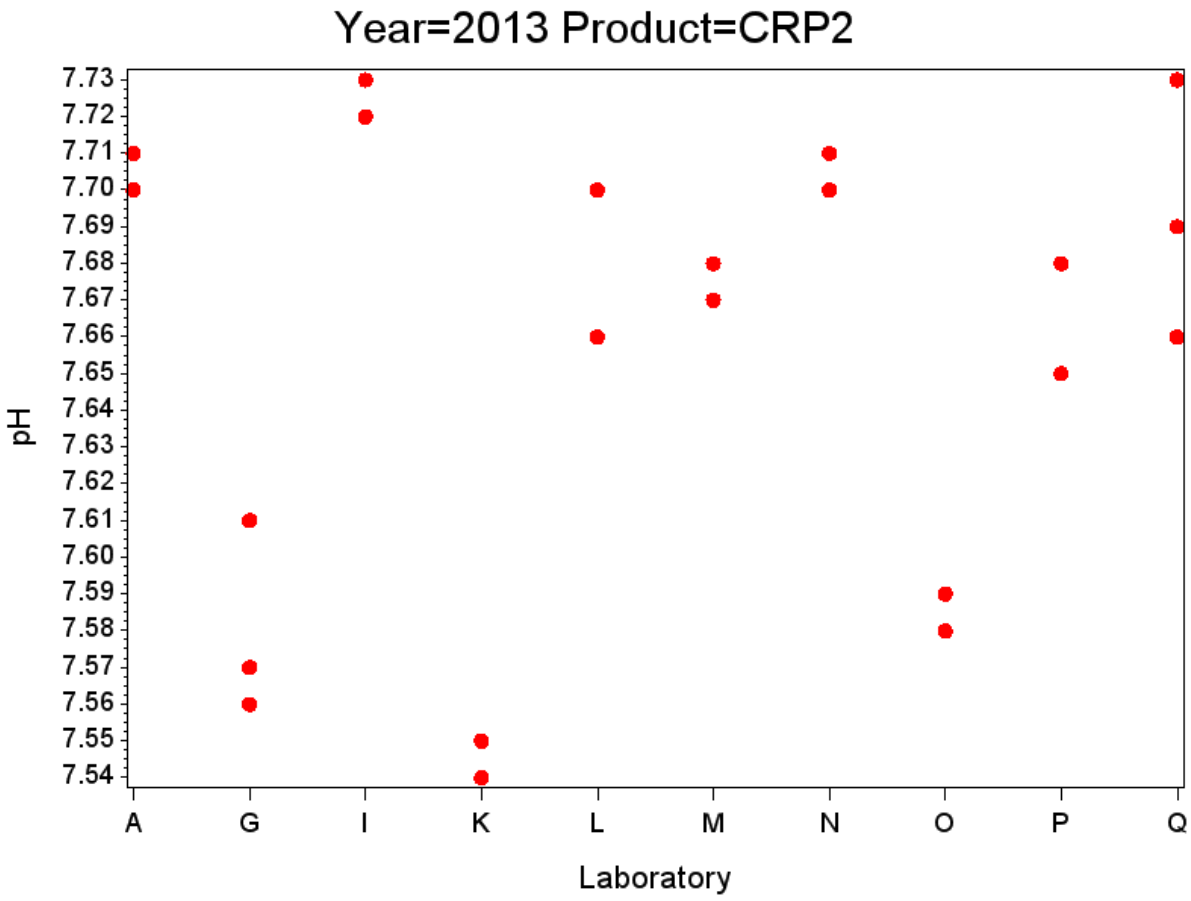


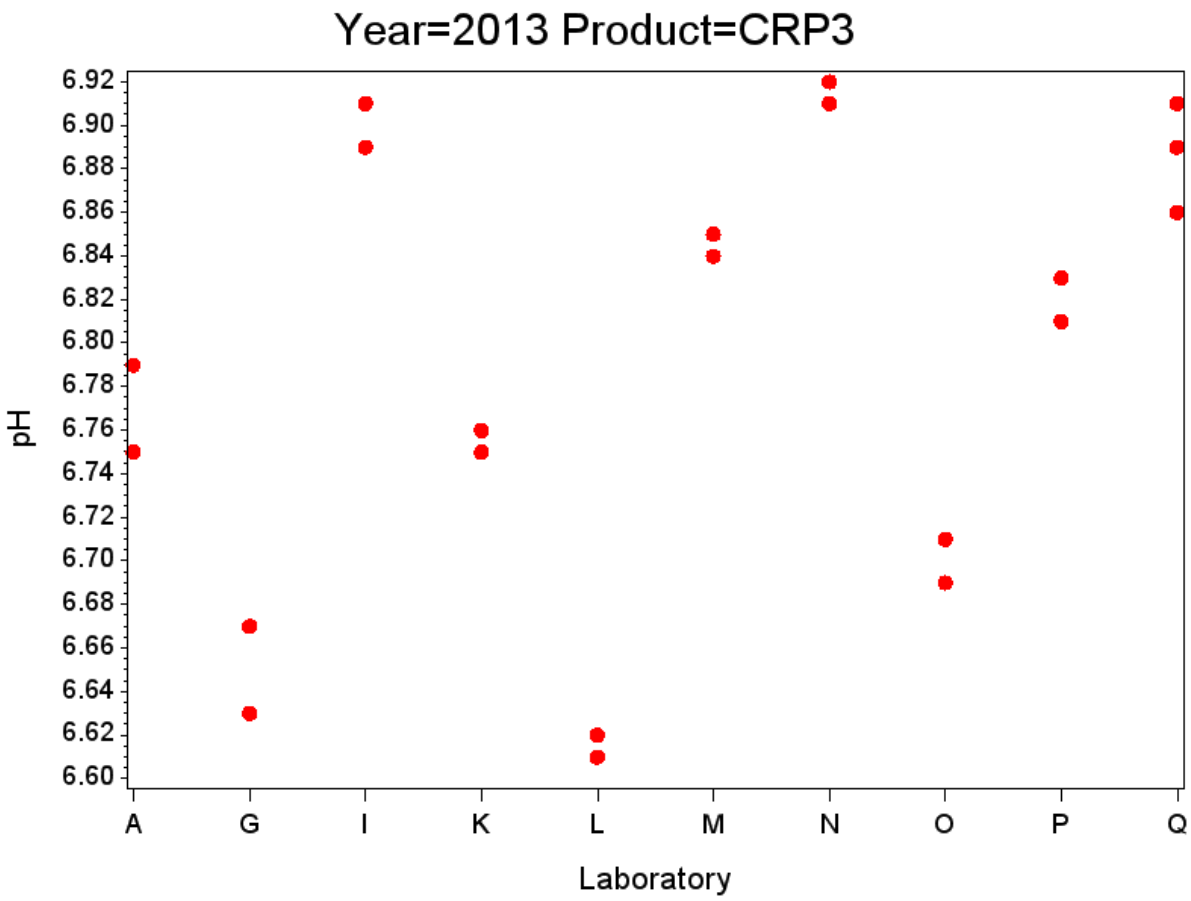


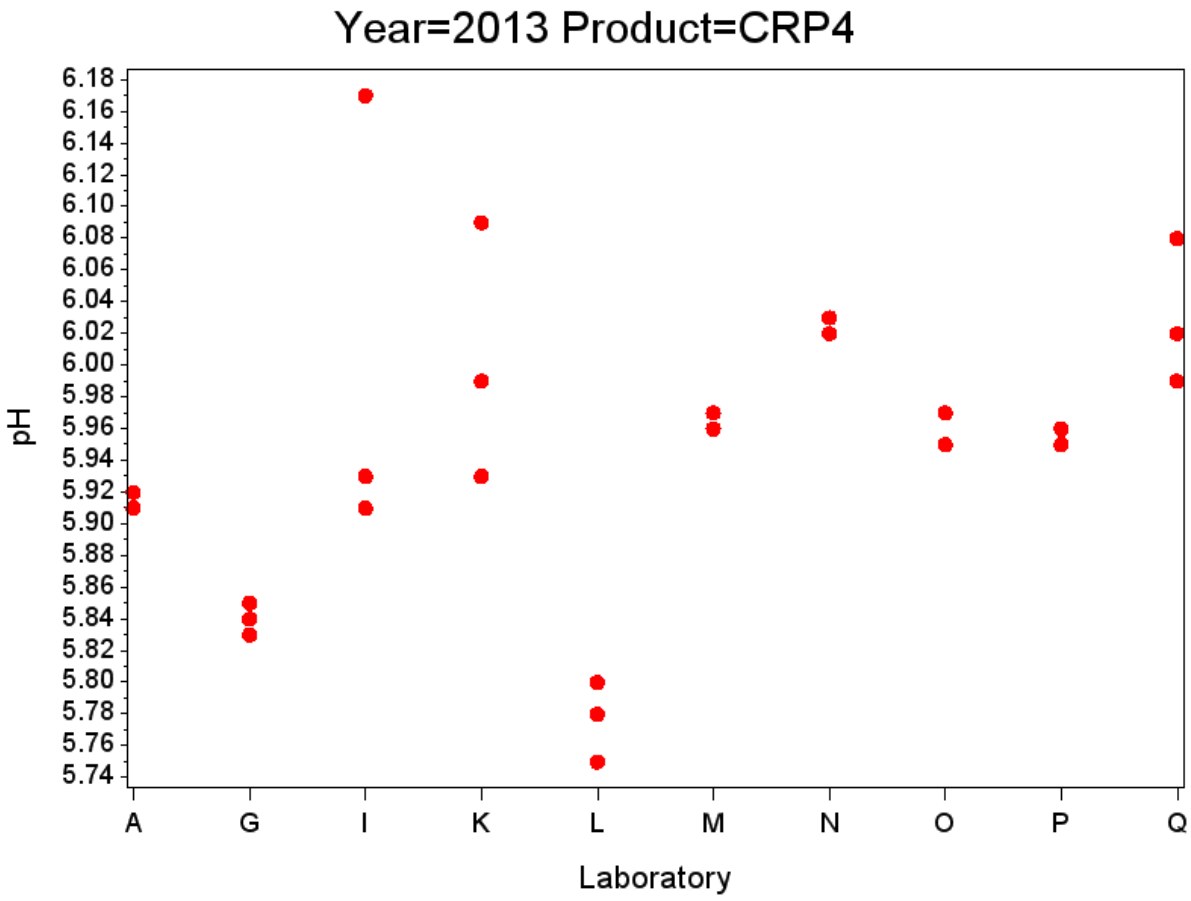

Year=2013 Product=CRP3
Nicotine (%)
2.34
2.32
2.30
2.28
2.26
2.24
2.22
2.20
2.18
2.16
2.14
2.12
2.10
2.08
2.06
2.04
A
G
I
K
L
M
N
O
P
Q
Laboratory

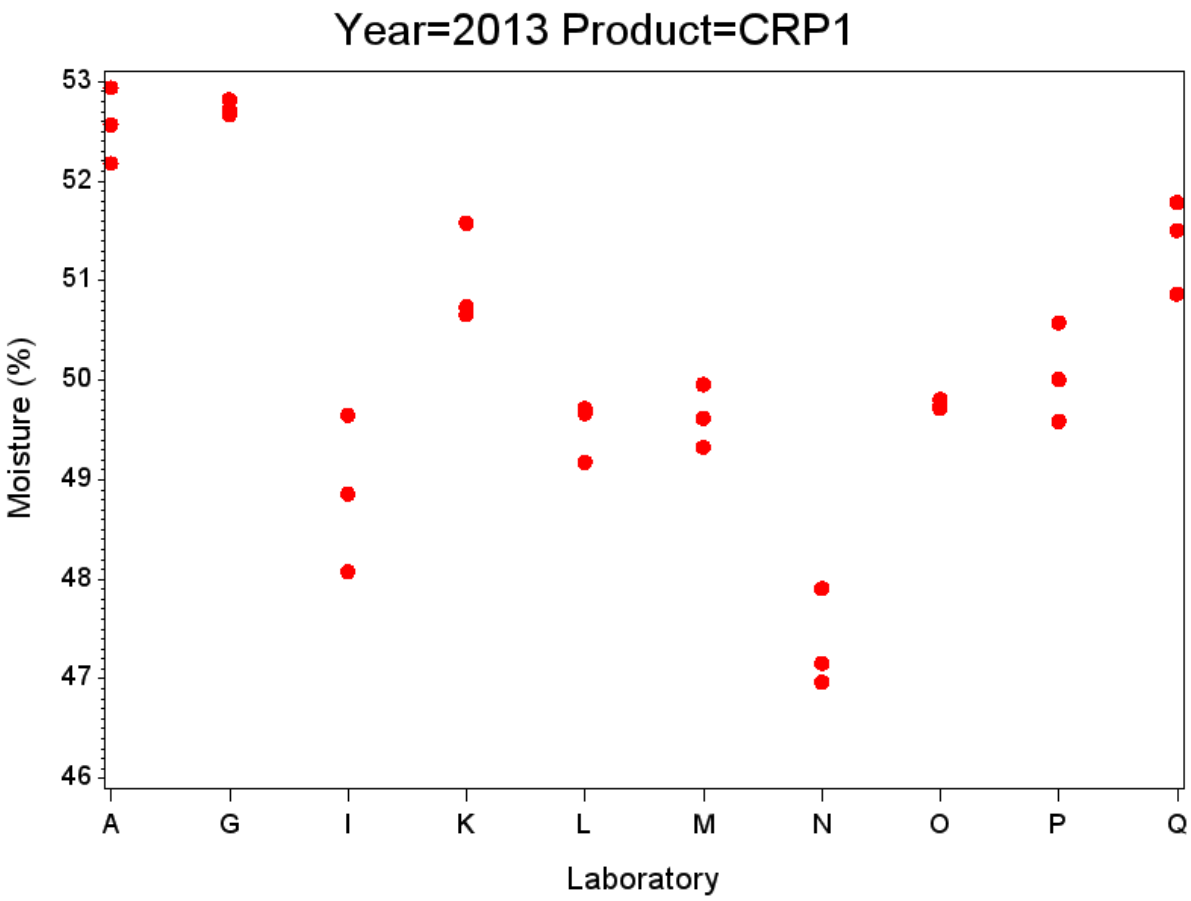

Year=2013 Product=CRP4
Nicotine (%)
1.24
1.22
1.20
1.18
1.16
1.14
1.12
1.10
1.08
1.06
1.04
1.02
1.00
0.98
A
G
I
K
L
M
N
O
P
Q
Laboratory

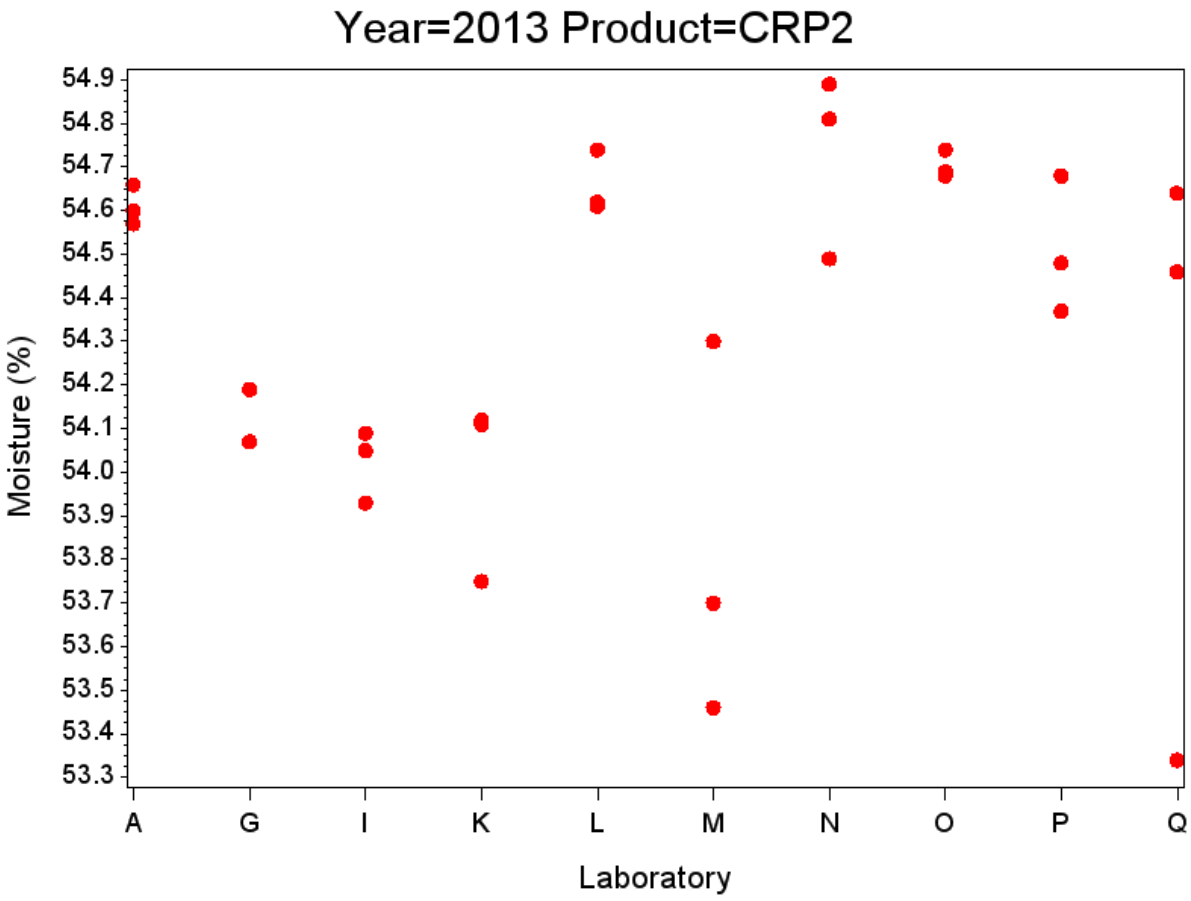

Year=2013 Product=CRP1
pH
8.02
8.00
7.98
7.96
7.94
7.92
7.90
7.88
7.86
7.84
7.82
7.80
7.78
7.76
7.74
7.72
7.70
7.68
7.66
7.64
7.62
7.60
7.58
A
G
I
K
L
M
N
O
P
Q
Laboratory

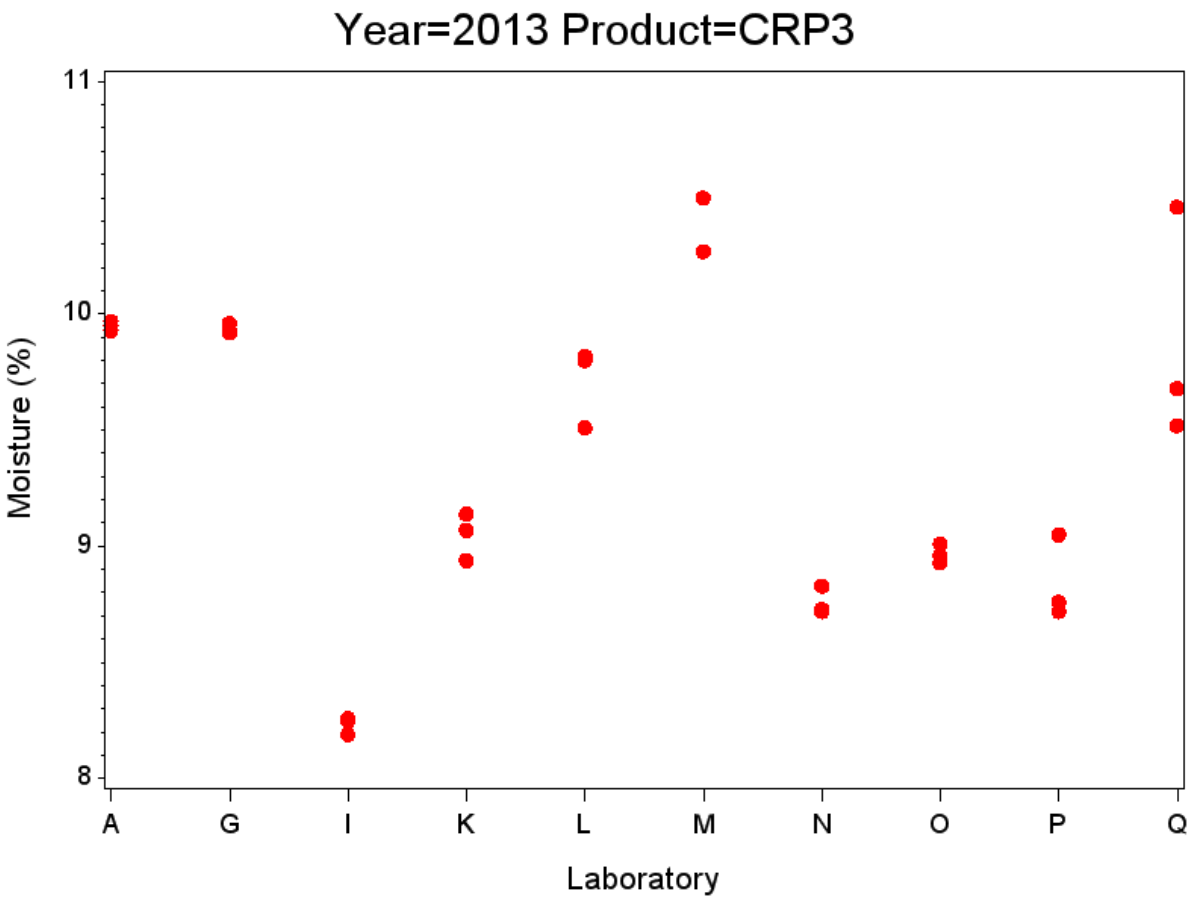

Year=2013 Product=CRP2
pH
7.73
7.72
7.71
7.70
7.69
7.68
7.67
7.66
7.65
7.64
7.63
7.62
7.61
7.60
7.59
7.58
7.57
7.56
7.55
7.54
A
G
I
K
L
M
N
O
P
Q
Laboratory

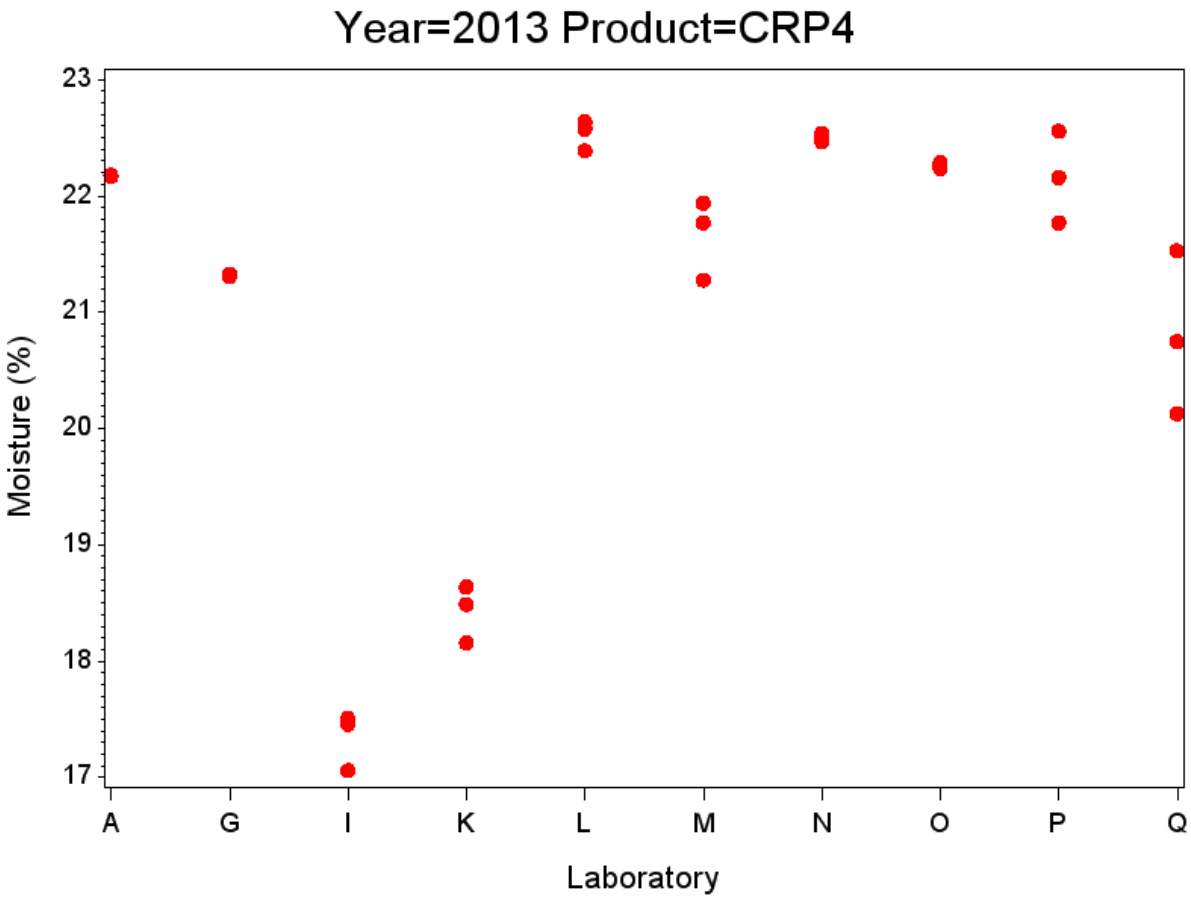

Year=2013 Product=CRP3
pH
6.92
6.90
6.88
6.86
6.84
6.82
6.80
6.78
6.76
6.74
6.72
6.70
6.68
6.66
6.64
6.62
6.60
A
G
I
K
L
M
N
O
P
Q
Laboratory

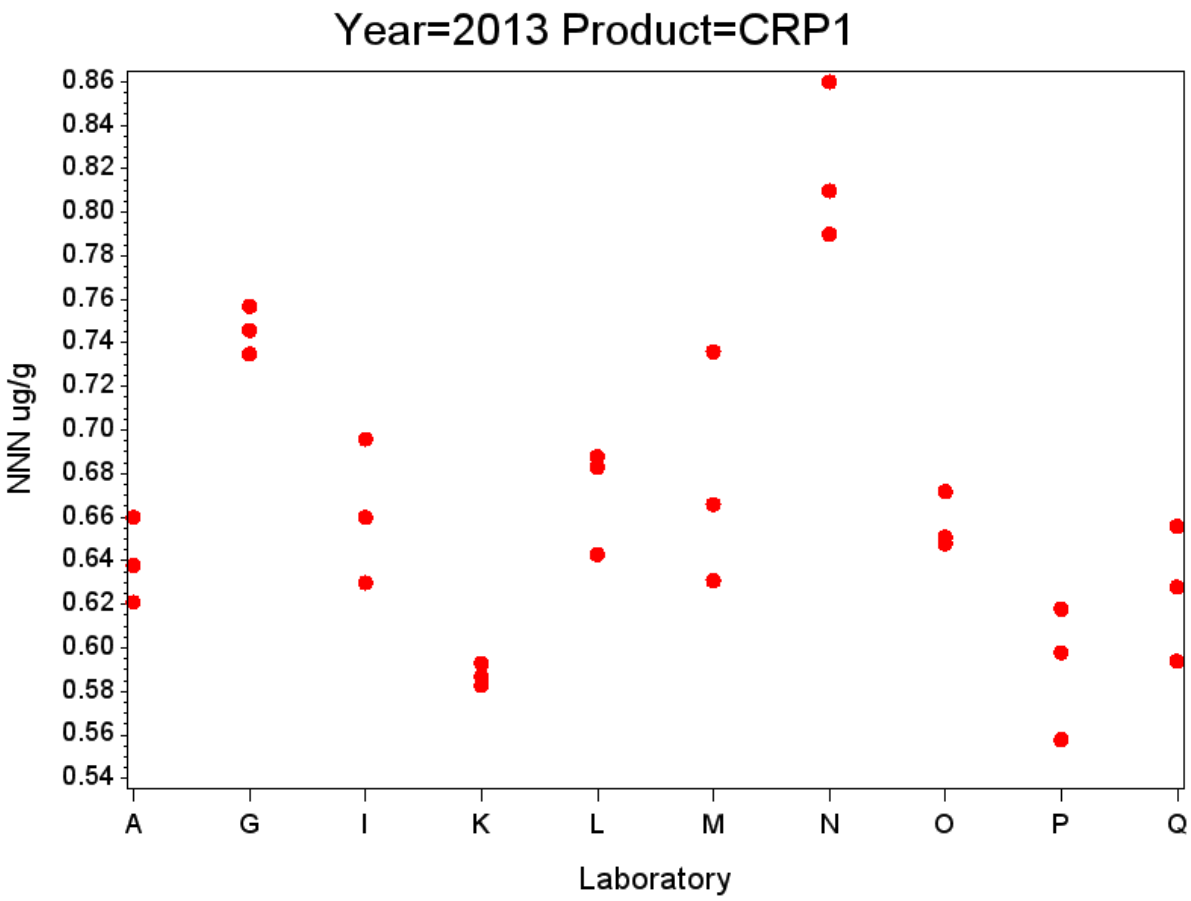

Year=2013 Product=CRP4
pH
6.18
6.16
6.14
6.12
6.10
6.08
6.06
6.04
6.02
6.00
5.98
5.96
5.94
5.92
5.90
5.88
5.86
5.84
5.82
5.80
5.78
5.76
5.74
A
G
I
K
L
M
N
O
P
Q
Laboratory

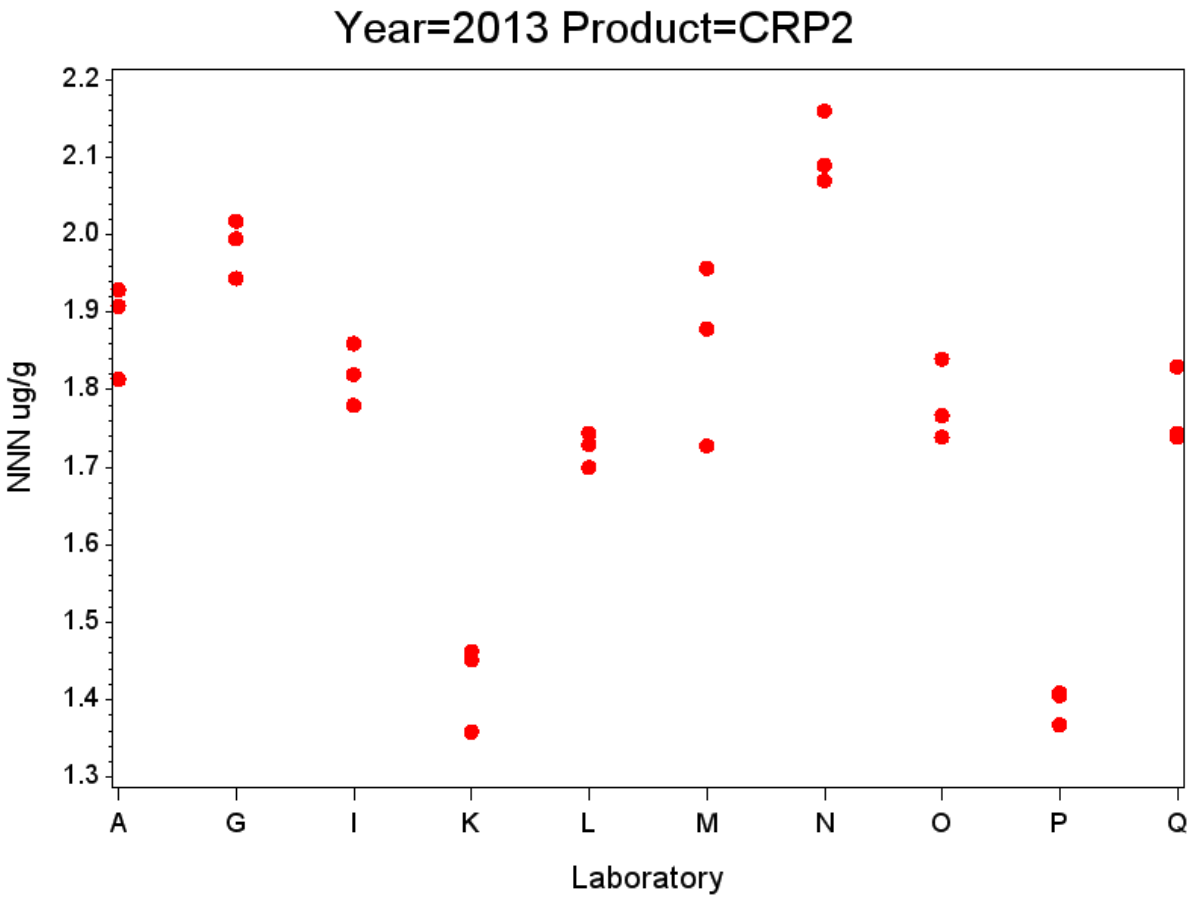

Year=2013 Product=CRP1
Moisture (%)
53
52
51
50
49
48
47
46
A
G
I
K
L
M
N
O
P
Q
Laboratory

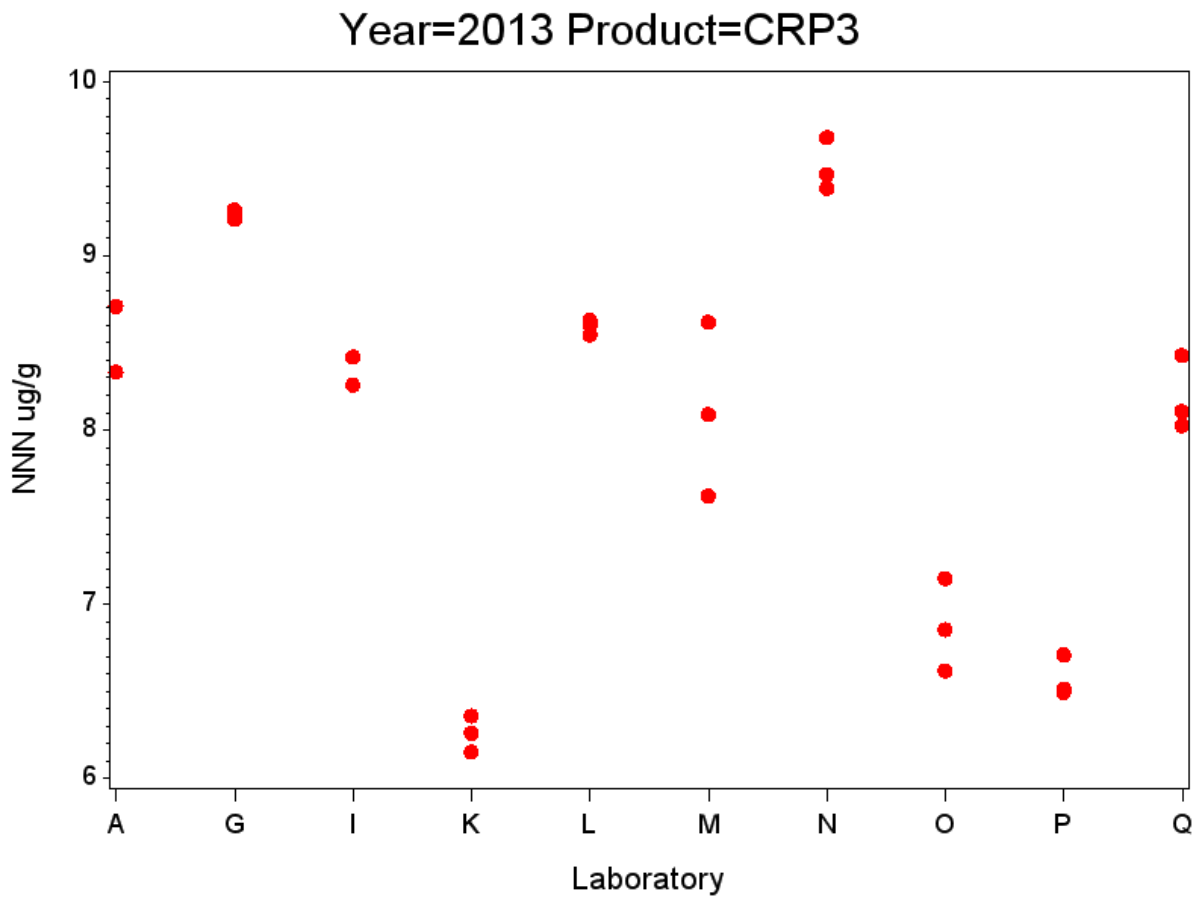

Year=2013 Product=CRP2
Moisture (%)
54.9
54.8
54.7
54.6
54.5
54.4
54.3
54.2
54.1
54.0
53.9
53.8
53.7
53.6
53.5
53.4
53.3
A
G
I
K
L
M
N
O
P
Q
Laboratory

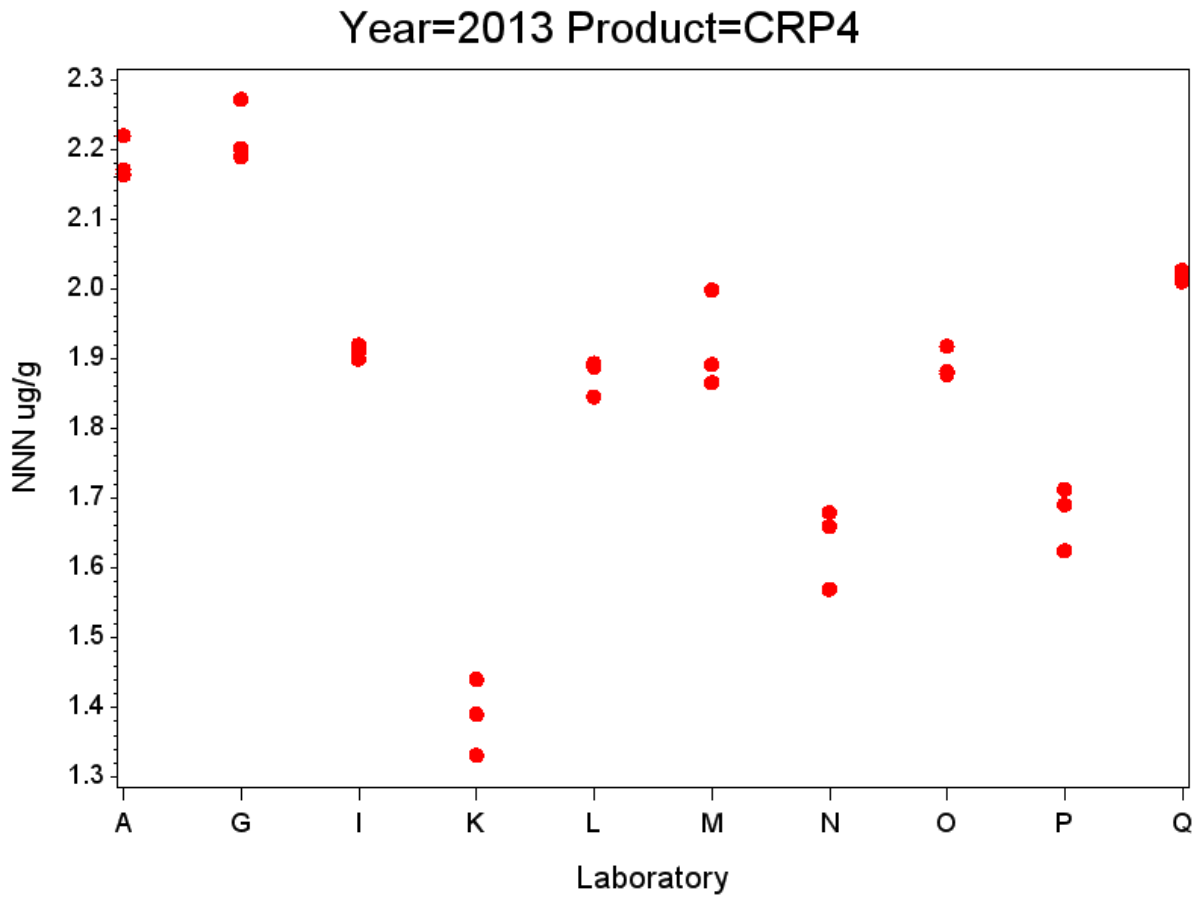

Year=2013 Product=CRP3
Moisture (%)
11
10
9
8
A
G
I
K
L
M
N
O
P
Q
Laboratory

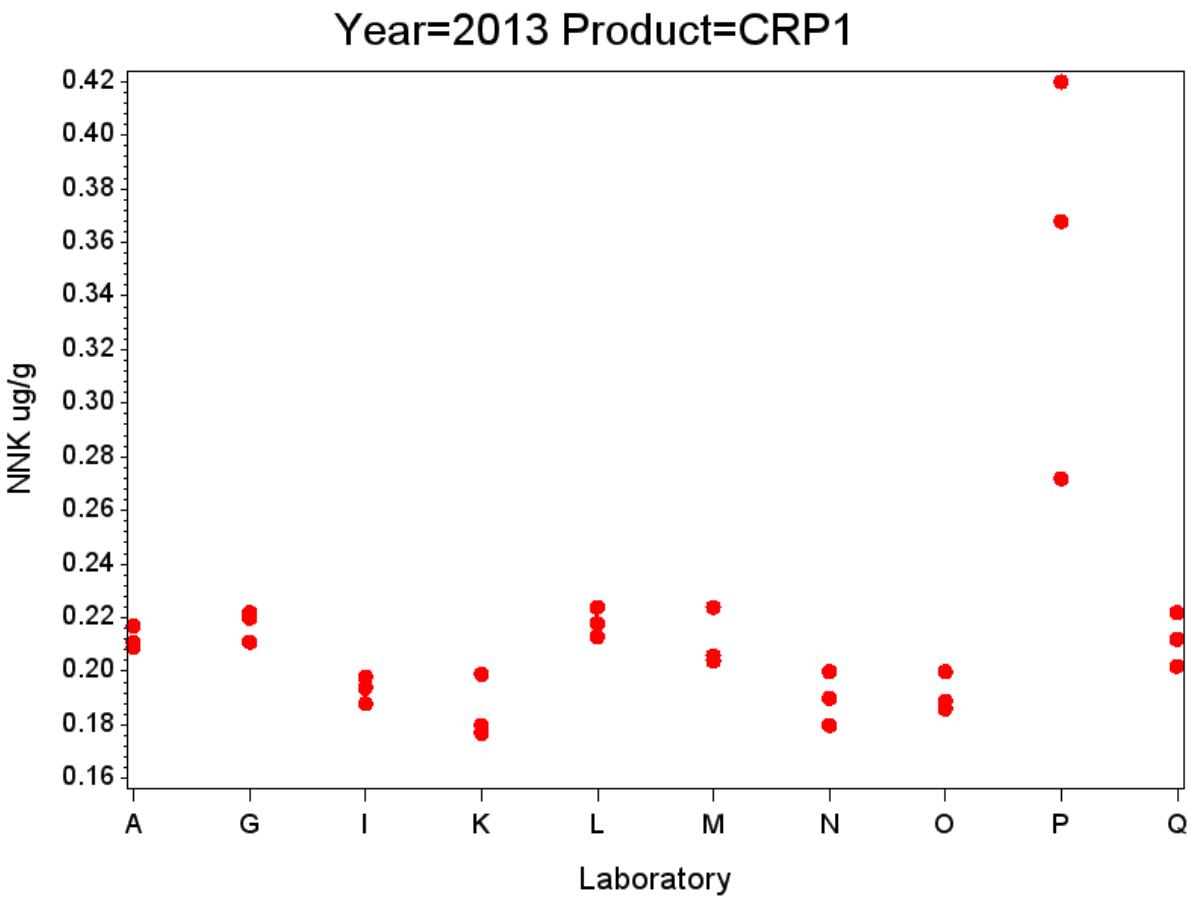

Year=2013 Product=CRP4
Moisture (%)
23
22
21
20
19
18
17
A
G
I
K
L
M
N
O
P
Q
Laboratory

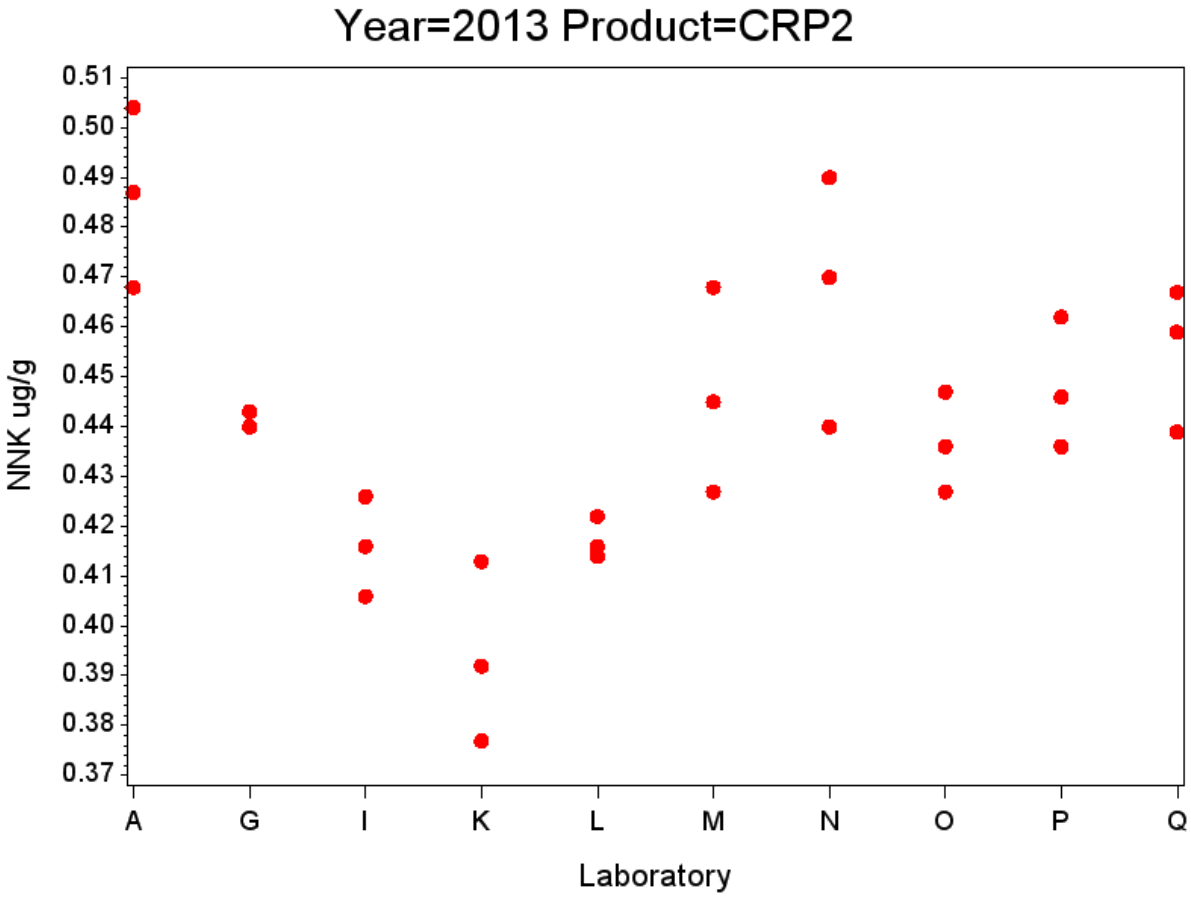

Year=2013 Product=CRP1
NNN ug/g
0.86
0.84
0.82
0.80
0.78
0.76
0.74
0.72
0.70
0.68
0.66
0.64
0.62
0.60
0.58
0.56
0.54
A
G
I
K
L
M
N
O
P
Q
Laboratory

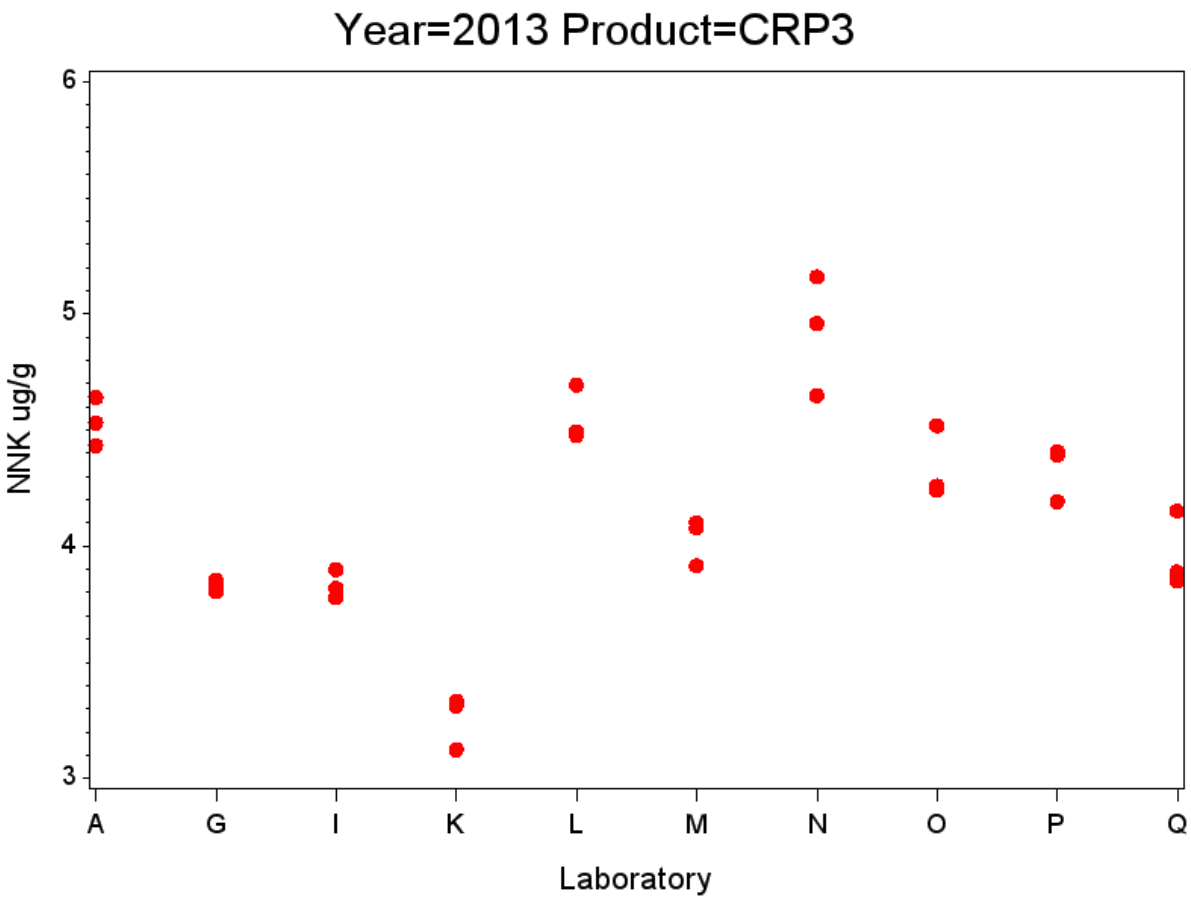

Year=2013 Product=CRP2
NNN ug/g
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
A
G
I
K
L
M
N
O
P
Q
Laboratory

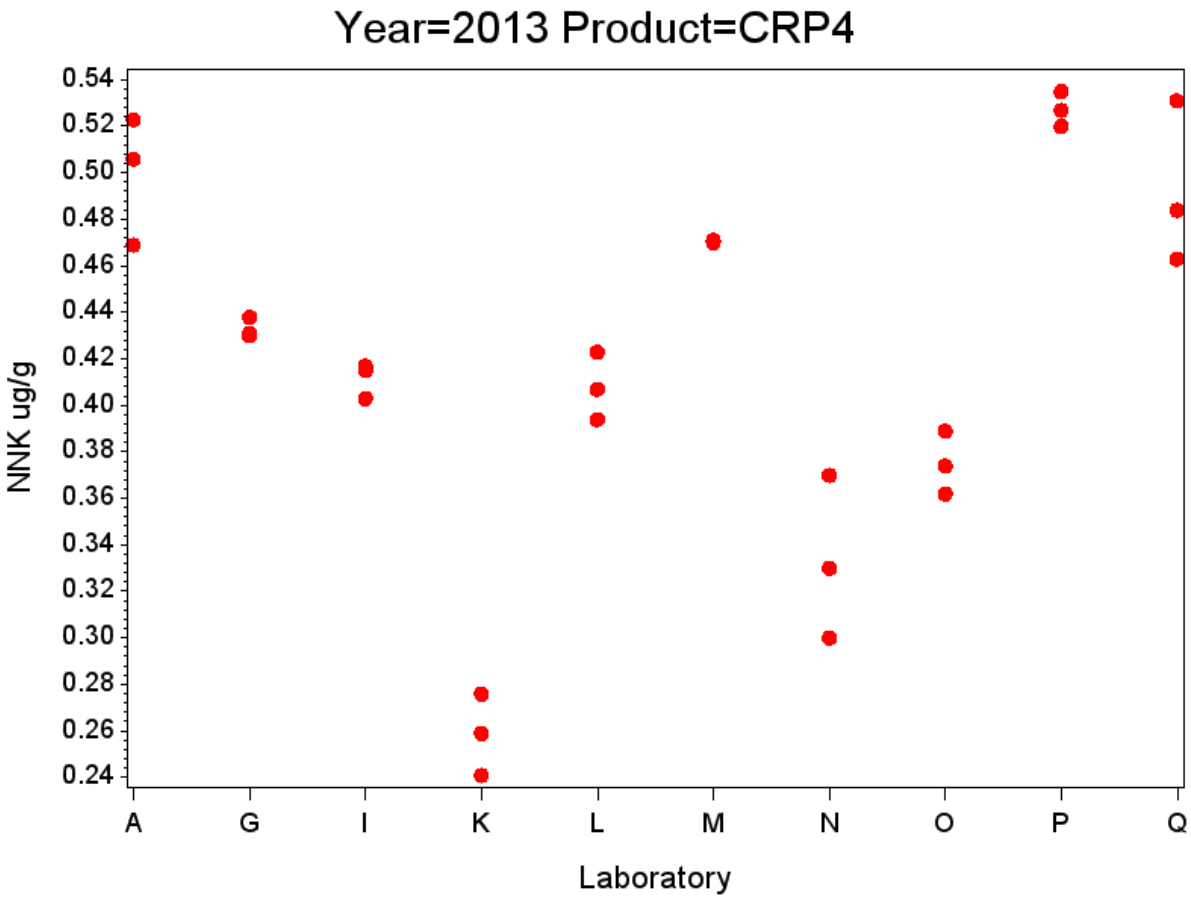

Year=2013 Product=CRP3
NNN ug/g
10
9
8
7
6
A
G
I
K
L
M
N
O
P
Q
Laboratory

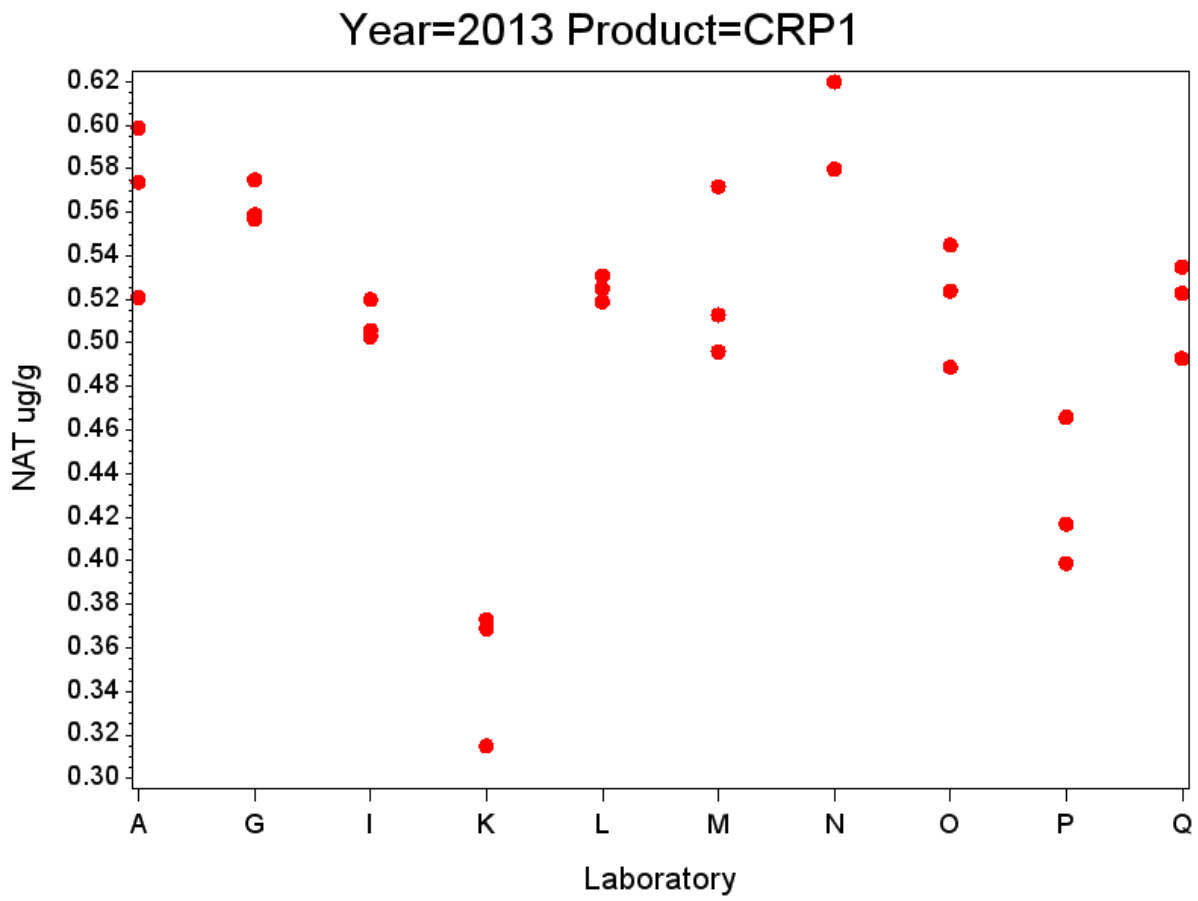

Year=2013 Product=CRP4
NNN ug/g
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
A
G
I
K
L
M
N
O
P
Q
Laboratory

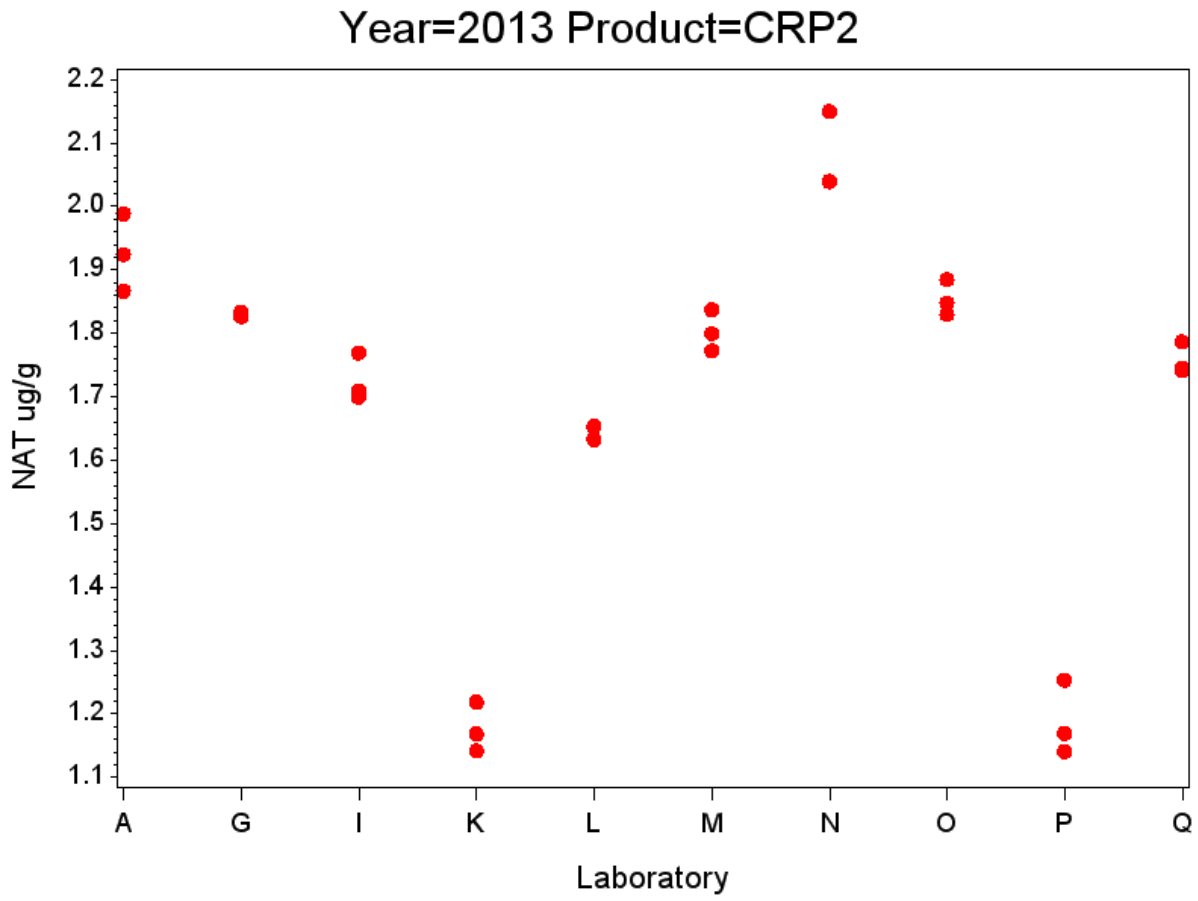

Year=2013 Product=CRP1
NNK ug/g
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
A
G
I
K
L
M
N
O
P
Q
Laboratory

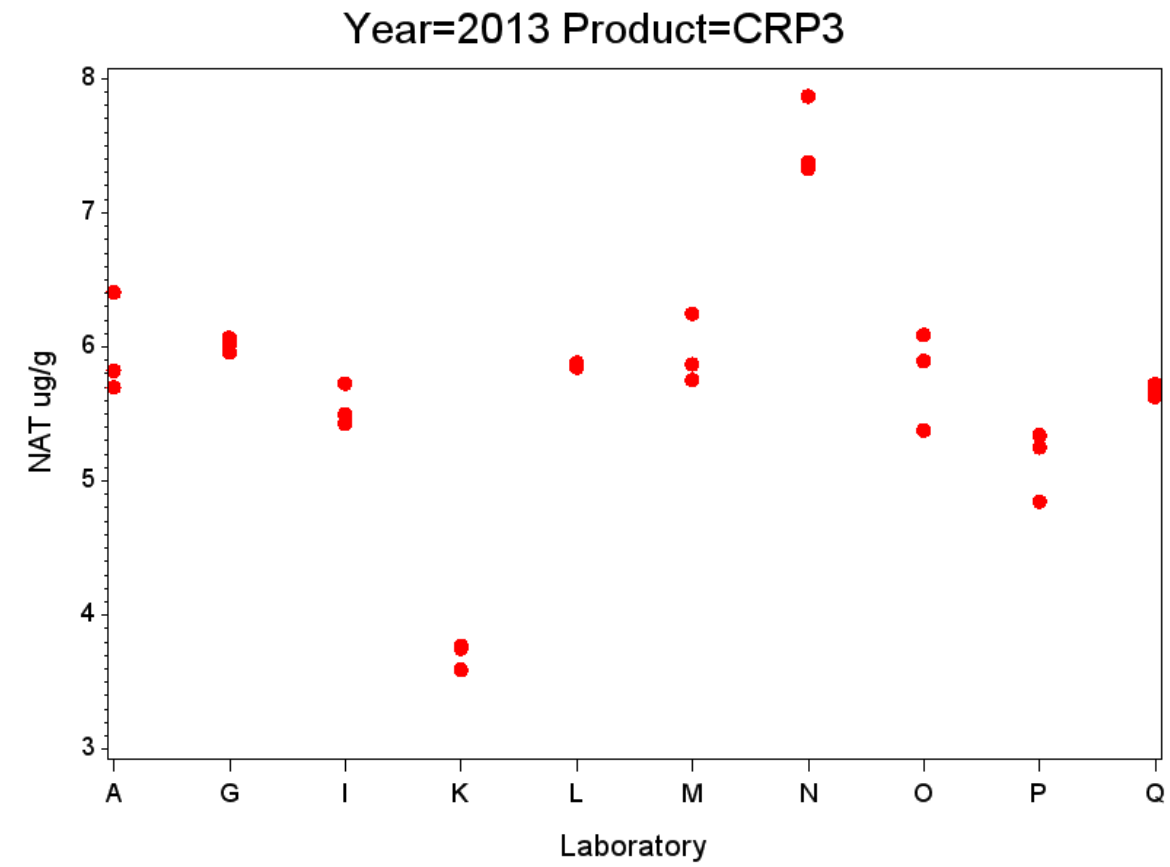

Year=2013 Product=CRP2
NNK ug/g
0.51
0.50
0.49
0.48
0.47
0.46
0.45
0.44
0.43
0.42
0.41
0.40
0.39
0.38
0.37
A
G
I
K
L
M
N
O
P
Q
Laboratory

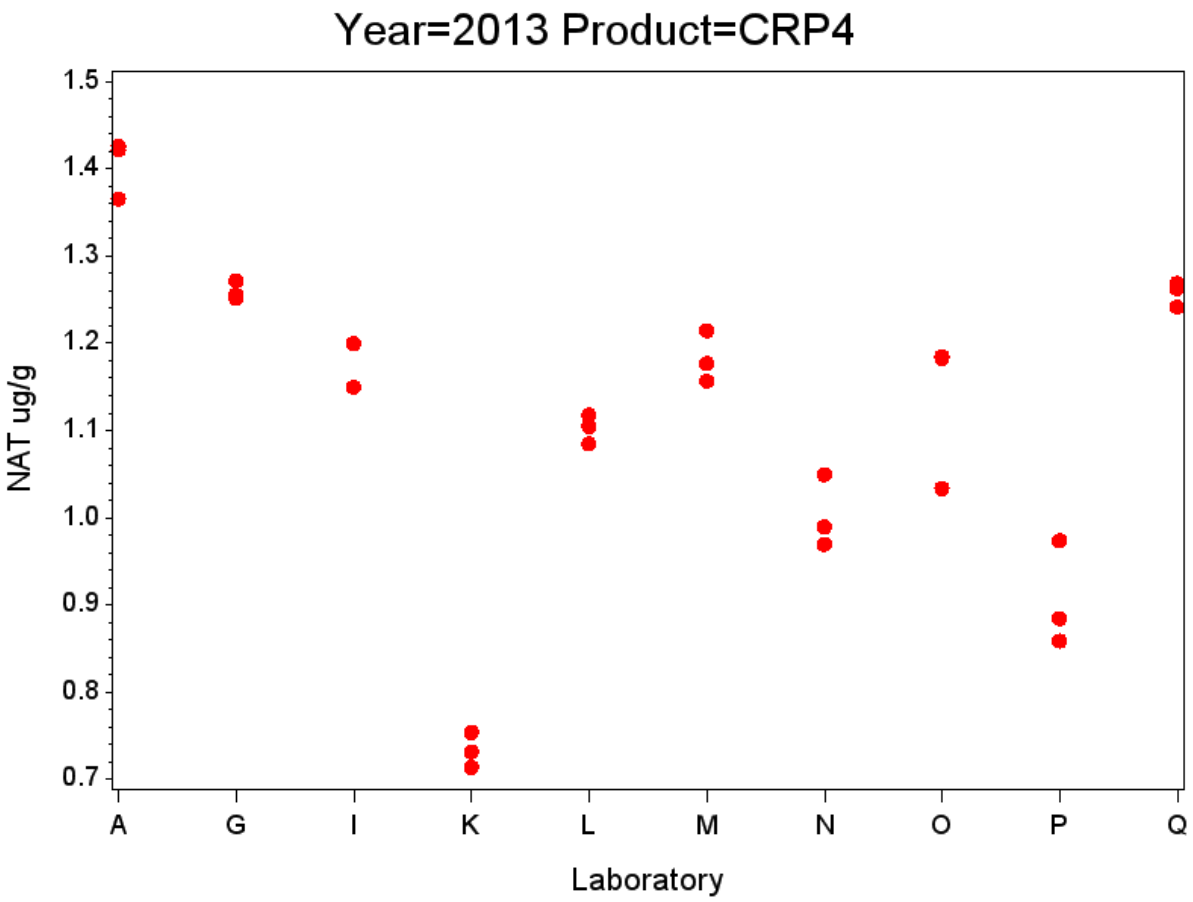

Year=2013 Product=CRP3
NNK ug/g
6
5
4
3
A
G
I
K
L
M
N
O
P
Q
Laboratory

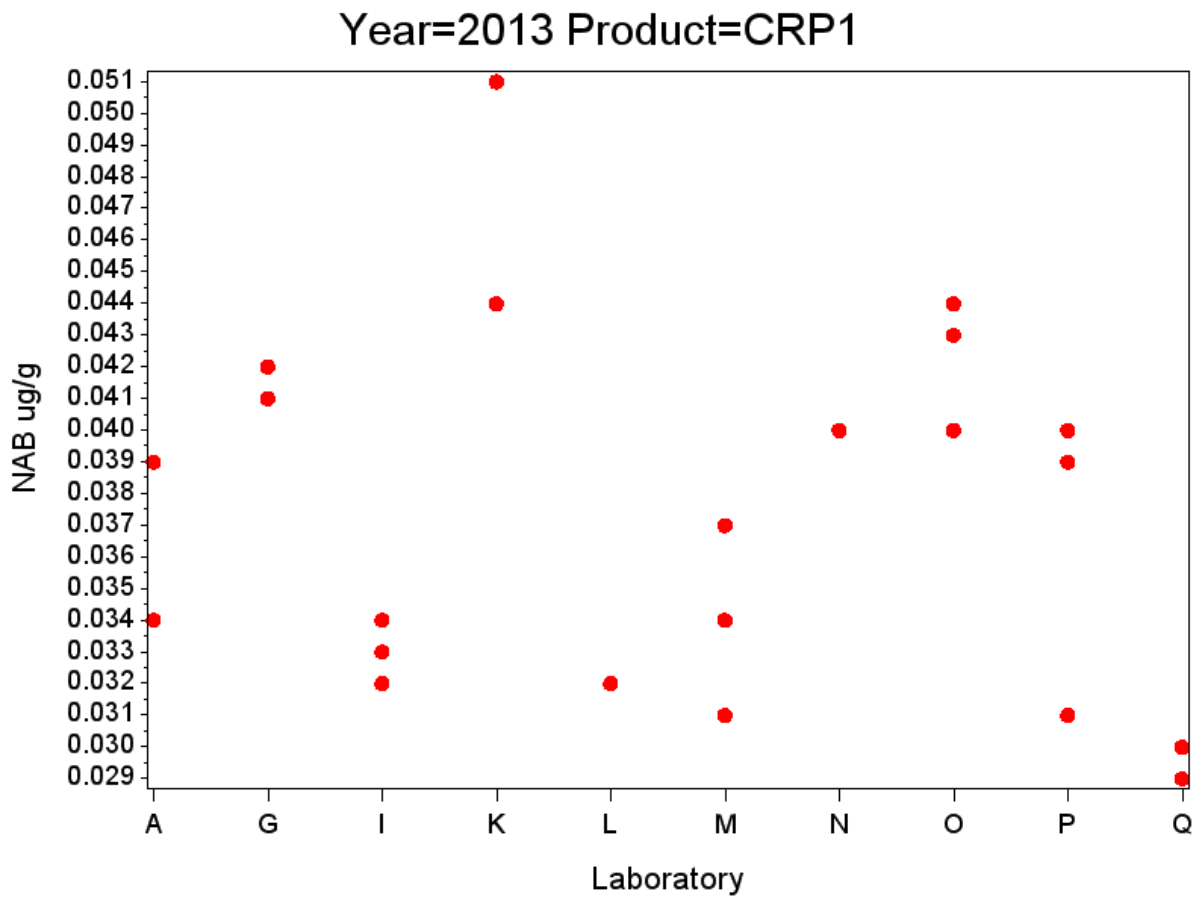

Year=2013 Product=CRP4
NNK ug/g
0.54
0.52
0.50
0.48
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
A
G
I
K
L
M
N
O
P
Q
Laboratory

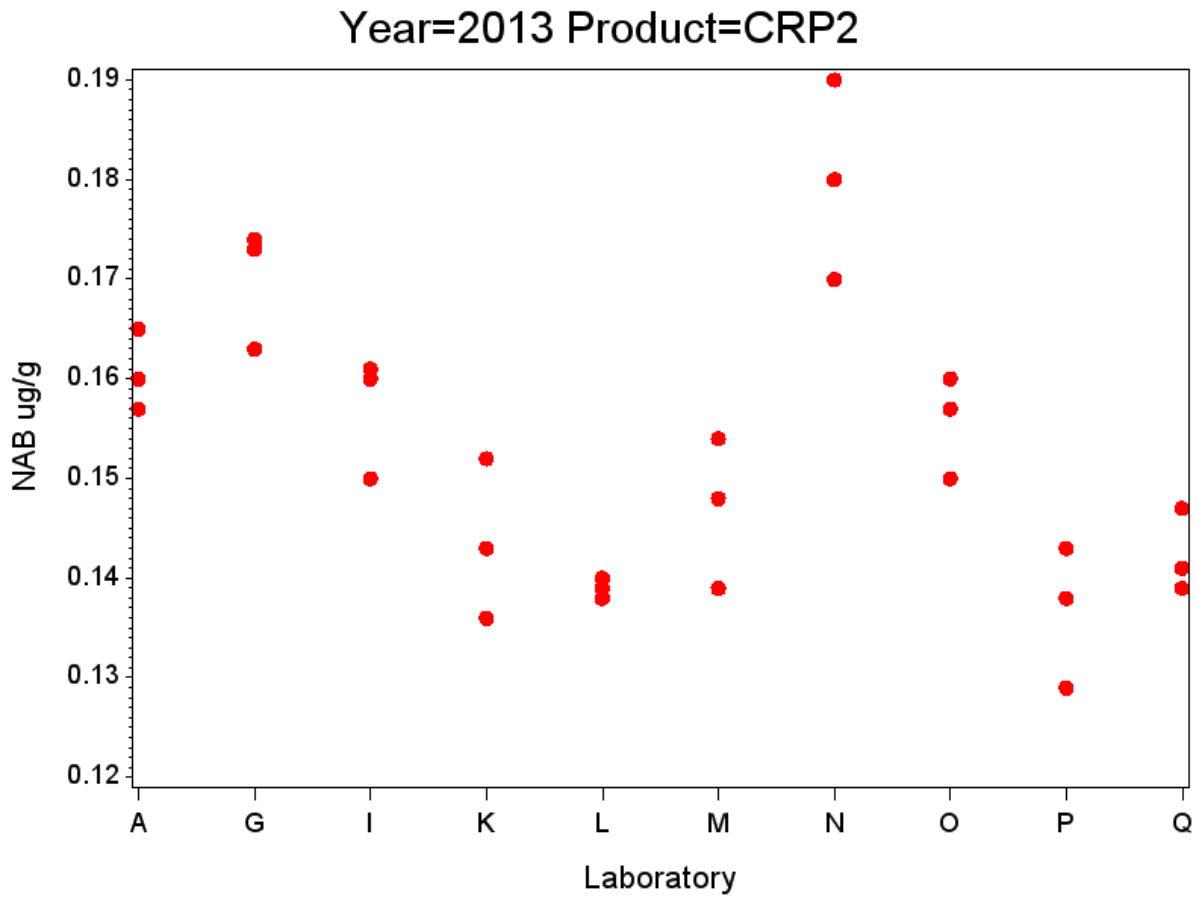

Year=2013 Product=CRP1
NAT ug/g
0.62
0.60
0.58
0.56
0.54
0.52
0.50
0.48
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
A
G
I
K
L
M
N
O
P
Q
Laboratory

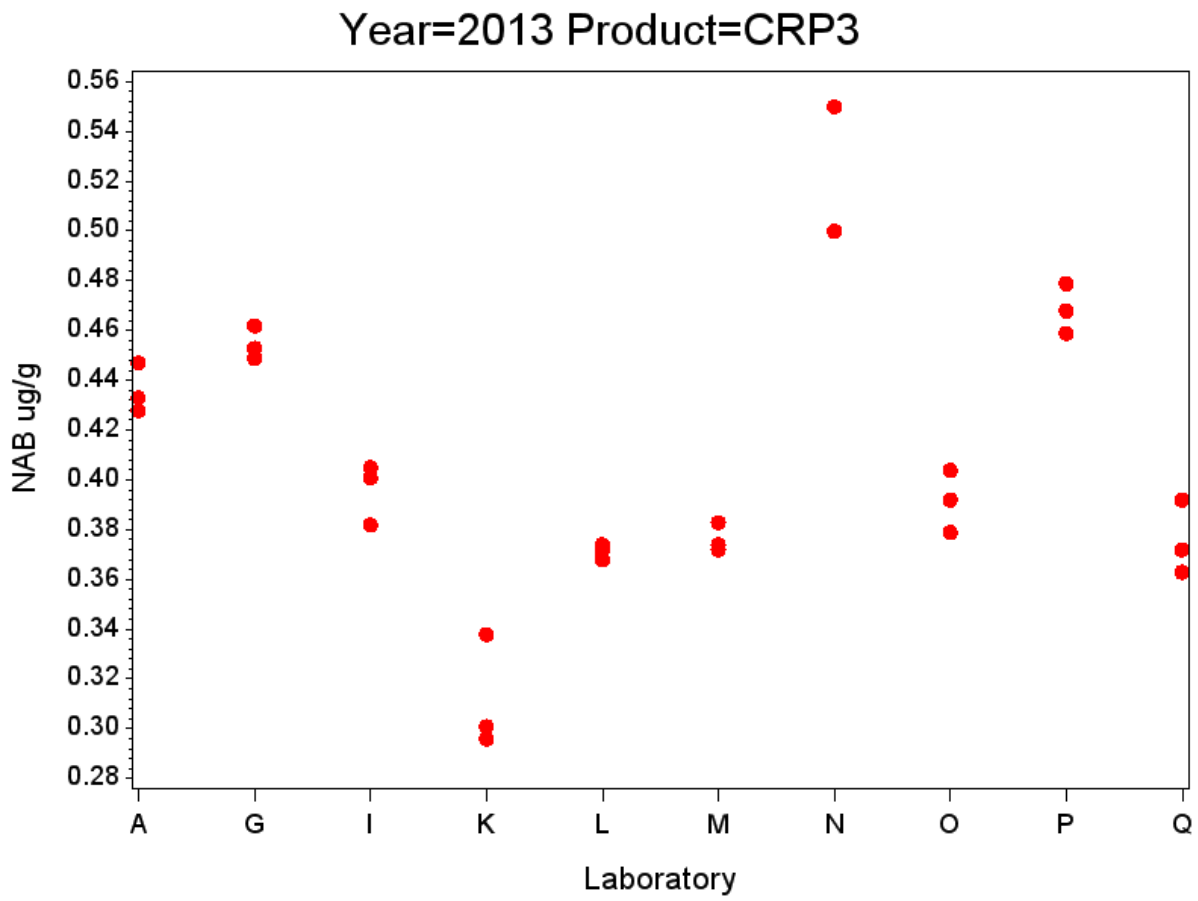

Year=2013 Product=CRP2
NAT ug/g
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
A
G
I
K
L
M
N
O
P
Q
Laboratory

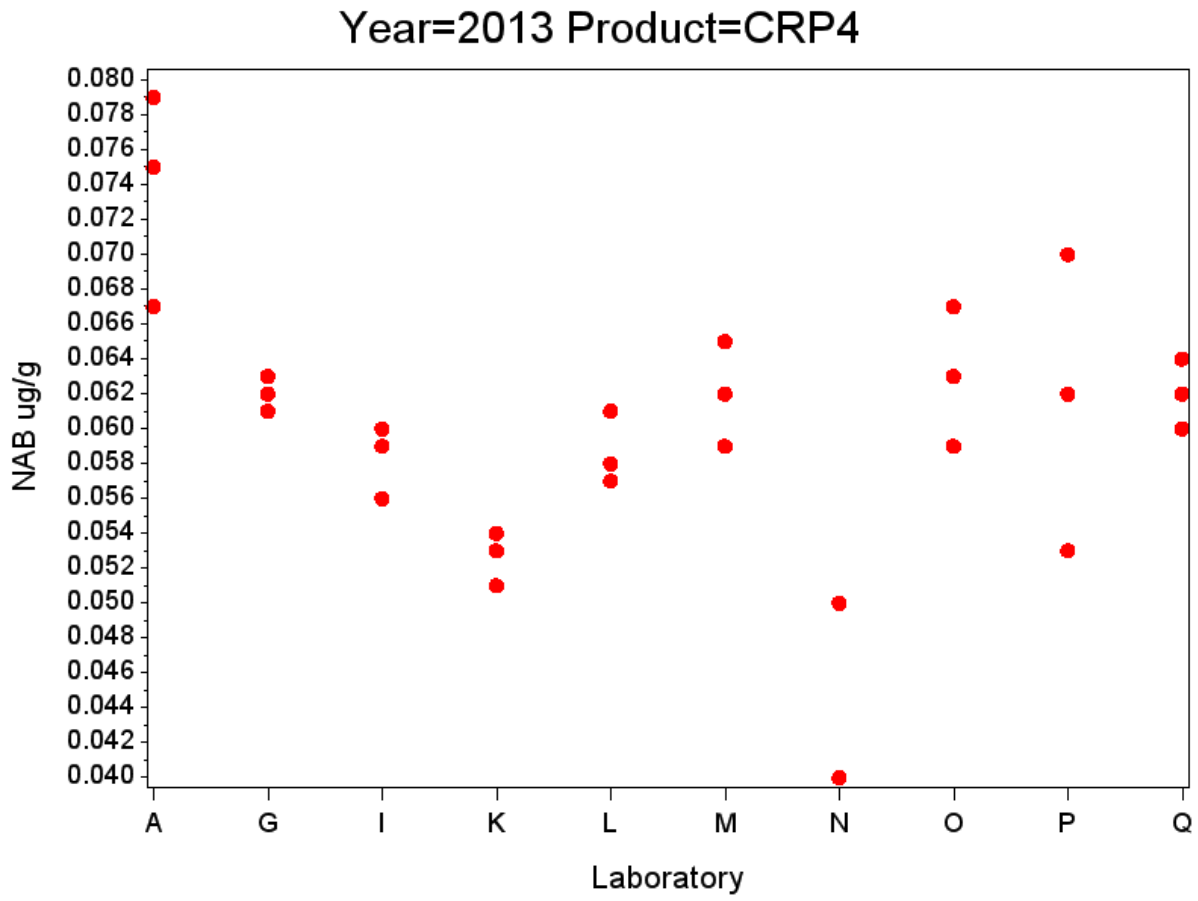

Year=2013 Product=CRP3
NAT ug/g
8
7
6
5
4
3
A
G
I
K
L
M
N
O
P
Q
Laboratory


Year=2013 Product=CRP4
NAT ug/g
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
A
G
I
K
L
M
N
O
P
Q
Laboratory

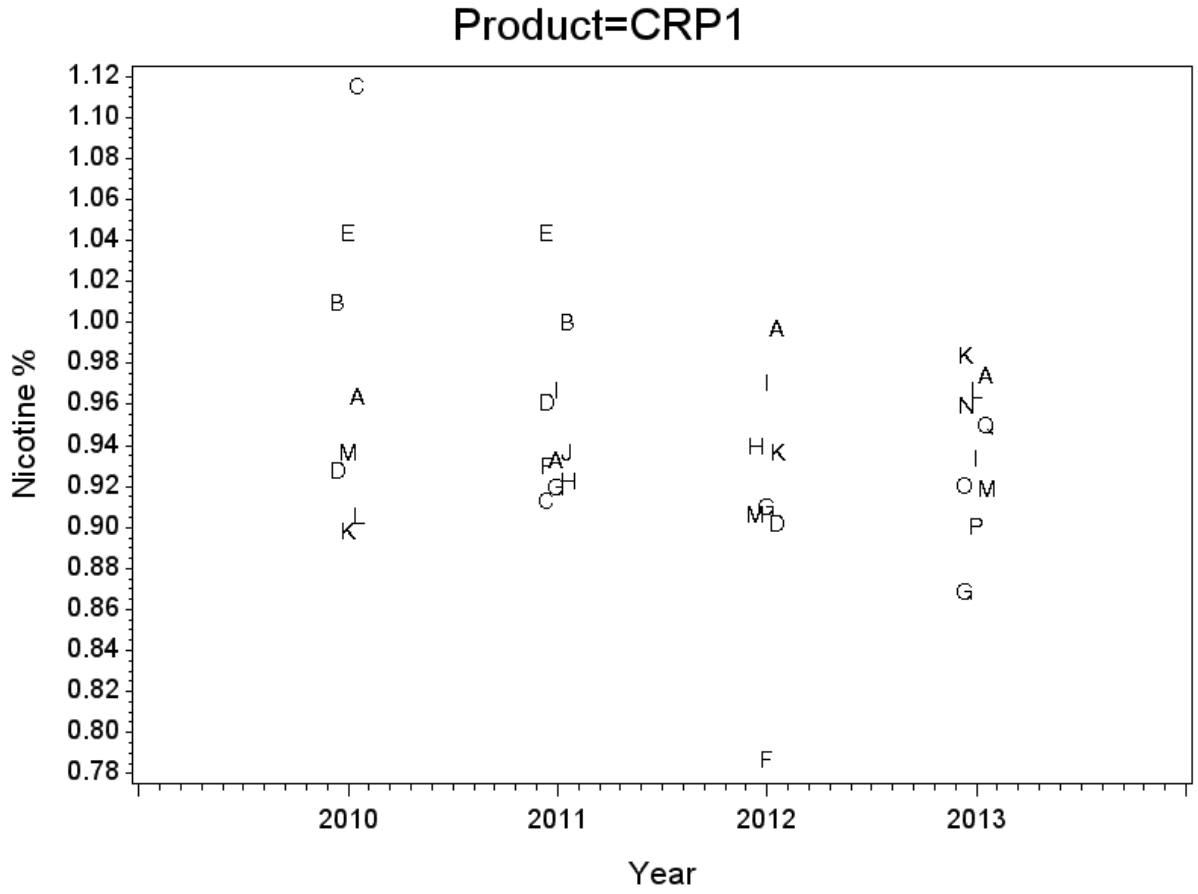

Year=2013 Product=CRP1
NAB ug/g
0.051
0.050
0.049
0.048
0.047
0.046
0.045
0.044
0.043
0.042
0.041
0.040
0.039
0.038
0.037
0.036
0.035
0.034
0.033
0.032
0.031
0.030
0.029
A
G
I
K
L
M
N
O
P
Q
Laboratory

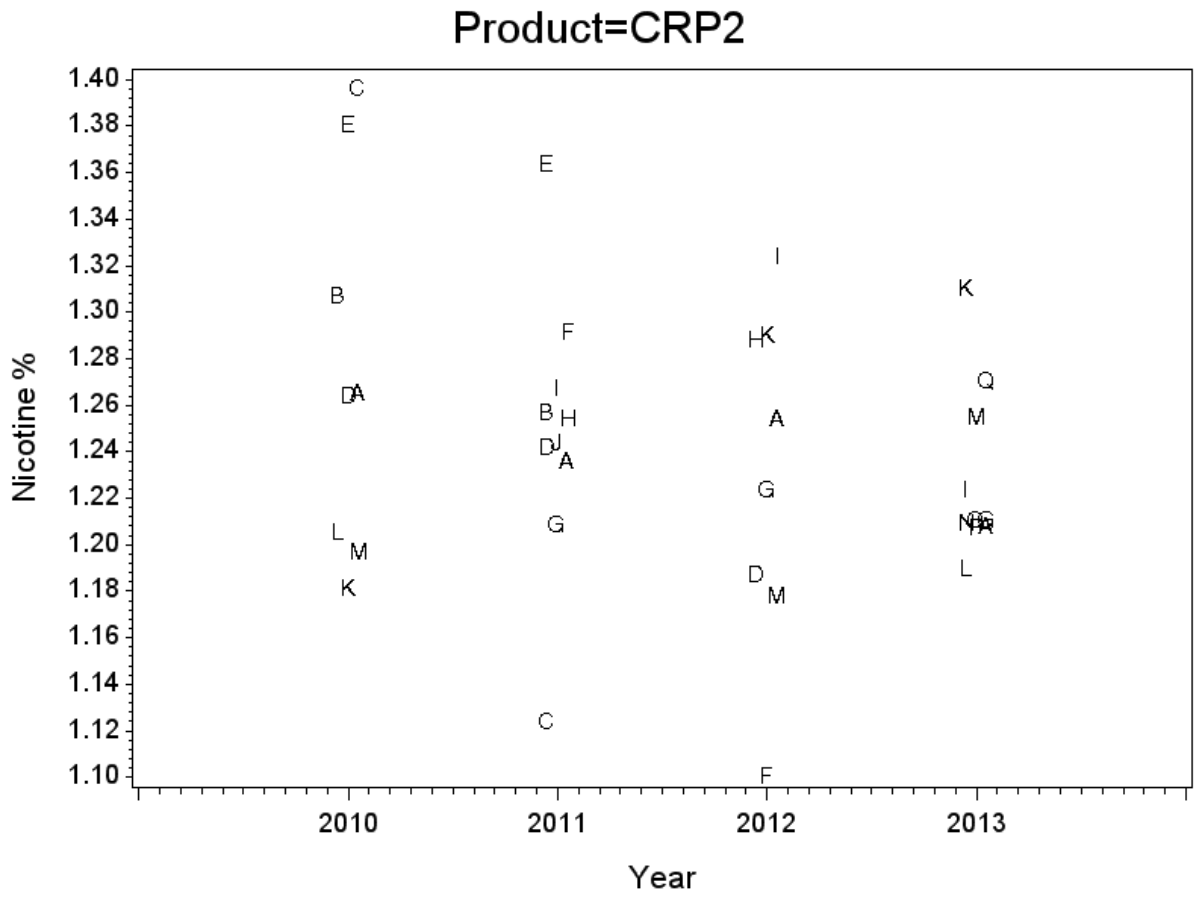

Year=2013 Product=CRP2
NAB ug/g
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
A
G
I
K
L
M
N
O
P
Q
Laboratory

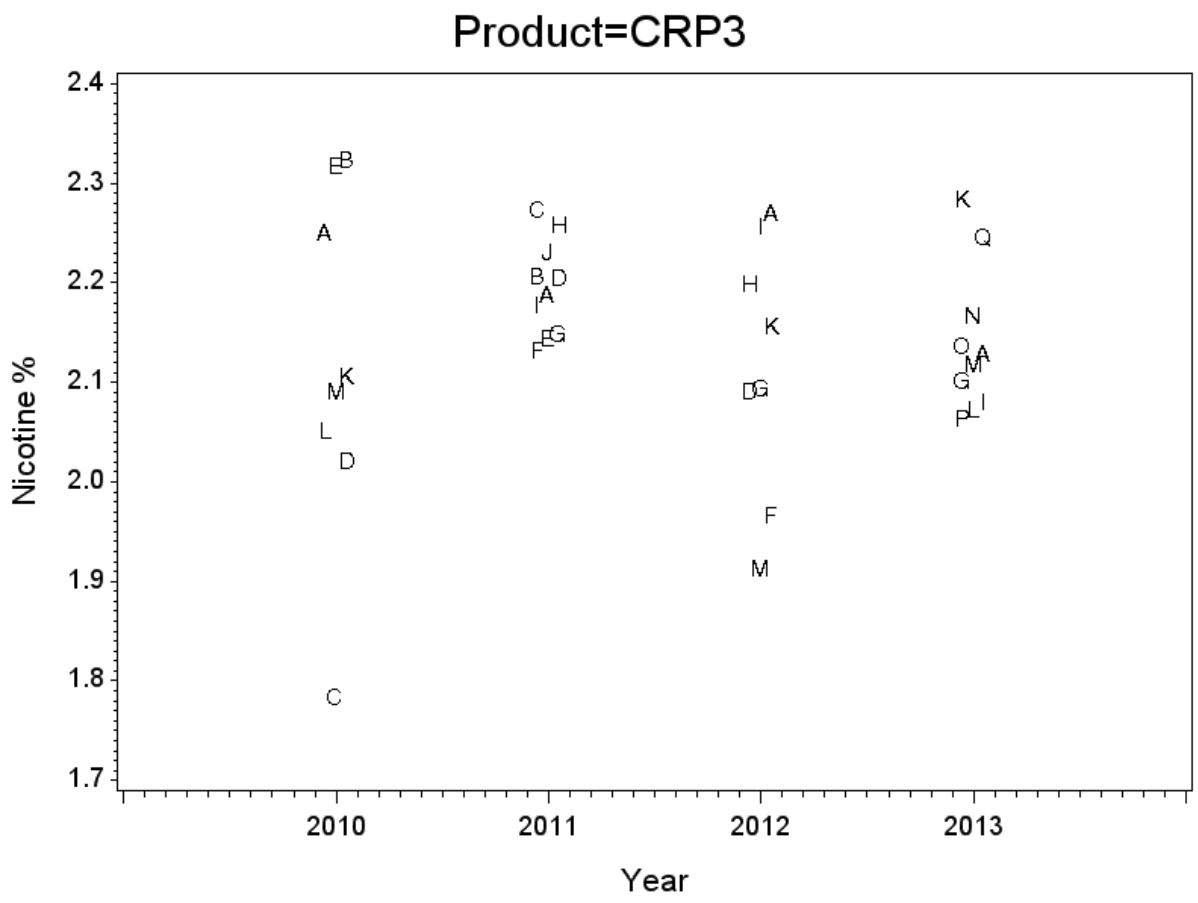

Year=2013 Product=CRP3
NAB ug/g
0.56
0.54
0.52
0.50
0.48
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
A
G
I
K
L
M
N
O
P
Q
Laboratory

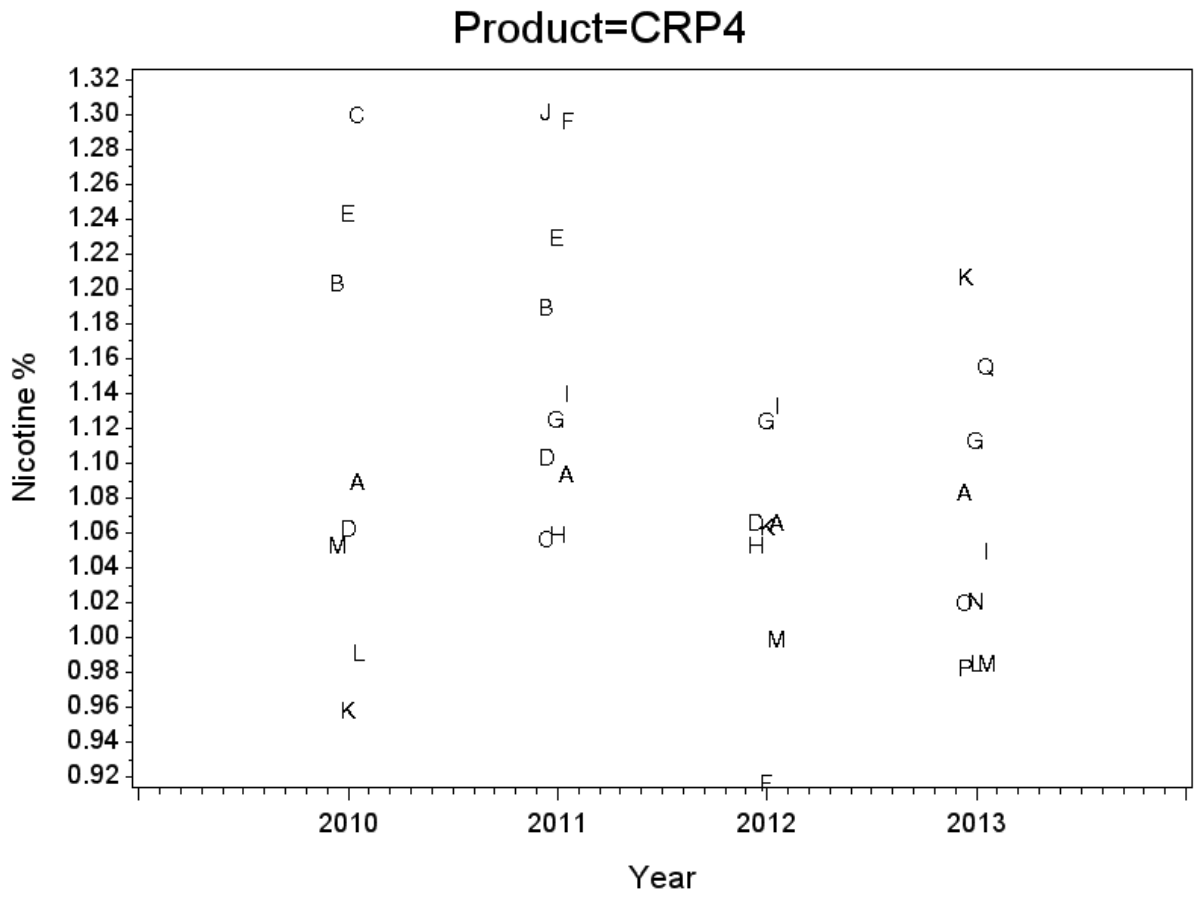

Year=2013 Product=CRP4
NAB ug/g
0.080
0.078
0.076
0.074
0.072
0.070
0.068
0.066
0.064
0.062
0.060
0.058
0.056
0.054
0.052
0.050
0.048
0.046
0.044
0.042
0.040
A
G
I
K
L
M
N
O
P
Q
Laboratory

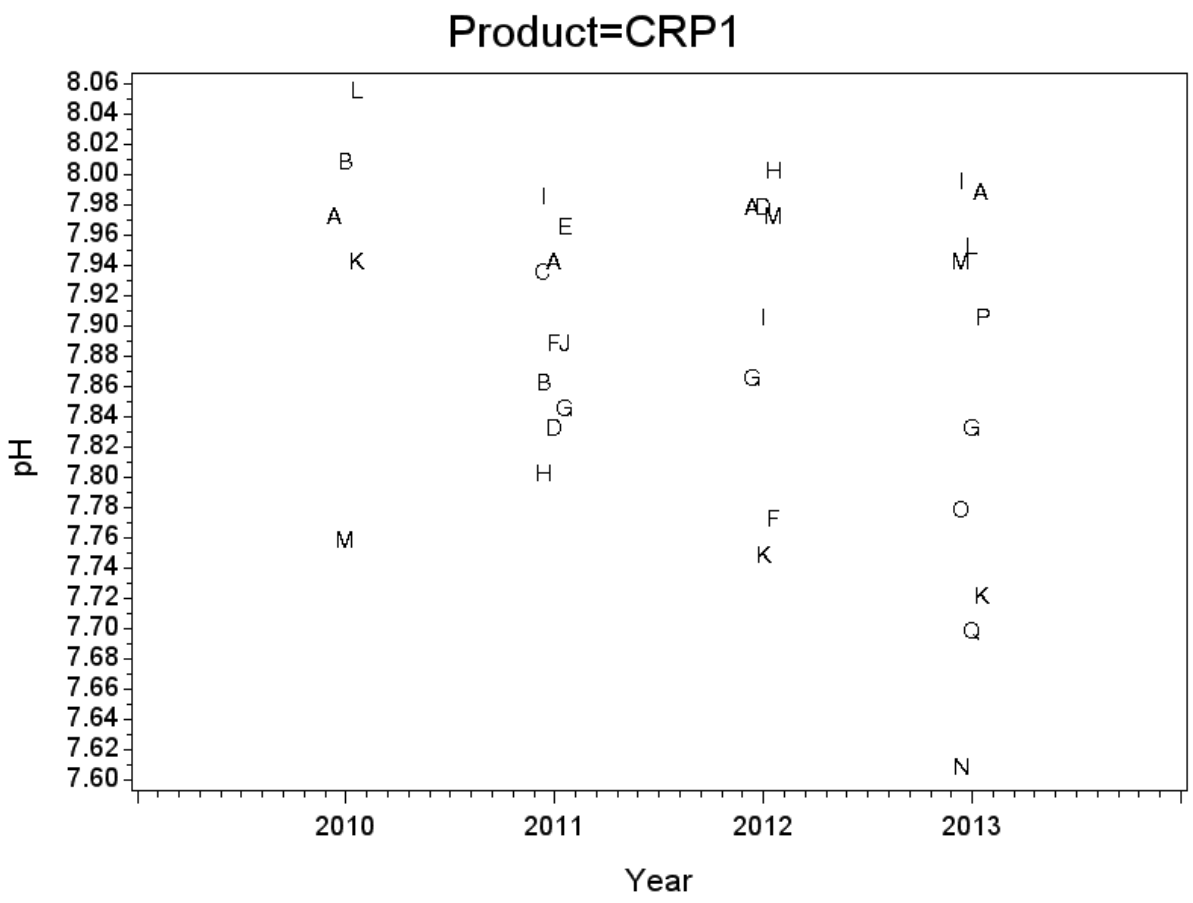
APPENDIX C: CRP Stability Assessment, Mean Data Plots, All Years

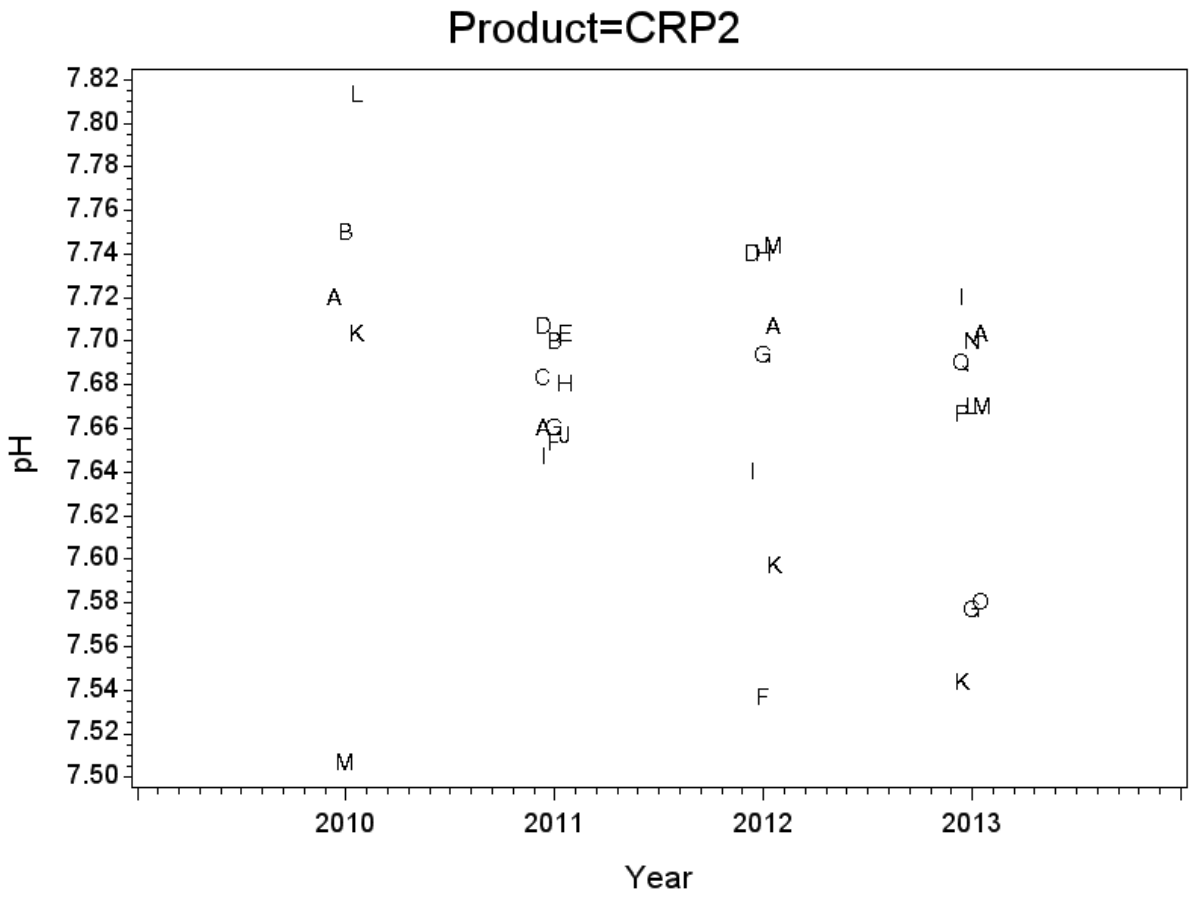


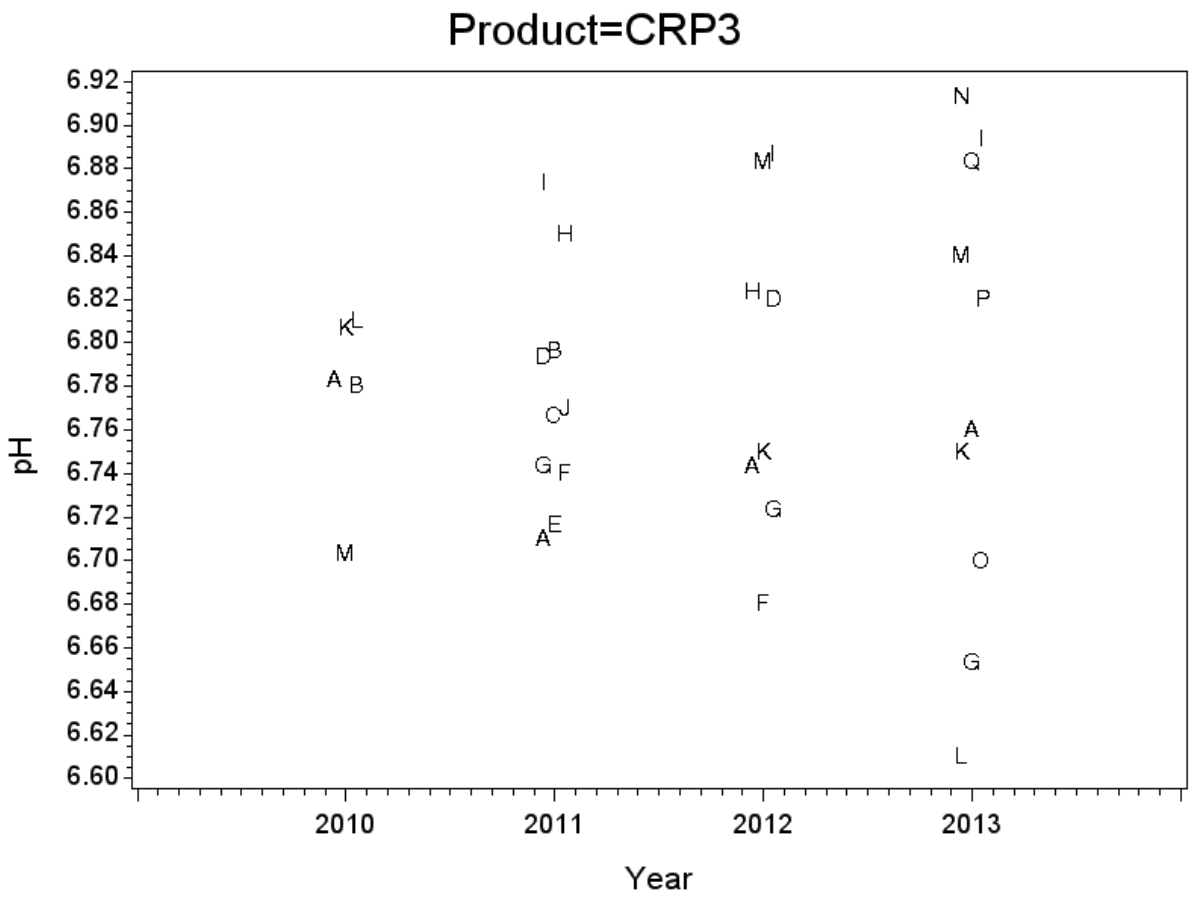


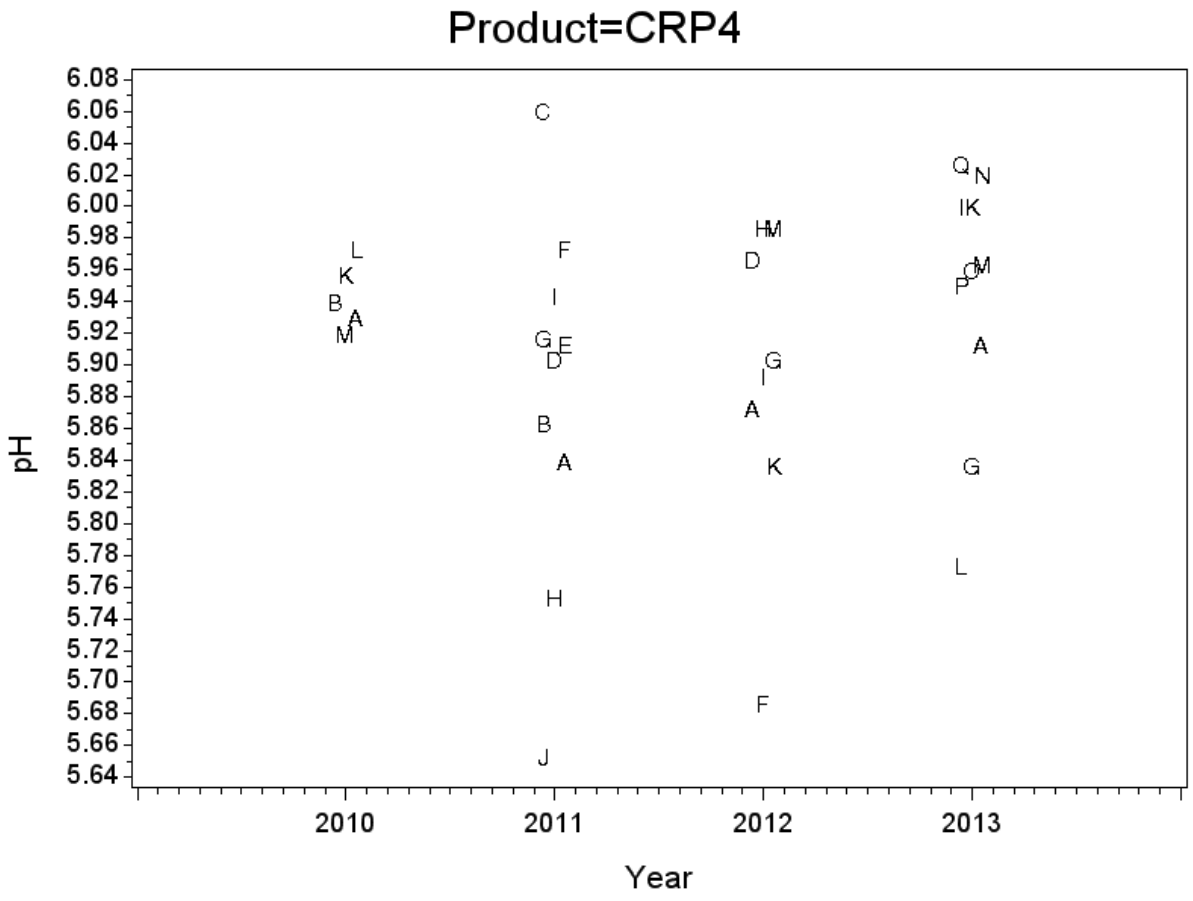

Product=CRP3
Nicotine %
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
2010
2011
2012
2013
Year

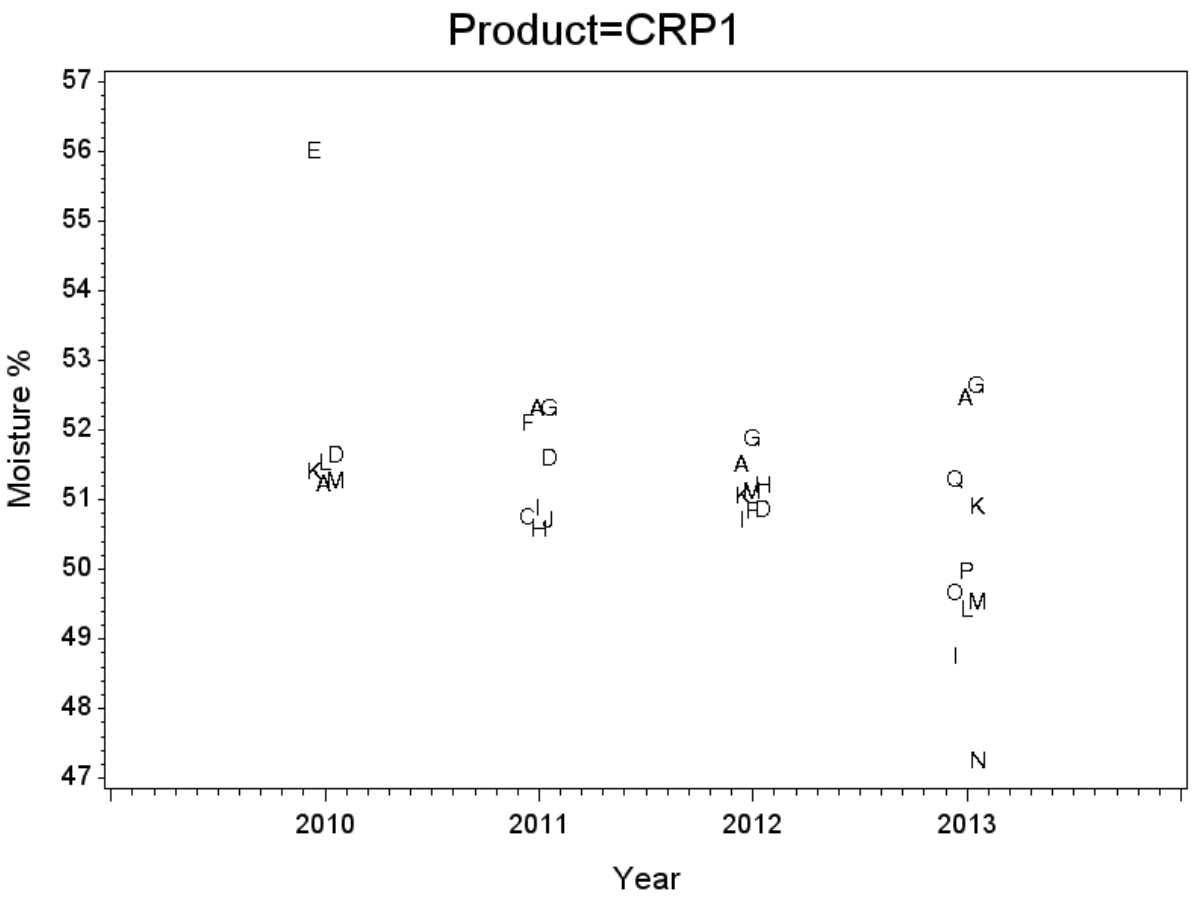

Product=CRP4
Nicotine %
1.32
1.30
1.28
1.26
1.24
1.22
1.20
1.18
1.16
1.14
1.12
1.10
1.08
1.06
1.04
1.02
1.00
0.98
0.96
0.94
0.92
2010
2011
2012
2013
Year

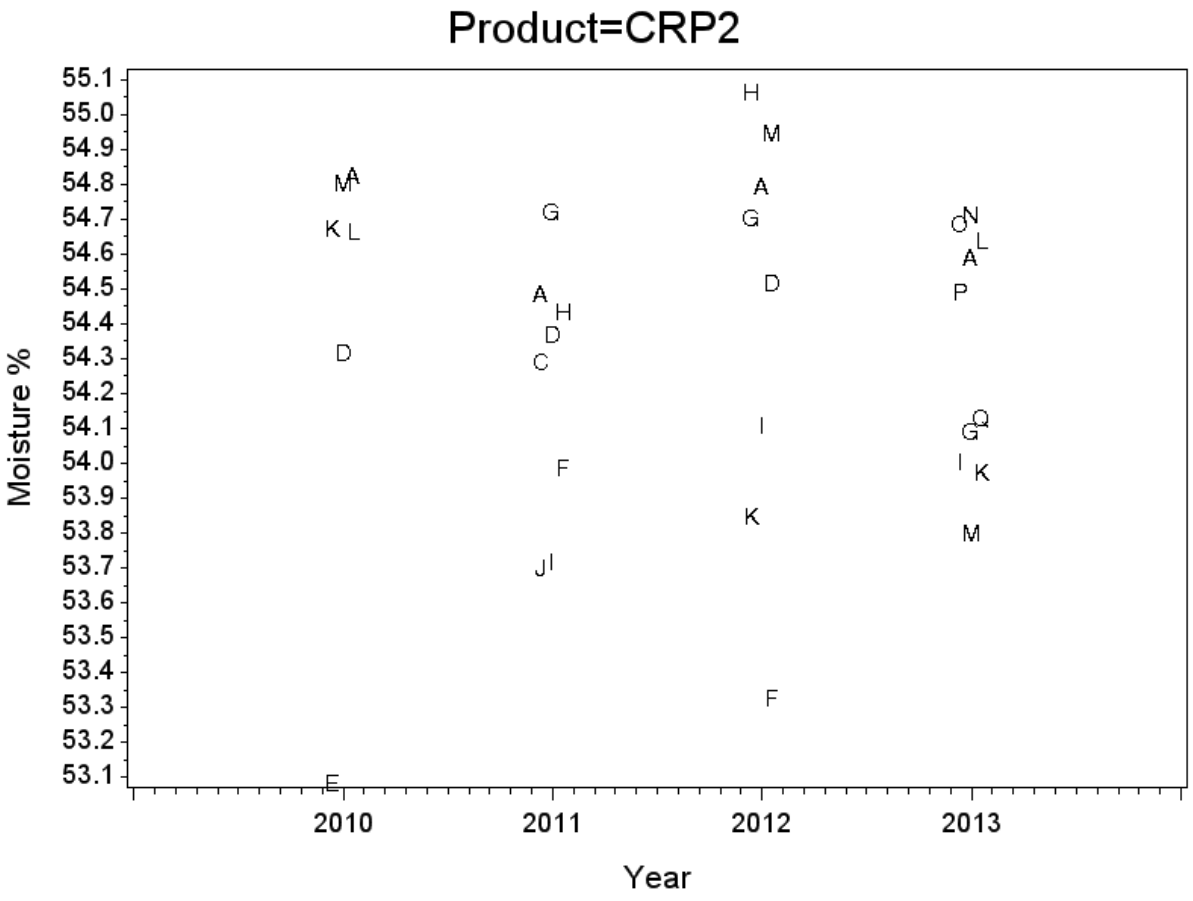

Product=CRP1
pH
8.06
8.04
8.02
8.00
7.98
7.96
7.94
7.92
7.90
7.88
7.86
7.84
7.82
7.80
7.78
7.76
7.74
7.72
7.70
7.68
7.66
7.64
7.62
7.60
2010
2011
2012
2013
Year

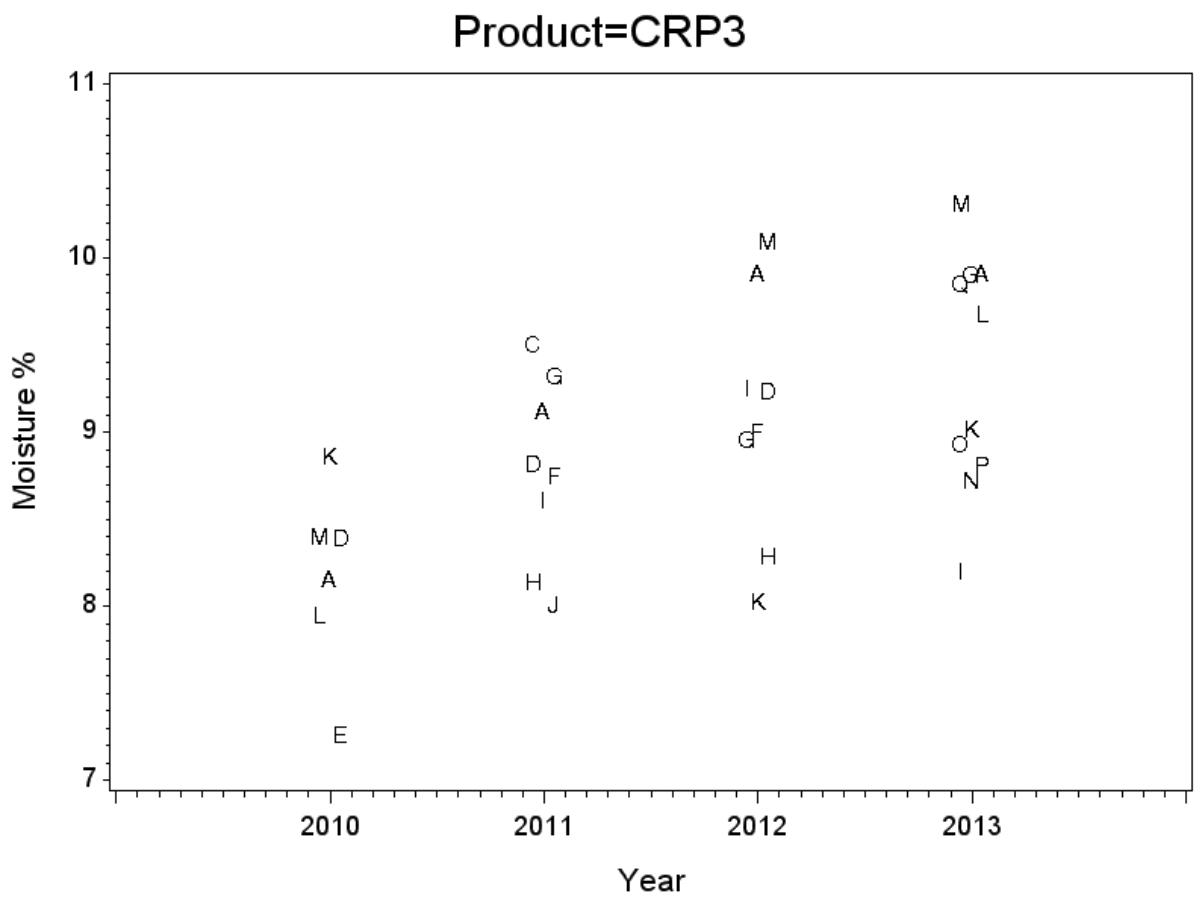

Product=CRP2
pH
7.82
7.80
7.78
7.76
7.74
7.72
7.70
7.68
7.66
7.64
7.62
7.60
7.58
7.56
7.54
7.52
7.50
2010
2011
2012
2013
Year

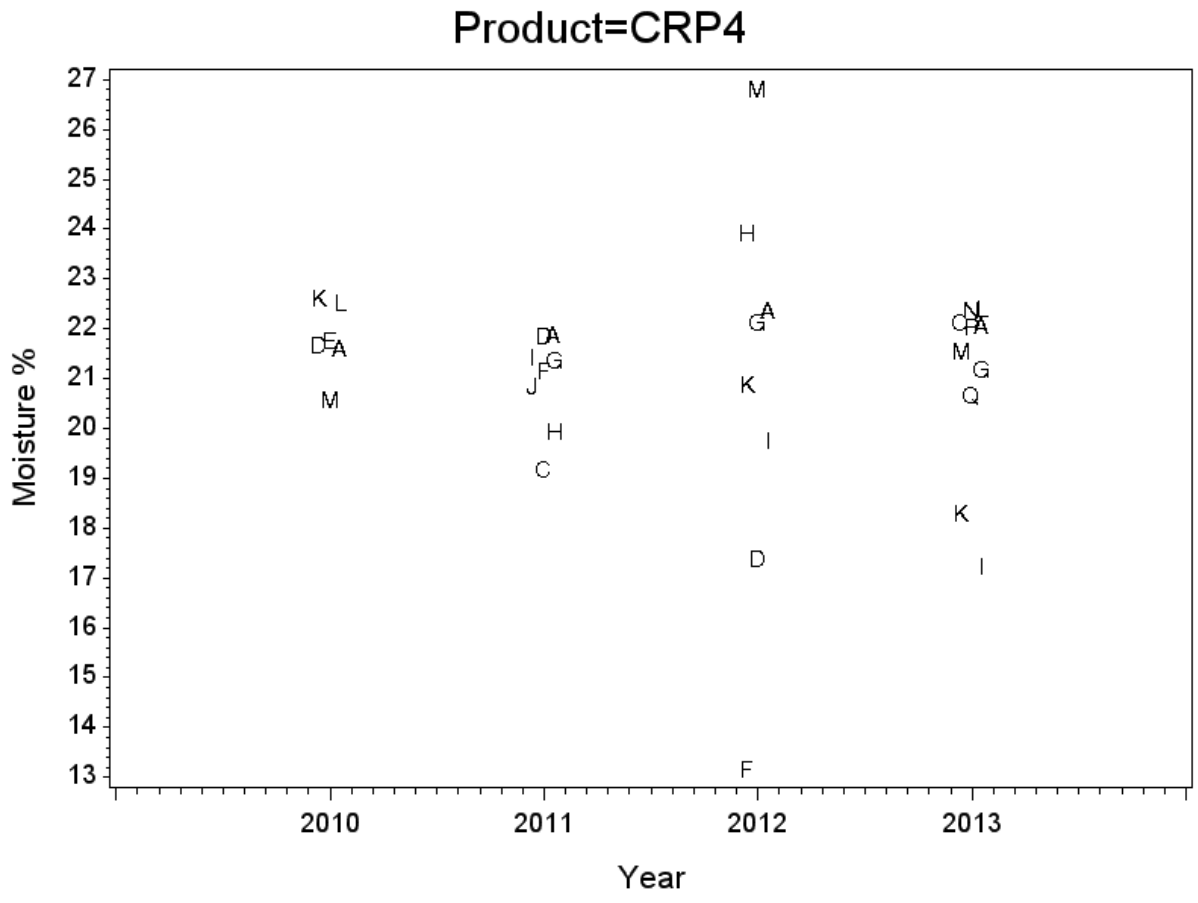

Product=CRP3
pH
6.92
6.90
6.88
6.86
6.84
6.82
6.80
6.78
6.76
6.74
6.72
6.70
6.68
6.66
6.64
6.62
6.60
2010
2011
2012
2013
Year


Product=CRP4
pH
6.08
6.06
6.04
6.02
6.00
5.98
5.96
5.94
5.92
5.90
5.88
5.86
5.84
5.82
5.80
5.78
5.76
5.74
5.72
5.70
5.68
5.66
5.64
2010
2011
2012
2013
Year


Product=CRP1
Moisture %
57
56
55
54
53
52
51
50
49
48
47
2010
2011
2012
2013
Year


Product=CRP2
Moisture %
55.1
55.0
54.9
54.8
54.7
54.6
54.5
54.4
54.3
54.2
54.1
54.0
53.9
53.8
53.7
53.6
53.5
53.4
53.3
53.2
53.1
2010
2011
2012
2013
Year


Product=CRP3
Moisture %
Year
2010
2011
2012
2013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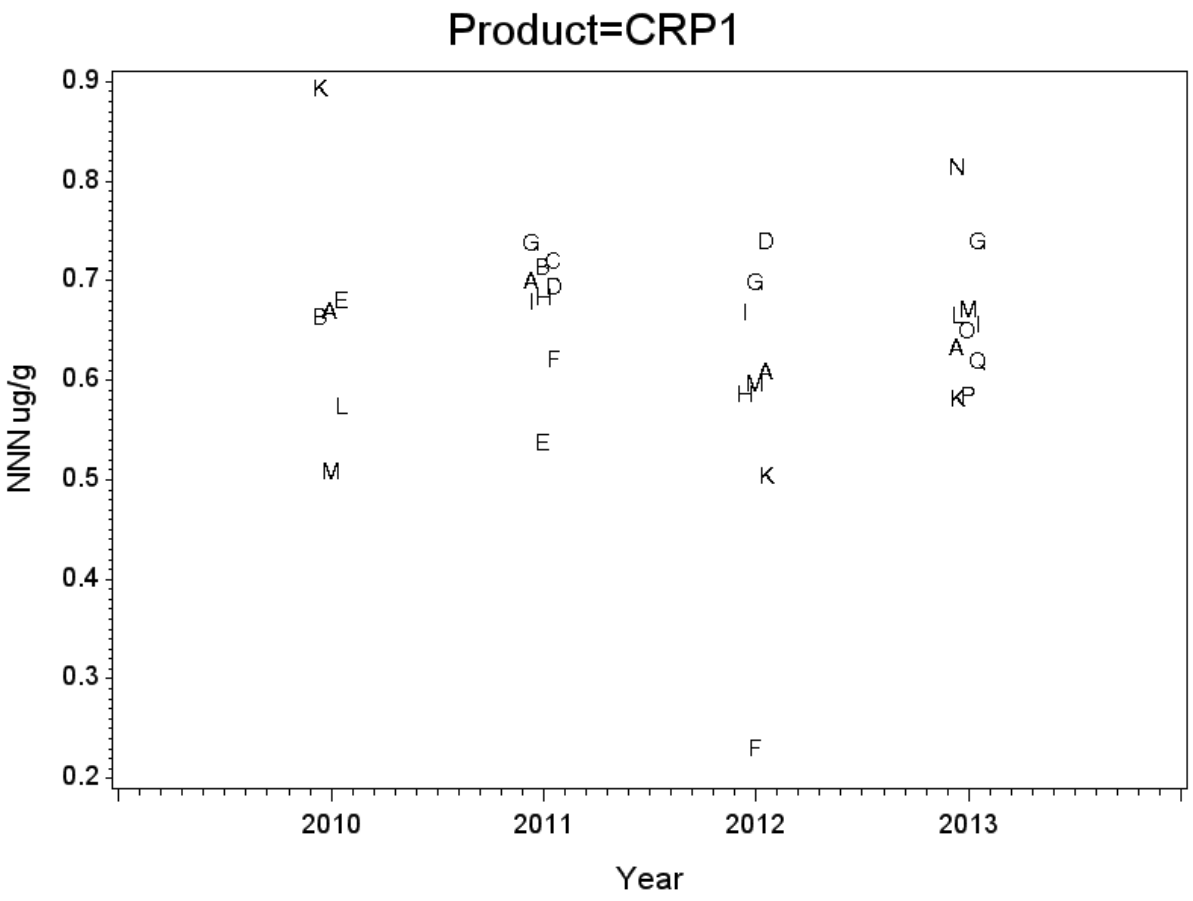
Moisture %
Year
2010
2011
2012
2013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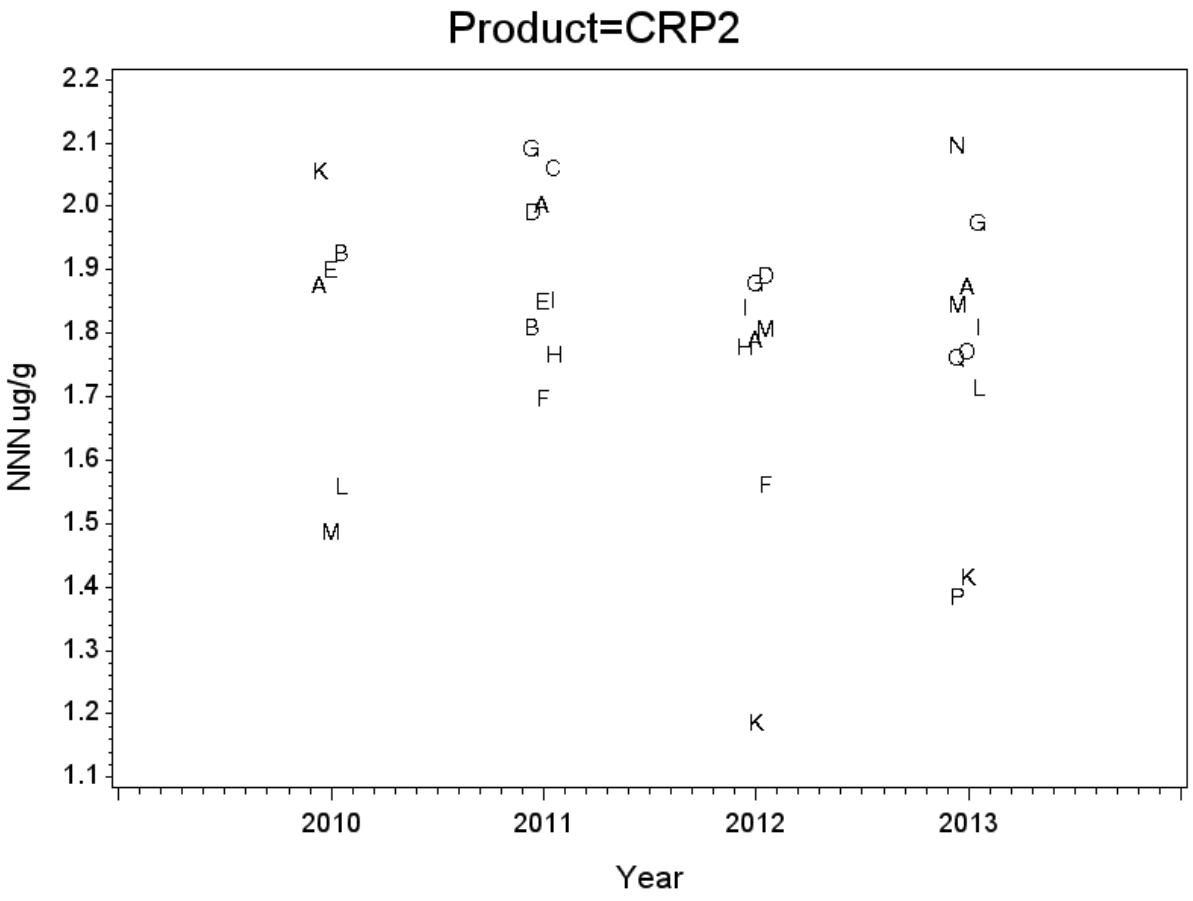
0.8
0.7
0.6
0.5
0.4
0.3
0.2
2010
2011
2012
2013
Year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2.1
2.0
1.9
1.8
1.7
1.6
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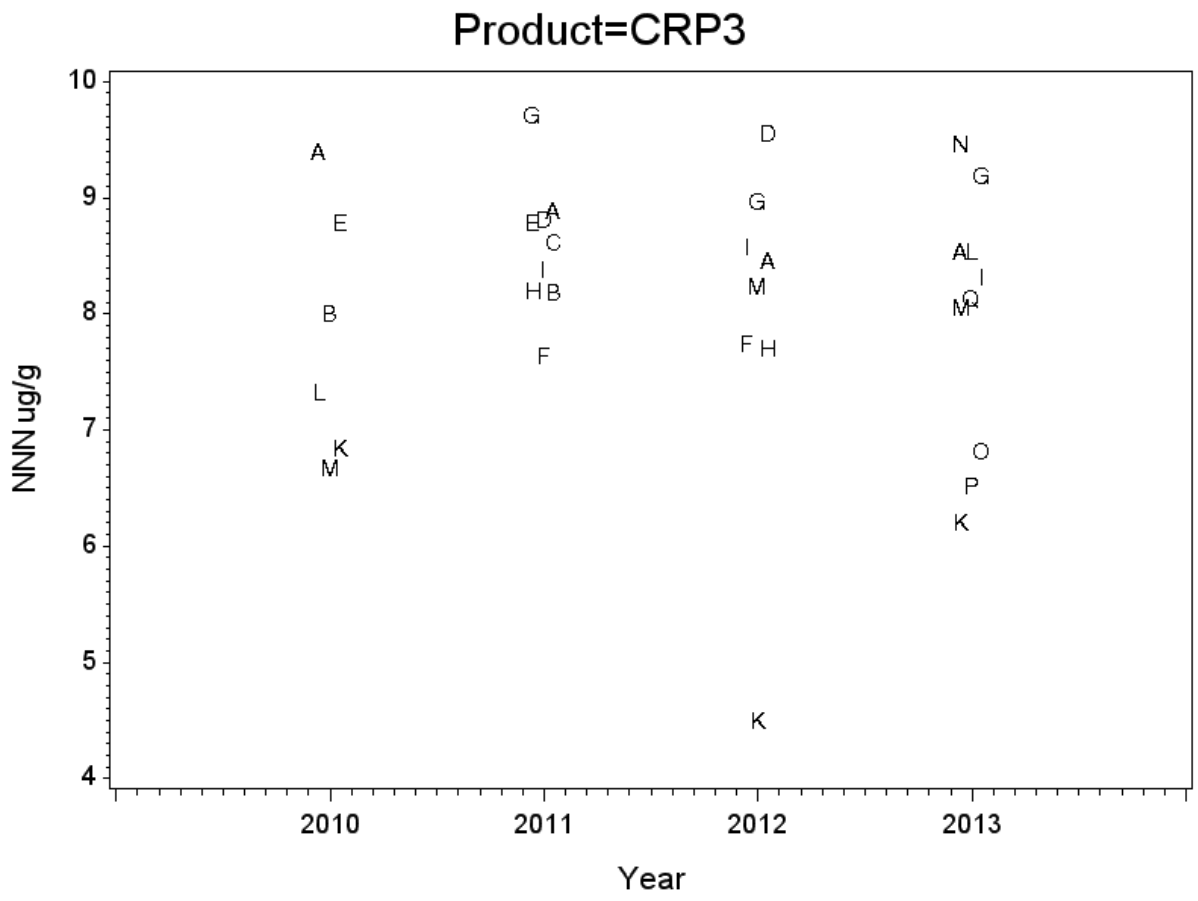
1.4
1.3
1.2
1.1
2010
2011
2012
2013
Year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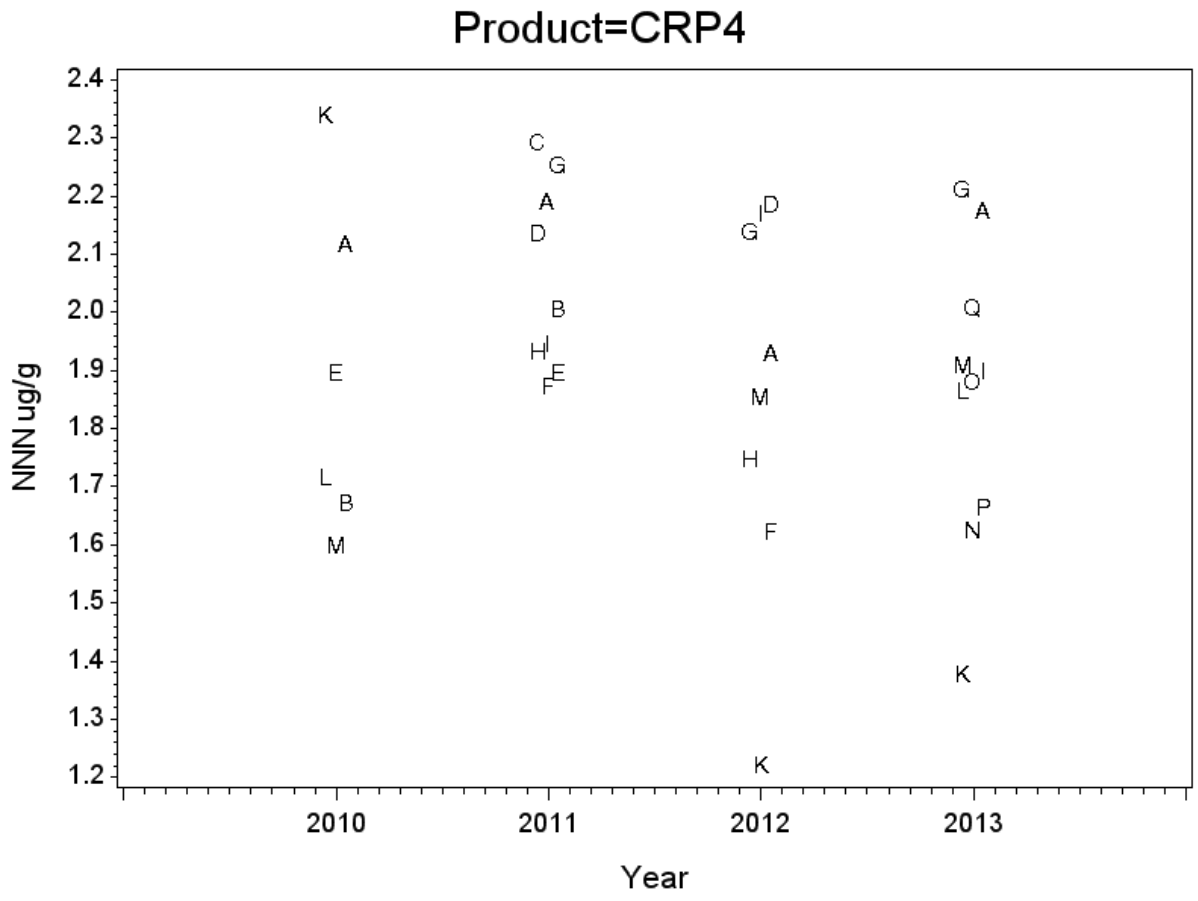
10
9
8
7
6
5
4
2010
2011
2012
2013
Year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2.2
2.1
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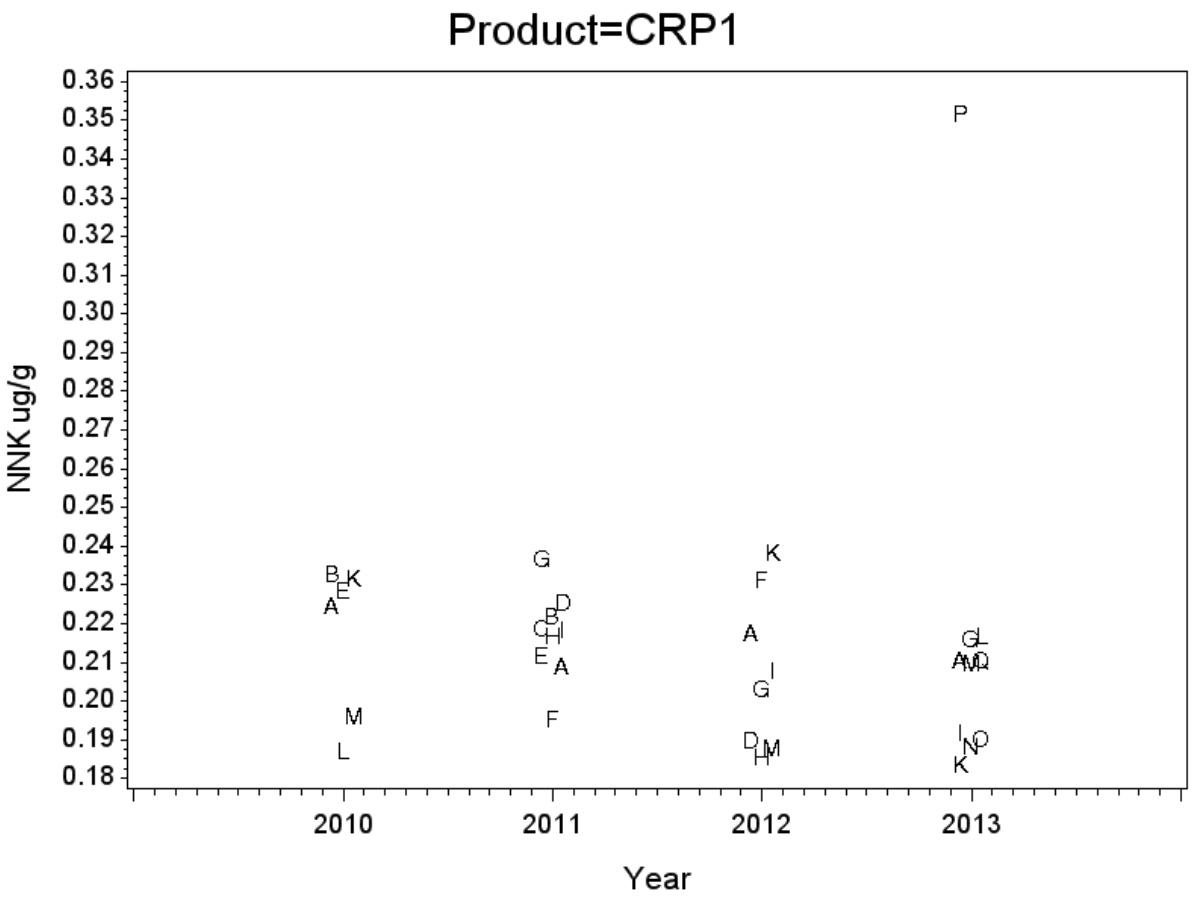
1.8
1.7
1.6
1.5
1.4
1.3
1.2
2010
2011
2012
2013
Year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0.34
0.33
0.32
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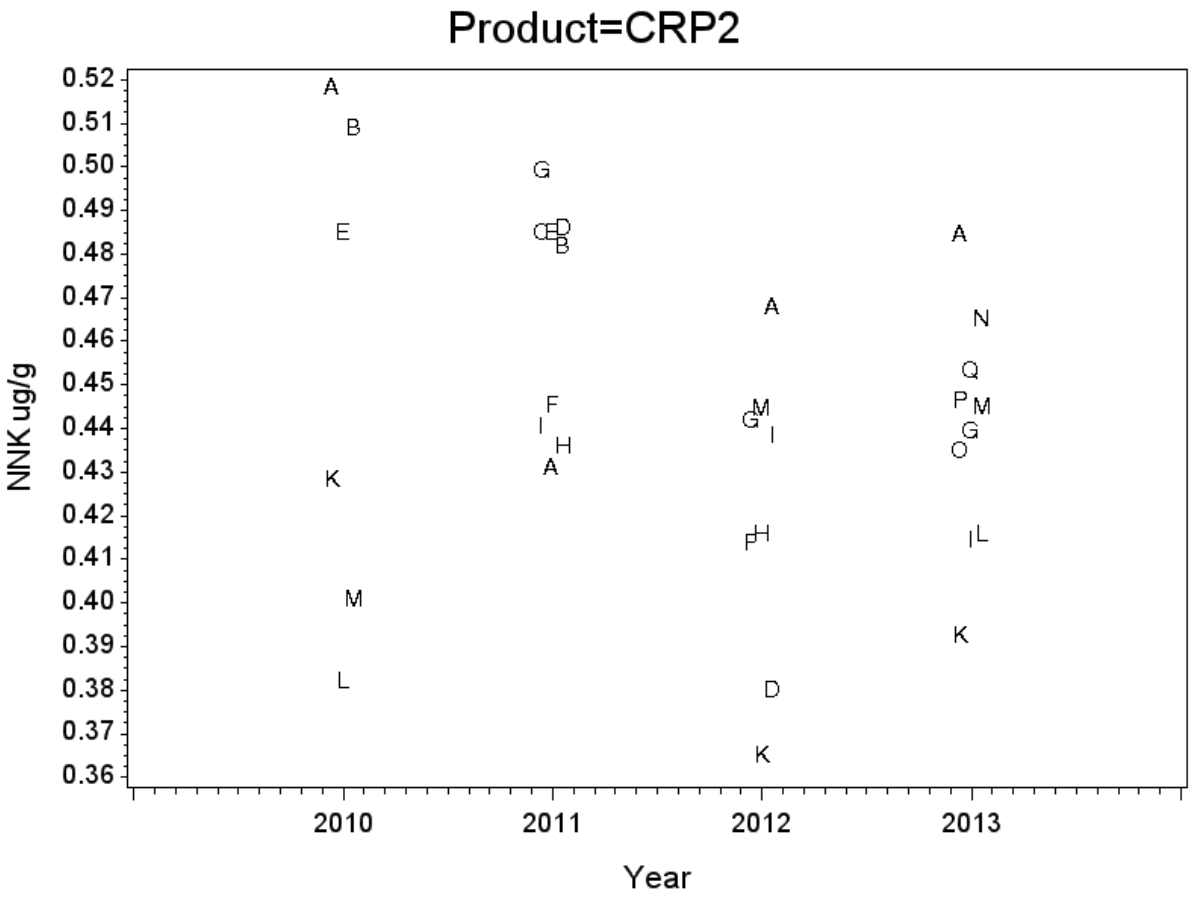
0.29
0.28
0.27
0.26
0.25
0.24
0.23
0.22
0.21
0.20
0.19
0.18
2010
2011
2012
2013
Year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0.50
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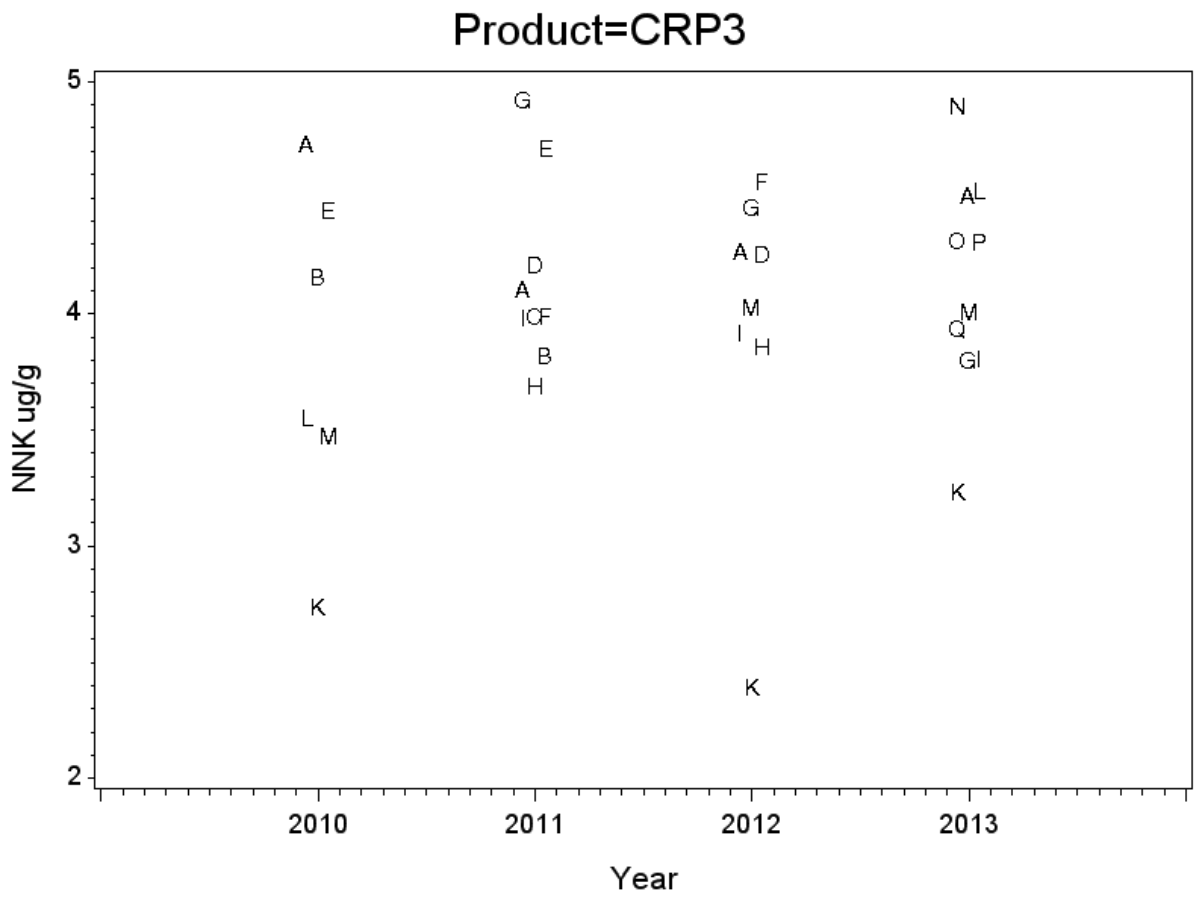
0.47
0.46
0.45
0.44
0.43
0.42
0.41
0.40
0.39
0.38
0.37
0.36
2010
2011
2012
2013
Year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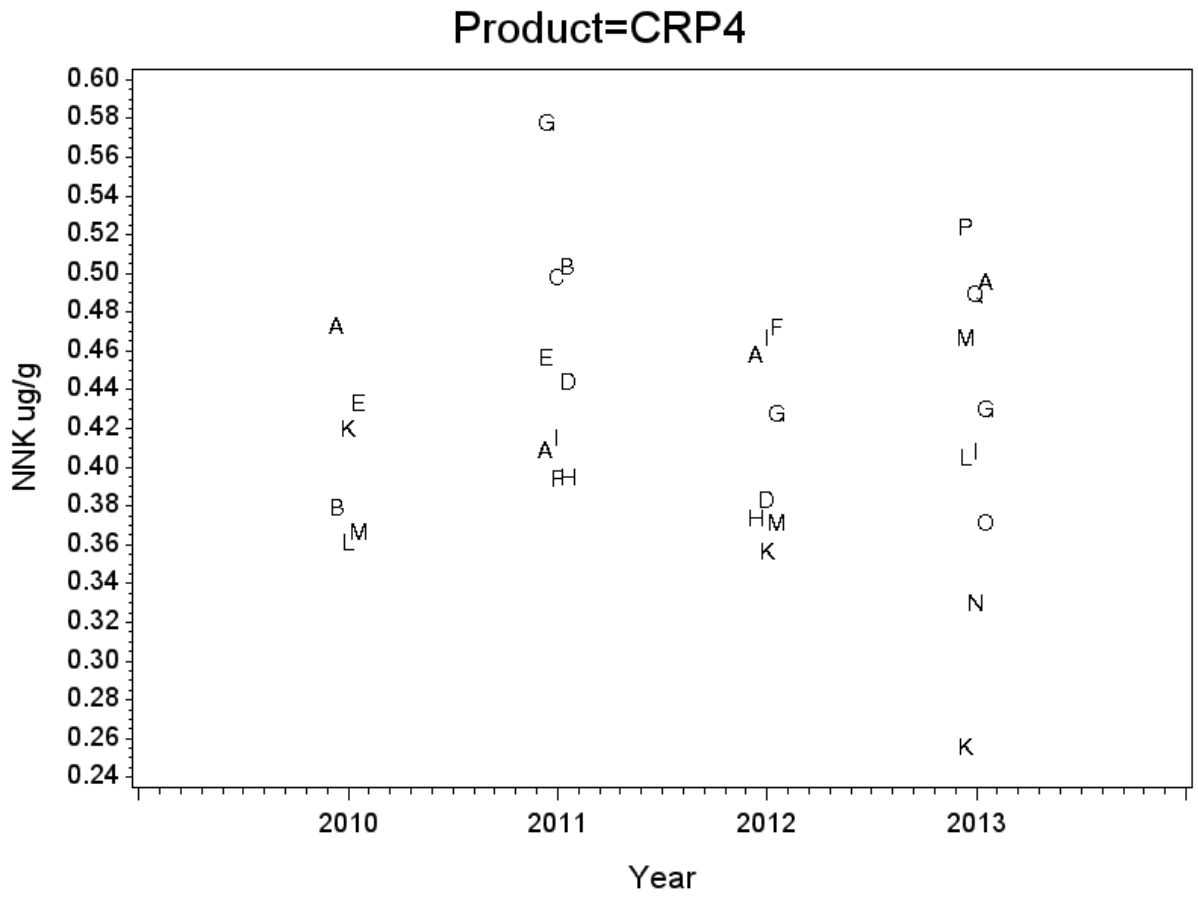
4
3
2
2010
2011
2012
2013
Year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0.46
0.44
0.42
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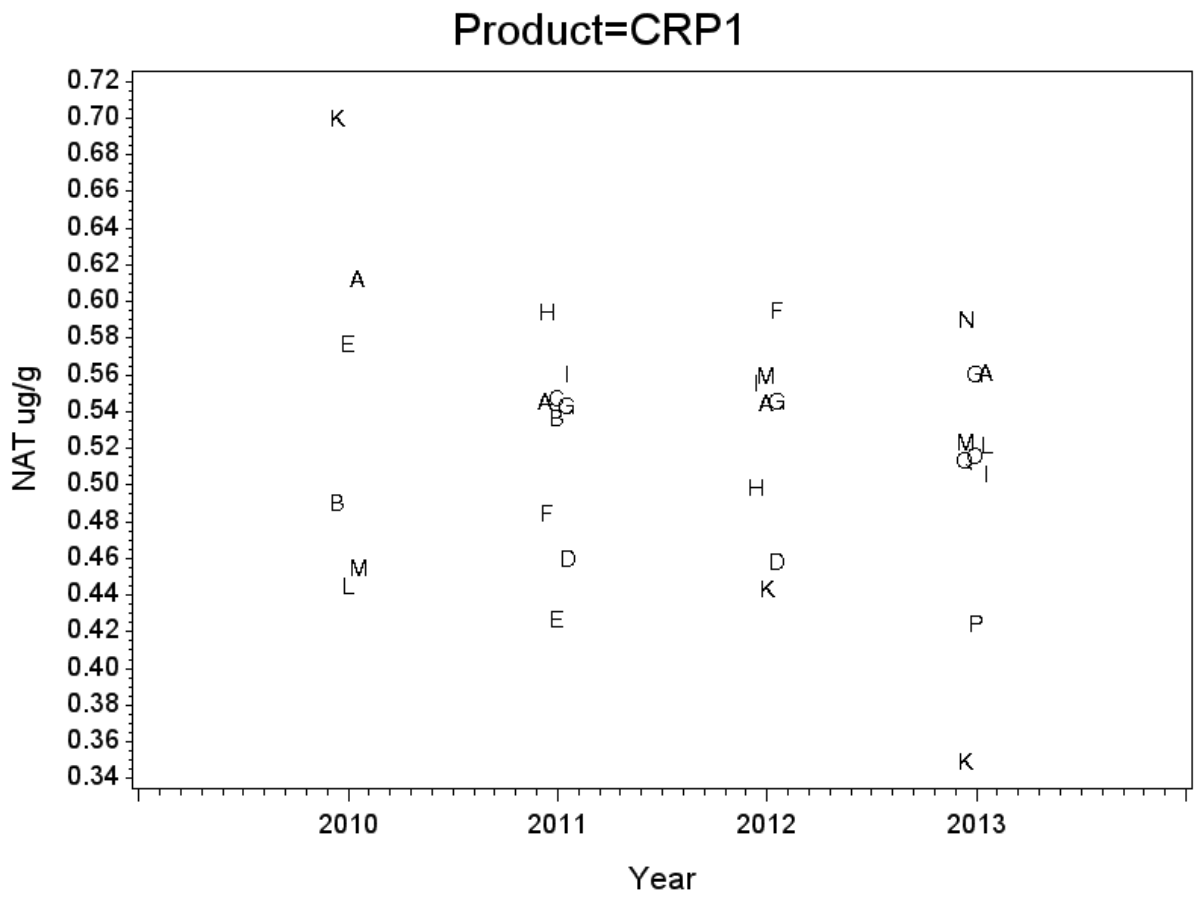
0.36
0.34
0.32
0.30
0.28
0.26
0.24
2010
2011
2012
2013
Year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0.58
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0.52
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0.46
0.44
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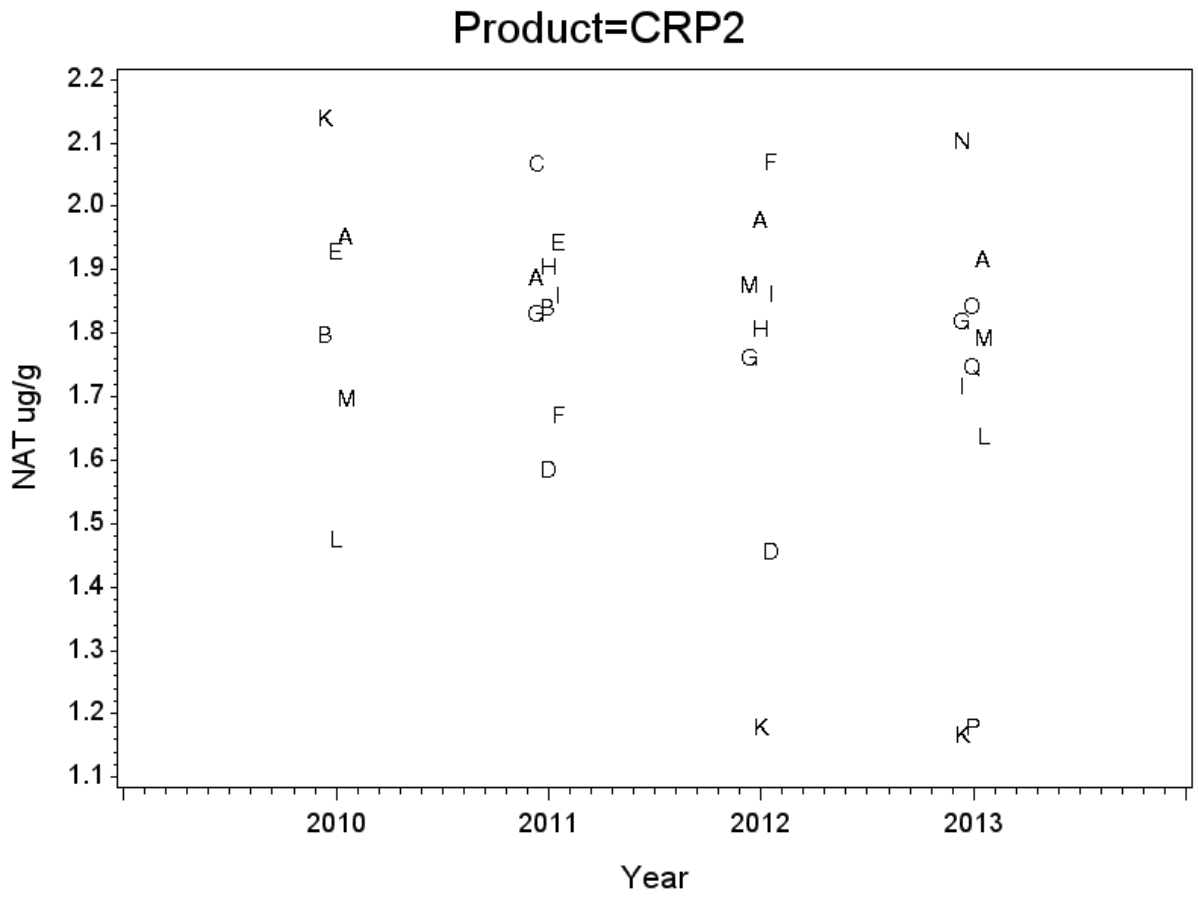
0.40
0.38
0.36
0.34
2010
2011
2012
2013
Year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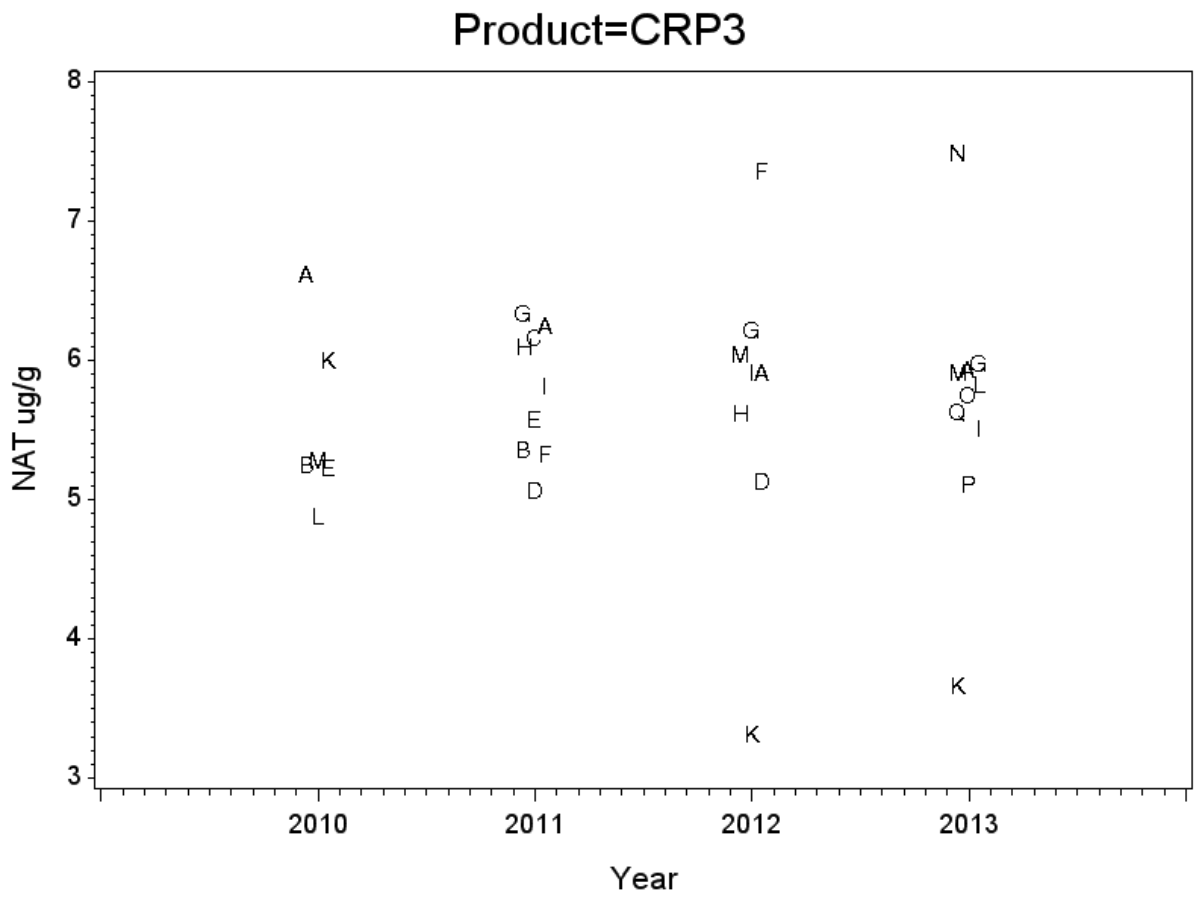
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
2010
2011
2012
2013
Year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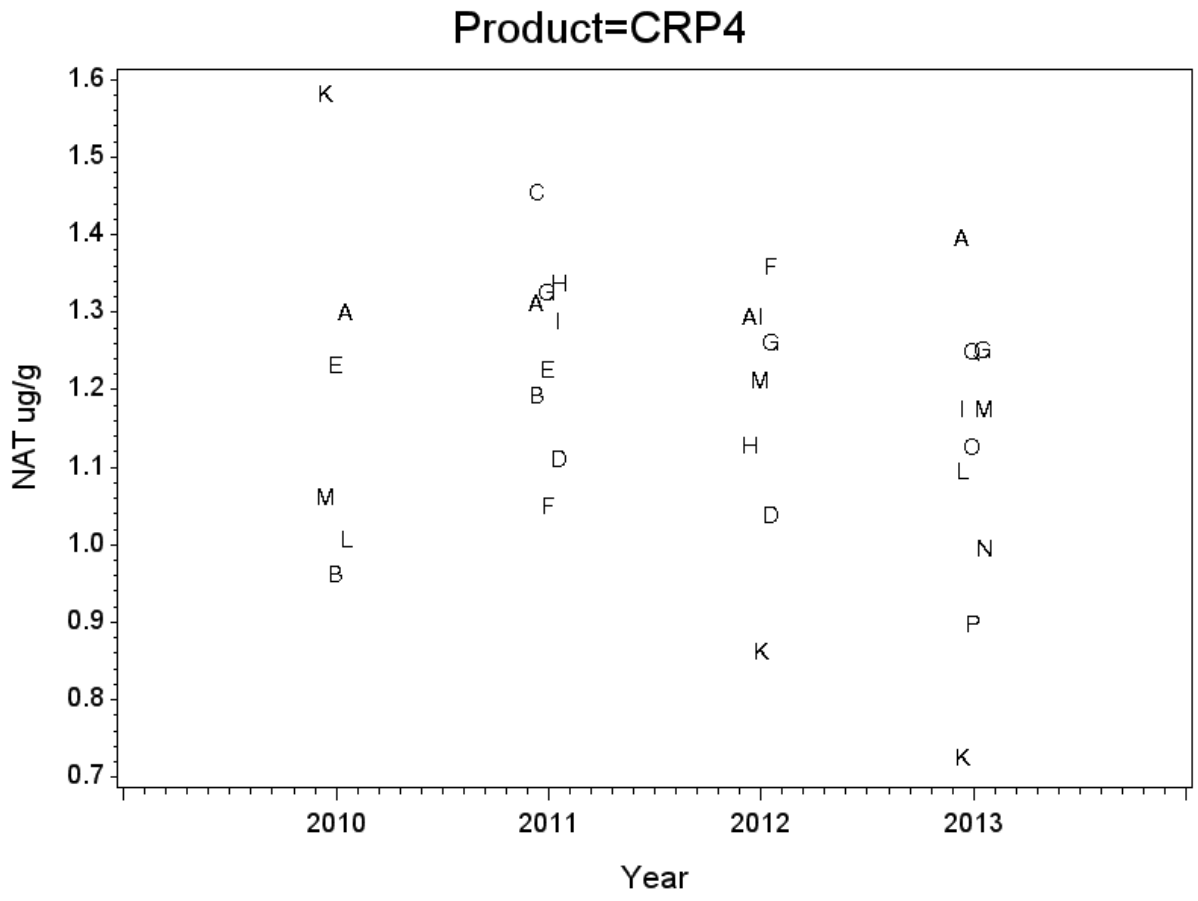
8
7
6
5
4
3
2010
2011
2012
2013
Year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NAT ug/g
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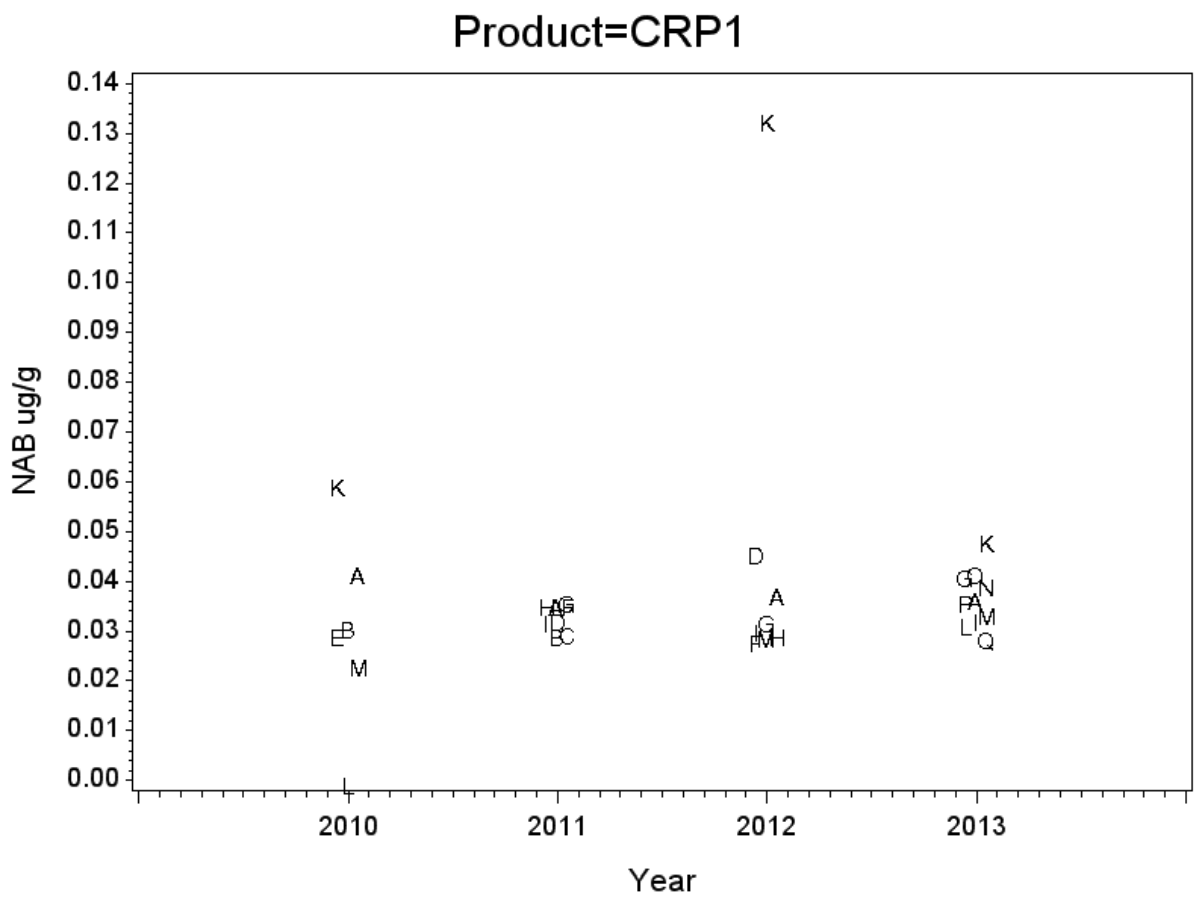
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
2010
2011
2012
2013
Year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0.11
0.10
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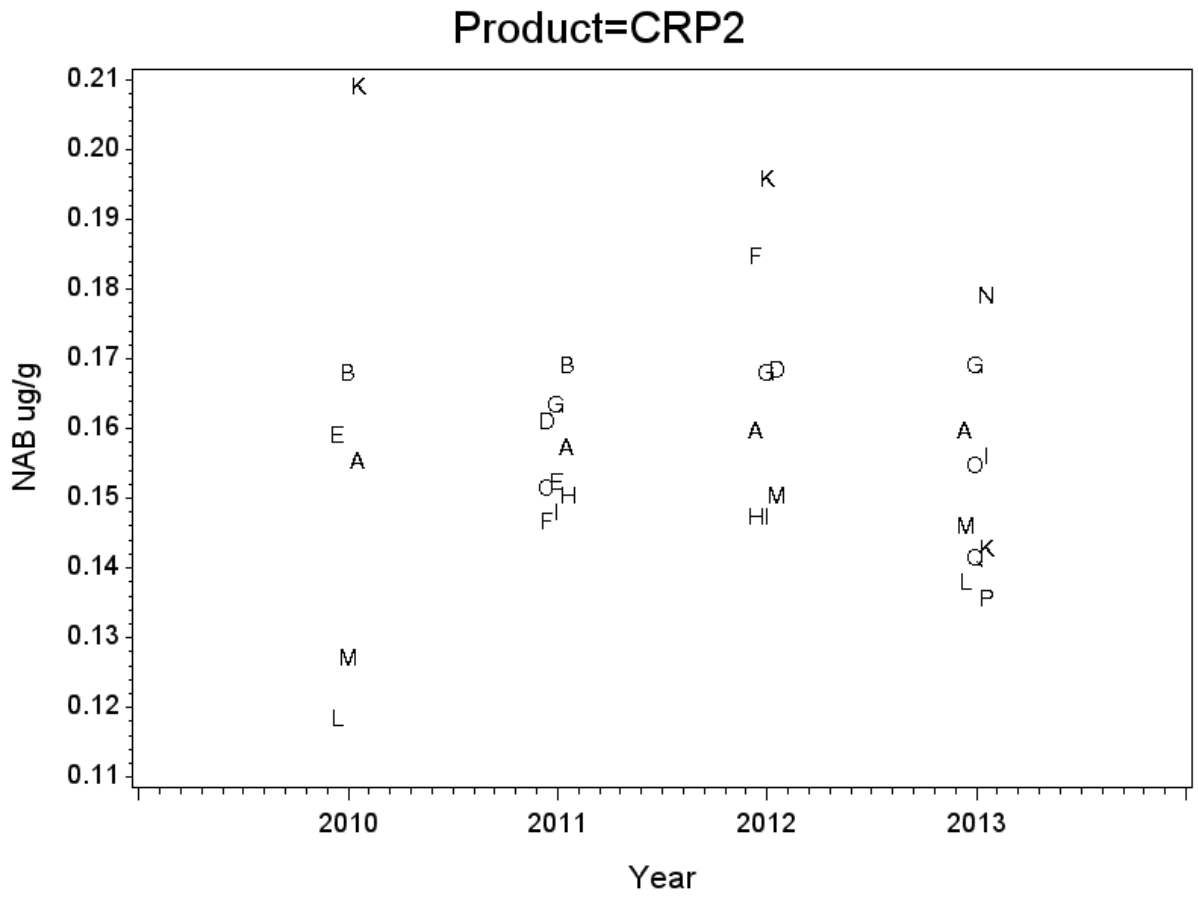
0.05
0.04
0.03
0.02
0.01
0.00
2010
2011
2012
2013
Year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0.16
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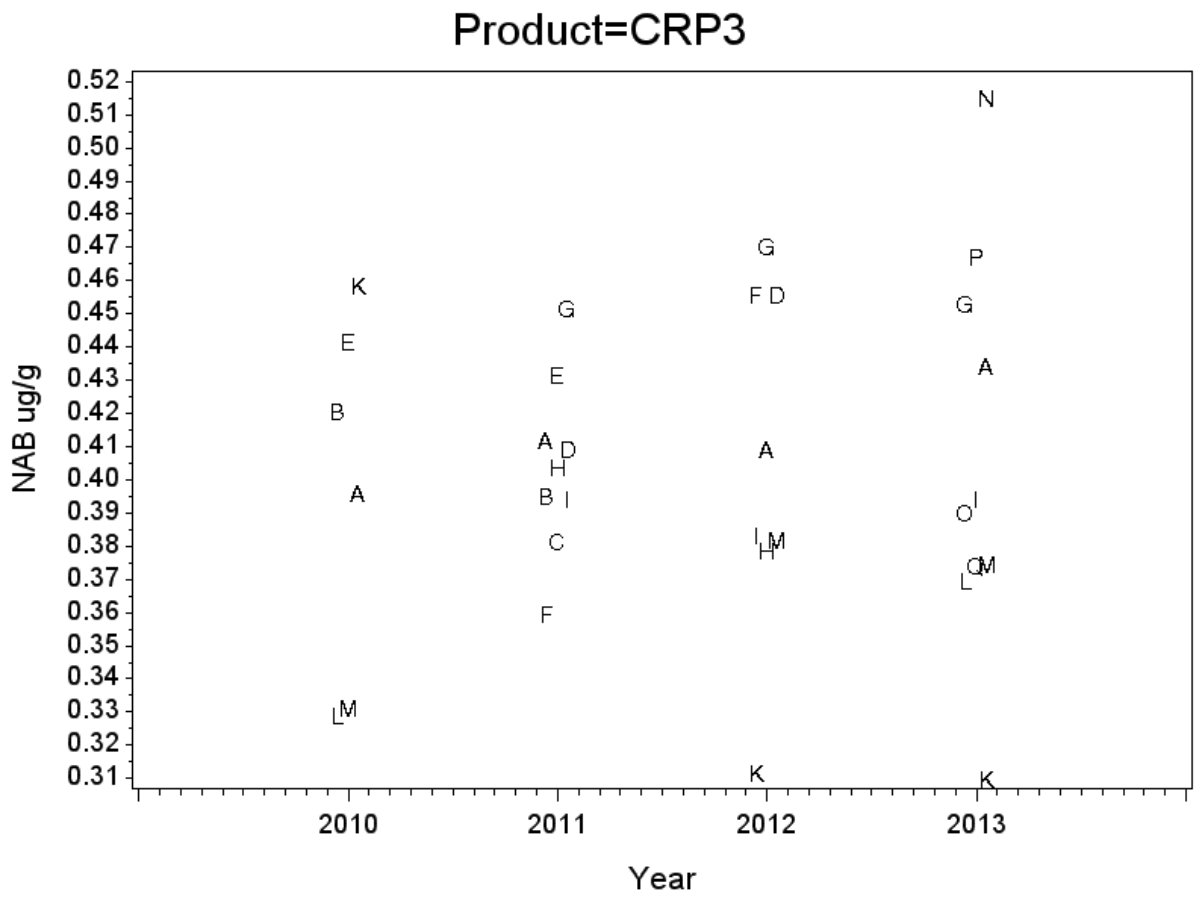
0.14
0.13
0.12
0.11
2010
2011
2012
2013
Year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0.42
0.41
0.40
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0.36
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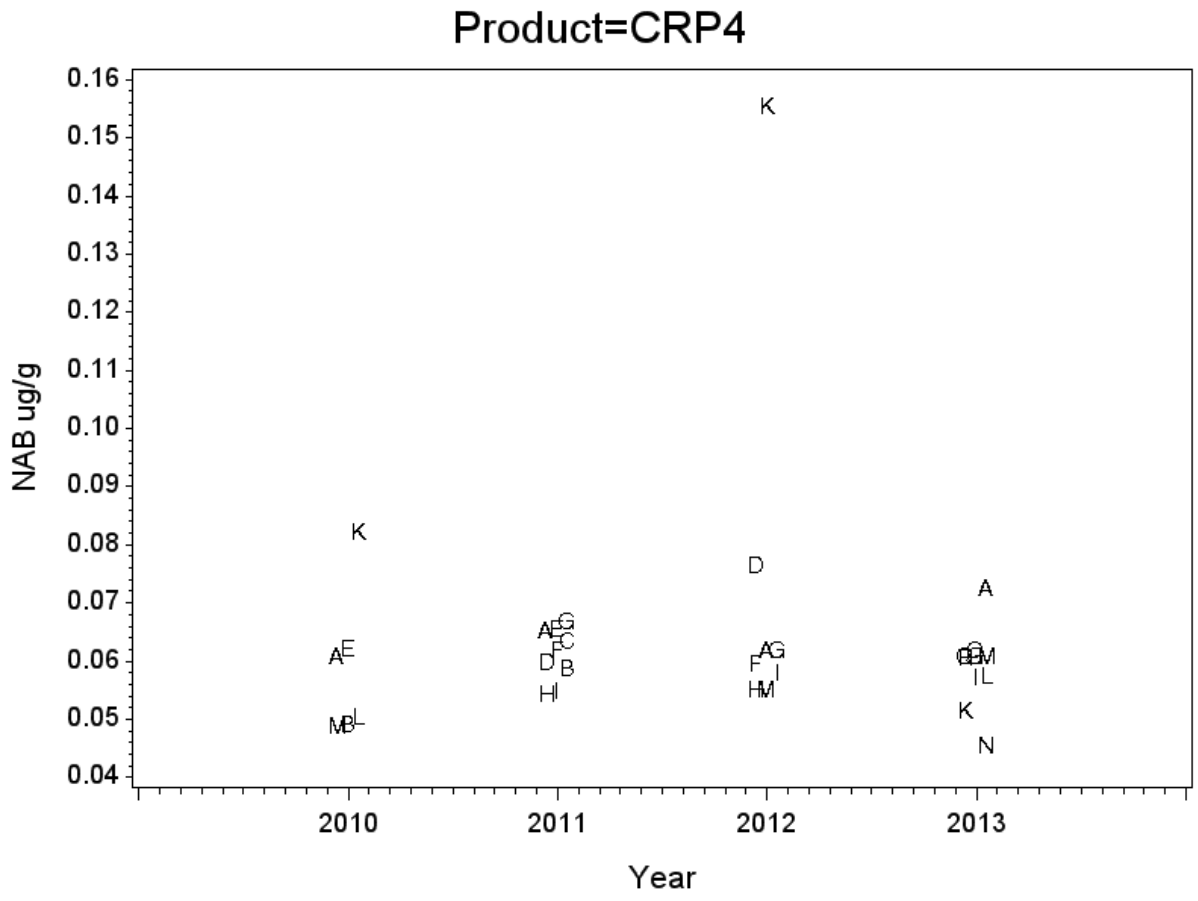
0.34
0.33
0.32
0.31
2010
2011
2012
2013
Year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0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
2010
2011
2012
2013
Year