Guidance on Qualifying Method for Determining Converted Fraction of Co-Processed with States	ing the Cellulosic Corn Kernel Fiber



Guidance on Qualifying an Analytical Method for Determining the Cellulosic Converted Fraction of Corn Kernel Fiber Co-Processed with Starch

Compliance Division
Office of Transportation and Air Quality
U.S. Environmental Protection Agency



Guidance on Qualifying an Analytical Method for Determining the Cellulosic Converted Fraction of Corn Kernel Fiber Co-Processed with Starch

Introduction

This document provides guidance on how to demonstrate that an analytical method for determining the cellulosic converted fraction of corn kernel fiber co-processed with starch at a traditional ethanol facility satisfies the applicable regulatory requirements. Public confidence in the volumes of cellulosic ethanol produced under the Renewable Fuel Standard (RFS) program is important to the integrity of the program. As such, affected stakeholders have sought clarification regarding what methods should be used to determine the cellulosic converted fraction. The intent of this guidance is to explain our interpretation of our regulatory requirements and to articulate clear criteria for the type of analysis and demonstrations that EPA believes would be an appropriate basis for registration under the program.

EPA evaluates registration applications on a case-by-case basis given the best available information and science relating to analytical methods for determining the cellulosic converted fraction of corn kernel fiber co-processed with corn starch. This guidance provides EPA's current view on the manner in which facilities should demonstrate the accuracy of such analytical methods and thus may satisfy the applicable registration requirements. However, any decisions on individual facilities' registration applications will be made in the context of each of those applications on the basis of the requirements of EPA's regulations. This guidance does not create any new requirements and may not apply to a particular situation based on the circumstances.

This guidance is organized into the following topics: (1) background and explanation of the relevant regulations; (2) EPA's interpretation of the regulatory term "reasonable accuracy"; (3) establishing voluntary consensus standard body (VCSB) methods that are consistent with the regulations; and (4) a summary of EPA's guidance for demonstrating that the results of an analytical method for calculating the cellulosic converted fraction are reasonably accurate using acceptable reference materials.

Background and Explanation of Regulations

In the 2014 Pathways II Final Rule, ¹ EPA added a pathway for the production of cellulosic ethanol from corn kernel fiber² and promulgated the regulations necessary to implement this pathway. The Agency stated that given variations in individual conversion processes, enzymes used, and other differences, the amount of finished fuel derived from the cellulosic content of corn kernels (i.e., the cellulosic converted fraction) can vary. For example, the process and enzymes used may be more effective in converting the sugars and starches in a feedstock than the cellulose or hemicellulose. In such a case, the cellulosic content of the feedstock may not be a good indicator of the amount of finished biofuel that is derived from

¹ 79 Federal Register 42128 (July 18, 2014).

² See id. at 42147–48; 40 CFR 80.1426, Table 1 row K (production of cellulosic ethanol from crop residue, including corn kernel fiber).

cellulosic materials. Furthermore, depending on the conversion process used, the amount of information needed to determine how much of the finished fuel is derived from the cellulosic content will also vary.³ Therefore, the regulations include requirements for registration, reporting, and recordkeeping related to calculating the cellulosic converted fraction to ensure a high degree of confidence that cellulosic biofuel RINs are appropriately generated.

The regulations at 40 CFR 80.1450(b)(1)(xiii)(B)(3) require a producer of renewable fuel seeking to generate cellulosic RINs who intends to produce a single type of fuel using two or more feedstocks converted simultaneously (such as corn starch and corn kernel fiber) to provide "chemical analysis data supporting the calculated cellulosic converted fraction (CF) and a discussion of the possible variability that could be expected between reporting periods per §80.1451(b)(1)(ii)(U)(1). Data used to calculate the cellulosic CF must be representative and obtained using an analytical method certified by a voluntary consensus standards body [VCSB],or using a method that would produce reasonably accurate results as demonstrated through peer reviewed references provided to the third-party engineer performing the engineering review at registration."

Further, EPA anticipated that the converted fraction may vary over time, and therefore established reporting requirements at 40 CFR 80.1451(b)(1)(ii)(U) that require recalculation and recertification to EPA of the cellulosic converted fraction at specified intervals (annually for low volume producers and every 500,000 gallons of cellulosic RINs generated for larger volume producers). The initial cellulosic converted fraction is based on the data submitted at registration and this upfront cellulosic converted fraction determination applies to RINs generated until a new cellulosic converted fraction allocation is available and reported. Given the natural variation in cellulosic content and conversion efficiencies, EPA recognized some variation would exist in the amount of biofuel that is derived from the cellulosic components of a feedstock. The regulations require that if the cellulosic converted fraction deviates from the previously calculated cellulosic converted fraction by 10 percent or more, a producer is required to alert EPA to this change in addition to updating the formula used to calculate RIN allocations.⁴ This regulatory requirement reflects EPA's recognition that variation within 10 percent of previously calculated numbers may result under normal operating conditions, but that larger variations raise significant concerns that the process or feedstock has significantly changed compared to what was approved at registration. As a practical matter, if EPA observes a high variability (i.e., over 10 percent) in the recertified cellulosic converted fraction, this may signal the need for additional inquiry with the producer to understand the cause of that variability.

Interpretation of the "Reasonable Accuracy" Requirement

This section provides a detailed explanation of how a producer of renewable fuel should demonstrate that an analytical method will produce reasonable accurate results. As there is currently no VCSB method for demonstrating how much of the cellulosic material is being converted into biofuel, ethanol producers have sought to use the second option in § 80.1450(b)(1)(xiii)(B)(3): calculating the cellulosic converted fraction using a non-VCSB method that would produce reasonably accurate results as demonstrated through peer reviewed

⁴ See id. at 42135.

³ *Id.* at 42134.

references. Some stakeholders have requested that EPA provide our interpretation of the peer reviewed reference requirement. EPA interprets this provision as requiring that peer reviewed references not only evaluate the potential performance of a non-VCSB analytical method but also demonstrate the accuracy of the results of that method. That is, the references must demonstrate to EPA that not only is the analytical method theoretically capable of producing accurate results, but that its application has, in fact, yielded a calculation of the cellulosic converted fraction that is reasonably accurate. Both criteria must be satisfied in order to demonstrate that the method used to calculate the cellulosic converted fraction would produce reasonably accurate results. This is consistent with the purpose of the additional registration and reporting requirements for fuels produced from co-processing two or more different feedstocks—to ensure the accurate assignment of RINs to the cellulosic versus non-cellulosic components of the finished fuel.⁵ That is, to uphold the integrity of RIN assignment and thus of the program, it is critical that the analytical method has been demonstrated to produce reasonably accurate results.

As explained further below, the wide degree of variability in the data EPA has reviewed alerted us to the fact that it is not possible, as a technical matter, to assess whether a method is accurately measuring how much of a cellulosic feedstock is converted into fuel without comparing the performance of the method to a known, representative reference material. EPA's concern about the lack of a benchmark against which to assess the performance of non-VCSB analytical methods led us to approach the National Institute of Standards and Technology (NIST) in August 2017 to pursue the development of a reference material containing both starch and cellulose. Such reference material would establish the "true value" of cellulosic content against which to evaluate the results of analytical methods and will allow peer reviewers and the Agency to determine whether those methods can produce reasonably accurate results for the cellulosic converted fraction. Accurate calculations of cellulosic conversion are necessary to apportion RINs correctly per the co-processing requirements under 40 CFR 80.1426(f)(3)(vi) and thus provide industry with a path forward on RFS registrations. A work group composed of NIST, the National Renewable Energy Laboratory (NREL), EPA, and industry was established to develop

evaluate the ability of particular analytical methodology to determine results that approach the true value.

⁵ 40 CFR 80.1426(f)(3)(vi) contains the requirements for assigning RINs to renewable fuels produced using two or more feedstocks processed simultaneously, including situations in which only one of the feedstocks is cellulosic. The formula in this provision for assigning RINs to the different components of the finished fuel incorporates the value for the cellulosic converted fraction. Thus, accurate assignment of RINs depends in part on accurate calculation of the cellulosic converted fraction.

⁶ A representative reference material per the National Institute of Standards and Technology (NIST) (see https://www.nist.gov/srm/srm-definitions) is a material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process. Uses may include calibrating a measurement system, assessing a measurement procedure, assigning values to other materials, and quality control. In this instance, EPA expects to evaluate the performance of non-VCSB methods based on their ability to return results consistent with the known cellulosic value for the reference material.

⁷ NIST, with stakeholder support, is developing representative reference materials using samples taken from a corn ethanol facility. The candidate reference materials were prepared from dried, ground and blended corn grain biomass intermediates before and after conversion to ethanol. Representative reference materials will allow the peer reviewers and the Agency to directly evaluate whether a non-VCSB method is able to achieve accurate results rather than relying on an opinion that the analytical steps in a method should be capable of doing so.

⁸ Accuracy describes how closely the measured value approximates its true value. A representative reference material that has been validated by an independent body is needed to provide a "true value" against which to

candidate materials and advance this process. NIST recently distributed the candidate materials for intra-laboratory analysis, an important step forward in the process.

While NIST is seeking input on both starch and cellulosic values of the candidate materials, there is an expectation within the workgroup that the first round of laboratory samples will focus predominantly on starch¹⁰ and that the cellulosic analysis will follow once an acceptable value has been determined for starch.¹¹ EPA believes it is not possible for an analytical method that is designed to focus on starch or some other non-cellulosic component(s), where the accuracy is determined for various components and not for cellulose directly by comparison to a representative reference material, to yield reasonably accurate calculations for cellulose. Peer reviewers and other outside parties have expressed concerns that resistant and retrograde starch present in samples due to feedstock handling or processing could impact laboratory results and cellulosic calculations. That is, measurements of starch may not themselves be accurate enough to be used to derive reasonably accurate estimates of the cellulosic converted fraction. This is especially true when cellulose is determined by mass balance via subtraction of a starch measurement because the variability of the starch measurements is likely to overwhelm the percent mass of cellulose that is converted. For a starch-based method in which cellulosic conversion is not measured directly, uncertainty in the starch measurement is propagated to the cellulose calculation. On average, the incoming corn feedstock is approximately 70 percent starch and 6 percent cellulosic fiber. Uncertainty of +/- 2 percent in the starch measurement translates to uncertainty of +/- 1.4 percent of total feedstock mass. Where industry expects approximately 2 percent total cellulosic corn mass conversion, carrying the +/- 1.4 percent uncertainty from the starch mass over to the 2 percent cellulosic mass yields an uncertainty in the calculation of cellulosic mass of +/- 70 percent. ¹² In general, EPA does not believe that a result with 70 percent uncertainty can be considered "reasonably accurate." Furthermore, the results of a recent EPA statistical analysis 13 indicate that it is possible, given the potential range of pre- and post-fermentation starch and cellulose values, that starch-based calculations could yield clearly nonsensical results for the cellulosic converted fraction, e.g., negative values and values in which over 100 percent of cellulosic mass is converted. These results again demonstrate that calculations based on starch reference values alone cannot ensure that resulting estimates of cellulosic conversion are reasonably accurate.

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⁹ Candidate materials are samples sent to laboratories for independent testing to help develop reference materials. For example, NIST has sent out candidate materials for testing at several laboratories and has requested reported values for starch and cellulose. Based on the quality of the data, statistical assessment, stability of the samples and other factors, NIST could proceed to develop "reference materials" from these "candidate materials."

¹⁰ For the purposes of this letter, we refer to a starch or "starch only" reference material or method as one that reports values for any component(s) other than cellulose, which may include starch, fats, proteins, and/or ash. Some proposed methods directly measure the conversion of starch and other, non-cellulosic components, and then subtract the starch values from the total to derive the cellulosic component of the finished fuel.

¹¹ It is our understanding that NIST also requested cellulosic values from labs participating in evaluation of the candidate reference material, but it is yet unclear whether participation and data quality will be sufficient to establish a reference value for cellulose from this "phase 1" effort.

¹² If starch is 70 percent of the mass and the coefficient of variation is +/- 2 percent, then the uncertainty surrounding the starch measurement is +/- 1.4 percent total mass. Industry has indicated that they expect 2 percent of the total mass which is fiber to be converted to cellulosic ethanol. Therefore, given just the variability on the starch measurement that is part of the analytical process to determine cellulose, there is a variability of 2 percent mass +/- 1.4 percent mass or +/-70 percent.

¹³ See Appendix.

Based on our work with NIST, on feedback received from labs such as NREL, the National Corn to Ethanol Research Center, and other technical experts in the ethanol industry, and on EPA's own modeling, we believe that reasonable accuracy should be demonstrated by validating that the results of a non-VCSB analytical method for calculating the cellulosic converted fraction are within 20 percent of the reported cellulosic value of a representative reference material.

First, the reference material and reported values should be representative of the mixture of starch and cellulose present in the feedstock. As explained above, based on work to date, EPA does not believe that the use of a reference material that only reports known values for starch (or for starch and other non-cellulosic components) can provide a reasonably accurate estimate of cellulose conversion when starch and cellulose are processed simultaneously. In our technical judgment, a reference material that is representative of the feedstocks actually being processed is necessary to accurately determine the cellulosic converted fraction, which is in turn needed to accurately apportion RINs. Second, while EPA evaluates the accuracy of results reported in registration applications on a case-by-case basis, as a general matter we currently would consider results within 20 percent of the known value for the cellulosic component of the representative reference material to be reasonably accurate. Such a benchmark is a corollary to assessing accuracy relative to reference material; once a "true value" has been established, a benchmark defines the universe of results around that value that should be deemed "reasonably accurate." Additionally, our experience reviewing results to date has indicated that a benchmark will be a useful tool and provide clarity to facilities aiming to achieve reasonably accurate results. In this instance, EPA's reporting regulations provide that deviations of up to 10 percent from a previously calculated cellulosic converted fraction are acceptable, i.e., that the additional reporting requirement is not triggered if deviation from the previous converted fraction is less than 10 percent. 14 While this benchmark may be a reasonable value to adopt in the context of calculating the cellulosic converted fraction, it is also reasonable to allow for some operational flexibility and/or natural variability that may be outside the control of the party. Therefore, given our current understanding we believe that reasonable accuracy is generally achieved for the purposes of 40 CFR 80.1450(b)(1)(xiii)(B)(3) when values are within 20 percent of the reported cellulosic component of representative reference material.

In summary, EPA believes that a representative reference material that contains and reports values for *both* starch and cellulose should be used to ensure that a non-VCSB test method is producing reasonably accurate results under the particular circumstances presented by co-processing starch and cellulose in the presence of resistant and retrograde starch. If the peer reviewed references demonstrate that a party using a non-VCSB analytical method has satisfied both criteria as outlined above, the application should be acceptable for registration under the pathway in the RFS program, assuming all other registration requirements are met.

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¹⁴ See 40 CFR 80.1451(b)(1)(ii)(U).

VCSB Methods for Determining the Cellulosic Converted Fraction

The preceding discussion addresses the use of a representative reference material to validate non-VCSB analytical methods under the second option in 40 CFR 80.1450(b)(1)(xiii)(B)(3). The same considerations regarding the use of a non-VCSB method under this second option are also relevant for purposes of the first option—obtaining the data used to calculate the cellulosic converted fraction using a VCSB-certified analytical method. This section addresses EPA's assessment of the current 15 ASTM effort to establish a VCSB method.

For the reasons laid out above—the need to accurately apportion RINs to the cellulosic and non-cellulosic components of the finished fuel consistent with RIN generation requirements and to uphold public trust in the integrity of the program—any VCSB analytical method should yield accurate calculations of the cellulosic converted fraction. In general, EPA believes that the collaborative development process and adoption of a VCSB-certified analytical method signifies that the method will produce results that are agreed to be sufficiently accurate to achieve the intended regulatory purpose. ¹⁶ However, it is EPA's understanding that the method currently being considered under the ASTM process—development of performance standards for validating starch conversion—is intended to serve as a VCSB method for calculating cellulosic conversion. Given our concerns as articulated in the previous section, EPA does not believe that such a method, based on direct measurements of non-cellulosic components only, can produce accurate results for cellulose, even if it has been deemed a "VCSB" method. Further, EPA may have concerns if the method was to provide that results within two standard deviations of the value reported for the reference material are acceptable and the NIST process yielded a product with a high variability (e.g., a reference material with a relative standard deviation of 30 percent), as there is the possibility of an absurd result being deemed an acceptable estimate of cellulosic conversion. EPA would not consider such an outcome to be sufficiently accurate to inform RIN allocation and thus does not believe that use of a method such as the one currently under consideration in the ASTM process would be consistent with the regulatory scheme.

Therefore, given EPA's current understanding of the potential accuracy of starch-based methods for calculating cellulosic conversion, if the current ASTM effort or another VCSB process results in certification of a starch-based/starch-only method intended for use under 40 CFR 80.1450(b)(1)(xiii)(B)(3), we recommend that parties relying on that method also include in their registration applications a demonstration that it produces reasonably accurate results according to the criteria in the previous section. EPA will continue to monitor the state of the evolving science in this field and will revisit this guidance as needed and update stakeholders as appropriate.

¹⁵ As of April 2019

¹⁶ EPA interprets the two options in 40 CFR 80.1450(b)(1)(xiii)(B)(3) as functional equivalents—two different ways of ensuring results are of sufficient accuracy to inform RIN allocation. Therefore, in the context of this regulatory provision, EPA believes a VCSB method should provide a reasonable degree of confidence in the results.

Summary of EPA's Guidance for Demonstrating Reasonable Accuracy

Peer reviews submitted with registration requests for adding the cellulosic pathway for co-processing corn kernel fiber and starch that rely on a non-VCSB analytical method to determine the cellulosic converted fraction must not only assess whether it is theoretically possible for the chosen method to measure cellulose accurately, but must also review the data to ensure that the method has actually provided reasonably accurate results. EPA recommends that peer reviews make this demonstration by documenting that the method returns cellulosic values for a representative reference material within 20 percent of the mean value reported for that material by an independent body such as NIST. If the references demonstrate that a party using a non-VCSB analytical method has satisfied the criteria as outlined above in this guidance, the registration application should be approvable, assuming all other registration requirements are met.

Appendix: EPA's Monte Carlo Evaluation of Starch-only Reference Materials for Cellulosic Ethanol from Corn Kernel Fiber

Executive Summary:

EPA conducted a Monte Carlo simulation to evaluate whether it would be reasonable for labs to use starch only values from representative reference materials currently under development by NIST to evaluate method accuracy for determining the cellulosic converted fraction. The Monte Carlo simulations showed that the error associated with starch measurements is large enough to produce expected cellulosic conversion results that could not be deemed to be reasonably accurate. Hence, EPA believes that demonstrating reasonable accuracy of cellulosic measurements will likely require a representative reference standard with a cellulosic value.

Background:

In 2014, EPA finalized regulations that added corn kernel fiber as an approved RFS pathway for producing cellulosic biofuel, provided that parties developed testing methods that could estimate the portion of the fuel derived from corn kernel fiber with "reasonable accuracy." However, over the last several years EPA has observed data showing very high variability in results reported for various facilities for the cellulosic converted fraction, where those results were obtained using non-VCSB analytical methods. EPA was also approached by technical experts in the space regarding concerns with starch assays, which are the basis of these non-VCSB methods. Based upon these observations, EPA approached NIST in August of 2017 to encourage NIST to develop representative reference materials to provide both starch and cellulosic values and which EPA could rely on to evaluate non-VCSB analytical methods. It is EPA's understanding that NIST has sent out materials for the first phase of reference material development – interlaboratory study and requested laboratories to report both starch and cellulosic values. However, it is also EPA's understanding that industry has expressed a desire to move forward based on the starch values first under "phase 1" and then cellulosic values for "phase 2."

In order to inform an assessment of whether current efforts underway with NIST, NREL and the ASTM workgroup can achieve reasonably accurate calculations of the cellulosic converted fraction, EPA applied a Monte Carlo simulation based on some preliminary data and feedback from industry. This analysis has enabled us to model simulations that present uncertainty and play them out in replication for validation. The remainder of this document discusses EPA's Monte Carlo assumptions, results and our preliminary conclusions from this analysis.

¹⁷ 40 CFR 80.1450(b)(1)(xiii)(B)(3) provides that data used to calculate the cellulosic converted fraction must be obtained using either a method certified by a voluntary consensus standards body (VCSB), or a method that would produce reasonably accurate results as demonstrated through peer reviewed references. As there is not currently a VCSB-certified method, efforts to date have focused on the second option.

EPA's Monte Carlo Simulations

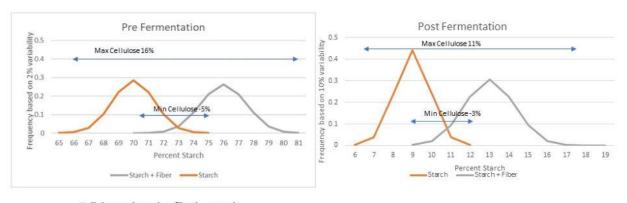
Monte Carlo simulations look at the probability of an outcome described by a normal distribution curve using a random number generator based on the mean and standard deviation of that distribution. The simulations let us evaluate all the possible outcomes for cellulosic conversion based on the normal distribution of measurements required as inputs to the calculation. Being able to evaluate a number of simulations allows for better decision making in evaluating whether analytical methods are robust enough for measuring cellulosic conversion.

A simplistic model of cellulose conversion that can be evaluated via Monte Carlo simulation is thus: Cellulose Converted = Cellulose Feedstock pre-fermentation – Cellulose in DDG post fermentation. We also need to make a few assumptions for our Monte Carlo scenarios as there is no direct measurement for cellulose. Cellulose, pre and post-fermentation, is derived from two or more measurements of other components in the corn kernel fiber. The first assumption for the Monte Carlo simulations is that the cellulosic determination is simplified to two measurements, one being the starch assay evaluated by the candidate reference material. Thus, the cellulosic component of the feedstock pre-fermentation is defined as some larger "measurement one" (M1) minus the initial starch content of the feedstock pre-fermentation (M_{starch pre}). The cellulosic content of the feedstock post-fermentation is similarly simplified as "measurement three" (M3) minus the residual starch content of the feedstock post-fermentation $(M_{\text{starch post}})$. Thus, our overall equation being modeled is CCF= $(M_1-M_{\text{starch pre}})-(M_3-M_{\text{starch post}})$. The second set of assumptions needed to perform the assessment of whether starch measurements are capable of accurately capturing the cellulosic converted fraction involves the level of starch contained in the pre and post-fermented corn and the variability associated with these starch measurements. EPA is basing this part of the analysis on preliminary data captured from calls with stakeholders working on the NIST reference materials. The Monte Carlo simulations thus assume an initial starch measurement of 70% starch by mass with a relative standard deviation of two percent. The post fermentation starch measurement used in these simulations was 9% by mass with a ten percent relative standard deviation. Lastly, we need to make a final assumption with regard to the cellulosic converted fraction to complete our analysis. Most parties that have approached the agency regarding this technology have claimed that corn naturally contains around 6% by mass of cellulose and that they are able to achieve a cellulosic converted fraction of one to three percent mass percent. In this analysis, EPA is assuming that there is 6% by mass fiber in the feedstock and that 2% by mass of the cellulosic material is converted into ethanol along with the starch that is fermented. With the assumptions outlined above, we can simplify our equation to CCF= $((S_{pre}+6)-S_{pre}))-((S_{post}+4)-S_{post})$ where "S" represents starch measurements pre and post-fermentation. Using the measurement data denoted above, this equation representing a theoretical 2% cellulosic converted fraction is thus; CCF= (76% + -2% - 70% + -2%) - (13% + -10% - 9% + -10%).

The scenario outlined above is an optimistic analysis that does not consider compounded variability from sampling and handling heterogenous material from large vessels at the facility nor more than two analytical steps required to derive the cellulosic converted fraction.

Nonetheless, we want to examine whether the variability in the starch measurements would preclude an accurate calculation of the cellulosic converted fraction when the only comparison available is to a known starch value. The Monte Carlo simulations will take a random value for each of the four measurements required to determine cellulose based on the starch performance data. We chose to run these Monte Carlo simulations in Microsoft Excel and the formula for the scenario presented above is: CCF = =((NORMINV(RAND(),76,1.52)-NORMINV(RAND(),70,1.4))-(NORMINV(RAND(),13,1.3)-NORMINV(RAND(),9,0.9))).

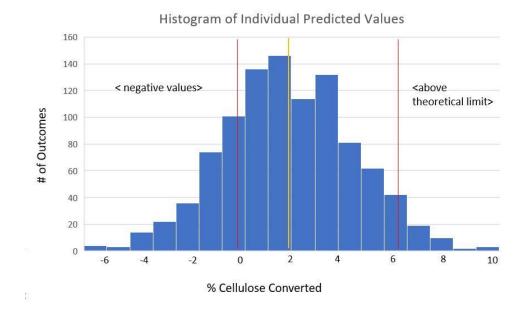
The normal distribution for pre and post-fermented starch measurements can be depicted graphically using the mean and standard deviations provided above. Using the expected value at three standard deviations allows one to predict a maximum and minimum cellulosic value for both pre and post fermentation.



Cellulose = (starch + fiber) – starch
Cellulosic conversion = Cellulose Pre Fermentation – Cellulose Post Fermentation

From the figure above, there are expected values for cellulose that would both exceed the theoretical value of cellulose in the feedstock and which are negative. These values indicate that fiber was not consumed in the process, but created; these results are nonsensical but theoretically possible due to the variability of the measurements. The figure above represents the assumptions that were input into the Monte Carlo simulations.

The results of the Monte Carlo simulations can be presented as a histogram of the expected cellulosic converted fraction values that result from the equation presented above. The following histogram represents individual results from 1000 simulations.



As can be viewed from the histogram results of the compiled Monte carlo simulations, a large proportion of absurd results (i.e. both negative and very high cellulosic conversions) are expected. The key findings from the Monte Carlo simulations include a high frequency (25-30%) of absurd results and a large number of negative cellulosic conversions (~20%). These expected outcomes are due to determining a small difference (i.e. the cellulose converted) from a large value (i.e., measurement of starch mass) with associated measurement error.

One option for dealing with measurement variability would be to employ composite sampling to produce a result closer to the population mean. Therefore, EPA expanded the Monte Carlo simulations to look at various numbers of samples composited into ten discrete sampling events.

	Sampling Events												Range	e	
# samples	1	2	3	4	5	6	7	8	9	10 me	an	stdev	min	ma	ex
10	2.26	1.65	3.35	2.37	3.03	3.04	2.41	1.83	2.09	1.25	2.33	0.67	1 1	1.25	3.35
20	1.87	2.40	2.96	2.30	2.62	3.13	3.09	1.60	1.68	1.49	2.31	0.63	1	1.49	3.13
50	1.55	1.74	2.15	2.10	2.31	2.65	2.30	2.33	2.35	2.24	2.17	0.32		1.55	2.65
100	1.63	1.88	2.43	1.78	2.35	2.43	2.10	2.26	2.31	1.97	2.11	0.29	1	1.63	2.43

However, the number of samples that are likely to be required to achieve reasonable accuracy (within +/- 20% of the accepted value) appears to be unrealistic for industry to execute (e.g. between 50 and 100 samples per event). Therefore, EPA believes that to show reasonable accuracy of cellulosic measurements, the path forward with corn kernel fiber will likely require a representative reference standard with a cellulosic value.