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

EPA United States Environmental Protection Agency

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Compliance Division
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Introduction

This document provides updated guidance on qualifying an analytical method for determining the cellulosic converted fraction of corn kernel fiber co-processed with starch in order to demonstrate compliance with the regulatory requirements in 40 CFR 80.1450(b)(1)(xiii)(B)(3).[1] 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 sought clarification from the EPA regarding what methods should be used to determine the cellulosic converted fraction of corn kernel fiber in the production of cellulosic ethanol. EPA first issued guidance on this topic in May 2019.[2] As the state of science has continued to evolve in this area, we are now updating our previous guidance. This document supplements the May 2019 guidance on qualifying an analytical method for determining the cellulosic converted fraction of corn kernel fiber co-processed with starch.

The intent of this guidance is to explain our interpretation of the regulatory requirements and to articulate clear criteria for the types of analysis and demonstrations that EPA believes can be accepted for registration under the program. This updated guidance reflects recent work conducted by the Department of Energy's (DOE) National Renewable Energy Laboratory (NREL) [3,4] to develop a public method which addresses the analytical concerns identified in the 2019 guidance and includes consideration of recently released reference materials by the National Institute of Standards and Technology (NIST).[5,6]

Based on the availability of the NREL method for determining the cellulosic converted fraction of corn kernel fiber co-processed with starch at a traditional ethanol facility and the

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[1] Requiring renewable fuel producers seeking to generate D-code 3 or 7 RINs who intend to produce a single type of fuel using two or more feedstocks converted simultaneously, where at least one of the feedstocks does not have a minimum 75% average adjusted cellulosic content to provide, *inter alia*, "[c]hemical analysis data supporting the calculated cellulosic Converted Fraction 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, 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."

[2] Environmental Protection Agency Guidance on Qualifying an Analytical Method for Determining the Cellulosic Converted Fraction of Corn Kernel Fiber Co-Processed with Starch, EPA-420-B-19-022. May 2019.

[3] Michel, Katie, Justin Sluiter, Courtney Payne, Ryan Ness, Brittany Thornton, Michelle Reed, Alexa Schwartz, and Ed Wolfrum. 2021. Determination of Cellulosic Glucan Content in Starch Containing Feedstocks. Laboratory Analytical Procedure (LAP), Issue Date: February 26, 2021. Golden, CO: National Renewable Energy Laboratory. NREL/TP-2800-76724. https://www.nrel.gov/docs/fy21osti/76724.pdf.

[4] Michel, Katie, Justin Sluiter, Courtney Payne, Ryan Ness, Brittany Thornton, Michelle Reed, Alexa Schwartz, and Ed Wolfrum (2021) Direct Determination of Cellulosic Glucan Content in Starch Containing Samples. Cellulose 28:1989-2002. https://doi.org/10.1007/s10570-020-03652-2.

[5] National Institute of Standards and Technology Reference Material 8644. Dried Corn Biomass Intermediate Before Conversion. June 23, 2021.

[6] National Institute of Standards and Technology Reference Material 8645. Dried Corn Biomass Intermediate After Conversion. June 23, 2021.

availability of NIST reference materials, this guidance describes three potential ways companies might satisfy the registration requirements:

1. Adoption of the DOE/NREL method,
2. Demonstration of reasonable accuracy by returning comparable cellulose values to the NIST reference materials using a non-voluntary consensus standards body (non-VCSB) method, or
3. Use of advanced analytical techniques; e.g. mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR), as identified by DOE/NREL in their analytical method and validation.

Our rationale for accepting NREL's method, as well as for the additional options based on NREL's method, for renewable fuel production facilities to use in demonstrating that they can measure cellulose with reasonable accuracy are discussed in this updated document.

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 elaborates upon EPA's current view on the manner in which facilities can demonstrate the accuracy of such analytical methods and thus satisfy the applicable registration requirements. However, it is not an exclusive list of ways in which facilities may satisfy those requirements and 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) background and overview of the 2019 guidance; (3) updates in the current guidance based on new science; (4) VCSB methods; and (5) a summary of EPA's current guidance.

Background and Explanation of Regulations

EPA's RIN generation regulations at 40 CFR 80.1426(f)(3)(vi) contain equations for assigning RINs to renewable fuel produced using two or more feedstocks processed simultaneously, including situations in which only one of the feedstocks is cellulosic. The formulas in this provision for assigning RINs to the different components of the finished fuel incorporate the value for the converted fraction of the feedstock which, for cellulosic feedstocks, is the cellulosic converted fraction. Thus, accurate calculations of cellulosic conversion are necessary to apportion RINs correctly per the co-processing and RIN generation requirements under 40 CFR 80.1426(f)(3)(vi).[7]

[7] "If a producer produces a single type of renewable fuel using two or more different feedstocks which are processed simultaneously, and each batch is comprised of a single type of fuel, then the number of gallon-RINs that shall be generated for a batch of renewable fuel and assigned a particular D code shall be determined according to the formulas in Table 4 to this section."

In the 2014 Pathways II Final Rule under the RFS program,[8] EPA added a pathway for the production of cellulosic ethanol from corn kernel fiber [9] 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. 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.[10] Therefore, the regulations included requirements for registration, reporting, and recordkeeping related to calculating the cellulosic converted fraction designed 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 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 converted fraction 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.[11] 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.

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[8] 79 Federal Register 42128 (July 18, 2014).

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

[10] *See* Id. at 42134.

[11] *See* id. at 42135.

Background and Overview of 2019 Guidance

At the time of the release of the 2019 guidance, there existed no publicly available analytical method to accurately measure cellulosic content in corn kernel fiber feedstock, nor did there exist available pre and post-processed corn kernel fiber reference materials with known baseline values for starch or cellulosic content. There was also not a VCSB analytical method for determining cellulosic content in instances of simultaneous conversion of starch and cellulose. Therefore, registrants and potential registrants relied on proprietary measurement methods to determine cellulosic content. Many of these methods did not measures cellulose content or conversion directly, but rather measured the starch content and then calculated the cellulose content via mass-balance subtraction.

However, in the 2019 guidance, EPA articulated that we did not believe it to be 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. We arrived at this conclusion based on numerous discussions with peer reviewers of proprietary methodologies and other outside parties that expressed concerns that resistant and retrograde starch present in samples due to feedstock handling or processing, as well as yeast contributions, could impact laboratory results and cellulosic calculations. That is, experts expressed that 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.

Based on this information and the then-current state of the science, EPA recommended in the 2019 guidance that parties seeking to satisfy the requirements of 40 CFR 80.1450(b)(1)(xiii)(B)(3) validate 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. As all producers were, at that time, utilizing proprietary methods, we stated that parties must provide peer reviewed references that evaluate a producer's analytical method and demonstrate to EPA that not only is the method capable of producing accurate results, but that its application has, in fact, yielded a calculation of the cellulosic converted fraction that is reasonably accurate. With the latest scientific advances including feedback from industry, EPA has updated the guidance as described below.

Updates in the Current Guidance Based on Scientific Advancements

This updated guidance integrates the latest scientific advancements on the measurement of corn kernel starch and fiber and presents three additional options for parties to demonstrate that the data they use to calculate the cellulosic converted fraction are obtained using an analytical method that should produce reasonably accurate results as demonstrated through peer reviewed references. This section provides a detailed explanation of how a producer of renewable fuel could demonstrate that an analytical method will produce reasonably accurate results. The three options presented here

are designed to provide producers additional flexibility in how they meet the regulatory requirements, which we believe is warranted based on the latest scientific advancements. For parties that do not wish to utilize one of these three additionally methods, the approach and recommendations in EPA's 2019 guidance remain valid as well.

As explained above, technical experts and EPA had identified resistant and retrograde starch remaining in samples as a source of uncertainty when attempting to measure cellulose. The analytical method that NREL published in January 2021 overcomes these starch quantification concerns by destroying all starch in the sample prior to quantification of cellulose. NREL applied advanced analytical techniques such as liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance spectroscopy (NMR) to validate the results of this approach, i.e., to validate that all the starch in samples had been destroyed, leaving behind only cellulose. EPA has evaluated NREL's methodology and believes that, if applied by renewable fuel producers as NREL intended, it will result in reasonably accurate cellulose measurements. Additionally, we also believe that other interested parties could apply the same advanced analytical techniques that NREL used to validate that all the starch in samples was destroyed in order to validate that their own methodologies accurately measure cellulose. We anticipate this approach will be appropriate if parties' analytical methods are conducted in a stepwise manner similar to NREL's approach, i.e., if the method is designed to remove starch (and other components) from samples prior to measuring cellulose.

Additionally, 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. The intent was for such reference material to establish a "true value" of cellulosic content against which to evaluate the results of analytical methods, which would allow peer reviewers and the Agency to determine whether those methods could produce reasonably accurate results for the cellulose measurements.[12]

In June 2021, NIST made publicly available a common reference material based on dried corn biomass; NIST also noted a non-certified starch content value for this reference material. This reference material may be used to determine the amount of starch present in feedstock and post conversion products, in support of accurate calculations of total cellulosic content.[13,14] While NIST did not provide a value for the cellulosic content of its reference material, NREL determined a cellulose value for the NIST reference material using its publicly available analytical method. Given that we believe NREL's method produces reasonably accurate results for cellulose measurements, we also believe it is reasonable for renewable fuel producers to use NREL's cellulose value for the NIST

[12] 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 evaluate the ability of particular analytical methodology to determine results that approach the true value.
[13] National Institute of Standards and Technology Reference Material 8645. Dried Corn Biomass Intermediate After Conversion. June 23, 2021.
[14] National Institute of Standards and Technology Reference Material 8644. Dried Corn Biomass Intermediate Before Conversion. June 23, 2021.

materials as a benchmark for comparison against alternative methods.[15] As the science progresses, a reference material with certified starch, cellulose, hemicellulose and lignin or total "cellulosic" values would be ideal to utilize in support of method validation, but this is not yet available.

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 previously determined that resistant and retrograde starch in samples meant that measurements of cellulose that were derived from measurements of starch could not be reasonably accurate. We therefore provided 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[16] of the reported cellulosic value of a representative reference material. Now, given NREL's work and public method, we are identifying three additional options for renewable fuel producers to demonstrate that their analytical methods result in cellulose measurements that are reasonably accurate. We have identified these additional options based on our determination that the NREL method satisfies the two key aspects of 40 CFR 80.1450(b)(1)(xiii)(B)(3): that the data used to calculate the cellulosic converted fraction must be obtained using an analytical method that would produce reasonably accurate results and that reasonable accuracy must be demonstrated through peer reviewed references. The three new options are: (1) facilities may adopt NREL's public method; (2) facilities can use NIST reference materials in their proprietary alternate methods to show EPA that the methods are capable of producing cellulosic results that are comparable to the cellulose values NREL assigned to the materials in the publication validating their public method; or (3) facilities attempting to register with other analytical methods can apply similar advanced analytical techniques as applied by NREL (e.g. NMR and mass spectrometry) to validate their methods and address EPA's concerns that starch is impacting the cellulosic measurements.

The information and analysis contained in the publication supporting NREL's analytical method indicates that the method is successful at destroying resistant and retrograde starch in samples such that the material remaining in the sample is cellulosic. Additionally, NREL's analysis and results indicate that cellulose is not lost in the process of destroying starch in the sample. As such, we believe it is reasonable to conclude that NREL's method is measuring cellulosic content with reasonable accuracy. Thus, renewable fuel producers properly applying NREL's method should also be able to obtain reasonably accurate measurements. Similarly, renewable fuel producers that apply similar advanced analytical techniques may be able to demonstrate that their analytical methods also remove all resistant and retrograde starch and retain cellulose in samples, which would allow them to demonstrate that those methods produce reasonably accurate cellulose measurements.

In the 2019 guidance, EPA explained that the reasonable accuracy of analytical methods should be demonstrated by validating their results against the reported cellulosic value of a representative reference material. While we believed, at that time, that an accepted consensus value for cellulose could be developed for the NIST materials, there is not currently such a value.

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[15] Michel, Katie, Justin Sluiter, Courtney Payne, Ryan Ness, Brittany Thornton, Michelle Reed, Alexa Schwartz, and Ed Wolfrum (2021) Direct Determination of Cellulosic Glucan Content in Starch Containing Samples. Cellulose 28:1989-2002. https://doi.org/10.1007/s10570-020-03652-2.

[16] The 20 percent covers approximately 3 standard deviations from the mean determined value for cellulose content based on the DOE/NREL analysis of the NIST reference material.

However, NREL has produced a cellulosic value for the NIST materials and, as described above, we believe the NREL method results in reasonably accurate cellulose measurements. Therefore, we are providing that renewable fuel producers may validate that their proprietary analytical methods produce reasonably accurate cellulose measurements by using such methods to measure the cellulose content of NIST materials and demonstrating that their measurements are comparable to NREL's value. We believe that a value could be deemed reasonably comparable to the NREL determined cellulose value for the NIST material, if the value was within 20 percent of the NREL ascribed value. This range covers approximately three standard deviations of the variance reported by NREL on their cellulosic measurement and therefore encompasses 95% of comparable values.

Renewable fuel producers must demonstrate that their analytical methods produce reasonably accurate results through peer reviewed references. In the 2019 guidance, EPA explained that we interpret 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 analytical method must be demonstrated to produce reasonably accurate results. NREL's development and publication of its public analytical method in a peer reviewed scientific journal, coupled with validation via advanced analytical techniques (e.g. NMR and mass spectrometry) satisfies this requirement.

Based on our experience evaluating NREL's publication of its public analytical method in a scientific journal, EPA believes that the reasonable accuracy of an analytical method may generally be demonstrated by the fact that it is peer reviewed and published in a scientific journal, provided that certain criteria are met. This interpretation is based on scientific rigor being upheld in the publication of a technical or academic journal article; in contrast, where such publication may be in a trade press, the Agency may find that necessary rigor to meet our peer review interpretation is insufficient. To ensure the peer review is meaningful, EPA believes the experts utilized in the peer review process should, (1) possess the necessary qualifications and expertise to serve as a peer reviewer on the topic, (2) be familiar with current progress and developed scientific methods on the topic and (3) satisfactorily review, in depth, all aspects of the proposed method and supporting analytics and calculations against the current science to guarantee accuracy of results.

EPA believes the facility registration step, where analytical results are combined with process data to yield cellulosic converted fraction data, is also important for demonstrating that the method can be applied in the field. 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. As part of facility registration, the professional engineer performing the facility inspection must also review the data and any analytical methods to ensure that the method can be applied in the field with reasonable precision (i.e. coefficient of variation less than 20% for reported cellulosic values).

In summary, EPA continues to believe that a representative reference material that contains and reports values for *both* starch and cellulosic constituents should ideally be used to ensure that a non-VCSB test method is producing reasonably accurate cellulose measurements. However, based on our evaluation of NREL's analytical method and NREL's provision of a cellulosic value for the

NIST materials, we now believe there are additional avenues for renewable fuel producers to demonstrate they have satisfied the regulatory requirements of 40 CFR 80.1450(b)(1)(xiii)(B)(3).

VCSB Methods for Determining the Cellulosic Converted Fraction

The preceding discussion addresses the use of NREL's public method or a representative reference material to validate non-VCSB analytical methods under the second part of 40 CFR 80.1450(b)(1)(xiii)(B)(3).[17] This section addresses EPA's assessment of ASTM method E3181-20 released in March 2020. [18] In brief, EPA does not consider ASTM method E3181-20 to be an analytic method that a party can use to determine cellulosic content, but rather, a practice or set of criteria that parties can use to evaluate the sampling procedures and analytic testing methods they may develop for this purpose.

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 including accurate representations of cellulose, hemicellulose and lignin excluding starch contributions. 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. ASTM standard practice E3181-20 is intended provide direction for statistical sample analysis, confidence and variance and for calculating starch, and subsequently cellulosic conversion via mass balance using any analytic test method (VSCB or otherwise). Given our concerns as articulated in the 2019 Guidance document, EPA does not believe that an analytic test method which calculates cellulosic content based on mass balance and direct measurements of non-cellulosic components only, primarily starch, without supporting analytical test assessments to address confounding measurements from resistant and retrograde starch, can produce accurate results for cellulose. In addition, EPA is concerned that the variability of the starch measurements is likely to overwhelm the percent mass of cellulose that is converted as starch measurement variability is propagated to the cellulose calculation.[19] ASTM E3181-20 notes this concern as well in section 5.1.3. While standard practice ASTM E3181-20 is not a VCSB analytic test method, it does provide additional guidance on determinations of precision and accuracy in evaluating analytic test methods and in executing an overall calculation procedure including sampling and statistical analysis.

EPA is not aware of other released VCSB methods at this time for cellulosic content determination. 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.

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[17] "...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."

[18] ASTM Method E3181-20 Standard Practice for Determination of the Converted Fraction of Starch and Cellulosic Content from a Fuel Ethanol Production Facility. August 2020.

[19] Environmental Protection Agency Guidance on Qualifying an Analytical Method for Determining the Cellulosic Converted Fraction of Corn Kernel Fiber Co-Processed with Starch, EPA-420-B-19-022. May 2019.

Summary of EPA's Guidance

In summary, based on the evolving state of the science, and since EPA believes that the NREL method overcomes the starch issues identified by concerned stakeholders, this guidance describes three additional options that renewable fuel producers may use to satisfy the regulatory requirements at 40 CFR 80.1450(b)(1)(xiii)(B)(3). To demonstrate reasonable accuracy in the calculation of the cellulosic converted fraction, applicants may, 1) Adopt the NREL method (and must return similar results to the NIST reference material; i.e. within three standard deviations as reported by NREL for their cellulose value); 2) Demonstrate reasonable accuracy by returning comparable cellulose values to the NIST reference materials using a non-VCSB method; i.e. within three standard deviations as reported by NREL for their cellulosic value of the NIST reference material; 3) Lastly, stakeholders may apply the same advanced analytical techniques; e.g. LC-MS and NMR, as identified by DOE/NREL in their analytical method and validation, to demonstrate that retrograde or resistant starch has not confounded the results. If the applicant demonstrates that the use of a non-VCSB analytical method is consistent with the description in this guidance, which is based on the current state of scientific developments to date, EPA anticipates that it can approve the registration application, following a case specific review, assuming all other registration requirements are met.