FDA U.S. FOOD & DRUG ADMINISTRATION

Technical Project Lead (TPL) Review of PMTAs (Toxicology)

Products Subject to this Review[1]	
Submission tracking numbers (STNs)	PM0000864, PM0000872, PM0000874, PM0000876, PM0000878, PM0000879
Common Attributes	
Submission date	July 29, 2020
Receipt date	July 29, 2020
Applicant	JUUL Labs Inc.
Product manufacturer	JUUL Labs Inc.
Application type	Standard
Product category	ENDS (VAPES)
Product subcategory[2]	Closed E-Liquid, Closed E-Cigarette
Cross-Referenced Submissions	
All STNs	MF0000243, MF0000276, MF0000425
PM0000864, PM0000872, PM0000874, PM0000876	MF0000363, MF0000402
Recommendation	
Issue marketing denial order letters for PM0000864, PM0000872, PM0000874, PM0000876, PM0000878, PM0000879	

Technical Project Lead (TPL):

Digitally signed by Kimberly R. Lindsey -S
Date: 2022.06.23 07:56:33 -04'00'

Kimberly Lindsey, MD, MA, DABS
Director, Division of Individual Health Science
Offfice of Science

Signatory Decision: Concur with TPL recommendation and basis of recommendation

Digitally signed by Matthew R. Holman -S
Date: 2022.06.23 08:11:31 -04'00'

Matthew R. Holman, Ph.D.
Director
Office of Science

[1] Tobacco product details, amendments, and dates provided in the Appendix. PMTA means premarket tobacco application.
[2] FDA referred to these products as ENDS Components during the course of review to facilitate processing. However, at the close of review the accurate category and subcategory are reflected in the TPL review and supersede those listed in primary discipline reviews.

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1. EXECUTIVE SUMMARY

Based on the information provided in these applications, as described in this Technical Project Lead review, I find that the applicant has not demonstrated that permitting the marketing of the new products in the premarket tobacco product applications (PMTAs) listed above ("new products" or "subject ENDS") would be appropriate for the protection of the public health (APPH). Accordingly, I recommend that marketing denial orders be issued for the new products.

Section 910 of the FD&C Act provides that FDA "shall deny an application" for a product to receive a PMTA marketing authorization if, "upon the basis of the information submitted to the Secretary as part of the application and any other information before the Secretary with respect to such tobacco product," FDA finds that "there is a lack of a showing that permitting such tobacco product to be marketed would be appropriate for the protection of the public health." Section 910(c)(2)(A). In assessing APPH, the statute requires FDA to consider the risks and benefits to the population as a whole, including both tobacco users and nonusers, and taking into account the increased or decreased likelihood that existing users of tobacco products will stop using such products and the increased or decreased likelihood that those who do not use tobacco products will start using such products. Section 910(c)(4).

As the statutory text makes clear, it is the applicant's burden to make a "showing"—with sufficient supporting information—that permitting the marketing of a new tobacco product would have a net benefit to public health based upon the risks and benefits to the population as a whole. Before determining that permitting the marketing of a new tobacco product would result in a net benefit to public health, FDA must weigh all potential public health benefits against all potential public health harms—including but not limited to the likelihood that the product will affect patterns of initiation and cessation of tobacco use. Concluding that there is a net benefit takes into account whether the applicant has provided sufficient information regarding product design, chemistry, stability, manufacturing controls including process controls and quality assurance procedures, toxicology, abuse liability, and other factors that can affect the product's risks to individual users and nonusers, including relative to those of other tobacco products. In June 2019, we issued a Guidance for Industry, Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems. Although not binding on FDA or the public, and an applicant can use an alternative approach if it satisfies the applicable legal requirements, the guidance gave FDA's current thinking on these applications. The guidance recommended "providing a full assessment of the toxicological and pharmacological profile associated with the new tobacco product." Guidance at 34. It noted the importance of providing, among other things: "[t]oxicological endpoints such as cytotoxicity, genotoxicity, carcinogenicity, and respiratory, cardiac, reproductive, and developmental toxicity"; "[i]nformation concerning substances that may be solvent extractable from the container closure system or leachable into the e-liquid when the e-liquid is in contact with the container closure system (e.g., information on whether toxic substances present in the container closure system can potentially transfer into the e-liquid or aerosol)"; "a conclusion as to whether there is a toxicological concern with respect to the ingredients, constituents… that will be delivered in the aerosol from the use." Guidance at 35. It noted that "where a thorough literature review does not address these points, these topics may need to be addressed in separate studies conducted by the applicant." Guidance at 35. Finally, the guidance recommended providing "an integrated summary discussing how permitting the marketing of the new tobacco product would be APPH from a toxicology perspective relative to any similar comparator tobacco products." Guidance at 37.

Without sufficient evidence of a product's toxicity risks, including in relation to the risks posed by other tobacco products, FDA cannot conduct a full evaluation of the overall risks and benefits of a new tobacco product. For example, FDA cannot adequately evaluate whether and to what extent relevant tobacco use behaviors would represent a public health benefit or a public health harm. Switching from one tobacco product to another tobacco product is not inherently beneficial or harmful. To illustrate, if a new tobacco product presents a greater level of risk than combustible cigarettes, switching from combustible cigarettes to the new product would represent a health harm to those who switch, not a health benefit. Only if FDA is able to conclude that a new tobacco product presents lower risk than combustible cigarettes can FDA consider that switching from combustible cigarettes to the new tobacco product represents a health benefit to those who switch.

In addition, FDA considers the risks of the new tobacco product in relation to other similar tobacco products (e.g., products in the same product category). For example, if an ENDS product poses lower risk than combustible cigarettes, but higher risk than other ENDS, the potential public health benefit from cigarette smokers switching could be offset by increased harm to users who choose the new tobacco product over less harmful ENDS products. These kinds of analyses, among other related considerations, would inform FDA's determination as to whether marketing of the product would have a net benefit to public health based upon the risks and benefits to the population as a whole. Other considerations could include how the product is actually used (e.g., nonuser initiation, dual use) and effects on nonusers (e.g., accidental or secondhand exposure).

As described in more detail in the deficiencies below, I found that the applicant did not provide sufficient information for me to assess the toxicological risks posed by the new products, and the information that was provided raised concerns. Among other deficiencies, the evidence provided was internally inconsistent; lacked key information; relied in significant part on methodological choices that, without adequate justification, ignored crucial indications of toxicity; and used a different methodology to assess the toxicity of the new products than it did for comparison products in key respects. As a result, I was unable to make any meaningful determinations regarding the risks of the new products relative to their benefits and compared to the risks of other tobacco products, including combustible cigarettes and other ENDS, which is necessary for me to make a determination as to likely net public health impact. Without such information, I am not able to adequately evaluate whether and to what extent relevant tobacco use behaviors (e.g., initiation with the new products, switching from combustible cigarettes to the new products, switching from other ENDS products, dual use of the new products with other tobacco products) would represent a public health benefit or a public health harm. Without the ability to make any meaningful determinations regarding the toxicological risks presented by the new products, the public health impact of the initiation, switching, and cessation data in the applications cannot be evaluated under the APPH standard, regardless of what those data otherwise show. In light of these uncertainties, it is possible that marketing of the new products would have a neutral impact, result in net public health benefit, or result in net public health harm. Because I do not have adequate information to fully evaluate the products' toxicological profile, and the evidence the applicant did submit raises substantial toxicity concerns, I cannot determine that these products have met the statutory standard. Therefore, the applicant has not met its burden of "showing" that permitting the marketing of the new products would be APPH as required by Section 910(c)(2)(A).

This review finds four deficiencies in these applications relating to all of the new products, i.e., the JUUL System:

Deficiency 1 relates to two genotoxic leachables identified as such by the applicant itself in its original PMTAs. These constituents were improperly identified and there is insufficient information to characterize their risks, i.e., the application lacks mainstream aerosol data, and an appropriate toxicological risk assessment for these constituents.

In the applicant's original submission for these PMTAs, the applicant identified the presence of two genotoxic leachables found to produce an excess cancer risk outside of generally accepted margins of "tolerable cancer risks." In response to FDA's Deficiency letter requesting additional information from the applicant, including mainstream aerosol yields of these leachables, the applicant revised the identity of these two genotoxic leachables. The information provided to support these revised identifications conflicts with the data the applicant previously submitted regarding the identification of these leachables, and raises questions about the true identity of the leachables and their toxicological impact. Furthermore, despite FDA's request (in its Deficiency letter) for mainstream aerosol data for these leachables, the applicant declined to provide this information.

Additionally, in response to FDA's Deficiency letter, the applicant evaluated these revised leachables using a new risk assessment (i.e., one that differed from the risk assessment submitted in the original PMTAs). The revised risk assessment presents concerns for two reasons. First, because the applicant has not established the identity of these revised leachables, the submitted risk assessment cannot be used to determine the toxicity of the new products. Second, there are significant methodological issues that preclude consideration of the findings in the revised risk assessment, including the use of a risk assessment method that does not have the specificity to evaluate the types of leachable chemicals typically studied in a leachables assessment (for example, polymers and heavy metals). As a result of all of the foregoing concerns, FDA was unable to perform an accurate and complete risk assessment of the new products.

Deficiency 2 concerns methodological issues impacting scientific validity of results provided in the applicant's in vitro micronucleus assay genotoxicity study comparing the genotoxic potential of the new products to other tobacco products. Specifically, the applicant unevenly applied acceptance criteria (including inconsistent cell counting) for the scoring and evaluation of positive and negative responses for the new products with the in vitro genotoxicity assays. The applicant-provided data identified instances in which the new products (PM0000872, PM0000874 and PM0000876 used with PM0000878 or PM0000879) induced positive or equivocal genotoxic responses. The positive genotoxic responses for the new products (using a cell count of 2000 cells) were then rejected by the applicant and the new products were further evaluated by counting an additional 2000 cells and rescoring the assay. The additional evaluation of positive responses runs counter to the assay guidelines as provided by the applicant, as these results indicated a "clearly positive" genotoxic response. Based on the assay guidelines as provided by the applicant, the positive genotoxicity responses identified for the new products should have been accepted and not subjected to an additional, more rigorous evaluation. Equivocal genotoxic responses may be further evaluated using expert judgment and/or further investigations (i.e., counting additional cells, if appropriate, or performing a repeat experiment that may have modified experimental conditions). The applicant evaluated equivocal responses for the new products by counting an additional 2000 cells "to clarify the response." The applicant did not provide a rationale or supporting statistical calculations to demonstrate why counting additional cells, in lieu of conducting a repeat or modified experiment, was appropriate and justified. The applicant did not count additional cells or further evaluate equivocal responses reported for the comparator products. These methodological issues impact the

scientific validity of the assay results provided by the applicant and preclude a full assessment of the new products' genotoxic potential.

Furthermore, the applicant evaluated the new and comparison products for genotoxic potential using different methods. For scientific validity, it is necessary that the new and comparison products are evaluated for genotoxic potential using a consistent methodology to ensure that accurate comparisons are made between the products. Specifically, the new products were evaluated using 2000 cells, or re-evaluated using 4000 cells if there was an equivocal or positive result. In contrast, all the comparison products were evaluated for genotoxic potential using a single assay at 4000 cells per concentration. Equivocal responses for the comparator products were not further evaluated, a method that differed from the method used for the JUUL System products without appropriate justification. In summary, the applicant's data showed unequal treatment of test articles, which impacted the scientific validity of the applicant's data, and precluded FDA from evaluating the genotoxicity of the JUUL System.

Deficiency 3 concerns findings from genotoxicity studies of PM0000872 (Menthol, 5% nicotine concentration), PM0000874 (Virginia Tobacco, 3% nicotine concentration) and PM0000876 (Virginia Tobacco, 5% nicotine concentration) showing the new products are likely to be genotoxic. As described above, data from the in vitro micronucleus assay showed positive genotoxic results for PM0000872 (Menthol, 5% nicotine concentration), PM0000874 (Virginia Tobacco 3%, nicotine concentration) and PM0000876 (Virginia Tobacco 5%, nicotine concentration) when these products were tested in accordance with applicant-provided assay guidelines, while the 3R4F combustible cigarette comparison product was scored equivocal or negative for genotoxicity. This indicates that PM0000872 (Menthol, 5% nicotine concentration), PM0000874 (Virginia Tobacco 3%, nicotine concentration) and PM0000876 (Virginia Tobacco 5%, nicotine concentration) may have increased genotoxic potential relative to the 3R4F combustible cigarette comparison product. The applicant rejected these valid assay results without justification, and conducted further assays using an additional 2000 cells per concentration. Based on this revised, albeit unjustified, methodology using 4000 cells per concentration, PM0000872 was found to be positive for genotoxicity, PM0000874 was found to be equivocal, and PM0000876 negative for genotoxicity. To address genotoxicity concerns for PM0000872 and PM0000874, the applicant conducted an in vivo toxicology study. The applicant did not attempt to address the initial positive genotoxicity result for PM0000876. Significantly, the in vivo study conducted for PM0000872 and PM0000874 was not bridged or otherwise extrapolated to the in vitro studies. Bridging is important to address inherent differences between in vitro and in vivo toxicological studies and show whether it is appropriate to supplant the in vitro findings with the in vivo findings. Further, an appropriate combustible cigarette comparison product was not included in the in vivo genotoxicity study conducted by the applicant to address the positive genotoxic and mutagenic responses seen in the in vitro assays for PM0000872 (Menthol, 5% nicotine concentration). Overall, the applicant did not provide a sufficient rationale, explanation or specific data to demonstrate that the negative in vivo genotoxic results should supplant the positive in vitro genotoxicity results. Thus, the information provided by the applicant indicates that PM0000872 (Menthol, 5% nicotine concentration), PM0000874 (Virginia Tobacco 3%, nicotine concentration) and PM0000876 (Virginia Tobacco 5%, nicotine concentration) have increased genotoxic potential relative to the 3R4F combustible cigarette comparison product.

Deficiency 4 concerns findings from mutagenicity studies of PM0000872 (Menthol, 5% nicotine concentration). PM0000872 was found to be positive for mutagenicity in the in vitro bacterial reverse mutation assay (Ames assay). The applicant-submitted data show that the aerosol

condensate produced from PM0000872 (Menthol 5%) with PM0000878 (JUUL Device), using standard puffing parameters, induced a significant mutagenic response when compared to the historical vehicle control group. The applicant did not provide sufficient additional information or a rationale to address the toxicological concerns regarding the mutagenic potential of PM0000872 (Menthol, 5% nicotine concentration).

Collectively, the issues noted above precluded a finding that any of the new products, i.e., the JUUL System, would be appropriate for the protection of the public health. All the deficiencies described above apply to the JUUL devices (PM0000878 and PM0000879) because the role of the devices is to deliver the e-liquid to the consumer in aerosol form. This means that the inability to perform a full and accurate toxicological evaluation of the new product e-liquids (PM0000864, PM0000872, PM0000874 and PM0000876) precludes the completion of a full and accurate toxicological evaluation of the JUUL devices (PM0000878 and PM0000879) as these devices play a critical role in the production and delivery of e-liquid to the product user.

The potential toxicological health risks of the new products, as described in the deficiencies above, preclude a finding that permitting the new products to be marketed would be appropriate for the protection of the public health. Even assuming the rest of the applications otherwise fully supported authorization, that finding could not outweigh the toxicological concerns identified in the applications and thus could not support a finding that the marketing of these products would be appropriate for the protection of the public health. Therefore, this TPL Review does not reach other aspects of the applications.

2. BACKGROUND

2.1. NEW PRODUCTS

The applicant submitted information for an electronic nicotine delivery system (ENDS) identified as the JUUL System. The JUUL System is a closed cartridge-based tobacco product developed by JUUL Labs, Inc., and is composed of two JUUL devices (both rechargeable, closed, cartridge-based devices) and four variants of JUULpods disposable cartridges, specifically:

- PM0000878 – JUUL Device
- PM0000879 – JUUL Locked Device
- PM0000864 – JUULpods Menthol (3.0%)
- PM0000872 – JUULpods Menthol (5.0%)
- PM0000874 – JUULpods Virginia Tobacco (3.0%)
- PM0000876 – JUULpods Virginia Tobacco (5.0%)

The JUULpods are pre-filled with approximately 0.7 mL of e-liquid. Both the JUUL Device and the JUUL Locked Device include a rechargeable battery and a USB charging dock accessory. The primary difference between the JUUL Device and the JUUL Locked Device is that the JUUL Locked Device requires a one-time age and identity verification before the device can be activated for use by consumers.

2.2. REGULATORY ACTIVITY

On July 29, 2020, FDA received these six PMTAs from JUUL Labs Inc. On August 5, 2020, FDA issued an Acceptance letter to the applicant. On March 26, 2021, FDA issued a Deficiency letter

for all of the PMTAs. On June 22, 2021, FDA received the applicant's response to the Deficiency letter.

Refer to the Appendix, Table 3 for a complete list of amendments received by FDA.

2.3. SCOPE OF REVIEW

The following discipline reviews were reviewed and considered for this TPL Review.

Table 1. Disciplines reviewed

Discipline	Cycle 1		Cycle 2	
	Reviewer(s)	Review Date	Reviewer(s)	Review Date
Regulatory	Donna Cheung	N/A	Donna Cheung	N/A
Toxicology	Matthew Hartog	3/24/2021	Matthew Hartog	6/16/2022
Environmental Science	William Brenner	3/26/2021	Thomas Creaven	6/15/2022

As noted above, the potential toxicological health risks of the new products preclude a finding that permitting the new products to be marketed would be appropriate for the protection of the public health. Even assuming the rest of the applications otherwise fully supported authorization, that finding could not outweigh the toxicological concerns identified in the applications and thus could not support a finding that the marketing of these products would be appropriate for the protection of the public health. Therefore, this TPL Review does not reach other aspects of the applications.

3. SCIENTIFIC REVIEW

3.1. COMPARISON PRODUCTS (FOR PURPOSES OF THE TOXICOLOGICAL EVALUATION)

3.1.1. Discipline key findings

Per the toxicology review:

- The University of Kentucky 3R4F reference cigarette was chosen by the applicant to provide combustible cigarette comparison data. The applicant compared mainstream aerosol Harmful and Potentially Harmful Constituents (HPHC) yields from the new products to mainstream smoke (MSS) HPHC yields from University of Kentucky 3R4F reference cigarette. The 3R4F reference cigarette was also used for *in vitro* cytotoxicity, mutagenicity and genotoxicity studies. The 3R4F reference cigarette was designed to be representative of the most popular cigarettes in the US cigarette market based on tobacco blend formulation. It is well characterized in literature and commonly used for research and standardization purposes. The toxicology review found the reference cigarette an appropriate comparison product.
- The applicant also compared mainstream aerosol HPHC yields of IQOS Regular and Menthol Heatsticks to the mainstream aerosol yields of the new products.

- The applicant tested and provided mainstream aerosol HPHC yields from the following Electronic Nicotine Delivery Systems (ENDS) products for comparison to the new products: Vuse Alto (Original flavor pod, 5% w/w nicotine, Menthol, 5% w/w nicotine), Blu PLUS+ (Classic Tobacco, 2.4% w/w nicotine, Menthol, 2.4% w/w nicotine), and NJOY Ace (Classic Tobacco, 5% w/w nicotine, Mint 5% w/w nicotine). These ENDS comparison products were also used for in vitro mutagenicity, cytotoxicity and genotoxicity studies. The applicant justified these comparison product selections based on their similarity to the new products (closed-system, pre-filled cartridge-based ENDS products) as well as comparable total puff counts, nicotine content, and e-liquid ingredients. The toxicology review concluded that the applicant's rationale for the selection of the ENDS comparison data is reasonable, and the products tested are appropriate comparison products.

3.1.2. Synthesis

As TPL, I agree with the toxicology review that the applicant's rationale for the selection of comparison products is appropriate for the purpose of conducting a toxicological evaluation. The rationale for selecting combustible cigarettes and other commercially available ENDS as comparison products is reasonable given their general product composition and design. This allows for a comparative toxicological evaluation of the JUUL System to a spectrum of tobacco products that are currently available on the US market.

3.2. PRODUCT CHARACTERIZATION

3.2.1. Discipline key findings

The following discussion is based on key findings provided in the discipline reviews:

3.2.1.1 Product design and composition

Per the toxicology review:

- The applicant states that JUUL Labs Inc. (JLI), the manufacturer of the JUUL System e-liquids, uses United States Pharmacopeia (USP) grade nicotine in all JUUL System e-liquids to minimize the presence of, and subsequent user exposure to, N-Nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which are known carcinogenic compounds in tobacco products. The applicant further states that the USP grade nicotine used in JUUL System e-liquids is predominantly non-protonated (or free-base).
- To characterize the risks associated with JUUL System's structural materials on potential interactions with the e-liquid and aerosol, the applicant provided extractable and leachable testing data. The extractable and leachable data for the JUULpods was evaluated by toxicology. The toxicology review identified two genotoxic and/or mutagenic leachables having a Margin of Exposure (MoE) less than one, and an excess cancer risk outside of generally accepted margins of "tolerable cancer risk". A full toxicology evaluation of these leachables could not be performed because the applicant did not provide data showing the mainstream aerosol yields of these two genotoxic and/or mutagenic leachables produced using the JUUL System. This was assessed as a deficiency by Toxicology and is discussed further in Section 3.3 and Section 3.4.

3.2.2. Synthesis

Per the toxicology review, there are two genotoxic and/or mutagenic leachables originating from the structural materials of the JUUL System. A full toxicology evaluation of these leachables could not be performed because the applicant did not provide mainstream aerosol yields for these two genotoxic and/or mutagenic leachables. (See section 3.3 for further discussion.) Toxicology assesses this as a deficiency. See sections 3.3 and 3.4.

3.3. TOXICANT EXPOSURE

3.3.1. Discipline key findings

The following discussion is based on key findings provided in the discipline reviews:

3.3.1.1 Toxicity

Per the toxicology review:

- **Identity of leachable constituents produced by the Juul System (PM0000864, PM0000872, PM0000874, and PM0000876 (Juulpods) with the devices PM0000878 and PM0000879) and their mainstream aerosol yield; methodological issues in the risk assessment provided to assess toxicity of these leachable constituents:** Following FDA's Cycle 1 review, FDA issued Deficiency 17[3] requesting that the applicant provide mainstream aerosol yields of the JUUL System for two leachable[4] constituents which had been analyzed and identified by the applicant as having an excess cancer risk outside of generally accepted margins of "tolerable cancer risk" or possessing some mutagenic and carcinogenic potential when inhaled (n-3-3-whole-pod-leach-tra-report.pdf; pg. 319 and 346). In its original submission, the applicant had acknowledged that these leachables were individually "considered a candidate target compound to monitor and evaluate in future analyses of the aerosol from the [applicant's] device" (n-3-3-whole-pod-leach-tra-report.pdf; pg. 220) and that "the limited nature of the available data precluded any firm conclusions on the tolerability of the two [leachables]" (n-3-3-whole-pod-leach-tra-report.pdf; pg. 221). Additionally, the applicant asserted the need for future monitoring of these two constituents during real time stability testing to better assess user exposure to these leachables (n-3-3-whole-pod-leach-tra-report.pdf; pg. 221). In response to Deficiency 17, the applicant stated that these two leachable constituents had only been tentatively identified in their initial analyses. The applicant then revised its original identification and re-identified these leachable constituents as different chemical compounds. Further, the applicant did not provide mainstream aerosol yields of the JUUL System for the original or re-identified leachables – it failed to provide information on the "tentatively identified" constituents in its initial application and, after follow up from FDA in a Deficiency letter, continued to fail to provide information on that initial constituent or the re-identified constituent. The applicant's response, including this failure to provide such data or information,

[3] See FDA's Deficiency Letter issued on March 26, 2021

[4] Leachables here are chemicals that migrate from the pod or device components into the e-liquid and may subsequently be inhaled by the consumer.

prevented FDA from conducting a full toxicological evaluation of the JUUL System. See section 3.4.1 for a detailed discussion of this issue.

- **Methodological issues in *in vitro* toxicology studies evaluating the new products (PM0000864, PM0000872, PM0000874, and PM0000876 (Juulpods) with the devices PM0000878 and PM0000879) and comparison products:** Following FDA's Cycle 1 review, FDA issued Deficiency 19 which requested that the applicant provide a discussion of the rationale for methodological differences in conducting *in vitro* toxicological studies of the Juul System for the new products (PM0000864, PM0000872, PM0000874 and PM0000876) and comparison products (i.e., ENDS and combustible cigarette comparators). The deficiency stated that "[t]his information is needed to ensure a valid toxicological evaluation of your PMTAs can be conducted, that the different cell counting and scoring methodologies used did not prevent the identification of genotoxic compounds, and that scientifically valid comparisons are made to the ENDS and combustible cigarette comparators." The applicant's response did not address FDA's concerns. See section 3.4.1 for a detailed discussion of this issue.
- **Genotoxicity of PM0000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) used with the devices PM0000878 and PM0000879[5]:** FDA's review of the *in vitro* and *in vivo* studies submitted by the applicant showed that PM0000872, PM0000874 and PM0000876 used with the devices PM0000878 and PM0000879 are genotoxic. Thus, following FDA's Cycle 1 review, FDA issued Deficiency 18 and Deficiency 19 which requested that the applicant provide additional data or an explanation regarding the finding of genotoxicity. The applicant provided new data and additional explanation which did not resolve the genotoxicity concerns stated in Deficiency 18 and Deficiency 19. See section 3.4.1 for a detailed discussion of this issue.
- **Mutagenicity of PM0000872 (Menthol 5%) used with the devices PM0000878 and PM0000879[6]:** FDA's review of the in vitro studies submitted by the applicant showed that PM0000872 used with the devices PM0000878 and PM0000879 is mutagenic. Thus, following FDA's Cycle 1 review, FDA issued Deficiency 18 which requested that the applicant provide additional data or an explanation regarding the finding of mutagenicity. The applicant provided additional explanation which did not resolve the mutagenicity concerns stated in Deficiency 18. See section 3.4.1 for a detailed discussion of this issue.
- **HPHC yields**: Toxicological evaluation of the mainstream aerosol yields of HPHCs included on the HPHC list, and other quantified chemical constituents found that levels of these compounds in the new products are not present at levels of concern. This toxicological evaluation was made by comparing the mainstream aerosol HPHC yields of the new products to the mainstream smoke HPHC yields of the 3R4F

[5] The aerosol condensates in these studies were produced using PM0000878 and bridged to PM0000879. Citation: h-1-1-5-locked-device-bridging-analysis.pdf. ; page 6 ("…the nonclinical toxicity studies performed with the JUUL Device [PM 0000878] are applicable to the JUUL Locked Device {PM 0000879})

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combustible cigarette comparator. A limitation of this toxicological evaluation for remaining non-HPHC chemical constituents measured in the mainstream aerosol of the new products is that the levels of propylene glycol (PG), menthol and several other constituents were not provided for the CC comparison product, therefore no comparisons to the new products can be made for these constituents. This limitation is outweighed by the total reduction in mainstream aerosol yields of the measured HPHCs in the new products versus the 3R4F CC comparison product, and by taking into account the available scientific literature suggesting reduced inhalation toxicity of PG relative to other listed HPHCs.

BOE data from clinical studies:

Per the toxicology review:

- Clinical studies evaluating nicotine pharmacokinetics found similar nicotine-concentration time profiles for all new tobacco products and comparison ENDS products, while the highest plasma levels of nicotine were observed after use of CC. The median time to reach maximum plasma nicotine concentrations was similar for the new tobacco products and the ENDS/CC comparison products. The total exposure to nicotine, based on area under the curve $_{0\text{-}60\text{-}baseline}$, following fixed usage of the new tobacco product was significantly lower when compared to usage of CC. This indicates that use of the new tobacco products and comparison ENDS products resulted in similar exposure to nicotine with a similar time needed to reach the maximum plasma nicotine concentration.

- The applicant-provided data in study PROT-00030 show that the study participants exclusively using PM0000876 resulted in an increased median urine total nicotine equivalents of 14.37% from baseline, which was larger than the reported change from baseline following exclusive use of UB CC. This finding suggests exclusive users of PM0000876 were exposed to more nicotine over the 6-day study period than users of UB CC. However, because this increase in nicotine exposure is not statistically significant, it is not ultimately a toxicological concern.

- Clinical studies measuring biomarkers of exposure (BOE) levels showed that after 6 days of exclusive use of the new products, CC users who switched to exclusive use of the new products had BOE levels similar to those in the tobacco product cessation group.

- A dual-use group that used either PM0000872 or PM0000874 and UB CC (cigarette consumption was limited to 50% of baseline amount) was associated with a significant reduction in levels of non-nicotine BOE. The BOE levels in this dual-use group remained elevated relative to the exclusive use of the new product and tobacco cessation groups, which abstained from smoking combustible cigarettes for the duration of the study.

- Exclusive users of PM0000864, PM0000872, PM0000874 and PM0000876 with PM0000878 were found to have significant reductions in urinary BOE as compared

to CC. While this suggests a reduced exposure to HPHCs compared to users of combustible cigarettes, a full evaluation of the applicant-provided risk assessment could not be performed due to the lack of data regarding the mainstream aerosol yield of the genotoxic/mutagenic leachables.

- Despite these findings regarding reductions in HPHCs and urinary BOEs, a full evaluation of the risk assessment for the use of any of the new JUULpod products (PM0000864, PM0000872, PM0000874, or PM0000876) with the JUUL devices (PM0000878 or PM0000879) could not be performed due to the remaining uncertainty regarding the identity of the genotoxic leachable constituents as well as the lack of data regarding the aerosolized yields of these constituents. As noted above, the applicant identified two genotoxic leachables associated with the JUULpod products (PM0000864, PM0000872, PM0000874, or PM0000876); however, the applicant did not provide data showing the mainstream aerosol yields of these leachables when the JUULpod products are used with the JUUL devices (PM0000878 and PM0000879) – either initially or when prompted by FDA in a Deficiency letter. Without this additional aerosol yield information, a full risk assessment of the JUUL System cannot be performed. As such, the study demonstrating reduced exposure to HPHCs, indicated by reduced urinary BOE, following use of the JUUL System should be interpreted with caution as the mainstream aerosol concentrations of these genotoxic leachables are unknown and these leachables were likely not captured in the BOE study.

3.3.2. Synthesis

Based on the toxicology review, the toxicological risk to the users of the Juul System cannot be fully evaluated because the applicant did not provide sufficient information to resolve FDA's concerns regarding (1) two leachable constituents, their mainstream aerosol yields, and the associated risk assessment for the new products PM0000864, PM0000872, PM0000874, PM0000876, PM0000878 and PM0000879 (i.e., the Juul System); (2) methodological issues in *in vitro* toxicology studies evaluating the new products (PM0000864, PM0000872, PM0000874, and PM0000876 (Juulpods) with the devices PM0000878 and PM0000879) and comparison products; (3) toxicology studies indicating the genotoxicity of PM0000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) used with the devices PM0000878 and PM0000879; (4) toxicology studies indicating the mutagenicity of PM0000872 (Menthol 5%) used with the devices PM0000878 and PM0000879. (See Section 3.4.1 for a detailed discussion of these issues)**.**

In the clinical studies, significant reductions in blood and urinary BOEs indicate that exposure to carcinogens and other toxicants present in cigarette smoke were greatly reduced with exclusive use of the new products compared to CC smoking. While it is theoretically possible for the decreased HPHC yields and reduced BOE levels to offset the risk posed by the genotoxic leachables, the applicant provided no data indicating if, and how much of, these leachables are transferred into mainstream aerosol. With unknown aerosol yields of these leachables and their disputed chemical identities, resulting in an unknown cancer potency and genotoxicity risk, it is not possible to do an offsetting evaluation for carcinogens (where decreased levels of one carcinogen potentially mitigate

increased levels of another).

3.4. HEALTH EFFECTS

3.4.1. Discipline key findings

The following discussion is based on key findings provided in the discipline reviews:

3.4.1.1 Toxicology

Per the toxicology review:

Identity of leachable compounds produced by the Juul System (PM0000864, PM0000872, PM0000874, and PM0000876 (Juulpods) used with the devices PM0000878 and PM0000879; mainstream aerosol yields of these leachable compounds in the Juul System; methodological issues in the risk assessment provided to assess toxicity of these leachable compounds in the aerosol yield of the Juul System

Identity of leachable constituents in PM0000864, PM0000872, PM0000874, and PM0000876 (Juulpods) used with the devices PM0000878 and PM0000879:

- In their original submission for these PMTAs, the applicant submitted a toxicological evaluation of identified leachable constituents (leachables) from their new products (PM0000864, PM0000872, PM0000874, PM0000876). These new products were subjected to accelerated aging under two conditions: 1) accelerated aging for 22 weeks at 30°C and relative humidity of 65%, equivalent to 9 months ambient conditions and 2) accelerated aging for 22 weeks at 40°C and relative humidity of 75%, equivalent to 18 months ambient conditions. In this toxicological evaluation, as noted above, the applicant identified the presence of two genotoxic leachables found to produce an excess cancer risk outside of generally accepted margins of "tolerable cancer risks." These leachables were identified by the applicant as Ethyl-4-hydroxyquinoline-3-carboxylate (EHQC) and Propylpyridine,1H-pyrrole-1-hexanoic acid,2,5-dihydro-2,5-dioxo-related compound (PHDC). The applicant supported the identification of these leachables using analytical chemistry data and mass spectrometry analysis. The applicant stated in their provided toxicological risk assessment that these leachables were individually "considered a candidate target compound to monitor and evaluate in future analyses of the aerosol from the [applicant's] device." The applicant did not comment on or provide quantitative data showing the levels of EHQC and PHDC in the mainstream aerosol generated from the new products.
- The applicant did not have specific toxicological data for the leachable EHQC. Therefore, the applicant evaluated toxicity associated with the chemicals 4-hydroxyquinolone and quinoline, which have certain structural similarities with EHQC. Applicant-provided information stated that 4-hydroxyquinolone was shown to induce micronuclei formation and genotoxicity in mice and that similar activity was observed for other quinoline and hydroxyquinoline compounds. EHQC is a hydroxyquinoline compound. Furthermore, quinoline is considered to be genotoxic based on extensive in vitro and in vivo toxicological studies.

Quinoline is classified in the EU as a category 2 mutagen (suspected of causing genetic effects) and a Category 1B carcinogen (may cause cancer). The US Environmental Protection Agency considers quinoline to be genotoxic (n-3-3-whole-pod-leach-tra-report.pdf; pages 105, 316). Quinoline was shown to induce mutations in bacterial mutation assays and induce genotoxicity, both in vitro and in vivo, through chromosomal damage and micronuclei formation. The applicant-provided risk assessment states that "[a]s a health precautionary approach for this assessment, it was assumed that EHQC poses an equivalent carcinogenicity hazard as quinoline" (n-3-3-whole-pod-leach-tra-report.pdf; page 106, 219-220, 317). The applicant-provided risk assessment of the leachable PHDC did not identify any specific toxicological data for this leachable. However, key molecular fragments of this leachable were evaluated and identified as potentially mutagenic, indicating that the leachable may possess mutagenic activity. The applicant-provided risk assessment states that "[a]s such, as a health-precautionary measure, this compound was assessed as a potential mutagen" (n-3-3-whole-pod-leach-tra-report.pdf; page 345).

- FDA's Deficiency letter (Deficiency 17) stated that these two leachables raised toxicological concerns and required additional information from the applicant, specifically mainstream aerosol yields of these leachables produced by the Juul System, in order to perform a complete toxicological evaluation of the new products.
- In their response to the Deficiency letter, the applicant did not provide mainstream aerosol yield data for these leachables. Rather, the applicant stated that the compounds which had been "tentatively identified" in the original application had now been "subsequently updated [by the testing lab] to complete identifications. ... upon further manual evaluation of the mass spectrometry (MS) and tandem mass spectrometry (MS/MS) spectra. . ."[7] and no longer present toxicological concerns. The applicant amended the previously provided chemical analyses, leachable identifications and risk assessments in the applicant's response to Deficiency letter. Specifically, the applicant revised the leachable EHQC to 1,8,9-trihydro-2-(3-carboxypropylamine-N-yl)-3-ethylcarboxylate-4-quinolone (TCEQ) and revised the leachable PHDC to Nornicotine, N-carboxyglycerol-5'-(methoxy-1-(p-hydroxybenzene-*O*4-yl-acetic acid)) (NNMA).
- The new leachables information provided by the applicant in response to FDA concerns raises questions about the true identity of the leachables and their toxicological impact. Specifically, applicant-provided data, i.e., the chemical structures, chemical formulas, and mass spectral data, do not support the revised identities of the leachables:
    - First, with respect to the leachable constituent EHQC, the applicant now states that what was first identified as the leachable EHQC has now been identified as TCEQ. This re-identification raises questions because in a separate document submitted by the applicant (i.e., a revised toxicology risk assessment that was provided by the applicant and submitted as part of the deficiency response (app-17-03-n-3-3-whole-pod-leach-tra-report.pdf; pages 105-6 and 306-7)), a leachable chemical

[7] Page 134 of Juul's response to the Deficiency letter

with the same structure as TCEQ is identified as a different chemical (4-((3-(ethoxycarbonyl)-4-oxo-1,4,6,7-tetrahydroquinolin-2-yl)amino)butanoic acid) (ETBA). This means that EHQC has been re-identified as two separate and distinct chemicals, i.e. TCEQ and ETBA.

- This revised toxicology risk assessment notes that the leachable initially evaluated as EHQC was re-identified as ETBA (app-17-03-n-3-3-whole-pod-leach-tra-report.pdf; page 216).
- Furthermore, this risk assessment of ETBA used the more appropriate Escher et al., 2010 risk assessment approach to evaluate toxicological concerns, and not the Carthew approach[8] that was used to evaluate TCEQ. No specific toxicity data was available for ETBA, however, based on the expected average daily exposure levels, the applicant-provided risk assessment finds that ETBA may be present at levels of toxicological concern. This risk assessment concludes that "[b]ased on the available information, a reassuring conclusion regarding consumer health risks [posed by exposure to ETBA] could therefore not be established" (app-17-03-n-3-3-whole-pod-leach-tra-report.pdf, page 307). This risk assessment of ETBA is in direct contradiction to the risk assessment provided for TCEQ in the applicant's response to the Deficiency letter (page 137) that found that no toxicological concerns are present. The risk assessment of TCEQ in the applicant's response to the Deficiency letter used the Carthew et al., 2009 approach (see explanation below). These conflicting data and multiple revisions by the applicant further undermine the applicant's revised identification of EHQC.

- Second, with respect to the leachable constituent, PHDC, the applicant's re-identification is not scientifically supportable for the following reasons:
  - The provided chemical structures and chemical formulas between the original and revised identification are inconsistent. The analysis and original identification of the leachable determined that the leachable had a chemical formula of $C_{18}H_{24}N_2O_4$ with an exact mass ranging from 332.1726 to 332.1757 Da. This original identification was made with an identification score[9] of 80.8 to 93.5. Following re-identification, the chemical formula was $C_{22}H_{26}N_2O_8$ with an exact mass ranging from 446.1654 to 446.1707 Da. This re-identification

[8] Carthew P, Clapp C, Gutsell S. Exposure based waiving: The application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. Food and Chemical Toxicology. 2009;47:1287-1295.

[9] The score "is used to determine the match quality and is a determination of how close the unknown spectrum matches the library spectrum. The score is calculated based on the database match using fragment and parent ions and their relative abundances. The scale is 0-100, with 100 being the best possible match. [redacted]" See [redacted] lab report (at n-3-4-[redacted] whole-pod-leachable-report-2.pdf, page 4 and app-17-02-n-3-4-[redacted] whole-pod-leachable-report-2.pdf, page 4).

was made with an identification score of 69.9 to 97.2. As the applicant stated that no new chemical analysis was performed and no new analytical data was acquired, the re-identification of the leachable represents significant changes in the purported chemical structure and formula of the leachable, which are reflected by the less precise library score for the "updated" identification of the leachable.

- The mass spectral data that was provided does not support the revised identification. The chemical structure and column retention time of the product ion for the "updated" identification of the leachable is not provided in the associated mass spectra, which prevents a conclusive comparison of the original and "updated" leachable chemical structures. The major fragment ion of the "updated" leachable (353.1136 m/z; $C_{19}H_{17}N_2O5$) does not correspond to any reported fragment or product ion of the originally identified leachable, which had a total mass of approximately 332.17 Da and a chemical formula of $C_{18}H_{24}N_2O_4$. There are no corresponding fragment ions in the "updated" mass spectral data that connect the original and "updated" identifications. There are no indications that change in exact mass of the product ion, from approximately 332.17 Da to 446.17 Da, can be explained by a neutral loss or other relevant mechanism.
- No explanation or additional information is provided by the applicant to describe the procedure used for the "manual evaluation" or how the "manual evaluation" of analytical data was conducted. No additional information describing how the "manual evaluation" that was used to "update" the identification of this leachable could have resulted in these changes to the chemical structure and chemical formula was provided by the applicant.
- FDA also identified an additional concern regarding one of the amended analytical reports provided by the applicant (app-17-02-n-3-4-[redacted]-whole-pod-leachable-report-2.pdf). In the amended report's summary "for LC-MS based on the chromatographic data" (page 7), it is stated that "[t]wenty three compounds were identified in the test article extracts…" However, in the original report, the corresponding section states that "[t]wenty two compounds were identified in the test article extracts…" (n-3-4-[redacted]-whole-pod-leachable-report-2.pdf; page 7). The chemical that was identified in the amended report, but was not present in the original report, is the leachable PHDC/NNMA. The applicant does not provide an explanation for how the genotoxic leachable was not detected in the original analysis, and after a "manual evaluation" of previously collected chemical analysis data, the leachable was detected in the new products. This raises additional concerns

regarding the reliability of the data, the correct identity of the leachable, and the genotoxic potential of the new products.

Issues in the risk assessment that were used to evaluate the toxicity of the revised leachables identified in the Juul System

- In their original submission, the applicant submitted a risk assessment of the JUUL System and discussed the appropriateness of two different risk assessment methods, one from Carthew *et al.,* (2009)[10] and one from Escher et al., 2010[11] (n-3-3-whole-pod-leach-tra-report.pdf; pages 174 and 408). When evaluating which risk assessment method is most appropriate to use for the assessment of leachable and extractable chemicals from a product, the applicant's initial submission identifies the Escher approach as most appropriate to assess the risk profile of the new products. In this discussion, the applicant states "[t]he Carthew *et al.* (2009) TTC [Threshold of Toxicological Concern] values were selected for evaluation of ingredients and non-targeted analytes in aerosol because the dataset of chemicals used to derive Carthew *et al.,* (2009) TTCs was deemed to be relevant to ingredients and aerosol constituents identified in the ENDS products. However, the TTC values proposed by Escher *et al.* (2010) would appear to be more representative for the extractables and leachables risk assessment, as the dataset used to derive these thresholds was composed of a range of 200 industrial chemicals." This risk assessment approach by Escher et al., 2010 identified two leachables (EHQC and PHDC) as being present at a level of toxicological concern. This evaluation was based on the expected average daily exposure to the leachables and the anticipated toxic potential of the leachables.
- As part of their response to FDA's Deficiency letter, in conjunction with providing revised identities for EHQC and the PHDC, the applicant submitted a revised toxicological risk assessment based on a different approach, the Carthew et al., 2009 approach, to evaluate the health risks posed by the two revised leachable constituents in an attempt to demonstrate that the revised leachables do not raise toxicological concerns. The applicant also used this revised risk assessment as justification for why they did not provide mainstream aerosol yields for these genotoxic leachables, despite FDA's request for such information in the Deficiency letter. In this revised risk assessment, the applicant modified their methodology to use an inappropriately conservative approach (i.e., the approach in Carthew et al., 2009) that likely understates the health risks posed to users of the new products. The dataset used to develop the Escher et al., 2010 risk assessment approach included industrial chemicals that are likely more representative of the types and classes of chemicals encountered in an assessment of leachables akin to those present in the aerosol yield of the JUUL System. On the other hand, the risk assessment approach used in Carthew et al., 2009 is based on an evaluation of consumer products ingredients, which consists of chemicals intentionally added to a product. As the

[10] Carthew P, Clapp C, Gutsell S. Exposure based waiving: The application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. Food and Chemical Toxicology. 2009;47:1287-1295.
[11] Escher SE, Tluczkiewicz I, Batke M, et al. Evaluation of inhalation TTC values with the database RepDose. Regulatory Toxicology and Pharmacology. 2010;58:259-274.

leachable chemicals "leach" from the components of the JUULpods and are not added intentionally to the product as ingredients, the Escher et al., 2010 method is thus more appropriate than the Carthew et al., 2009 method which is geared towards the risk assessment of added ingredients. Because the Carthew et al., 2009 method specifically focused on ingredients, it does not have the specificity needed to evaluate the types of leachable chemicals typically studied in a leachables assessment (for example, polymers, heavy metals). Additionally, the dataset used to derive the Carthew et al., 2009 approach and evaluate subsequent health risks does not include chemicals relevant to the assessment of leachables. This is demonstrated by the risk assessment approach used in Carthew et al., 2009 specifically excluding genotoxic carcinogens, *in vivo* mutagens, heavy metals and polymers from the risk assessment "as they were not considered representative of the ingredients that are, or could be used, in aerosols for consumer use." Furthermore, the applicant limited this revised risk assessment to assessing systemic toxicity and does not include local toxic effects, which have a lower threshold for occurrence. When evaluating the revised leachables using the Escher et al., 2010 approach, which is more appropriate to assess the risk profile of the new products and was previously used by the applicant to evaluate their new products, these leachables are present in the e-liquids at a level of toxicological concern. Therefore, regardless of the identity of these leachables (that is, whether they are the two the applicant identified in the original submission, or the two the applicant "re-identified" in response to the Deficiency letter), Toxicology would require data on the mainstream aerosol yield of these leachables to fully evaluate the potential for toxicity and/or adverse health outcomes in users of the new products.

- A second risk assessment provided by the applicant in response to FDA's Deficiency letter addressed the re-identification of the leachable EHQC (app-17-03-n-3-3-whole-pod-leach-tra-report.pdf). In this risk assessment EHQC was re-identified as ETBA, as opposed to TCEQ. This additional risk assessment, which the applicant chose not to discuss and simply appended to its response to FDA's Deficiency letter, identified the Escher et al., 2010 approach as the most appropriate risk assessment approach for toxicological evaluation of leachables (i.e., EHQC, ETBA, TCEQ) (app-17-03-n-3-3-whole-pod-leach-tra-report.pdf; page 164 and 385). This risk assessment found ETBA, which was re-identified from EHQC, to be present at a level of toxicological concern in the new products. This is in direct contrast to the other risk assessment provided by the applicant, where the Carthew et al., 2009 approach was used to evaluate the re-identified leachables TCEQ, previously EHQC, and NNMA, previously PHDC (response-to-deficiency-letter.pdf; page 137 and 138). This risk assessment found that TCEQ and NNMA were not present at a level of toxicological concern in the new products. The applicant states that the use of the Carthew et al., 2009 approach to evaluate TCEQ and NNMA is consistent with the risk assessment approach used in their original submission. This statement is inaccurate as the originally submitted risk assessments used the Escher et al., 2010 approach (n-3-3-juulpod-material-toxicologic-risk-assessment.pdf; page 28). The Escher et al., 2010 and Carthew et al., 2009 approaches are not interchangeable and have

inherent differences in risk tolerance, meaning that Carthew et al., 2009 will likely understate risk of toxicity from leachables relative to Escher et al., 2010.

Mainstream aerosol yield of these leachables in the Juul System:

- FDA finds that there is potential for there to be toxic leachables in the mainstream aerosol yield of the Juul System, regardless of whether the actual leachables in the new products are those identified in the applicant's original submission or instead those subsequently identified in the applicant's response to FDA's Deficiency letter. As stated in the PMTAs, the Juulpods (PM0000864, PM0000872, PM0000874 and PM0000876) are designed to function as a system with the unlocked (PM0000878) or locked (PM0000879) devices for the production and delivery of aerosol to the product user. The applicant describes the new products as the JUUL System. These leachables would be expected to be delivered to the product users via the new product JUUL devices PM0000878 or PM0000879. Specifically, the devices (PM0000878 and PM0000879) are responsible for aerosolizing and delivering the e-liquid to the user. HPHCs, and other toxic constituents including leachable constituents within the e-liquid can be transferred into the aerosol via the device.[12] The device functional parameters (i.e., coil temperature, power delivery and maximum puff duration) control and mediate the transfer of these genotoxic leachables into the mainstream aerosol; as such, the JUUL devices mediate user exposure to these genotoxic leachables.
    - Thus, the applicant needed to have provided sound information to support the proper identification of these leachable constituents, and further, provided mainstream aerosol yield data for the new JUULpod tobacco products (PM0000864, PM0000872, PM0000874 and PM0000876) used with the new JUUL devices (PM0000878 or PM0000879) for these properly identified leachable constituents so that FDA can perform a complete and accurate toxicological risk assessment of all of the new products. This concern is reflected in Deficiency 1 of this review.
    - In summary, there is an unknown quantity of two genotoxic leachables contained in the mainstream aerosol produced using the JUUL System. The identity of these leachables is currently disputed. A correct identification of these leachables is needed to properly evaluate their potential toxicological properties and determine what their carcinogenic potency, and subsequent carcinogenic risk, is likely to be. While the applicant reports decreased HPHC yields and reduced BOE levels that could potentially be used to offset the risk posed by these genotoxic leachables, the applicant provided no data indicating if, and how much, of these leachables are transferred into mainstream aerosol. With unknown aerosol yields of these leachables and their disputed chemical identities, resulting in an unknown cancer potency and genotoxicity risk,

[12] HPHCs, and other toxic constituents including leachable constituents within the e-liquid, can also be produced as a result of the device functional parameters. However, there is no evidence to suggest that occurred here.

it is not possible to do an offsetting evaluation for carcinogens (where decreased levels of one carcinogen mitigate increased levels of another). FDA cannot draw a definitive conclusion regarding the toxicological risk of the JUUL system, or any of the individual components of that system, without a complete data set containing aerosol yields for the leachables and a conclusive determination of their chemical identity.

Methodological issues with the *in vitro* genotoxicity studies evaluating the genotoxicity of the new products (PM0000864, PM0000872, PM0000874, and PM0000876 (Juulpods) used with the devices PM0000878 and PM0000879) and the comparison products.

- The applicant states in their submitted technical summary report for the *in vitro* genotoxicity study that "[t]he in vitro MN [micronucleus] assay was performed using the human lymphoblastoid TK6 cell line and the recommendations of OECD TG 487" (n-3-1-2-mn-testing-technical-summary-liquid.pdf, page 5 and n-3-1-2-mn-testing-technical-summary-condensate.pdf; page 6). The applicant's study protocol for the in vitro micronucleus genotoxicity study states that the study would (1) "be in general accordance with the OECD Guideline Number 487 (adopted July 29, 2016) (n-3-1-2-micronuc-men-5-rpt-03420reva-report.pdf; page 39), (2) "[m]icronucleus frequencies will be analyzed in at least 1000 mononucleated cells per culture (at least 2000 mononucleated cells per concentration), when available (n-3-1-2-micronuc-men-5-rpt-03420reva-report.pdf; page 45), and (3) "Cases which do not fit clearly into the positive or negative criteria may be judged equivocal…In these cases the Study Director, based on sound scientific judgement, may take additional factors into consideration in evaluating the test results (n-3-1-2-micronuc-men-5-rpt-03420reva-report.pdf; page 46). However, despite test articles of the new products meeting your assay acceptability criteria for a clearly positive response, and the OECD Guidelines stating that "[t]here is no requirement for verification of a clear positive or negative response (page 14 of the guidelines), the applicant retested these new products using additional cells. Additionally, the procedure used in the applicant-provided in vitro genotoxicity study utilized a different methodology for evaluating and scoring the JUULpod e-liquid and aerosol from PM0000864, PM0000872, PM0000874 and PM0000876, produced using the JUUL device, versus the ENDS and CC comparison products.
    - Treatment of assays relating to the new products: The applicant-provided data identified instances in which the new products induced positive or equivocal genotoxic responses. The positive genotoxicity responses for the new products (using a cell count of 2000 cells) were then rejected by the applicant and the new products were further evaluated by counting an additional 2000 cells and rescoring the assay. The additional evaluation of positive responses runs counter to the assay guidelines as provided by the applicant, as these results indicated a "clearly positive" genotoxic response. Significantly, test articles with "clearly negative" genotoxic responses based on a 2000 cell count did not undergo further evaluation and rescoring. It appears that the applicant chose to increase the statistical power of the assay (using

4000 cells rather than 2000 cells) for unfavorable data showing a positive result for genotoxicity while retaining the 2000 cell count method mandated by the OECD guidelines when a result negative for genotoxicity was obtained. Additional and more stringent criteria (i.e., rejecting "very weak" or "very small" clearly positive genotoxicity responses) were applied to samples that were identified as clearly positive for genotoxicity within the in vitro micronucleus assay. Equivocal genotoxic responses may be further evaluated using expert judgment and/or further investigations (i.e., counting additional cells, if appropriate, or performing a repeat experiment that may have modified experimental conditions). The applicant evaluated equivocal responses for the new products by counting an additional 2000 cells "to clarify the response." The applicant did not provide a rationale or supporting statistical calculations to demonstrate why counting additional cells, in lieu of conducting a repeat or modified experiment, was appropriate and justified. The applicant did not count additional cells or further evaluate equivocal responses reported for the comparator products. These shifting standards created a situation where positive genotoxic responses were placed under enhanced scrutiny relative to negative responses.

- Treatment of assays relating to the comparison products: In the original application, data were submitted from *in vitro* toxicological studies to demonstrate a lack of risk in comparison to other tobacco products, including other ENDS products and combustible cigarettes. However, the methods and criteria used were inconsistent between the new and comparison products. Specifically, for the new products, as discussed above, more stringent acceptance criteria were used to evaluate samples that were identified as either equivocal or positive for genotoxicity. By contrast, the ENDS and combustible cigarette comparison products were evaluated for genotoxicity based on a 4000 cell count assay, and no data from a 2000 cell count based assay was provided by the applicant. Additionally, equivocal responses for the comparator products were not further evaluated by the applicant. Not having the same data set for comparison products as for new products prevents a comparative evaluation of the new versus comparator products. This lack of a complete data set also prevents an evaluation of how the comparison products would have been affected by the two rounds of scoring that the applicant provided for the new products; i.e., genotoxicity scores changing from "clearly positive" to "clearly negative." The concerns regarding the use of this inconsistent methodology for evaluating the comparison products were conveyed to the applicant in the FDA Deficiency letter. In response to this deficiency, the applicant states "JLI recognizes that there were differences in the number of cells scored for the new tobacco products and the comparators" (page 154 of response to deficiency) and "JLI recognizes it would have been preferable to evaluate all micronucleus assay results in the same manner…" (page 158 of response to deficiency).

- In order to explain their departure from their submitted study protocol, the applicant stated that the counting of additional cells is needed to assist in establishing the biological relevance of the results. However, no rationale is provided for how this selected approach would yield the new information that is needed to assess the biological relevance of these assay results, and why it was necessary to reject valid assay results indicative of genotoxicity in order to assess the biological relevance of the assay results for select new products. It is also clear from the revision of the genotoxicity assessments that the counting of additional cells was not limited to the evaluation of biological relevance, but was also used to reassess several new products with a more stringent procedure (i.e. rejecting "very weak" or "very small" clearly positive genotoxicity responses).
    - The applicant states that the increase in cell counting may provide greater statistical power to distinguish between results, however no statistical power calculations or other calculations were provided by the applicant. These calculations are needed to demonstrate that this magnitude of an increase, from 2000 to 4000 cells per concentration, is appropriate for the statistical analysis being performed. This information is needed to show that the procedural modification would not adversely impact the validity of the assay. The applicant-provided new data in the response to the deficiency (Figure 5, page 162 of the deficiency response) shows that the increased counting of cells, from 2000 to 4000 cells per concentration, identified a similar %MN. This suggests that sufficient sampling and statistical power to detect a significant difference were likely present under the initial conditions of the assay and that increasing the number of cells counted was not necessary to identify genotoxic responses.
- In summary, the genotoxicity data, as provided, is unreliable and the methodology used to produce the data raises significant concerns regarding the quality of the data and accuracy of the results. These concerns prevent a conclusive evaluation of the toxicological risks posed by the new products from being performed. An adequate rationale was not provided by the applicant to explain how the modified cell counting and micronucleus scoring procedure used in the applicant-provided study did not adversely impact the ability to correctly identify genotoxic versus non-genotoxic test articles. No calculations were provided by the applicant to demonstrate that these select changes to the statistical power of the assay did not impact the scientific validity of the assay. No rationale was provided by the applicant to explain how these modified procedures would assist in evaluating the biological relevance of the genotoxicity assay results. Therefore, based on the applicant's methodologies to assess genotoxicity, FDA is unable to perform a complete toxicological evaluation and risk assessment of the new products without additional information. The inconsistent use of assay acceptance criteria resulted in unequal treatment of test articles within the genotoxicity assay (depending on whether or not those test articles

resulted in positive or negative results for genotoxicity), which impacts the scientific validity of the assay. Finally, the methods and criteria used were inconsistent between the new and comparison products, including more stringent acceptance criteria used to evaluate samples that were identified as either equivocal or positive for genotoxicity. This prevents FDA from performing a conclusive evaluation of the new products and accurately assessing the biological significance and relevance of the *in vitro* micronucleus assay results.

- The toxicological concerns regarding the JUUL devices (PM0000878 and PM0000879) are two-fold. Genotoxic constituents within the new product e-liquids (PM0000864, PM0000872, PM0000874 and PM0000876) are being transferred into the mainstream aerosol via the JUUL devices (PM0000878 and PM0000879) resulting in the production of genotoxic constituents within the mainstream aerosol. The JUUL devices (PM0000878 and PM0000879) are ultimately responsible for producing the mainstream aerosol, which contains genotoxic constituents, and for conveying these genotoxic constituents to the product user. This means that the inability to perform a full and accurate toxicological evaluation of the new product e-liquids (PM0000864, PM0000872, PM0000874 and PM0000876) precludes the completion of a full and accurate toxicological evaluation of the JUUL devices (PM0000878 and PM0000879) as these devices play a critical role in the production and delivery of genotoxic constituents to the product user.

The genotoxicity of PM000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) used with the devices PM0000878 and PM0000879

- Data submitted from in vitro studies demonstrated PM0000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) are genotoxic. PM0000872, PM0000874 and PM0000876 were evaluated for genotoxic potential and found to be positive for genotoxicity under the initial conditions of the assay (i.e., using 2000 cells per concentration). These initial positive genotoxicity results were rejected by the applicant without an adequate scientific rationale or justification, and the new products were re-evaluated for genotoxicity using additional cells. No explanation was provided to address how this rejection of valid assay results, which met the pre-defined assay acceptance criteria listed by the applicant, does not adversely impact the scientific validity of the assay.
- PM0000872 (Menthol 5%) was evaluated for genotoxic potential and was found to be positive for genotoxicity under two assay conditions, using either 2000 cells or 4000 cells per concentration. Therefore, regardless of whether the 2000 cells per concentration methodology described in OECD guidance, or the 4000 cells per concentration methodology used by the applicant in the re-test, is used, the applicant's data indicate the genotoxicity of the new product. PM0000874 (Virginia Tobacco 3%) was initially found to be positive for genotoxicity under the initial assay conditions, using 2000 cells per concentration, and after re-evaluation using 4000 cells, was found to be equivocal for genotoxicity. PM0000876 (Virginia Tobacco 5%) was initially found to be positive for genotoxicity under the initial assay conditions, using 2000

cells, and after re-evaluation using 4000 cells, was found to be negative for genotoxicity. No additional information or in vivo study data was provided by the applicant to further evaluate the reported genotoxicity of PM0000876 (Virginia Tobacco 5%).

- In an attempt to address the genotoxicity concerns and positive genotoxicity findings of the in vitro micronucleus assay, the applicant provided in vivo data to show that PM0000872 and PM0000874 did not induce genotoxicity. The applicant did not provide bridging data (e.g., precise measurements of condensate/e-liquid constituents *in vitro*, pharmacokinetic parameters/tissue levels of constituents *in vivo*, dose-response relationships *in vitro* and *in vivo*) to compare the in vitro and in vivo genotoxicity assessments of PM0000872 and PM0000874, and did not include a combustible cigarette comparator within the in vivo genotoxicity study.
    - This bridging information should have been provided to demonstrate that HPHC and other toxicant levels are similar between the studies and, more importantly, that the in vivo study is a fair representation of the in vitro study it is being compared against, in this case, an in vitro study that produced positive genotoxicity results. A characteristic of in vivo studies is that due to dilution associated with increased areas of exposure and body mass not present within in vitro systems, increased exposure concentrations are needed in an in vivo study to produce the same HPHC concentrations at the tissue level, where the toxicity occurs, relative to the HPHC concentrations present in the in vitro system. If a sufficiently high exposure concentration is not used for the in vivo study, the tissue levels of HPHCs and other toxicants will be reduced relative to the in vitro study, and the in vivo study will appear to give a negative result for a genotoxic compound. Conversely, if an excessively high exposure concentration is used for the linked in vivo study, the specific toxic mechanisms that produced the positive in vitro genotoxicity result will be affected and the presentation of toxicity will be altered (i.e., cells that underwent chromosomal damage and formed micronuclei in vitro may undergo cell death pathways in vivo due to excessive exposure to toxicants). Bridging information would also have been important for assessing the biological relevance of the results, which is a specific concern regarding the positive genotoxicity results from the in vitro genotoxicity assay. There are inherent differences between *in vitro* and *in vivo* toxicological assays, with *in vitro* assays being well suited for assessing precise toxicological mechanisms[13], such as genotoxicity. The differing strengths and weaknesses of *in vitro* and *in vivo* toxicological assays make it inappropriate to discard *in vitro* results in light of conflicting *in vivo* data.
    - Additionally, even putting the lack of bridging data aside, a combustible cigarette comparator is needed within the in vivo study to make relative comparisons on the genotoxicity of the new products.

[13] Shukla, S., Huang, R., Austin, C., and Xia, M. (2010). The future of toxicity testing: A focus on in vitro methods using a quantitative high throughout screening platform. Drug Discov Today 15(23-24), 997-1007.

- Furthermore, the applicant-submitted data for the in vivo DNA damage/Comet assay was highly variable and also lacked a combustible cigarette comparator. In one instance of the DNA damage/Comet assay, the standard deviation (an indicator of variability) of a PM0000872 exposed group is 62% of the mean, while the filtered air control group standard deviation is ≈68% of the reported mean. This high variability was also observed in DNA damage/Comet assays using PM0000874. In one example, the standard deviation of a PM0000874 exposed group is ≈107% of the mean, while the filtered air control group standard deviation is ≈68% of the reported mean. While relatively large standard deviations are commonly observed in DNA damage/Comet assays, the high variability of the data can compromise the statistical analysis and can limit the conclusions that can be drawn.[14]
- With the absence of a combustible cigarette comparator group, the relative risk of genotoxicity following use of the new tobacco product versus combustible cigarettes is unclear. These conflicting in vitro versus in vivo genotoxicity assay results and lack of a suitable comparator in vivo prevent a conclusive genotoxic assessment of PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%). These toxicological concerns were conveyed in FDA's Deficiency letter to the applicant and were not adequately addressed in the applicant's response to the Deficiency letter. In response to FDA's Deficiency letter, the applicant provided "final" versions of the previously submitted in vivo genotoxicity and mutagenicity studies (app-18-01-n-3-2-rpt-04746reva-me-5-genotox-report.pdf and app-18-02-n-3-2-rpt-04743reva-prot-00903reva-report.pdf). These "final reports" did not contain new information or additional explanations regarding the conduct or design of the in vivo genotoxicity study. In their response, the applicant states "although positive in vitro data could indicate intrinsic genotoxic properties of a drug, appropriate in vivo data determine the biological significant of these in vitro signals in most cases." This is accurate and underscores the need for proper bridging between the linked in vitro and in vivo genotoxicity studies, as well as the necessity of including an appropriate comparator when evaluating relative genotoxic responses and determining the biological significance of in vitro results.
- The applicant submitted data from an additional in vitro genotoxicity assay using a different CC comparator (1R6F combustible cigarette; app-19-02-rpt-03529-rev-a-1r6f-condensate-mn.pdf). This study demonstrated a positive genotoxic response in 2 of 3 assay conditions, as opposed to the previously reported equivocal response for the 3R4F CC comparator. No explanation of the differing results was provided. The ability to draw conclusions from this additional in vitro genotoxicity study is limited as the assay only included the 1R6F CC comparator and no other products. The applicant also cites the 2010 Surgeon General's Report *How Tobacco Smoke Causes Disease: The Biology and Behavioral*

[14] Langie, S., Azqueta, A., and Collins, A. (2015). The comet assay: past, present, and future. Front Genet 6(266), 1-3.

Basis for Smoking-Attributable Disease to show that, contrary to what was demonstrated in their study using the 3R4F CC, "condensate from cigarette smoke is mutagenic in a variety of systems." The inability of the applicant to replicate this positive genotoxicity result calls into question the reliability of the assay methodology and the quality of the provided data. This situation demonstrates the need for inclusion of an appropriate comparator when making relative toxicity assessments between conflicting in vitro and in vivo studies. The applicant demonstrated understanding of the importance of comparator data when evaluating the new products in vitro and this same standard for comparison should have been carried through into the in vivo genotoxicity studies that were designed to dispute and override positive in vitro genotoxicity results.

- The Juulpods (PM0000864, PM0000872, PM0000874 and PM0000876) are designed to function as a system with the unlocked (PM0000878) and locked (PM0000879) devices for the production and delivery of aerosol to the product user. The applicant describes the new products as the JUUL System. These leachables are expected to be delivered to the product users via the new product JUUL devices PM0000878 and PM0000879. Specifically, the devices (PM0000878 and PM0000879) are responsible for aerosolizing and delivering the e-liquid to the user. HPHCs, other toxic constituents including leachable constituents, and mutagenic and genotoxic constituents within the e-liquid can be transferred into the aerosol via the device. The device functional parameters (i.e., coil temperature, power delivery and maximum puff duration) control and mediate the transfer of these constituents into the mainstream aerosol. As such, the applicant needed to have provided data from a repeated in vivo genotoxicity study using a relevant and justifiable exposure concentration of aerosol from PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3% with PM0000878 or PM0000879 and smoke from the 3R4F combustible cigarette comparison product, or provided scientific data and a rationale to address the conflicting genotoxicity results for PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%) from the in vitro and in vivo genotoxicity study data the applicant provided. Furthermore, the applicant needed to have provided scientific data and a rationale to address the positive in vitro genotoxicity score from the initial genotoxicity assay of PM0000876 (Virginia Tobacco 5%) using 2000 cells. Without this data, the toxicological impacts of the Juul System cannot be fully reviewed by FDA.
- In summary, the genotoxicity data, as provided, indicate that the new products PM0000872, PM0000874 and PM0000876 are potentially more genotoxic than the CC comparator. While there may be other explanations for the unexpected in vitro genotoxicity result for the CC comparator (scored equivocal instead of positive), the data indicates positive genotoxicity results for PM0000872, PM0000874 and PM0000876. This means, based on the applicant-provided data, users of PM0000872, PM0000874 and PM0000876 with the devices PM0000878 or PM0000879 may be at greater risk for genotoxicity relative to users of CC.

The mutagenicity of PM000872 (Menthol 5%) used with the devices PM0000878 and PM0000879

- Data from the in vitro bacterial reverse mutation assay was submitted to identify if a test article is able to induce DNA mutations. This data demonstrated that PM0000872 (Menthol 5%) is mutagenic. The submitted data show that the aerosol condensate produced from PM0000872 (Menthol 5%), using PM0000878 with standard puffing parameters, induced a significant mutagenic response when compared to the historical vehicle control group (Ames assay; n-3-1-1-ames-men-5-rpt-03408-reva-report). The mean (± Standard Deviation) revertant colonies per plate were reported as 50 (12) and 48 (6) at the test article concentrations of 3.13 µL/plate and 6.25 µL/plate, respectively. The corresponding historical vehicle control data for the bacterial reverse mutation assay reports the mean revertant colonies per plate as 15 (6). According to your study guidelines, the criteria for a positive mutagenic response include a three-fold increase in TA98 revertants seen in two or more successive concentrations, or a repeatable response at a single concentration. The submitted data met these criteria for a positive response.
- In response to the deficiency describing the toxicological concerns regarding the mutagenicity of PM0000872 (5% Menthol), the applicant stated that they respectfully disagree with this conclusion. The applicant states that the results indicate the new product has only a 2-fold increase in the mean revertants/plate when compared to the triplicate vehicle concurrent control cultures. The mean (± Standard Deviation) revertant colonies per plate were 25 (3) for the triplicate vehicle concurrent control cultures in the applicant-submitted data. Because the submitted result for the vehicle concurrent control is nearly two standard deviations larger than the corresponding historical vehicle control (i.e., 25 (3) vs. 15 (6)), this indicates that the applicant-provided data for the vehicle control group (i.e., 25 (3)) may not be representative of historical data (i.e., 15 (6)); therefore, comparisons to this control group should be made with caution.
- The JUULpods, including PM0000872 (Menthol 5%), are designed to function as a system with the unlocked (PM0000878) and locked (PM0000879) devices for the production and delivery of aerosol to the product user. The applicant describes the new products as the JUUL System. The devices (PM0000878 and PM0000879) are responsible for aerosolizing and delivering the e-liquid to the user. HPHCs, other toxic constituents including leachable constituents, and mutagenic and genotoxic constituents within the e-liquid can be transferred into the aerosol via the device. The device functional parameters (i.e., coil temperature, power delivery and maximum puff duration) control and mediate the transfer of these constituents into the mainstream aerosol.
- Given the foregoing, the applicant needed to have provided additional data, information or a scientific rationale for PM0000872 as used with the JUUL devices PM0000878 and PM0000879 to demonstrate that these results from the in vitro bacterial reverse mutation assay are not biologically relevant or biologically significant. This could have included data and results from a repeated in vitro bacterial reverse mutation assay, a discussion of the mutations induced within the TA98 strain by test articles and their in vivo relevance, the

anticipated mutagenic and detoxification responses that would occur in vivo following exposure to PM0000872 (Menthol 5%) and how the assay results from PM0000872 (Menthol 5%) can be interpreted in relation to suitable comparison products (i.e., combustible cigarettes). In the absence of this additional information needed to establish the in vivo relevance of this in vitro mutagenicity assay result, the applicant-provided information demonstrate that PM0000872 (Menthol 5%) is mutagenic.

3.4.2. Synthesis

The applicant has not provided sufficient information for FDA to do a complete toxicological evaluation, including a toxicological health risk assessment. Per the toxicology review, the aerosols generated by the new products (PM0000864, PM0000872, PM0000874, and PM0000876) when used with PM0000878 and/or PM0000879 have not been accurately identified and characterized. As noted in the toxicology reviews, data for the new products lack yields in mainstream aerosol for the two leachable constituents first identified by the applicant as EHQC and PHDC, and after subsequent revision through the applicant's response to the Deficiency letter, identified as TCEQ and NNMA, respectively.

The applicant initially identified EHQC and PHDC as having an excess cancer risk outside of generally accepted margins of "tolerable cancer risk" or possessing some mutagenic and carcinogenic potential when inhaled and recognized them as candidate target compounds "to monitor and evaluate in future analyses" of the aerosol from the [applicant's] device. The applicant stated, "With respect to remaining uncertainties, the limited nature of the available data precluded any firm conclusions on the tolerability of two compounds, namely EHQC and [PHDC]." FDA issued a Cycle 1 deficiency for this issue, given the need for evaluation of these compounds during real-time stability testing. FDA requested that the applicant provide additional information regarding the identification of these leachable constituents present in the new products, and the mainstream aerosol yields of phenol[15] during use of the new products.

In the response to this deficiency, as noted above, the applicant revised its initial identification and toxicological risk assessment of the leachable chemicals generated by the JUUL System, identifying EHQC and PHDC now as 1,8,9-trihydro-2-(3-carboxypropylamine-N-yl)-3-ethylcarboxylate-4-quinolone [TCEQ] and Nornicotine, N-carboxyglycerol-5'-(methoxy-1-(p-hydroxybenzene-*O*4-yl-acetic acid)) [NNMA] and providing toxicological risk information relevant to these newly identified chemicals.

This additional information does not adequately address the toxicology concerns regarding the leachable chemicals that were described in the deficiency. The additional information is inadequate as the revised chemical structures, chemical formulas and identifications of two of the leachables in the applicant-provided additional information are incongruous with the originally provided information. The revised identifications of these two leachables are not supported by the conflicting mass spectral data and revised chemical analyses provided by the applicant. See discussion above in section 3.4.1.

[15] The applicant's response to FDA's Cycle 1 Deficiency letter resolved FDA's concerns relating to the HPHC phenol.

Moreover, the applicant provided a revised risk assessment which used an inappropriate approach that likely understates the health risks posed to users of the new products. See discussion in section 3.4.1. FDA's toxicological risk assessment, using the more appropriate approach provided by the applicant in their original submission, found that these leachables, both the original and revised identifications, are present at a level of toxicological concern. Because these leachables, regardless of their original or revised identification, are present at a level of toxicological concern, data showing their concentrations in mainstream aerosol generated by the new products (PM0000864, PM0000872, PM0000874, and PM0000876) when used with PM0000878 and/or PM0000879 is needed for a complete toxicological risk assessment. Data showing the mainstream aerosol yield of these leachables from the new products and a combustible cigarette comparison product were not included in the applicant-provided additional information and are needed to fully assess the toxicological risks posed by exposure to these potentially health hazardous leachables. These unresolved issues have been identified as deficiencies to be conveyed to the applicant (See section 5).

In addition to the two re-identified leachables that raise toxicological concerns and the lack of mainstream aerosol yield data for the leachables, I also agree with the toxicology review that the applicant used inconsistent methodology to carry out in vitro micronucleus assay genotoxicity testing and did not provide an adequate rationale to explain how this departure from their submitted protocol did not adversely impact the ability to correctly identify genotoxic versus non-genotoxic test articles. See discussion in section 3.4.1. above. Therefore, FDA is unable to perform a complete toxicological evaluation and risk assessment of the new products without additional information. This issue remains a toxicology concern and is identified as a deficiency to be conveyed to the applicant (see section 5 of this memorandum).

I also agree with the toxicology review that the applicant has also provided in vitro study data demonstrating that the proposed new product PM0000872 (i.e., Menthol 5%) is mutagenic and that the new products PM0000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) are genotoxic. Data from the in vitro bacterial reverse mutation assay (Ames assay), which evaluates whether compounds are capable of inducing genetic mutations, shows that PM0000872 induced a significant mutagenic response. Data from the in vitro micronucleus assay which evaluates whether compounds are capable of inducing genotoxicity shows that PM0000872, PM0000874 and PM0000876 induced significant genotoxic responses. Although the applicant provided data in an attempt to show that PM0000872 and PM0000874 did not induce genotoxicity in vivo using the micronucleus assay and Comet assay for DNA damage following inhalation exposure to PM0000872 and PM0000874 used with PM0000878, the assay results were highly variable and may not reliably indicate the occurrence of DNA damage. Additionally, no comparison data were provided (e.g., comparison data to the 3R4F CC) for the in vivo study and no bridging data were provided in order to make comparisons between the in vivo and in vitro results. No additional data or information were provided by the applicant to address the positive in vitro genotoxicity response initially reported for PM0000876 (Virginia Tobacco 5%). Notably, the in vitro micronucleus study identified PM0000872, PM0000874 and PM0000876 as potentially more genotoxic than the 3R4F CC comparison product, which induced an equivocal response in the assay. As TPL, I agree with toxicology in that this deficiency remains unresolved, as the negative genotoxicity result from the in vivo study here does not outweigh results from an in vitro study. Differences in study design and

execution can cause changes in the concentrations of HPHCs and other health hazardous constituents, dose-response relationships, toxicokinetics and toxicodynamics, which can produce conflicting results between in vitro and in vivo studies. Despite FDA's request for such information in the Deficiency Letter, the applicant did not provide additional information describing these factors and other bridging information addressing the 3R4F CC comparison product in these studies. Therefore, this issue remains a toxicological concern that may impact individual and population health and is identified as a deficiency to be conveyed to the applicant (See sections 3 and 5 of this memorandum).

To summarize, these toxicology deficiencies preclude an APPH finding because:

- There is an unknown quantity of two genotoxic leachables contained in the mainstream aerosol produced using the JUUL System. The identity of these leachables is currently disputed. A correct identification of these leachables is needed to properly evaluate their potential toxicological properties and determine what their carcinogenic potency, and subsequent carcinogenic risk, is likely to be. While the applicant reports decreased HPHC yields and reduced BOE levels that could potentially be used to offset the risk posed by these genotoxic leachables, the applicant provided no data indicating if, and how much, of these leachables are transferred into mainstream aerosol. With unknown aerosol yields of these leachables and their disputed chemical identities, resulting in an unknown cancer potency and genotoxicity risk, it is not possible to do an offsetting evaluation for carcinogens (where decreased levels of one carcinogen mitigate increased levels of another). FDA cannot draw a definitive conclusion regarding the toxicological risk of the JUUL system, or any of the individual components of that system, without a compete data set containing mainstream aerosol yields for the leachables and a conclusive determination of their chemical identity. Additionally, the applicant did not provide information on the presence of these leachables in comparator products, therefore no information is available to determine if these reported leachables in the new products represent a unique adverse health risk relative to other commercially available CC or ENDS comparator products.
- The genotoxicity data, as provided, is unreliable and the methodology used to produce the data raises significant concerns regarding the quality of the data and accuracy of the results. These concerns prevent a conclusive evaluation of the toxicological risks posed by the new products from being performed. A major public health concern regarding the use of tobacco products is based on carcinogenicity and chronic illnesses that cause irreparable damage to one's health. Therefore, having reliable genotoxicity and toxicological data with conclusive results is critically important to assessing the public health impact of using these new products versus other currently marketed CC or ENDS comparator products.
- The genotoxicity data, as provided, indicate that the new products PM0000872, PM0000874 and PM0000876 used with the devices PM0000878 or PM0000879 are potentially more genotoxic than the CC comparator. While there may be other explanations for the unexpected in vitro genotoxicity result for the CC comparator (scored equivocal instead of positive as expected based on existing literature), the data indicates positive genotoxicity results for PM0000872, PM0000874 and PM0000876, which, on its own, raises toxicological concerns.
- The mutagenicity data, as provided, indicate that the new product PM0000872 used with the devices PM0000878 or PM0000879 is mutagenic. This finding raises

toxicological concerns regarding this product. Adequate information to address these toxicological concerns was not provided by the applicant.

4. ENVIRONMENTAL DECISION

4.1. DISCIPLINE FINDINGS

Environmental science concluded that the environmental assessments for all PMTAs qualified as a type of Categorical Exclusion under 21 CFR 25.35(b) because they may not be introduced or delivered for introduction in interstate commerce. As TPL, I agree with this conclusion.

4.2. ENVIRONMENTAL CONCLUSION

Under 21 CFR 25.35(b), issuance of an order under section 910(c) of the Federal Food, Drug, and Cosmetic Act that a new tobacco product may not be introduced or delivered for introduction into interstate commerce (i.e., a marketing denial order, MDO) falls within a class of actions that are ordinarily categorically excluded from the preparation of an environmental assessment (EA) or environmental impact statement (EIS). To the best of our knowledge, no extraordinary circumstances exist that would preclude application of this categorical exclusion. FDA concludes that categorical exclusion is warranted and no EA or EIS is required.

5. CONCLUSION AND RECOMMENDATION

Under section 910(c)(2)(A) of the FD&C Act, FDA must deny a PMTA if it finds that there is a lack of a showing that permitting the new tobacco product to be marketed would be appropriate for the protection of the public health. Based on the information provided in the applications and, as described in this Technical Project Lead review, I find that the applicant has not demonstrated that permitting the marketing of the new products in the PMTAs listed above would be APPH.

As TPL, I recommend that MDOs be issued for the pods and the devices. The recommendation for the MDO is based on the toxicology deficiencies of the pods (PM0000864, PM0000872, PM0000874 and PM0000876), as well as the devices (PM0000878 and PM0000879) as outlined below in this section and in Section 5.1.

This review finds four deficiencies in these applications. Deficiency 1 relates to two potentially genotoxic leachables and their mainstream aerosol yields in all new products. Deficiency 2 relates to methodological issues impacting the scientific validity of the in vitro micronucleus assay genotoxicity testing carried out by the applicant. Deficiency 3 relates to the genotoxic potential of PM0000872, PM0000874, and PM0000876. Deficiency 4 relates to the mutagenic potential of PM0000872.

5.1. DEFICIENCIES

The following deficiencies should be conveyed to the applicant:

1. **Identity of leachable constituents produced by the Juul System (PM0000864, PM0000872, PM0000874, PM0000876, PM0000878, PM0000879); mainstream aerosol yields of these leachable constituents in the Juul System; issues in the risk assessment provided to assess toxicity of these leachable constituents in the mainstream aerosol yield of the Juul System**

You have not provided proper identification of leachable constituents (leachables) in the new products nor have you provided mainstream aerosol yield data for these leachables generated by the new products (PM0000864, PM0000872, PM0000874, and PM0000876) when used with PM0000878 and/or PM0000879. As a result, FDA cannot perform an accurate and complete risk assessment of the new products.

In your original submission for these PMTAs, you submitted a toxicological evaluation of identified leachables from your new products PM0000864, PM0000872, PM0000874, PM0000876 when used with PM0000878 and/or PM0000879. The devices (PM0000878 and PM0000879) are responsible for aerosolizing and delivering the e-liquid to the user. HPHCs and mutagenic and genotoxic constituents from the JUULpods can be transferred into the mainstream aerosol via the devices. The device functional parameters (i.e., coil temperature, power delivery and maximum puff duration) control and mediate the transfer of these genotoxic leachables into the mainstream aerosol. In this toxicological evaluation, you identified the presence of two genotoxic leachables found to produce an excess cancer risk outside of generally accepted margins of "tolerable cancer risks." These leachables were identified by you as Ethyl-4-hydroxyquinoline-3-carboxylate (EHQC) and Propylpyridine,1H-pyrrole-1-hexanoic acid,2,5-dihydro-2,5-dioxo-related compound) (PHDC). Further, you declined to provide testing results of these leachables in the mainstream aerosol generated under intense and non-intense use of your new tobacco products. Nor did you provide a comparison with similar testing for suitable comparison products.

In response to FDA's Deficiency letter, you revised the identity of the leachable EHQC to 1,8,9-trihydro-2-(3-carboxypropylamine-N-yl)-3-ethylcarboxylate-4-quinolone (TCEQ) and revised the identity of the leachable PHDC to Nornicotine, N-carboxyglycerol-5'-(methoxy-1-(p-hydroxybenzene-O4-yl-acetic acid)) (NNMA). The information you provided to support these revised identifications (revised chemical structures, chemical formulas and mass spectral data) is incompatible with chemical analysis and mass spectral data you previously submitted regarding the identification of these leachables. Additionally, information you provided elsewhere in the response to FDA's Deficiency letter identifies the revised leachable TCEQ as a different chemical, (4-((3-(ethoxycarbonyl)-4-oxo-1,4,6,7-tetrahydroquinolin-2-yl)amino)butanoic acid) (ETBA) (app-17-03-n-3-3-whole-pod-leach-tra-report.pdf; pages 105 and 306). This conflicting data further undermines your revised identification of EHQC as TCEQ. You continued to decline to provide testing results of these leachables in the mainstream aerosol.

In addition, in response to the Deficiency letter, you provided a new risk assessment indicating that these revised leachables are not present at levels of toxicological concern. However, there are two overarching concerns with this submitted risk assessment. First, because you have not established the identity of these revised leachables, the risk assessment that you submitted evaluating these leachables cannot be used to determine the toxicity of the new products. Second, we find significant methodological issues that preclude our consideration of your findings. The risk assessment you provided uses a less conservative approach (Carthew et al., 2009[16]) than what was used in the original risk assessment, (Escher et al., 2010[17].) When the

[16] Carthew P, Clapp C, Gutsell S. Exposure based waiving: The application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. Food and Chemical Toxicology. 2009;47:1287-1295.
[17] Escher SE, Tluczkiewicz I, Batke M, et al. Evaluation of inhalation TTC values with the database RepDose. Regulatory Toxicology and Pharmacology. 2010;58:259-274.

original, more conservative, approach was used, these leachables were found to be present at a level of toxicological concern. In your original risk assessment, you stated that the more conservative risk assessment approach used in Escher et al., 2010 "would appear to be more representative for the extractables and leachables risk assessment" as this risk assessment evaluated industrial chemicals, whereas the risk assessment approach used in Carthew et al., 2009 is based on an evaluation of consumer products ingredients. We agree that the dataset used to develop the Escher et al., 2010 risk assessment approach included industrial chemicals that are likely more representative of the types and classes of chemicals encountered in an assessment of leachables akin to those present in the aerosol yield of the JUUL System. The risk assessment approach used in Carthew et al., 2009 is based on an evaluation of consumer products ingredients, which consists of chemicals intentionally added to products. As the leachable chemicals "leach" from the components of the JUULpods and are not added intentionally to the products as ingredients, the Escher et al., 2010 method is more appropriate than the Carthew et al., 2009 method which is geared towards the risk assessment of added ingredients. Because the Carthew et al., 2009 method specifically focuses on ingredients, it does not have the specificity needed to evaluate the types of leachable chemicals typically studied in a leachables assessment (for example, polymers and heavy metals). Additionally, the dataset used to derive the Carthew et al. 2009 approach and evaluate subsequent health risks does not include chemicals relevant to the assessment of leachables; it specifically excludes genotoxic carcinogens, in vivo mutagens, heavy metals and polymers from the risk assessment, "as they were not considered representative of the ingredients that are, or could be used, in aerosols for consumer use." You demonstrated in the provided mainstream aerosol HPHC yield data for the new products that the aerosol generated using PM0000864, PM0000872, PM0000874, and PM0000876 with PM0000878 contains genotoxic constituents, in vivo mutagens and heavy metals. Therefore, Carthew et al., 2009's less conservative approach used in your revised risk assessment of these leachables is inappropriate for evaluation of the new products, as this approach does not accurately model the potential health risks associated with use of the new products.

You have not explained why the less conservative approach used in Carthew et al., 2009 is appropriate to perform a risk assessment of these revised leachables. Additionally, you did not provide an explanation for why the revised risk assessment was limited to assessing systemic toxicity and did not include local toxic effects, which have a lower threshold for occurrence. Overall, your additional risk assessment has not adequately addressed the toxicology concerns regarding these leachable constituents, and you did not provide an explanation for using a less conservative approach than the approach used in your initial risk assessment.

In order for FDA to perform a full toxicological evaluation of these leachable constituents, the correct identity of these leachables needs to be determined. Accordingly, you needed to have:

a) Provided consistent and non-conflicting information to support your identification of these leachable constituents. These data are needed to ensure that an accurate toxicological risk assessment is performed.
b) Provided a toxicological risk assessment of these identified leachable constituents that used an appropriate methodology consistent with your original approach. This is needed to ensure the health risks associated with use of the new products are accurately evaluated using a reasonably conservative risk assessment methodology.
c) Provided testing results of these two leachable constituents in the mainstream aerosol generated from appropriately aged new JUULpod products (PM0000864, PM0000872,

PM0000874, and PM0000876) with the new JUUL devices (PM0000878 or PM0000879) under intense and non-intense use conditions, and a comparison with similar testing for suitable comparison products. These aged JUULpod products should undergo 1) accelerated aging for 22 weeks at 30°C and relative humidity of 65%, equivalent to 9 months ambient conditions and 2) accelerated aging for 22 weeks at 40°C and relative humidity of 75%, equivalent to 18 months ambient conditions. For example, the new products used to generate the aerosol should have been prepared in the same manner (i.e., the same parameters to simulate shelf aging) as previously done to evaluate leachable constituents. These data are needed to assess the toxicological risks associated with the presence of these leachable constituents in the mainstream aerosol generated from the new product and enable FDA to determine relevant health risks to consumers of the new products.

2. Methodological issues impacting scientific validity of results provided from your in vitro micronucleus assay genotoxicity study comparing the genotoxic potential of the new products to other tobacco products

You submitted in vitro toxicological studies to assess the genotoxic potential for the new products (PM0000864, PM0000872, PM0000874, PM0000876, PM0000878 and PM0000879) in comparison to other tobacco products. These in vitro studies utilized both the e-liquids and aerosol condensates. However, the methodology you used in the assays to evaluate in vitro genotoxicity (i.e., the in vitro micronucleus assay) raises concerns regarding the scientific validity of the assay results due to unjustified deviations from the guidelines that you selected in order to conduct the in vitro micronucleus assay. Specifically there was: (a) uneven application of acceptance criteria (including inconsistent cell counting) for the scoring and evaluation of positive and negative genotoxic responses and (b) use of different methodologies to evaluate the new products and the comparison products.

The devices (PM0000878 and PM0000879) are responsible for aerosolizing and delivering the e-liquid to the user. HPHCs, mutagenic and genotoxic constituents within the e-liquid can be transferred into the aerosol via the device. The device functional parameters mediate and control the delivery of these toxic constituents to the user and are a critical factor in assessing user exposure to genotoxic constituents. This means that the inability to perform a full and accurate toxicological evaluation of the new product e-liquids (PM0000864, PM0000872, PM0000874 and PM0000876) precludes the completion of a full and accurate toxicological evaluation of the JUUL devices (PM0000878 and PM0000879) as these devices play a critical role in the production and delivery of genotoxic constituents to the product user.

(a) Uneven application of acceptance criteria (including inconsistent cell counting) for the scoring and evaluation of positive and negative genotoxic responses for the new products

In your study protocol, you stated that the in vitro micronucleus assay was performed according to Organization for Economic Cooperation and Development (OECD) guidelines as described in OECD Test Guideline 487 (TG 487) for the in vitro micronucleus assay. TG 487 describes the purpose and principles of the assay, the methodology used to conduct the in vitro micronucleus assay, acceptability criteria for the acceptance of assay results, and criteria for the evaluation and interpretation of assay results. The acceptability criteria in TG 487 sets standards needed for accepting the results of the assay. Within the acceptance criteria in TG 487, there is no

requirement to verify a clearly positive or clearly negative response. You initially evaluated the new products for genotoxic potential using 2000 cells per concentration as required by TG 487, but diverged from this required cell count when there was a positive result for genotoxicity. Specifically, if a new product yielded a negative result, this negative result was accepted as final. However, if a positive result was produced for the new product, the assay result was rejected and the new product was re-evaluated using 4000 cells per concentration. Equivocal responses for the new products were further evaluated by counting an additional 2000 cells "to clarify the response" however, you did not provide a rationale or supporting statistical calculations to demonstrate why counting additional cells, in lieu of conducting a repeat or modified experiment, was appropriate and justified. This inconsistent methodology used to evaluate the genotoxic potential of the new products raises concerns as your select re-evaluation of new products yielding positive genotoxicity results caused assay results to be changed to lower risk categories (i.e., negative or equivocal for genotoxicity).

Furthermore, your inconsistent testing methodology and select application of more rigorous criteria for the evaluation of the new products that were found to be positive for genotoxicity is not supported by TG 487 or by your submitted study protocol. Based on these criteria, the positive genotoxicity responses identified for the new products should have been accepted and not subjected to an additional, more rigorous evaluation. We note that in response to this toxicological concern (raised by FDA in Cycle 1, Deficiency 19 of FDA's Deficiency letter), you state that, in specific situations, the number of cells counted and scored for micronuclei formation was increased to establish the biological relevance of the results. However, assessing the biological relevance of the genotoxicity assay results does not require rejecting these valid results and you did not provide a justification or explanation for why these results were rejected. You rejected genotoxicity results for clearly positive responses that you subjectively deemed to be "very weak" or "very small", despite the fact that the responses met all assay acceptance criteria described in TG 487 and your provided study protocol. In lieu of rejecting clearly positive responses for assays conducted in line with the guidelines set out in your study protocol, you could have discussed toxicologically relevant factors (e.g., the presence of detoxification pathways in vivo that are absent within this in vitro model system) that may mitigate the occurrence of toxicity in vivo. However, you did not address such toxicologically relevant factors in your response, or explain how the counting (use) of additional cells and re-evaluation of the assay results is an appropriate method to assess the biological relevance of a positive genotoxicity response. The inconsistent use of assay acceptance criteria resulted in unequal treatment of test articles within the genotoxicity assay, which adversely affects the scientific validity of the assay, thereby preventing accurate and meaningful toxicological conclusions on the genotoxic potential of the new products from being made.

(b) Use of a different methodology to evaluate the comparison products.

Additionally, the data you provided from the in vitro micronucleus assay shows that the new products were not evaluated using the same methodology as the comparison products. The new products, as described above, were evaluated using 2000 cells, or re-evaluated using 4000 cells if there was a equivocal or positive result. In contrast, all the comparison products were evaluated for genotoxic potential using a single assay at 4000 cells per concentration without appropriate justification. Furthermore, you did not count additional cells or further evaluate equivocal responses reported for the comparator products.

The differences in methodology created situations where there may be meaningful differences in statistical power between the genotoxicity assays. Statistical analysis of the assay results is a key component of evaluating the genotoxic potential of a test article, therefore differences in statistical power can directly affect the ability to correctly identify positive or negative genotoxic test articles. As you state in your response, "[s]coring 4,000 cells instead of 2,000 cells may provide greater statistical power to distinguish between a weak positive response and potentially false positive results." However, you did not provide statistical power calculations for your study. This information is needed to demonstrate that the increased counting of cells for select groups is appropriate for the statistical analyses being performed and to show that these modifications do not adversely impact the validity of assay. This is necessary information as the affected statistical analysis is a key deciding factor in determining whether or not the product being evaluated is identified as being positive or negative for genotoxicity. However, even if this additional information describing the statistical power of the analysis was provided, significant concerns regarding inconsistencies in the assay methodology and rejection of valid assay results remain. You provided new data in response to Deficiency 19 of FDA's Deficiency Letter to show that the increased counting of cells, from 2000 to 4000 cells per concentration, identified a similar percentage of micronuclei formation, which indicate similar genotoxic responses. This suggests that sufficient sampling and statistical power were present at 2000 cells per concentration to accurately assess the genotoxic potential of the new and comparison products. Furthermore, you state that "[s]ince all the [in vitro micronucleus] studies were conducted consistent with the OECD TG 487 guidelines, the differences in the number of cells scored for some of the test articles and comparison test samples do not impact the scientific validity of the assay and the ability to correctly identify genotoxic versus non-genotoxic test articles." In contrast to what you stated, there is a conflict with the OECD guidelines as OECD only states the minimum standards to evaluate and score a single test article. OECD guidelines emphasize that when multiple cell cultures are evaluated, the same number of cells from each culture must be scored. The methodology used to evaluate the genotoxic potential of a group of test articles needs to be comparable to generate reliable, valid results and is necessary when making relative comparisons of genotoxic potential between test articles. As demonstrated in your submitted data, your assessment of genotoxic potential for the new products varied significantly as additional cells were counted.

For scientific validity, it is necessary that the new and comparison products are evaluated for genotoxic potential using a consistent methodology to ensure that accurate comparisons are made between the products. For example, from the data you provided, it is unknown if any of the comparison products would have been positive for genotoxicity at 2000 cells per concentration and unknown if the new products that were found to be negative at 2000 cells per concentration would have produced a positive response at 4000 cells per concentration. You did not provide an adequate scientific justification or explanation for why the differences in methodology for the in vitro genotoxicity evaluation of the new products and the comparison products does not impact the scientific validity of the assay. The inability to adequately compare the in vitro genotoxicity assay results between the new and comparison products prevents a complete and accurate toxicological evaluation of the new products.

In summary, the unequal treatment of test articles is demonstrated in your data by the acceptance of negative genotoxicity responses for the new products without further investigation while positive genotoxicity responses were further evaluated using more rigorous criteria (i.e., rejecting "very weak" or "very small" responses). Additionally, the unequal

treatment of new and comparison products within your in vitro genotoxicity assay and your rejection of valid assay results prevents FDA from performing a conclusive toxicological evaluation of the new products.

Accordingly, in order for FDA to evaluate the genotoxicity of the Juul System you needed to have:

a) Specifically, addressed how the differences in assay methodology do not impact the scientific validity of the assay or cause differences in the ability to correctly identify genotoxic versus non-genotoxic test articles, or
b) Provided data comparing the genotoxic potential of the new products and the comparison products using a consistent methodology.

This information is needed to ensure that an accurate and complete toxicological evaluation of the new products can be conducted, that the different cell counting and scoring methodologies you used did not prevent the identification of genotoxic compounds, and that scientifically valid comparisons are made between the new and comparison products.

3. **Regarding the Genotoxic Potential of PM0000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) used with the devices PM0000878 and PM0000879:**

You submitted data from the in vitro micronucleus assay (n-3-1-2-micronuc-men-5-rpt-03420reva-report.pdf, n-3-1-2-micronuc-vt-3-rpt-03425reva.report.pdf, n-3-1-2-micronuc-vt-5-rpt-03399reva-report.pdf) to demonstrate that PM0000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) do not induce genotoxic responses, and that PM0000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) are relatively less genotoxic than a combustible cigarette comparison product. However, your data demonstrate that PM0000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) induced clearly positive genotoxic responses under the initial conditions of analyzing 2000 cells per concentration used in this assay. You did not provide an adequate rationale or justification to address why these initial genotoxicity assay results from valid assays were rejected. The 3R4F combustible cigarette comparison product was found to be equivocal for genotoxicity. These results indicate that PM0000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) with PM0000878 and PM0000879 may be relatively more genotoxic than the combustible cigarette comparison product.

In an attempt to address this positive genotoxicity result for PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%), you provided data from an in vivo genotoxicity study. You did not provide additional information or in vivo study data to further evaluate the genotoxicity of PM0000876 (Virginia Tobacco 5%). The in vivo study evaluated the potential for PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%) to induce DNA damage in vivo, assessed using the Comet assay, and to induce genotoxicity in vivo, assessed using the micronucleus assay. The data you provided in the DNA damage/Comet assay indicated that PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%) had negative responses for both induction of DNA damage and genotoxicity in vivo. However, the results were highly variable and may not

reliably indicate the occurrence of DNA damage.[1818] Additionally, the in vivo study did not include a combustible cigarette comparison product; therefore, no comparisons of genotoxic potential between PM0000872 (Menthol 5%) or PM0000874 (Virginia Tobacco 3%) and a combustible cigarette can be made using your provided in vivo data. The inclusion of a combustible cigarette comparison product within the in vivo genotoxicity study is needed to perform a complete toxicological evaluation of PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%), as your in vitro genotoxicity study indicated PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%) with PM0000878 and PM0000879 may be more genotoxic than the 3R4F combustible cigarette comparison product. The relative genotoxicity of PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%) with PM0000878 and PM0000879 versus a combustible cigarette comparison product needs to be conclusively addressed in order to perform a complete toxicological evaluation of PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%).

It is not scientifically sufficient or adequate to accept the negative genotoxicity results from the in vivo genotoxicity study without an explanation or justification for why the positive in vitro genotoxicity results should be considered biologically insignificant or irrelevant. Differences in the design and execution of in vitro and in vivo studies can cause changes in the concentrations of HPHCs and other health hazardous constituents within the test system (i.e., cell culture or animal model), which will affect dose-response relationships, tissue level exposure to hazardous constituents, toxicokinetics and toxicodynamics. Differences in the biological system used to evaluate genotoxicity in vitro and in vivo (i.e. availability of detoxification pathways, occurrence of bioactivation, contributions of gender and/or species-specific effects) may also contribute to producing conflicting results between in vitro and in vivo studies. You did not provide a justification or explanation addressing these toxicologically relevant factors and how they pertain to the conflicting genotoxicity results of PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%) with PM0000878 and PM0000879 in your additional information. A comparison of the in vitro and in vivo studies using additional information or bridging data from scientific literature is needed to put the positive in vitro genotoxicity result for PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%) with PM0000878 and PM0000879 in the context of the in vivo biological system.

Therefore, you needed to have:

a) Provided data from a repeated in vivo genotoxicity study using a relevant and justifiable exposure concentration of aerosol from PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%) with PM0000878 and/or PM0000879 and smoke from the 3R4F combustible cigarette comparison product. Or,
b) Provided scientific data and a rationale to address the conflicting genotoxicity results for PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%) from the in vitro and in vivo genotoxicity study data you provided. These data can include measurements of HPHCs and other chemical constituents from PM0000872 (Menthol 5%) and PM0000874 (Virginia Tobacco 3%) with PM0000878 and the 3R4F combustible cigarette comparison product to compare the cell and tissue levels of potentially hazardous constituents between the positive in vitro and negative in vivo genotoxicity studies, an assessment of relevant pharmacokinetic/toxicokinetic parameters, detoxification

[18] While relatively large standard deviations are commonly observed in the DNA damage/Comet assays, the high variability of the data can compromise the statistical analysis and can limit the conclusions that can be drawn (Langie et al., 2015).

mechanisms, metabolic and bioactivation pathways, and/or an evaluation of dose-response relationships for relevant HPHCs and other health hazardous constituents that may be present within the in vitro and in vivo genotoxicity assays.

c) Provided scientific data and a rationale to address the positive in vitro genotoxicity score from the initial genotoxicity assay of PM0000876 (Virginia Tobacco 5%) using 2000 cells. This assay met the acceptance criteria listed in your study protocol, however, you rejected the positive genotoxicity result and re-evaluated PM0000876 (Virginia Tobacco 5%). You did not provide an adequate rationale or justification to address why these initial genotoxicity assay results from valid assays were rejected. You also did not provide an explanation as to why this rejection of valid assay results does not adversely impact the scientific validity of the assay.

This additional information is needed to evaluate the biological significance and relevance of the positive in vitro genotoxicity result for PM0000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) with PM0000878 and PM0000879. The devices (PM0000878 and PM0000879) are responsible for aerosolizing and delivering the e-liquid to the user. HPHCs, mutagenic and genotoxic constituents within the e-liquid can be transferred into the aerosol via the device. The device functional parameters mediate and control the delivery of these toxic constituents to the user and are a critical factor in evaluating the genotoxicity of the new products. This information is also needed to assess the risk of genotoxicity associated with use of PM0000872 (Menthol 5%), PM0000874 (Virginia Tobacco 3%) and PM0000876 (Virginia Tobacco 5%) with PM0000878 and PM0000879, which received positive results for in vitro genotoxicity, relative to the 3R4F combustible cigarette comparison, which received an equivocal result for in vitro genotoxicity and was not included within the in vivo genotoxicity study.

4. Regarding the Mutagenic Potential of PM0000872 (Menthol 5%) used with the devices PM0000878 and PM0000879:

You submitted data from the in vitro bacterial reverse mutation assay (Ames assay; n-3-1-1-ames-men-5-rpt-03408-reva-report) to identify if a test article is able to induce DNA mutations. Your submitted data show that the aerosol condensate produced from PM0000872 (Menthol 5%) using the devices PM0000878 and PM0000879, using standard puffing parameters, induced a significant mutagenic response when compared to the historical vehicle control group. According to your study guidelines, the criteria for a positive mutagenic response include a three-fold increase in TA98 revertants seen in two or more successive concentrations, or a repeatable response at a single concentration. Your data submitted indicates that PM0000872 (Menthol 5%) used with the devices PM0000878 and PM0000879 induced a mutagenic response. The devices (PM0000878 and PM0000879) are responsible for aerosolizing and delivering the e-liquid to the user. HPHCs, mutagenic and genotoxic constituents within the e-liquid can be transferred into the aerosol via the device. The device functional parameters mediate and control the delivery of these toxic constituents to the user and are a critical factor in evaluating the mutagenicity and genotoxicity of the new products. Thus, the information set out below is needed to assess the risk of mutagenicity associated with use of PM0000872 (Menthol 5%), used with the devices PM0000878 and PM0000879.

You also needed to have:

a) Provided additional data, information or a scientific rationale for PM0000872 as used with the JUUL devices PM0000878 and PM0000879 to demonstrate that these results from the in vitro bacterial reverse mutation assay are not biologically relevant or biologically significant. This could have included data and results from a repeated in vitro bacterial reverse mutation assay, a discussion of the mutations induced within the TA98 strain by test articles and their in vivo relevance, the anticipated mutagenic and detoxification responses that would occur in vivo following exposure to PM0000872 (Menthol 5%) and how the assay results from PM0000872 (Menthol 5%) can be interpreted in relation to suitable comparison products (i.e., combustible cigarettes). This additional information is needed to assess the risk of mutagenicity posed by PM0000872 (Menthol 5%) and will establish the in vivo relevance of this in vitro mutagenicity assay result.

6. APPENDIX

Table 2. New Products Subject to Denial Order

Common Attributes of PMTAs[19]	
Submission date	July 29, 2020
Receipt date	July 29,2020
Applicant	JUUL Labs Inc.
Product manufacturer	JUUL Labs Inc.
Product category	ENDS (VAPES)
Attributes	**New Product**
STN	**PM0000864**
Product name	JUULpods (Menthol 3.0%)[20]
Product subcategory	Closed E-Liquid
Package type	Cartridge
Package quantity	1 Cartridge
Characterizing flavor	Menthol
Nicotine concentration	0.7 mL
E-liquid volume	3.0%
PG/VG ratio	30/70
Additional property	Blister Pack
STN	**PM0000872**
Product name	JUULpods (Menthol 5.0%)[20]
Product subcategory	Closed E-Liquid
Package type	Cartridge
Package quantity	1 Cartridge
Characterizing flavor	Menthol
Nicotine concentration	0.7 mL
E-liquid volume	5.0%
PG/VG ratio	30/70
Additional property	Blister Pack
STN	**PM0000874**
Product name	JUULpods (Virginia Tobacco 3.0%)[20]
Product subcategory	Closed E-Liquid
Package type	Cartridge
Package quantity	1 Cartridge
Characterizing flavor	Tobacco
Nicotine concentration	0.7 mL
E-liquid volume	3.0%
PG/VG ratio	30/70
Additional property	Blister Pack

[19] We interpret package type to mean container closure system and package quantity to mean product quantity within the container closure system, unless otherwise identified.

[20] Brand/sub-brand or other commercial name used in commercial distribution.

STN	PM0000876
Product name	JUULpods (Virginia Tobacco 5.0%)[20]
Product subcategory	Closed E-Liquid
Package type	Cartridge
Package quantity	1 Cartridge
Characterizing flavor	Tobacco
Nicotine concentration	0.7 mL
E-liquid volume	5.0%
PG/VG ratio	30/70
Additional property	Blister Pack
STN	**PM0000878**
Product name	JUUL Device
Product subcategory	Closed E-Cigarette
Package type	Box
Package quantity	1 ENDS Device
Characterizing flavor	None
Length	[redacted]
Diameter[21]	N/A
Wattage	[redacted]
Battery Capacity	200 mAh
E-liquid volume	0.7 mL
Nicotine concentration	N/A
PG/VG ratio	N/A
Additional Properties	Width: [redacted] Depth: [redacted] Color: Slate Universal Serial Bus (USB) Charging Dock

[21] Applicant provided depth as an alternative for diameter given the product proportions.

STN	PM0000879
Product name	JUUL Locked Device
Product subcategory	Closed E-Cigarette
Package type	Box
Package quantity	1 ENDS Device
Characterizing flavor	None
Length	[redacted]
Diameter[21]	N/A
Wattage	[redacted]
Battery Capacity	256 mAh
E-liquid volume	0.7 mL
Nicotine concentration	N/A
PG/VG ratio	N/A
Additional Properties	Width: [redacted] Depth: [redacted] Color: Slate Universal Serial Bus (USB) Charging Dock

Table 3. Amendments Received

Submission Date	Receipt Date	Amendment	Applications being amended	Reviewed	Brief Description
November 30, 2020	November 30, 2020	PM0004306	All STNs	Yes	Response to November 9, 2020 Inspection Request Letter
June 22, 2021	June 22, 2021	PM0004760	All STNs	Yes	Response to March 26, 2021 Deficiency letter