



**Technical Memorandum**

**Technical Review and Analysis  
of FDA Report:  
“Evaluation of e-cigarettes”**





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### **Technical Review and Analysis of FDA Report: “Evaluation of e-cigarettes”**

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## Summary

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A recent report and press release issued by the Food and Drug Administration (FDA) assert that NJOY e-cigarette cartridges contain harmful levels of carcinogens and toxic chemicals. It was further implied that these chemicals are not found in other FDA-approved nicotine-containing products. Subsequently, a technical review and scientific analysis of the FDA's report was performed by scientists at Exponent. Results of the review identified the following limitations:

- ❖ The report failed to present standard protocols for proper study design with regards to the testing of the referenced control device, documenting the number of samples tested either within or across tests, or presenting statistical analyses when quantifiable results were obtained.
- ❖ The chemical content of similar nicotine-containing FDA-approved products was not completely described with respect to the presence of tobacco-specific nitrosamines (TSNAs) and other tobacco-associated impurities that have also been found in nicotine replacement therapy (NRT) devices at similar, if not higher, levels.
- ❖ In the lots that were tested by the FDA, none of the key chemicals of concern in this study such as TSNAs and tobacco-associated impurities were able to be quantifiably measured in the liquid of NJOY's cartridges because they were all below the limits of quantification (LOQ).
- ❖ All of the tobacco-associated impurities found in the NJOY products were **“present but at less than the level of the Nicotrol® inhaler [manufacturer] specification”** according to the FDA report.
- ❖ There is no indication in the published scientific literature that cotinine or  $\beta$ -nicotyrine are carcinogenic or have toxicity ratings of concern. These were the only tobacco-associated impurities found in trace levels in the vapor phase of (some of) NJOY's products.
- ❖ The report does not reflect the actual dose of nicotine delivered to the user from the “control” Nicotrol® inhaler device when used as recommended by the manufacturer (6–16 cartridges/day or 24–64 mg of nicotine, 50 mcg/100 mL puff). By comparison, NJOY devices delivered 46 mcg/100 mL in the highest-strength cartridge tested, according to the FDA report.
- ❖ Data presented in the report does not adequately support the opinion that users of NJOY products would actually be exposed to TSNAs and tobacco-specific impurities in the vapor phase during normal device use; and if exposed, that those levels would be a health concern as compared to other FDA-approved products.

In summary, the report “Evaluation of e-cigarettes” suffers from several limitations, that taken together result in it failing to adequately support the FDA claims of potential adverse health consequences from the use of NJOY e-cigarette products tested as compared to other FDA-approved nicotine containing products.

## Background

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On July 22<sup>nd</sup>, 2009 the Food and Drug Administration (FDA) issued a press release warning of the use of e-cigarettes based on results from their laboratory tests that showed detectable levels of “*carcinogens and toxic chemicals*” in products from two leading e-cigarette manufacturers: NJOY and Smoke Everywhere (FDA 2009). Specifically, it was noted that diethylene glycol was found in one Smoke Everywhere cartridge tested, and that tobacco-specific nitrosamines (TSNAs), and tobacco-associated metabolites or “*impurities*” were found in both manufacturer product lines. Additionally, the FDA reported that one e-cigarette product made by NJOY did not deliver consistent amounts of nicotine between similarly-labeled cartridges, and that more nicotine was delivered from the “high” cartridge as compared to the Nicotrol® 10 mg inhaler, a FDA-approved nicotine containing device.

Upon learning of the FDA report, NJOY contracted Exponent’s Health Science practice to perform an independent third-party review and analysis of FDA’s experimental methods and scientific findings. This review was performed with the intent of evaluating whether or not FDA’s findings show a clear indication of health risks to the users of NJOY’s products as compared to users of FDA currently-approved nicotine delivery products such as the Nicotrol® inhaler and Nicorette® gum.

## Review and Analysis of FDA's Report

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### Study Design

The FDA tested for the presence of key chemicals in the liquid contained in the whole cartridges, and in small aliquots of the vapor generated from the cartridges by two separate techniques. Analyte detection techniques included gas chromatography followed by mass spectrometry (GC/MS)<sup>1</sup>, liquid chromatography followed by mass spectrometry (LC/MS), and high performance liquid chromatography with ultraviolet analyte detection (HPLC/UV). With respect to NJOY's products, four types of cartridges were tested: (1) menthol high, (2) menthol medium, (3) regular medium, and (4) regular low. Not all cartridges were tested in each analysis. Nicotine and its major metabolite, cotinine, were the only chemicals able to be quantifiably measured by any of the experimental techniques used in the analysis.

In the whole cartridge liquid, the FDA assayed for nicotine, four major TSNA's [N-nitrosonicotine (NNN), N-nitrosoanabasine (NAB), N-nitrosoanatabine (NAT) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)]; tobacco-specific impurities [(cotinine, nicotine-N-oxide, nornicotine, anatabine, anabasine, pseudooxynicotine, myosmine,  $\beta$ -nicotyrine, and 1-methyl-3-nicotinoylpyrrolidine (MNP)]; and diethylene glycol (DEG).

In the vapor phase, the FDA assayed for nicotine and tobacco-specific impurities using both the sparging apparatus and a heated head space vapor method that was meant to simulate the temperatures achieved by the electronic cigarette devices.

The FDA-approved Nicotrol® inhaler was used as a "control" for some test methods.

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<sup>1</sup> GC/MS - A method that combines gas-liquid chromatography (the separation and analysis of compounds that can be vaporized) and mass spectrometry (a technique to determine the elemental composition of a molecule) to identify and estimate the concentration of substances within a test sample.

## Results

### Whole Cartridge: Tobacco-Specific Nitrosamines by LC-MS/MS

All four TSNAs were detected in all four of the NJOY cartridges tested by whole cartridge liquid analysis. As indicated by Table 1 legend “A” the detection was below the limit of quantification (LOQ) for each of the TSNAs: NAB (LOQ = 21 ppb); NAT (LOQ = 21 ppb); NNK (LOQ = 75 ppb); NNN (LOQ = 24 ppb). The limit of detection (LOD) for these chemicals using their techniques was not given. All values for the NJOY cartridges were found to be below the specified limit of quantification given above. The Nicotrol® inhaler “control” was not tested for the presence of these compounds.

### Whole Cartridge: Tobacco-Specific Impurities by GC-MS and GC-MS/MS<sup>2</sup>

Liquid in whole cartridges was screened for possible tobacco-specific impurities: cotinine, nicotine-N-oxide, nornicotine, anatabine, anabasine, pseudooxynicotine, myosmine,  $\beta$ -nicotyrine, and 1-methyl-3-nicotinoylpyrrolidine (MNP). Nicotine-N-oxide, nornicotine, anatabine, pseudooxynicotine, and MNP were not detected in any of the NJOY samples. Cotinine, anabasine, myosmine, and  $\beta$ -nicotyrine were detected in whole cartridge liquid analysis of all four NJOY cartridges tested. However, none of these detection levels were quantified. The level of quantification was not provided for these chemicals in the table legend. The limit of detection (LOD) for cotinine, anabasine, myosmine, and  $\beta$ -nicotyrine was 20 ppb, 10 ppb, 69 ppb, and 170 ppb, respectively.

Additionally, the Table 1 legend “B” indicated that the levels of cotinine, anabasine, myosmine, and  $\beta$ -nicotyrine found in the liquid of the NJOY cartridges were “present, but at less than the level of the Nicotrol® inhaler specification.” The specification was listed as not more than (NMT) 0.5% for three of these compounds: cotinine, myosmine, and  $\beta$ -nicotyrine. Based upon reference to these manufacturing specifications, it is not clear whether or not the Nicotrol®

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<sup>2</sup> GC-MS/MS – This technique is performed by adding a second phase of mass fragmentation. Tandem mass spectrometry (MS/MS) is a more powerful technique that offers a higher sensitivity and selectivity for target compound analysis found in very low levels.

inhaler device was actually tested for the presence of these compounds or whether the manufacturer's specifications were relied upon as a valid source of quantification.

### **Whole Cartridge: Nicotine content by HPLC-UV**

Nicotine levels were measured in the liquid of all four NJOY cartridges by two different extraction techniques. Values were similar using each technique. The highest amount of nicotine detected from the NJOY menthol high cartridge was 6.76 mg. This is the amount available for extraction from an 18 mg NJOY nicotine cartridge using the FDA techniques as stated in the report. The amount of nicotine extractable from the Nicotrol® device was apparently not tested. Manufacturer's inserts for this product claim that 4 mg of nicotine is extractable from a 10 mg inhalant cartridge.

### **Simulated Use: Nicotine and Tobacco-Specific Impurities by Head Space GC-MS (HSGC-MS) and Sparging Apparatus**

$\beta$ -nicotyrine and cotinine were the only tobacco impurities (other than nicotine) detected by both of the vapor analyses. Two NJOY cartridges were tested in the sparging vapor analyses; menthol high and menthol medium. "Trace" amounts of cotinine were found in the menthol high cartridge. This designation "trace" is not defined. Cotinine was not detected in the vapor of the menthol medium NJOY cartridge.  $\beta$ -nicotyrine was detected, but not quantified, in the vapor of three NJOY cartridges tested using the head space technique; menthol high, menthol low, and regular high. Nicotine levels were found to vary between three different menthol high cartridges tested by the sparging technique. Quantified nicotine levels of the three menthol high cartridges were 43.2, 34.9, and 26.8 mcg/100 mL puff. Also, the menthol medium cartridge was found to have 10.6 mcg nicotine/100 mL puff. The Nicotrol® inhaler device was described to have 15.2 mcg/100 mL puff; however, there was no mention in the methods section of the report of the Nicotrol® inhaler being tested with the same apparatus as the e-cigarette. The LOQs and LODs were not specified for either of the vapor phase techniques or for any of the impurities. Use of standards to establish LODs and LOQs was also not discussed. Additionally, there was no mention of testing for any of the TSNAs in the vapor phase analyses of these same products.

## Whole Cartridge: Diethylene Glycol and Ethylene Glycol by GC/MS

All four NJOY cartridges were found negative for the presence of diethylene glycol.

## Discussion

### Tobacco-Specific Nitrosamines

TSNAs are common constituents of tobacco extracts and have been reported in scientific studies to be present in FDA-approved nicotine replacement therapy (NRT) devices. TSNAs are also normal constituents of conventional cigarette smoke. The FDA reported finding non-quantifiable but detectable levels of these compounds in the liquid of NJOY's cartridges but did not test for the presence of these compounds in the vapor phase. The levels of these compounds in the NJOY cartridges were below the LOQs listed for these compounds (21–75 parts per billion). However, in the methods section of the FDA report, the sensitivity of this assay is described as being sensitive to 40 parts per trillion (Wu et al. 2008). Further, the report states the results of the testing were quantifiable in this methods section. “The assumption was made that recovery of TSNAs from the E-cigarette cartridge assembly was as good as that published by Wu, et al., and quantitative”. Elsewhere in the report (Table 1), it states that the results were not quantifiable and were below the limit of quantitation. It is difficult to know, due to the conflicting statements, but it may be that 40 parts per trillion is actually the limit of detection in this assay. If this is the case, then the values of the TSNAs obtained by the FDA may be as low as parts per trillion and as high as just below the limits of quantitation listed in the report; ppb.

For comparison purposes, the levels of these compounds in mainstream and sidestream smoke from conventional cigarettes is on the order of parts per million (Hoffman et al. 1978). In Hoffman's study, the tobacco of 5 different cigarettes contained between 0.22 and 7.0 ppm ( $\mu\text{g/g}$ ) of the carcinogenic NNN, 0.13 and 0.74 ppm of the carcinogenic NNK and 0.44–3.2 ppm of NAB.

The levels of the carcinogen NNN in Nicorette® 4 mg gum are approximately 2.0 ppb ( $\text{ng/g}$ ) dry gum weight. The carcinogen NNK was found in the NicoDerm CQ® 4 mg patch at levels

of 8 ppb (ng/g) dry patch weight (Stepanov et al. 2006). Osterdahl et al. found up to 380 ppb (ng/g) of TSNA per gram of Nicorette® chewing gum (Osterdahl et al. 1990). The researchers also detected the presence of these compounds in the saliva of Nicorette users, confirming that these substances were being passed into the saliva of users of this product. All the saliva samples obtained during Nicorette® chewing contained NNN and NAT/NAB at levels ranging from 0.42 to 19 ppb (ng/g) and from 1.3 to 46 ppb (ng/g), respectively. No TSNA could be detected in saliva samples collected just before Nicorette® chewing. The only NRT found not to contain TSNA that was tested was the Commit® 2mg lozenge (Stepanov et al. 2006). The Nicotrol® inhaler was not tested by either group above, or by the FDA for the presence of TSNA in the liquid or the vapor (aerosol).

TSNAs were also not tested for in the vapor of NJOY's products. It is the vapor that the users of these products are exposed to, not the liquid. Nitrosamines are known to volatilize poorly into the vapor phase (Hoffman et al. 1979). Therefore, it is speculative as to whether they are actually bioavailable to the user of e-cigarette through normal use of the device. In contrast, FDA-approved NRT products, Nicorette and NicoDerm CQ, do not volatilize their products for delivery so it is quite possible, and indeed shown in the case of Nicorette gum, that users may be receiving measurable amounts of these compounds through normal use of these products (Osterdahl et al. 1990). NJOY is not aware of any testing that indicates that TSNA are detected in the vapor phase of their products. Based on the results of the FDA analysis of the whole cartridge liquid, if there is any TSNA in the vapor phase of NJOY products, the levels would be predicted to be on the order of less than the LOQ for each substance.

### **Tobacco-Specific Impurities**

Cotinine, anabasine, myosmine, and  $\beta$ -nicotyrine were each detected in whole cartridge liquid analysis of all four NJOY cartridges tested, but at levels lower than those found in the Nicotrol® inhaler device and below the manufacturer specifications for the devices. Table 1 legend "B" indicated that the levels of cotinine, anabasine, myosmine, and  $\beta$ -nicotyrine when the GC-MS/MS technique was applied were "present but at less than the level of the Nicotrol® inhaler specification." Furthermore, in the Nicotrol® inhaler control sample, cotinine, myosmine, and

$\beta$ -nicotyrine were detected individually at not more than (NMT) 0.5%. The limit of quantification was not provided for these compounds.

It is not clear whether or not the liquid in the Nicotrol® device was tested for the presence of these compounds or whether the manufacturer's specifications were relied upon as a valid source of quantification. Due to the lack of numerical values reported it seems that the manufacturing specifications may have been used as a possible source of the quantitation of levels of impurities in the Nicotrol® inhaler device.

$\beta$ -nicotyrine and cotinine were the only tobacco impurities (other than nicotine) detected in NJOY cartridges by both of the vapor analyses. Information in the available scientific literature regarding these two compounds indicates that neither have been found to be carcinogenic or particularly toxic by standard [Ames] tests. Cotinine is a weak metabolite of nicotine and has been shown to lack mutagenic or carcinogenic potential through several published Ames Toxicity assays. Other studies have shown that the activity of cotinine is several hundred-fold less than nicotine in behavioral studies using rats (HSDB 2005). One in-vitro study of  $\beta$ -nicotyrine has shown that it can inhibit the class of enzymes responsible for the metabolism of nicotine to cotinine, thus slowing the metabolism of nicotine (Denton et al. 2004), but it currently has no established toxicity profile.

## **Nicotine**

Nicotine levels were measured in the liquid of all four NJOY cartridges by two different extraction techniques. Values were similar using each technique. The highest amount of nicotine detected from the NJOY "menthol high" cartridge was 6.76 mg. This is the amount available for extraction from an 18 mg NJOY nicotine cartridge according to the FDA report, Table 1. The amount of nicotine extractable from the Nicotrol® device using these techniques was not tested in this report. Manufacturer's package inserts for this product claim that 4 mg of nicotine is deliverable from a 10 mg inhalant cartridge.

Nicotine levels were found to vary between three different menthol high cartridges tested by the sparging technique. Quantified nicotine levels of the three menthol high cartridges were 43.2,

34.9, and 26.8 mcg/100 mL puff. The menthol medium cartridge was found to have 10.6 mcg nicotine/100 mL puff. The Nicotrol® inhaler device was described to deliver 15.2 mcg/100 mL puff. There was no mention in the methods section of the report, however, of the Nicotrol® inhaler being tested with the same apparatus as the e-cigarette.

For comparison of nicotine delivered to the user by an FDA approved device, the Nicotrol® inhaler inhalation package comes to the user as 168 10 mg cartridges. Recommended dosage is 6–16 cartridges daily. The manufacturer recommends an intensive inhalation regimen (80 deep inhalations over 20 minutes), which releases on the average 4 mg of the nicotine content of each cartridge. If each breath is approximately 100 cc or 100 mL in volume as is typically assumed, then 4 mg/80 (100 mL) breaths would yield 0.05 mg nicotine/puff or 50 mcg per puff, which is actually more than the highest amount of nicotine delivered by the NJOY menthol high cartridge according to FDA tests. Note that the maximum recommended daily dose of the Nicotrol® inhaler is 40 mg. For the 10 mg inhaler, recommended dosing is 6–16 cartridges a day. Thus, if 4 of the 10 mg is delivered, as is stated in the packet insert, then the use of 16 cartridges (the maximum recommended dose) would yield 64 mg of nicotine per day.

## **Study Design and Presentation of Results**

One of the weaknesses in the FDA study was the lack of study controls. The FDA-approved Nicotrol® inhaler was presented as a “control” for their studies, but was only used for some of the experiments. The device was never tested for the presence of the same “carcinogens and toxic chemicals” that were found in some of the e-cigarette cartridges. When it was indicated that the Nicotrol® inhaler device did contain some levels of tobacco associated impurities, it was never discussed in the report or even mentioned in the results section. These tobacco-specific impurities in the Nicotrol® inhaler were also not discussed in comparison to the values obtained for NJOY’s or Smoke Everywhere’s products. With respect to the TSNAs, the report did not mention that these substances are found in nicotine gum, the patch, nasal spray, and lozenges in concentrations that are at the very least similar to, or higher than those found in the NJOY cartridges (Stepanov et al. 2006; Osterdahl et al. 2004). Specific levels were difficult to approximate from the non-quantitative “detect” and “non-detect” results shown in the report

because LODs were given in the absence of LOQs and vice versa. Therefore, with respect to TSNAs, it is possible that the values are as low as parts per trillion and as high as parts per billion.

Information was not presented regarding standards and quality control parameters. Limited information was given on the number of cartridges tested for each experiment within a group and the number of times each cartridge was tested within an experiment. Furthermore, no statistical analysis was presented.

Key carcinogenic and toxic chemicals that were found in the liquid phase were not examined in the vapor phase. From a human health exposure perspective, it would have been beneficial to determine if the same chemical constituents found in the liquid phase analysis were also found in the vapor phase, especially considering that the vapor phase is the pathway of exposure to the e-cigarette user.

## **Concluding Statement**

The detection of trace and non-measurable levels of TSNAs and tobacco-associated impurities in the liquid, rather than the vapor phase of NJOY's products, at levels that are many orders of magnitude below conventional cigarettes, and at or below FDA-approved nicotine containing products, should be considered as indicators of the regulatory acceptability of the NJOY products rather than reason for concern. When considering the relative potential health risks posed by these trace levels, it is worth noting that the approved NRTs, which have been shown to contain these substances, were not judged to contain levels sufficient to warrant toxicity information or reference to these substances in their own product literature.

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