



Study to Determine Presence of TSNAs in NJOY Vapor

December 9, 2009

Ben Thomas Group, LLC
11200 Westheimer Rd. Suite 900
Houston, Texas 77042
(832) 814-6789 Cell
(713) 243-8871 Fax

Tobacco-Specific Nitrosamines (TSNAs) in NJOY Electronic Cigarettes

Report to:

Scottera, Inc. dba NJOY

December 9, 2009

NJOY, Inc. has requested Dr. Ben Thomas (President and Principal of Ben Thomas Group, LLC) to review the report issued by ANALYZE, Inc. concerning the levels of common tobacco-specific nitrosamines (TSNAs) that might be associated with the electronic cigarettes marketed by the NJOY company. NJOY also requested my assessment of the risk, if any, posed by the TSNAs to users of NJOY electronic cigarettes. This report summarizes my review and evaluation. Based on the findings of the ANALYZE chemists, little or no TSNAs are present in the aerosol ("smoke") to which a consumer would be exposed. Moreover, based on our review of the toxicology information relating to TSNA, we conclude that TSNAs do not raise health concerns from use of the NJOY product. The following comments are pertinent:

1. Professional Background

Appended for your information is a copy of my resume. I am an expert on health and environmental issues, with over 35 years of professional experience. I received my B.S. degree in biology from Tulane University, and my M.S. and Ph.D. degrees in pathology from the University of Texas Graduate School of Biomedical Sciences at Houston (at the M.D. Anderson Cancer Center). I was subsequently named a Rosalie B. Hite Postdoctoral Fellow in biochemistry at M.D. Anderson, where I investigated the mechanisms of toxicity and carcinogenicity.

Following my postdoctoral training, I accepted a position as a toxicologist with the Shell Oil Company, where I was responsible for the toxicological issues associated with oil products, aromatics, olefins, solvents, metals, radiation, synfuels, and other products and processes. I represented Shell in various industrial trade associations, and chaired the Toxicology Committee of the American Petroleum Institute (API), the API Benzene Toxicology Task Force; the API Neurotoxicity Task Force; the Butadiene Toxicology Research Task Group of the Chemical Manufacturers Association (now the American Chemical Council); among others.

In 1990, I joined the consulting industry, and have worked in that capacity since that time. I am appointed at the rank of Adjunct Professor to the faculty of the University of Texas Health Science Center at Houston, where I teach in the areas of toxicology, pathology, and health risk. In addition to my consulting and teaching activities, I am the Chief Operating Officer of CleanBlue Water LLC, a company dedicated to providing reliable and safe drinking water to small communities around the world.

2. Overview of Issue

NJOY Inc. is the distributor of a line of electronic cigarettes that use a microelectronic heating element to vaporize a small amount of nicotine dissolved in propylene glycol, glycerol and water. As these vapors cool, they condense to form a fine aerosol (tiny droplets of liquid suspended in the air) that visually resembles smoke and are inhaled by the user.

In July 2009, the US Food and Drug Administration (FDA) announced that an analysis of the ingredients of two leading brands of electronic cigarettes had detected known carcinogens and toxic chemicals to which users could potentially be exposed. The toxicants cited included four TSNAs – N-nitrosornicotine (NNN); N-nitrosoanabasine (NAB); N-nitrosoanatabine (NAT); and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).

Because of the FDA findings, NJOY retained [REDACTED] to evaluate the TSNA content of the NJOY electronic cigarettes. [REDACTED] retained ANALYZE to collect the appropriate samples and conduct the analytical studies.

I have been asked by NJOY to evaluate the data from ANALYZE, and to consider the health implications of the TSNAs to the users of the NJOY products.

3. Technical Approach

This analysis is based on the information contained in the ANALYZE report, and on the available published scientific literature relating to the toxicity of TSNAs as identified through the PUBMED database of the U.S. National Library of Medicine.

In particular, I have critically evaluated the sampling and analytical methods developed and used by the ANALYZE chemists in order to confirm that the reported data are of a quality to allow me to draw valid and supportable conclusions. For purposes of health evaluation, only data related to TSNAs in generated aerosols (i.e., the material to which a user would be exposed) is considered here. It should be noted that no TSNAs were detected in the liquid cartridges by ANALYZE, although they suggested that propylene glycol or other constituent present in the liquid might be inhibiting their analysis

Similarly, I have also critically evaluated the relevant toxicological studies to assess what is known and reported about the health effects of TSNAs.

4. ANALYZE Report

Using a method based on that used by the FDA (Westenberger 2009), ANALYZE collected samples of the aerosols generated from four types of NJOY cartridges – Traditional Light; Traditional Ultra Light; Menthol Regular; and Menthol Light. In order to accurately measure air flow rates, ANALYZE forced breathing quality compressed air through the front end (battery end) of the electronic cigarette at a nominal flow of 3.5 SCFH. The aerosol was generated in 3-second “puffs” and was directed through a series of three capture flasks, each containing methylene chloride to dissolve any TSNA from the aerosol into the solvent. Studies using reference TSNA standards indicated that recoveries using this sampling method were 75% or greater (note: these recoveries are acceptable for this method),

The TSNA content of the aerosol samples (collected in methylene chloride) was determined by drying the methylene chloride under nitrogen, then redissolving the residue into a small amount of ammonium acetate. The TSNA in the ammonium acetate solution were then analyzed by a Liquid Chromatography – Mass Spectrometry/Mass Spectrometry (LC-MS/MS) method as described by Wu et al. (2008). Studies with reference TSNA standards determined the Limit of Detection (LOD) for each TSNA in the aerosol to be approximately 1.2 – 1.5 ng/L.

It should be emphasized that the LOD is determined empirically by the laboratory, and is defined as “the minimum concentration at which a compound can be detected reliably.” The LOD is not the same as a Quantitation Limit (defined as “the minimum concentration at which a compound can be quantified reliably”). The Quantitation Limit is often ten-times the LOD or more – that is, in order to be reliably quantified, a TSNA would have to be present in the aerosol at a concentration of 12 – 15 ng/L or higher.

The studies conducted by ANALYZE were technically appropriate and appear to have been well done with regard to data quality and the conclusions that they reach. They sampled the aerosols generated by the NJOY electronic cigarettes, and tested for the presence of NNN, NAB, NAT, and NNK. Of the four TSNA, only NAT was present in the collected aerosols. ANALYZE's best estimate was that the concentration of NAT in the aerosol samples were 2 – 5 ng/L (i.e., detectable, but not reliably quantifiable). NNN, NAB and NNK were not detected in the NJOY aerosols (i.e., technically, below the LOD; although the lack of demonstrable peaks in the chromatograms argues that these TSNA are totally absent in the NJOY products).

5. Toxicology of NAT

A review was conducted of the published scientific literature relating to the toxicity of TSNA, as identified through the PUBMED database of the U.S.

National Library of Medicine. The toxic and carcinogenic potential of NAT and other TSNAs was evaluated in the rat by Hoffman et al. (1984). This was the only relevant study identified in the published literature that evaluated the systemic toxicity and carcinogenicity of NAT following long-term exposure. In the study, 60 subcutaneous injections of NAT (total doses of 1, 3 and 9 mmol/kg) did not produce any change of body weight, nor any decreased survival in treated rats. In marked contrast to NNN and NNK, NAT did not produce tumors in any tissue (i.e., NAT was not toxic and not carcinogenic in this bioassay).

Comprehensive reviews of the toxicology of TSNAs have been compiled by Hecht (1998) and by the International Agency for Research on Cancer (IARC 2008).

6. Summary and Conclusion

In summary, of the four TSNAs evaluated by ANALYZE, only NAT was detected at low levels in the aerosol samples from the NJOY electronic cigarettes. NAT was tested by Hoffman et al. (1984) and was shown to be non-toxic and non-carcinogenic in rats receiving a combined subcutaneous dose of up to 9 mmol/kg. Based on the above, there is no evidence that carcinogenic TSNAs are present in the aerosol from NJOY electronic cigarettes. Thus, it is my conclusion that the TSNAs do not pose a health risk to the users of the electronic cigarettes distributed by NJOY.



Ben Thomas, Ph.D.
December 11, 2009

References:

- ANALYZE, Inc. Materials Characterization Report. (Submitted to [REDACTED], October 21, 2009).
- Hecht SS (1998). Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. Chem Res Toxicol 11(6): 559-603.
- Hoffman D, Rivenson A, Amin S & Hecht SS (1984). Dose-response study of the carcinogenicity of tobacco-specific N-nitrosamines in F344 rats. J Cancer Res Clin Oncol 108: 81-86.
- International Agency for Research on Cancer (2008). Some tobacco-specific nitrosamines. IARC Monograph Series 89: 421-583.

US Food and Drug Administration (22 July 2009). FDA News Release – FDA and Public Health Experts Warn About Electronic Cigarettes.

Westenberger BJ (2009). CDER/OPS/OTR, Division of Pharmaceutical Analysis, FDA. [cited by ANALYZE]

Wu J, Joza P, Sharifi M, Rickert WS & Lauterbach JH (2008). Anal Chem 80: 1341-1345. [cited by ANALYZE]

Ben Thomas, Ph.D.

11200 Westheimer Rd, Suite 900

Houston, Texas 77042

[bthomas@benthomasgroup.com]

C: (832)-814-6789

F: (713)-243-8871

Professional Profile

Dr. Ben Thomas brings more than 35 years of professional experience in the fields of toxicology, pathology, risk mitigation, regulatory negotiation, litigation support, strategic planning, program development, and program management. Ben received his bachelor degree in biology (chemistry minor) from Tulane University, and his master and doctoral degrees in pathology from the University of Texas Graduate School of Biomedical Sciences at Houston. He was named a Rosalie B. Hite Postdoctoral Fellow at the University of Texas M.D. Anderson Cancer Center, where he conducted research on the mechanisms of chemical toxicity and carcinogenicity. Dr. Thomas worked as a toxicologist for 12 ½ years with the Health, Safety & Environment department of Shell Oil Company. During that time, he was active in national and international health and environmental research programs, and at one point was overseeing more than \$40 million in biomedical research. He chaired the Toxicology Committee of the American Petroleum Institute (API), the API Benzene Toxicology Task Force, the API Gasoline in Groundwater Task Force, the Toxicology Research Task Group on 1,3-Butadiene of the Chemical Manufacturers Association (now the American Chemical Council), and other industrial research panels. Ben joined the consulting industry in 1990, and is internationally recognized for his health and environmental expertise. He is well respected in the regulatory community, and was appointed as a member of the Science Advisory Panel of the National Urban Air Toxics Research Center (NUATRC), that was created by the Clean Air Act Amendments of 1990. In addition to his consulting and corporate work, Dr. Thomas holds an academic appointment as Professor (adjunct) at the University of Texas Health Science Center at Houston.

Credentials and Professional Honors

Ph.D., Pathology, University of Texas Health Science Center at Houston, 1973

M.S., Pathology, University of Texas Health Science Center at Houston, 1971

B.S., Biology, Tulane University, 1969

Rosalie B. Hite Postdoctoral Fellow, University of Texas M.D. Anderson Hospital & Tumor Institute, Houston (1974–1977); Sigma Xi

Employment History

President, Ben Thomas Group, LLC, 2009-Present
Senior Managing Scientist, ██████████, 2005-2009
Principal and Vice President, RAM Group, 2003–2005
Senior Scientist, Conestoga-Rovers & Associates/RAM Group, 2001–2003
Principal and Vice President, RAM Group, 1999–2001
Adjunct Professor (Toxicology/Risk Assessment), University of Texas Health Science Center, 1996–present
Principal and Executive Vice President; Compliance Solutions, Inc., 1995–1999
Principal/Senior Science Advisor; ENVIRON Corporation, 1993–1995
Regional Program Manager/Director of Toxicology and Risk Management, ENSR Consulting & Engineering, 1990–1993
Staff Toxicologist; Shell Oil Company; Health, Safety & Environment, 1977–1990

Publications

- Saraf S, Thomas B (2007). Biodiesel: a feedstock quandary. *Hydrocarbon Processing* 2007(9): 132-134.
- Saraf S, Thomas B (2007). Influence of feedstock and process chemistry on biodiesel quality. *Transactions of the Institution of Chemical Engineering: Part B, Biofuel Special Issue* 85: 360–364.
- Zheng N, Thomas B. Causal relationships between occupational and environmental exposures and injury: general causation vs. specific causation. *J Environ Occup Med* 2005; 22:181–183. [Chinese].
- Cox LA, Jr, Thomas FB. A generic PBPK modeling tool for rapidly developing PBPK models. *Toxicologist* 1992; 12(1):188 (Abstract 684).
- Thomas FB, Halder CA, Holdsworth CE, Cockrell BY. Hydrocarbon nephropathy in male rats. Part II. Temporal and morphologic characterization of the renal lesions. In: *Proc. Symposium on Hydrocarbon Toxicity*, University of Surrey, Surrey, England, 1984.
- Thomas FB, Thomas DM, Shewach DS, Furlong NB. Substrate specificity of aryl hydrocarbon (benzo(a)pyrene) hydroxylase. pp. 669–683. In: *Polynuclear Aromatic Hydrocarbons, 3rd International Symposium on Chemistry and Biology—Carcinogenesis and Mutagenesis*. Jones PW, Leber P (eds.), 1979.
- Thomas FB, Furlong NB. A simple radioassay of benzo[a]pyrene activation: Observations on the covalent interactions of benzo[a]pyrene with protein. *Anal Biochem* 1976; 72:546–551.

Tessmer CF, Hrgovcic M, Thomas FB, Wilbur J, Mumford DM. Long-term serum copper studies in acute leukemia in children. In: *The Year Book of Cancer 1974*. Clark RL Cumley RS (eds.), 1974.

Furlong NB, Thomas FB, Mintz CG. A probable mechanism for apurinic acid inhibition of DNA synthesis. *FEBS Letters* 1974; 41:256–258.

Tessmer CF, Hrgovcic M, Thomas FB, Fuller LM, Castro JR. Serum copper as an index of tumor response to radiotherapy. *Radiology* 1973; 106–112.

Hrgovcic M, Tessmer CF, Thomas FB, Fuller LM, Gamble JF, Shullenberger CC. Significance of serum copper levels in adult patients with Hodgkin's disease. *Cancer* 1973; 31:1337–1345.

Tessmer CF, Krohn W, Johnston D, Thomas FB, Hrgovcic M, Brown BW. Serum copper in children (6–12 years): An age correction factor. *Am J Clin Pathol* 1973; 60:870–878.

Hrgovcic M, Tessmer CF, Thomas FB, Ong PS, Gamble JF, Shullenberger CC. Serum copper observations in patients with malignant lymphoma. *Cancer* 1973; 32:1512–1514.

Thomas FB. Copper binding proteins in normal human serum and in Hodgkin's disease. Dissertation, The University of Texas Health Science Center at Houston, Graduate School of Biomedical Sciences, 1973.

Tessmer CF, Hrgovcic M, Brown BW, Wilbur J, Thomas FB. Serum copper correlations with bone marrow. *Cancer* 1972; 30:293–297.

Tessmer CF, Hrgovcic M, Thomas FB, Wilbur JR, Mumford DM. Long-term serum copper studies in acute leukemia in children. *Cancer* 1972; 30:358–365.

Thomas FB. Serum copper relation to estrogen administration: A comparative study. Thesis, The University of Texas Health Science Center at Houston, The Graduate School of Biomedical Sciences, 1971.

Pienta RJ, Tessmer CF, Thomas FB. Effect of murine oncogenic viruses on serum copper levels. *Cancer Res* 1969; 10:69 (Abstract).

Book Chapters

Thomas FB, Simpson BJ. Application of short-term assays by the petroleum industry to identify skin carcinogens. pp. 393–399. In: *Skin Carcinogenesis: Mechanisms and Human Relevance*. Slaga TJ, Klein-Szanto AJP, Boutwell RK, Stevenson DE, Spitzer HL, D'Motto B (eds.), 1989.

Thomas FB. Toxicological effects of benzene. pp. 7–15. In: *Benzene in Florida Groundwater: An Assessment of the Significance to Human Health*. American Petroleum Institute, Washington, DC, 1986.

Sussman RM, Thomas FB, Irons RD. Risk assessment issues presented by 1,3-butadiene. pp. 177-214. In: *Risk Assessment in the Chemical Industry*. Chemical Manufacturers Association, Washington, DC, 1985.

Hrgovcic M, Tessmer CF, Brown BW, Wilbur JR, Mumford DM, Thomas FB, Shullenberger CC, Taylor HG. Serum copper studies in the lymphomas and acute leukemias. *Progr Clin Cancer* 1973; 5:121–153.

Presentations

Saraf S & Thomas B. Biodiesel: current trends and opportunities. Invited paper, presented at the South Texas Section of American Institute of Chemical Engineers (AIChE), Houston, TX, May 3, 2007.

Thomas B. Health risks associated with PFOA. Invited paper, presented at the Mealey's PFOA/C-8 Science, Risk, and Litigation Conference, Philadelphia, PA, October 24, 2005.

Zheng N, Thomas B. Does aluminum welding fume cause clinically significant pneumoconiosis and lung cancer? – An analysis of specific causation. Poster presented at the 44th Annual Meeting of the Society of Toxicology, New Orleans, LA, 2005.

Bobst S, Zheng N, Thomas B. Real world toxicology: A framework for evaluating tort claims in the courtroom. Poster presented at the 44th Annual Meeting of the Society of Toxicology, New Orleans, LA, 2005.

Zheng N, Thomas B. Causal relationships between occupational and environmental chemical exposures and diseases in toxic tort cases. Presented, 3rd International Academic Conference on Environmental and Occupational Medicine, Shanghai, China, 2004. [Chinese].

Thomas B. MTBE and benzene toxicology. Panel discussion with Scott R and Mehlman M at Mealey's MTBE & UST Litigation Conference; Marina del Rey, CA, November 4, 2002.

Zheng N, Thomas B. Development of generic soil and groundwater cleanup standards for sodium chlorate. Invited paper, 3rd National Conference of the Chinese Society of Toxicology, Beijing, China, 2001. [Also presented to Fudan University and Beijing University School of Public Health] [Chinese].

Thomas B. A toxicologist's perspective of MTBE. Invited paper, presented at the Petroleum Marketing Attorneys Meeting, Washington, DC, April 4, 2000.

Thomas B. Toxicology of methyl tertiary-butyl ether (MTBE) – an update. Invited paper, presented at Mealey's UST and MTBE Litigation Conference, Amelia Island, FL, November 16, 1999.

Thomas B. Evaluation of health issues associated with E&P wastes. Invited paper, presented at the Louisiana Gulf Coast Oil Exposition, Lafayette, LA, October 29, 1999.

Thomas B. MTBE – toxicology and the use of animal data to prove causality. Invited paper, presented to the 2nd Annual Appellate Judges and Lawyers Symposium: Scientific Methodology and the Admissibility of Expert Testimony, The University of Kansas, Law and Organizational Economic Center, Lawrence, KS, May 13–15, 1999.

Thomas B. Kekulé's devils and E&P waste. Invited paper, presented at the Society of Petroleum Engineers, Evangeline Section, Environmental Issues Forum, Lafayette, LA, February 22, 1999.

Thomas B. Toxicological issues in the chemical processing industry. Invited paper; presented at the 1st Annual Symposium of the Mary Kay O'Connor Process Safety Center, George Bush Presidential Conference Center; College Station, TX, May 30–31, 1998.

Thomas B. The toxicology of methyl tertiary-butyl ether (MTBE). Invited paper, presented at Mealey's Underground Storage Tank Conference, Amelia Island, FL, June 9, 1998.

Thomas B. The toxicological significance of chemicals in water supplies (or what is clean water). Invited paper, presented at the ELA Seminar on New Ground-Water Supply Issues, Houston, TX, April 16, 1998.

Thomas B, Handley B. Offsite consequence analysis: the public connection. Invited paper, presented at the Hazard Assessment/Offsite Consequence Analysis Session, Petro-Safe 98, Houston, TX, January 28, 1998.

Thomas B, Handley B. Risk-based corrective action. Training course for the TNRCC certification of Corrective Action Project Managers. Texas Natural Resource Conservation Commission, contract through the Texas A&M Engineering Extension Service, 1997.

Thomas FB, Plunkett L, Libicki SB & Kappleman WB. The comprehensive assessment of risks due to emissions from hazardous waste incinerators. Training course for the Louisiana Department of Environmental Quality, Baton Rouge, LA, June 26–28, 1995.

Thomas B, Plunkett L, Wojciak J. Strategic approaches to implementing the Texas Water Commission's Risk Reduction Rules. Presented at the ENVIRON Workshop on the Risk Reduction Rules, Houston, TX, June 17, 1993.

Thomas B. Risk characterization. Invited paper, presented to the Association for the Environmental Health of Soils, Houston, TX, May 12, 1993.

Thomas B, Thompson R, Lu C. The risk-based remediation of total petroleum hydrocarbon contamination of soils. Invited paper, presented at Petro-Safe '93, Houston, TX, January 27, 1993.

Cox LA, Jr., Thomas FB, Woodrow JO. Decisions with unknown consequences: a random valuation model. Presented at the Annual Meeting of the Society for Risk Analysis, San Diego, CA, December 9, 1992.

Thompson RA, Woodrow JO, Thomas FB. Risk based prioritization of remediation options. Presented at the Annual Meeting of the Society for Risk Analysis, San Diego, CA, December 7, 1992.

Thomas B, Brassow C. The TWC Risk Reduction Rules and remediation of soil contamination. Presented at How to Classify and Clean up or Dispose of Solid Waste, sponsored by Texas Environmental Education Services, Houston, TX, October 16, 1992.

Thomas FB. Multi-media risk assessment. Invited paper, presented to the Association for the Environmental Health of Soils, Houston, TX, July 1992.

Thomas FB. Risk assessment and the Clean Air Act Amendments. Presented at the ENSR Breakfast Seminar, Houston, TX, May 2, 1991.

Thomas FB. The evolving Material Safety Data Sheet. Invited paper, presented to the Dallas Bar Association, Environmental Law Section, Dallas, TX, March 28, 1991.

Thomas FB. Multi-media risk assessment. Invited paper, presented at the Bridgestone/Firestone Environmental Affairs Domestic Conference, May 8, Nashville, TN, 1991.

Thomas FB. Application of toxicology, epidemiology and industrial hygiene. Invited paper, presented at the "Toxic Tort Litigation" Course sponsored by the Continuing Legal Education Committee of the Houston Bar Association, October 11, Houston, TX, 1991.

Thomas FB. The science and art of risk assessment. Invited paper, presented at Marathon Oil Company's Health, Environment and Safety Conference, Houston, TX, October 10, 1990.

Thomas FB. Science vs. compliance: the argument for QA's involvement in science. Invited paper, presented at the Annual Meeting of the Society of Quality Assurance, Orlando, FL, October 3, 1990.

Thomas FB. The toxicology of 1,3-butadiene. Invited paper, presented to the American Petroleum Institute Toxicology Committee, Toronto, Ontario, September 2, 1990.

Thomas FB. Air toxics impacts on human health and the ecology. Presented at Air Toxic Compliance Conference, Executive Enterprises, Inc., Houston, TX, June 11–12, 1990.

Thomas FB. Toxicological overview of ethylene oxide, butadiene, gasoline, polyolefin manufacturing, and composites. Invited paper, presented at the American Occupational Health Association (AOHA) Conference, Houston, TX, May 3, 1990.

Von Burg R, Lakin M, Thomas B, Egan B. Public interest group use of your SARA 313 data. Invited paper, presented at the Annual Meeting of the National Petroleum Refiners Association, San Antonio, TX, March 25–27, 1990.

Thomas FB, Hulse M. Compounds in asphalt cement fumes and their health effects. Invited paper, presented to the International Society for Asphalt Pavements, Baltimore, MD, November 9, 1989.

Thomas FB. Neurotoxicology. Invited paper, presented to the Gulf Coast Section of the American Industrial Hygiene Association, Houston, TX, March 8, 1984.

Thomas FB. Neurotoxicology. Invited paper, presented to the Deep South Section of the American Industrial Hygiene Association, New Orleans, LA, October 31, 1983.

Thomas FB. Evaluation of the neurotoxic potential of hexacarbon solvent mixtures in the Sprague-Dawley rat. Invited paper, presented to the Mid-West Regional Chapter of the Society of Toxicology, Chicago, IL, May 12, 1983.

Lington AW, Lewis SC, Thomas FB, Granville GC, Cragg ST, Spencer PS. The neurotoxic activity of commercial hexane mixtures in the male rat (Part I). Presented to the Annual Meeting of the Society of Toxicology, 1983.

Lington AW, Lewis SC, Thomas FB, Granville GC, Cragg ST, Spencer PS. The neurotoxic activity of commercial hexane mixtures in the male rat (Part II). Presented to the Annual Meeting of the Society of Toxicology, 1983.

Thomas FB. Applications of Sephadex G-200 chromatography to the study of the copper binding components of human serum. Presented to the Southeastern Texas Section of the American Chemical Society, Houston, TX, 1974.

Mintz CG, Thomas FB, Furlong NB. Approaches to the in-vitro synthesis of ara-C apurinates. Presented to the Southwest Section of the American Association for Cancer Research, November 8-9, New Orleans, LA, 1974.

Thomas FB, Furlong NB. Biochemical studies on the role of DNA polymerase in benzo[a]pyrene carcinogenesis. Presented to the Southwest Section of the American Association for Cancer Research, November 8-9. New Orleans, LA, 1974.

Thomas FB. Atomic absorption spectrophotometry in the clinical laboratory. Presented to the Annual Convention of the Texas Society of Medical Technologists, Houston, TX, May 12, 1972.

Academic Appointments

- Professor (Adjunct Faculty), University of Texas Health Science Center at Houston, and The University of Texas M.D. Anderson Cancer Center at Houston (1996-Present)

Research Experience

- Chairman, Toxicology Work Group, Asphalt Institute (1989–1990)
- Chairman, Toxicology Committee, American Petroleum Institute (1987–1989)
- North American representative to the Butadiene Steering Committee, International Institute of Synthetic Rubber Producers (IISRP) (1986–1990)
- Chairman, 1,3-Butadiene Toxicology Research Task Group, Chemical Manufacturers Association (now American Chemical Council) (1985–1990)
- Chairman, Neurotoxicology Task Force (PS-29), American Petroleum Institute (1980–1985)
- Chairman, Benzene Toxicology Task Force (PS-7), American Petroleum Institute (1977–1990)

Science Advisory Boards/Panels

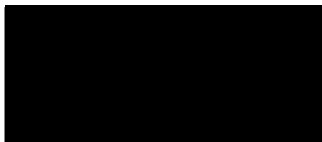
- Science Advisory Board, National Urban Air Toxics Research Center (1991–1993)

MATERIALS CHARACTERIZATION REPORT

Report No.: 0910.14

Date: October 21, 2009

Customer:



Customer P.O.: 0900923.000 E0T0

Samples: Njoy Smokeless Cigarette Solution Cartridges:

- Traditional Light Mfg Date June 2009
- Traditional Ultra Light Mfg Date June 2009
- Menthol Regular Mfg Date June 2009
- Menthol Light Mfg Date June 2009

Nicotrol[®] Inhaler (Pharmacia & Upjohn Co)

- BDC 0009-5400-01 LB024A 01/2012

Objective: The objective of the proposed study is to detect and quantify any *Tobacco Specific Nitrosoamines* (TSNA's) in the liquid formulated product and vapor phase produced by Njoy e-cigarette units. The compounds of interest are those reported in the FDA study (Westenberger – May 4, 2009)¹: N-nitrosonicotine (NNN), N-nitrosoanabasine (NAB), N-nitrosoanatabine (NAT) and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK). Using the same vapor phase capture technique, the TSNA content of the vapor produced by the Nicotrol[®] Inhaler was examined for comparison.

Please visit us at our web site www.analyzeinc.com

Tel: 480.814.8200
Fax: 480.814.8201
www.analyzeinc.com

318 South Brassfield Lane • Chandler, Arizona 85224

ANALYZE
THE MATERIALS CHARACTERIZATION EXPERTS

SUMMARY

1. The concentrations of the four target TSNA compounds (NNN, NAB, NAT and NNK) were measured in the liquid phase and the vapor phase generated from four Njoy cartridge products (Traditional Light, Traditional Ultra Light, Menthol Regular and Menthol Light). In addition, the TSNA concentration in the vapor phase from a Nicotrol[®] Inhaler was determined for comparison.
2. The LC-MS/MS analytical method to assay TSNAs employed for this study was adapted from that reported by J. Wu, P. Joza, M. Sharifi, W.S. Rickert and J.H. Lauterbach in *Anal. Chem.*, **2008**, *80*, 1341-1345.
3. The vapor capture method employed for this study was adapted from that reported by the FDA to trap nicotine and related compounds released from activation of e-cigarettes [B.J. Westenberger, CDER/OPS/OTR, Division of Pharmaceutical Analysis, FDA, May 4, 2009].
4. The limit of detection for the four TSNAs in the chromatography mobile phase is ca. 0.5 ppb.
5. No TSNAs were detected in the liquid extraction of one cartridge analyzed from each of the four Njoy sample types. The limits of detection were <55 ppb in solution. There is a significant matrix effect which affects the chromatography and mass spectroscopy. This matrix effect effectively limits the amount of propylene glycol and glycerol in the analysis solution and results in a further dilution of the TSNA concentration with concomitant rise in LOD.
6. All four target TSNA compounds (NNN, NAB, NAT and NNK) were observed to travel with vapor produced by a simulated Njoy matrix when placed in the filament assembly. It can be concluded from this result that any TSNAs present in Njoy solutions have the potential to be transferred to a second location through activation of the Njoy unit.

7. One of the four TSNA compounds, NAT, was observed in the vapor produced from each of the four Njoy products analyzed in this study. The concentrations of this compound in the vapor (expressed as nanograms per liter) are listed in Summary Table I.

Summary Table I – NAT Concentration in Sample Vapor

Sample ID	Concentration NAT in Vapor (ng/L)		
	Trial 1	Trial 2	Avg.
Traditional Light	2.7	2.3	2.5
Traditional Ultra Light	7.3	2.8	5
Menthol Regular	1.1	2.4	2
Menthol Light	4.0	2.4	3
Nicotrol [®] Inhaler	---	0.9	0.9*

Further method development is required to validate the results from the Nicotrol[®] Inhaler listed here.

8. NNN, NAB and NNK were not detected in the vapor collected from the Njoy sample solutions. The limits of detection for these compounds are <2 ng/L in vapor, which translates to <30 ppb in the sample solution. It should be noted that these LOD values were obtained from a simulated sample matrix and not from an actual Njoy solution. While these values are likely similar to that of the Njoy products, compositional variations between the simulated matrix and the Njoy product may give rise to differences in these concentrations.
9. In addition to NAT, very low intensity signals associated with NAB and NNN (in one of two trials) were detected from the Nicotrol[®] Inhaler unit. The magnitudes of these signals were below the values required for reliable integration and quantitation. The capture method used to acquire all results from the Nicotrol[®] Inhaler vapor was optimized for the Njoy unit. Since there many differences between the two delivery systems, further method development is likely needed to optimize the capture system for the Nicotrol[®] Inhaler and validate the data obtained from this device.

10. A number of general considerations to this study are listed here:

- For the vapor capture from all Njoy samples, it was noted that the intensity of the signal from the deuterated internal standard compounds was considerably lower in capture flask 1 than in flasks 2 and 3. Likewise, several cases occurred when the NAT signal intensity was greater in flask 2 than in flask 1. These results strongly suggest that a suppression effect occurred in flask 1 which is likely due to the higher concentrations of other sample matrix components; e.g., propylene glycol. However, the comparison of analyte signal to internal standard signal, which was also suppressed, should compensate for this effect.
- For several of the sample trials, the NAT signal was detected in capture flask three. While the total intensity of this signal did not exceed 16% in all cases but one, there is a possibility that some NAT escaped from the vapor capture apparatus. This fact, combined with the 78% recovery of NAT in the evaporation study, necessitates the allowance of ca. 35% error for all NAT values.
- The ability to concentrate the capture solution volume by allowing the volatile methylene chloride solvent to evaporate results in the low levels of TSNA that can be detected in the vapor phase experiments. However, due to the 75 – 83% recoveries in the evaporation study for the other three TSNA target compounds, an adjustment of +17-25% for the TSNA LODs may be warranted.
- At the conclusion of all sample analyses, the Njoy units were still able to generate visible vapor. Therefore, the sample cartridges were not exhausted. All TSNA concentrations were based on the initial five liters of vapor produced by the unit. It is unknown if the TSNA concentration in the vapor produced by the Njoy units is consistent over time or if the amount of TSNA entrained in the vapor changes as a function of usage.
- The volume of vapor collected and used in all calculations should be considered an estimate. Each 'puff' was performed manually with some variation (perhaps \pm 10-20%) in total vapor volume inevitably exists between samplings.

INTRODUCTION

The Njoy units (battery and filament apparatus) and solution cartridges were received from M Neilson in August, 2009:

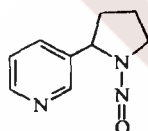
- Traditional Light Mfg Date June 2009
- Traditional Ultra Light Mfg Date June 2009
- Menthol Regular Mfg Date June 2009
- Menthol Light Mfg Date June 2009

One Nicotrol[®] Inhaler System containing 168 cartridges and 5 mouth pieces was also received:

- NDC 0009-5400-01 LB024A 01/2012

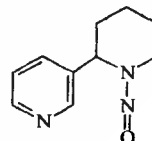
The objective of the proposed study is to detect and quantify any *Tobacco Specific Nitrosoamines* (TSNAs) in the vapor phase produced by Njoy ecigarette and Nicotrol[®] units. The compounds of interest are those reported in the FDA study (Westenberger – May 4, 2009)¹: N-nitrosocotinine (NNN), N-nitrosoanabasine (NAB), N-nitrosoanatabine (NAT) and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK).

N-Nitrosocotinine (CAS 80508-23-2)



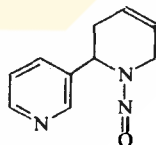
$C_9H_{11}N_3O$
177.20
177.090212
C 61.0% H 6.3% N 23.7% O 9.0%

N-Nitrosoanabasine (CAS 1133-64-8)



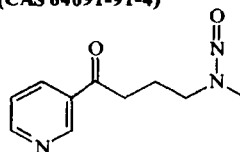
$C_{10}H_{13}N_3O$
191.23
191.105862
C 62.8% H 6.9% N 22.0% O 8.4%

N-Nitrosoanatabine (CAS 71267-22-6)



$C_{10}H_{11}N_3O$
189.21
189.090212
C 63.5% H 5.9% N 22.2% O 8.5%

4-(Methylnitrosamino)- 1-(3-pyridyl)-1-butanone (CAS 64091-91-4)



$C_{10}H_{13}N_3O_2$
207.23
207.100776
C 58.0% H 6.3% N 20.3% O 15.4%

Standards used for analyte identification and quantitation were obtained from Toronto Research Chemicals, Inc. and Sigma Aldrich. All standards used are listed here:

<u>Standard</u>	<u>Supplier</u>
• N'-Nitrosonornicotine Cat# N535000 Lot# 7-MDB-87-1	Toronto Research Chemicals
• 4-(methylnitrosoamine)-1-(3-pyridinyl)-1butanone Cat# 78013-10MG Lot# 0001435887	Sigma Aldrich
• N-Nitrosoanabasine Cat# N524250 Lot# 3-RSA-80-2	Toronto Research Chemicals
• N-Nitrosoanabasine (d4) Cat# N524252 Lot# 4-ELZ-86-2	Toronto Research Chemicals
• N-Nitrosoanatabine Cat# N524750 Lot# 8-MDB-106-1	Toronto Research Chemicals
• N'-Nitrosonornicotine (d4) Cat# N535002 Lot# 7-MDB-62-1	Toronto Research Chemicals

Method Development

Instrumental Method Development

The LC-MS/MS analytical method to assay TSNA's employed for this study was adapted from that reported by J. Wu, P. Joza, M. Sharifi, W.S. Rickert and J.H. Lauterbach in *Anal. Chem.*, **2008**, *80*, 1341-1345.

Analyte Detection. The capability of the Varian 1200 tandem quadrupole (with an intermediate collision cell) mass spectrometer to detect the four target TSNA compounds and the two deuterated TSNA internal standard compounds was verified by infusing standard solutions containing each analyte directly into the mass spectrometer. The standard solutions were made by diluting stock solutions (made in acetonitrile) with the intended capture solvent: 100 mM ammonium acetate. Resulting standard solution concentrations ranged between 500 – 250 ppb of target analyte.

Electrospray Ionization (ESI) was used for solvent introduction; the solutions were thusly monitored for signals with mass/ charge ratios (m/z) corresponding to the protonated target molecules. In all cases, signals with m/z values corresponding to the target analyte were detected, a positive result for instrument sensitivity to each compound. Full scan mass spectra from the direct infusion of the standard solutions are displayed in Figures 1a-c:

Figure 1a – ESI Direct Infusion Mass Spectra of NNN and NAB

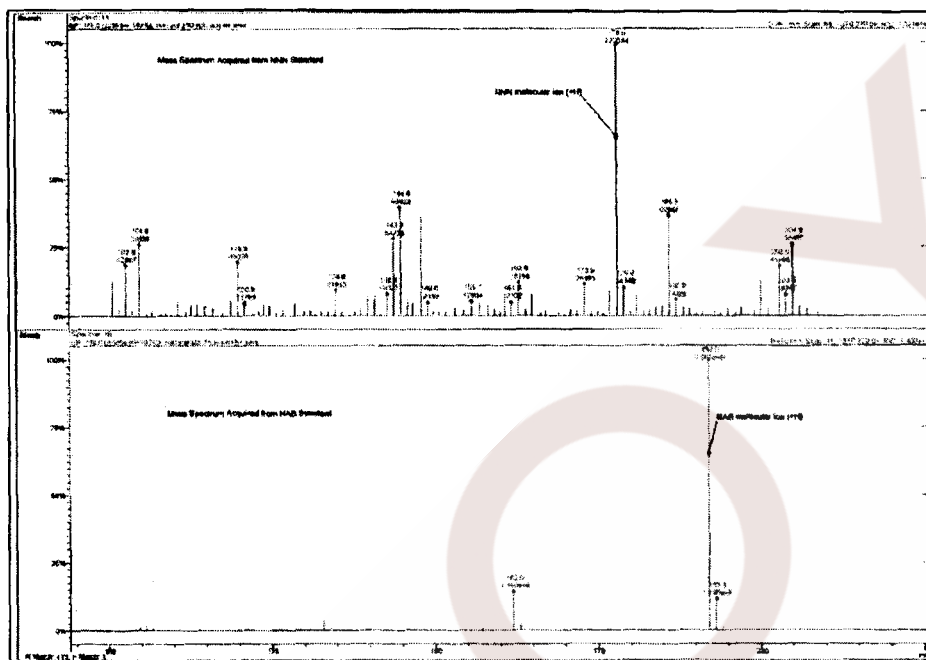


Figure 1b – ESI Direct Infusion Mass Spectra of NAT and NNK

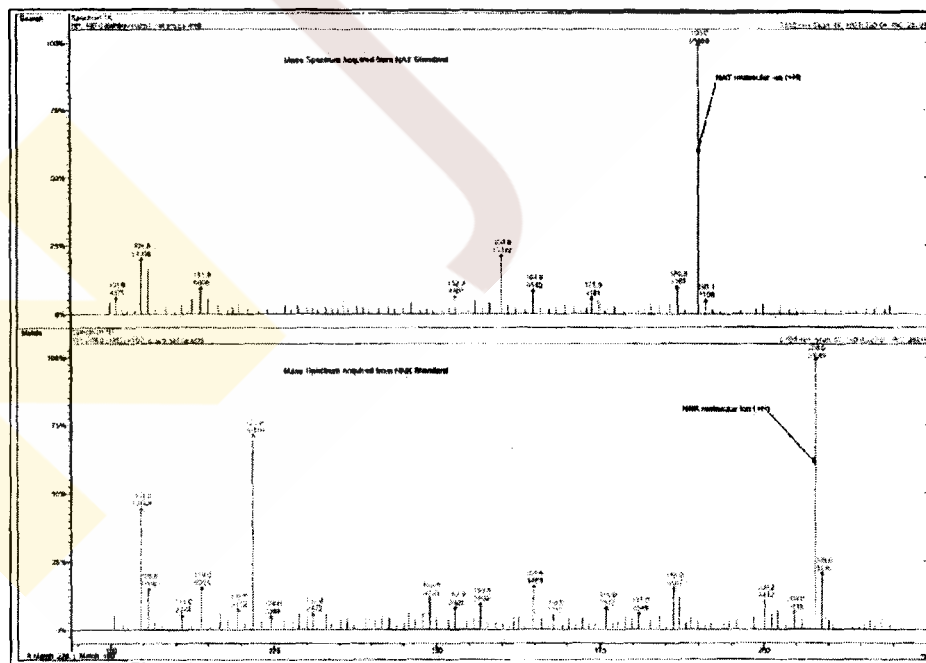
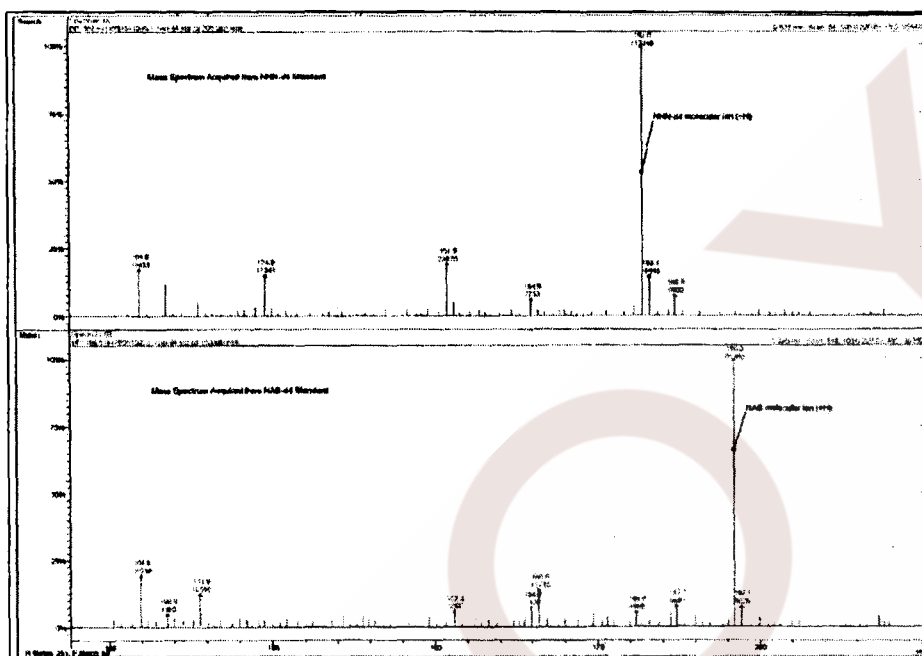


Figure 1c – ESI Direct Infusion Mass Spectra of NNN-d₄ and NAB-d₄

High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS/MS). HPLC is an analytical technique for the separation of organic compounds. Analytes separate according to their interaction with a stationary phase (column chromatography) and identified qualitatively based on their relative retention times within the column and quantitatively through integration of detector signal intensity, which is proportional to the analyte concentration. Detection is accomplished with a soft ionization mass spectrometer (MS) that is focused to the ion of interest based on a molecular formula or in full mass sweep if the ion need be selected since a reference standard is not available. Further structural information may be gathered by collision induced fragmentation generating an additional mass spectrum termed MS/MS; with chromatography termed HPLC-MS/MS. As previously described, infusion-MS (or MS/MS) data may be gathered for component verification if suitable chromatographic separation data is not available. A solution of the analyte is injected/infused directly into the MS bypassing the chromatographic separation.

Instrumental Conditions. All analyses were conducted using an Agilent 1100 HPLC system fitted with an auto-sampler, and Chemstation software. The solvent flow was plumbed into a Varian 1200 tandem quadrupole mass spectrometer equipped with a collision cell for secondary fragmentation. The analytical conditions by which all standard and sample chromatograms were acquired are listed below:

Column:	Waters Xterra MS C18 2.5 um (2.1X50mm)	
Eluent Phase A:	0.1% HOAc in Water	
Eluent Phase B:	0.1% HOAc in Methanol	
Mobile Phase:	<u>Time (min)</u>	<u>%B</u>
	0	25
	0.5	25
	2.5	5
	4.25	75
	5.0	5
	6.0	5
Solvent Flow Rate	0.15 ml/min	
Temperature:	60 °C	
Injection Volume	10 µL	
Run Time:	6 minutes	

Positive Polarity

Needle Voltage:	5000 V
Shield Voltage:	600 V
Nebulizing Gas:	51 psi (N ₂)
Drying Gas:	150 °C, 21 psi
Housing:	50 °C
Capillary Voltage:	27 V
Detector:	Scan <i>m/z</i> 100- 220 (for direct infusion ESI)

SIM Conditions (for all HPLC-MS/MS)

Target Compound	Parent Ion (m/z)	Fragment Ions (collision energy)
NNN	178	148(-7.5V), 120(-16V)
NAB	192	162(-8.0V), 133(-18V)
NAT	190	160(-7.5V), 106(-14V)*
NNK	208	122(-10V), 106(-18.5V)*
NNN-d ₄	182	152(-8.0V)
NAB-d ₄	196	166(-9.0V)

* Ion fragment 106 was excluded from all analyses run on the second HPLC column used in this analytical study due to co-elution of NAT and NNK.

TSNA Standards in Mobile Phase. All chromatograms were acquired in MS/MS mode. The signals corresponding to all secondary fragments of each target compound were combined, producing individual chromatograms for each compound. The peaks were integrated and the resulting areas were used to calculate the response factor at various concentrations for each compound/ internal standard pair. Figures 2a-d are stacked plot comparison of SIM chromatograms produced by individual analyte solutions of various TSNA concentrations (ca. 2.2 – 0.3 ppb) diluted in mobile phase. These analyses were used to establish lower limits of detection for the target compounds individually *in the mobile phase* without the presence of sample matrix.

Figure 2a – Chromatograms of NNN Solutions for LOD in Mobile Phase

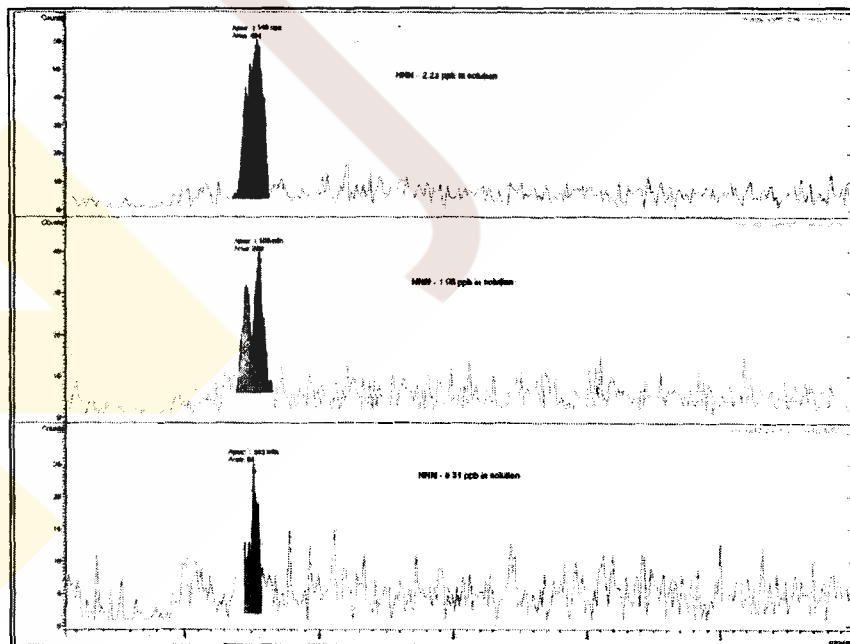


Figure 2b – Chromatograms of NAB Solutions for LOD in Mobile Phase

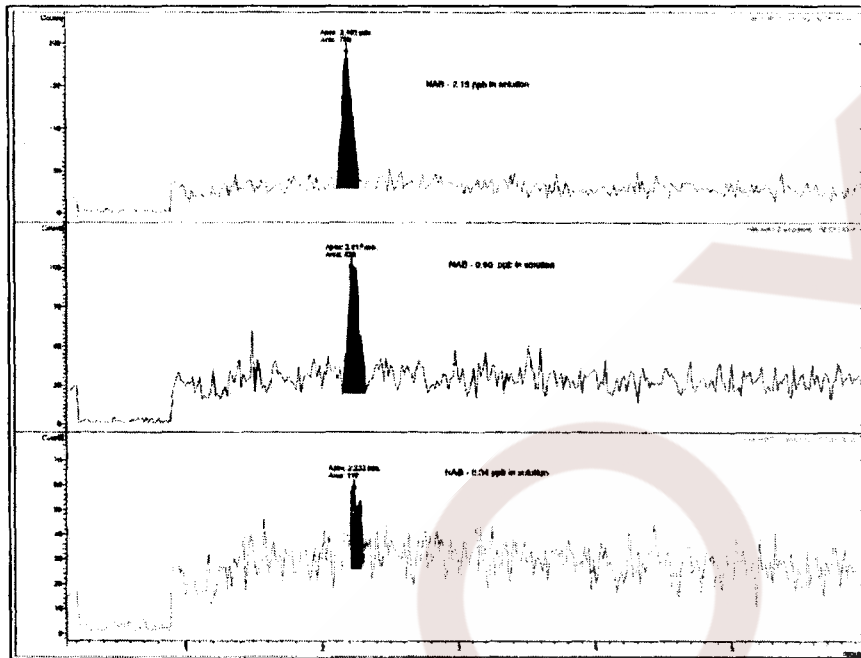


Figure 2c – Chromatograms of NAT Solutions for LOD in Mobile Phase

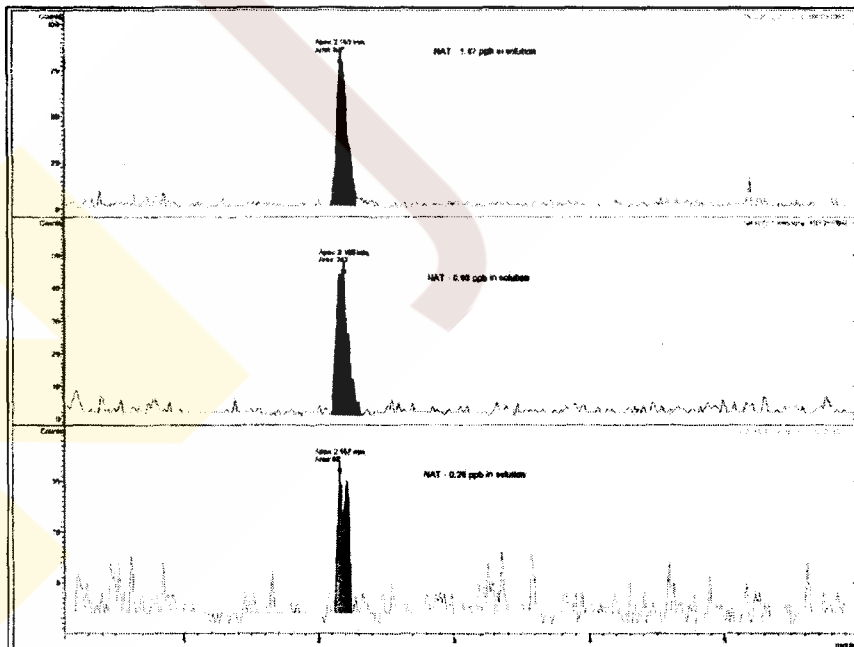
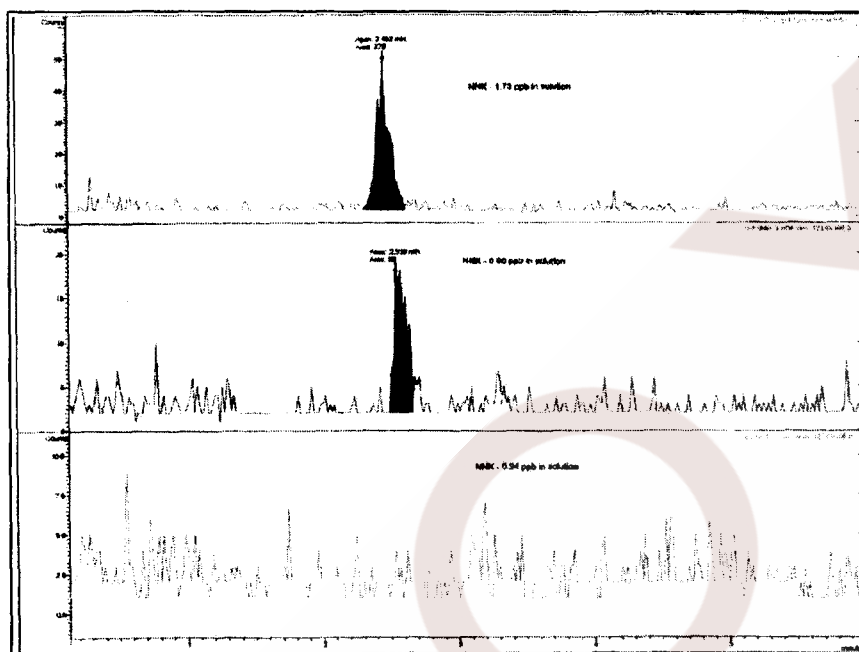
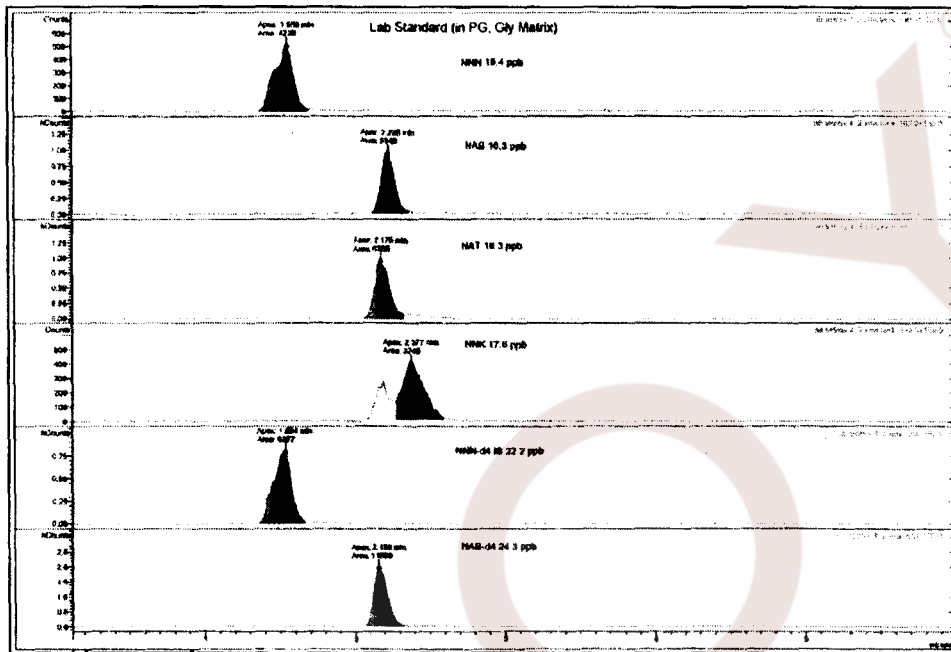


Figure 2d – Chromatograms of NNK Solutions for LOD in Mobile Phase

While the limits of detection (LOD) varied with specific TSNA, the average LOD is ca. 0.5 ppb.

TSNA Standards in Mobile Phase. In order to mimic the Njoy formulation, a lab matrix was made containing 60 wt-% water, 30 wt-% propylene glycol, 10 wt-% glycerol and 0.8 wt-% nicotine. The individual standards were added to this lab matrix to produce TSNA concentrations of ca. 10-25 ppb. A representative chromatogram is shown in Figure 3.

Figure 3 – Chromatograms Produced by Analysis of Standard Solution (Lab Matrix)

The retention times of each compound were influenced by the presence and concentration of other components of the sample matrix. Although retention time shifts were observed, identification of each compound was achieved by a) comparison with the elution time of deuterated standards and b) selective ion monitoring for secondary ion fragments exclusive to the target compounds. Table I lists all compounds and their approximate retention times.

Table I – Chromatography Results for Target Compounds

Compound ID	Retention Time (approx)	Chromatogram position in Displayed Stacks
NNN	1.4 – 1.6	top
NAB	2.1 – 2.3	second
NAT	2.1 – 2.3	third
NNK	2.2 – 2.5	fourth
NNN-d4	1.5	second to bottom
NAB-d4	2.2	bottom

A gradual increase of pressure within the liquid chromatograph was observed throughout the course of the study. This pressure exceeded usable limits just before the second set of sample analyses was performed; a second Waters Xterra MS C18 2.5 μ m (2.1X50mm) column was obtained and implemented into the system. Subtle differences in chromatographic separation were observed between columns; NAT and NNK were resolved by the first column used but co-

eluted on the second column. Due to this co-elution, the use of the common fragment ion m/z 106 for detection and quantitation was no longer viable and was excluded from all data from the second set of analyses on all samples. This exclusion altered the response factors; a different set of response factors were therefore applied to the first and second samplings of the Njoy and Nicotrol products. These response factors are listed in Tables IIa-b.

Due to structural similarity, NNN-d₄ is the chosen internal standard for NNN, NAB-d₄ is the chosen internal standard for NAB and NAT. The internal standard for NNK was NNN-d₄ based on retention time and similarity of response intensity.

Equation 1

$$\text{Response Factor} = \frac{\text{Area of Analyte} \times \text{Concentration of Internal Standard}}{\text{Area of Internal Standard} \times \text{Concentration of Analyte}}$$

Table IIa - Response Factors used for First Samplings

Compound ID	RF with NNN-d ₄	RF with NAB-d ₄
NNN	0.8210	---
NAB	---	1.0767
NAT	---	0.6274
NNK	0.7087	---

Three standard solutions (ca. 2, 5 and 20 ppb) were used to study the trend in response factors over various TSNA concentrations. For all analytes, the standard deviation of the response factors produced over this concentration range were <0.1. While good consistency was observed, the average value of the response factors acquired from the *least* concentrated solutions were eventually chosen for sample analysis due to the low levels of analyte detected in the sample extract solutions. All values are included in the Appendix Tables.

Table IIb - Response Factors used for Second Samplings

Compound ID	RF with NNN-d ₄	RF with NAB-d ₄
NNN	0.8654	---
NAB	---	1.0703
NAT	---	0.4913*
NNK	0.6189*	---

* The large variation between the RF values from the first and second column for NAT and NNK result in the modification of the ions included in the analyte signal (e.g., exclusion of m/z 106).

A solution of known concentration was run by the above listed conditions and used to verify the accuracy of the calculated response factors for each TSNA target compound. Table III lists all check standard recoveries:

Table III - Check Standard Recoveries

Compound ID	Check Standard Recovery (%)
NNN	112 ± 3
NAB	103 ± 4
NAT	108 ± 3
NNK	114 ± 7

A solution of only the two internal standards was run to insure that none of the four target analytes were contained in these standard solutions and would therefore contaminate sample extracts by addition. No signals corresponding to the target TSNA were detected, a representative chromatogram from this study is included in the Calibration Appendix.

Liquid Solution Extraction Method Development

One cartridge from each of the following sets was extracted into a buffered aqueous solution (100 mM ammonium acetate) to determine the presence of TSNA's in the as received liquid:

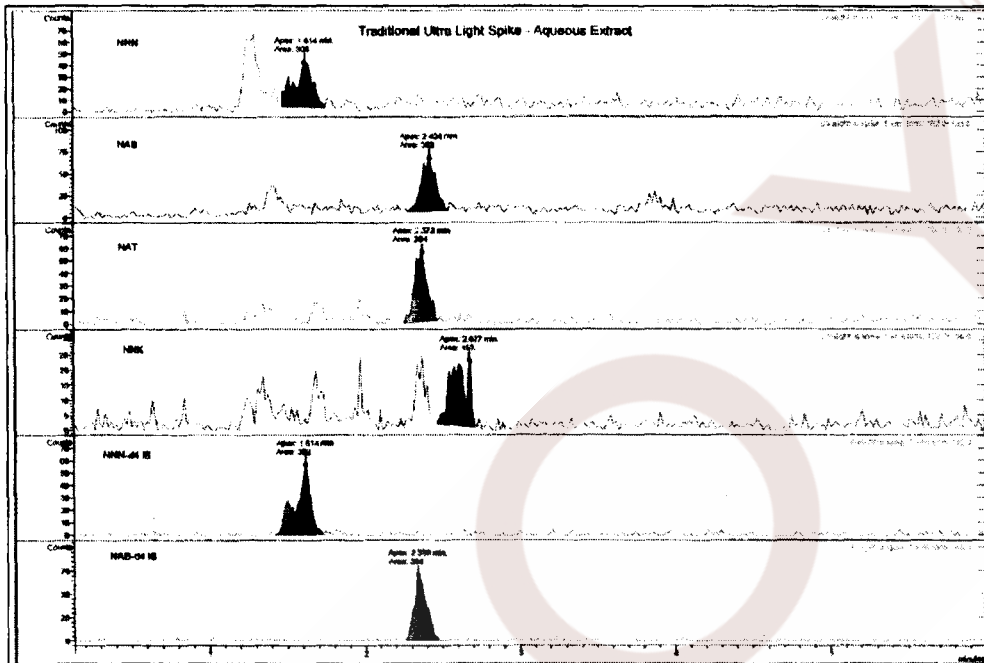
- Traditional Light Mfg Date June 2009
- Traditional Ultra Light Mfg Date June 2009
- Menthol Regular Mfg Date June 2009
- Menthol Light Mfg Date June 2009

The quantity of liquid solution in one 'Traditional Ultra Light' cartridge was determined by weighing the unit (after removed of outer casing, silicone plug and mouth piece) before and after removal of the liquid solution. The liquid was removed by pressing the saturated sponge onto a paper towel until no further moisture was observed. The inner casing was wiped dry with a Kimwipe. The cartridge component parts were then re-weighed and the difference was taken as the solution mass. The resulting mass (1.06 grams) was then used as a representative mass value for all extracted samples; determination of individual solution masses was not practical due to loss during handling.

Procedure. A 250 ml Erlenmeyer flask was silanized to deactivate the glass surface. A Njoy cartridge was then disassembled and all component parts in contact with the liquid solution were placed in the Erlenmeyer flask (sponge, inner casing and silicone plug). A volume of extraction solvent (10 ml of 100 mM ammonium acetate) was added to the flask and the solution was shaken on an automated wrist shaker for 30 minutes. A known quantity of internal standard solution was then added and the solution was analyzed for TSNA content by LC-MS/MS.

Determination of lower limit of detection in solution extract. A known amount the target TSNA's were spiked into a liquid extract of one Traditional Ultra Light cartridge. Figure 4 is the chromatogram produced by the analysis of this spiked sample solution. The peaks representing the elution of the target compounds are of low intensity. The limits of detection for the target compounds (LOD) by this extraction technique were determined from this analysis.

Figure 4 – Liquid Extract of Traditional Ultra Light Solution – Spiked with TSNA Target Compounds



While the target analytes were detected, the signal intensity of each was less than would be expected for the quantity of compound added. There is likely some matrix effect or loss during transfer/extraction of these compounds. The limit of detection for the four TSNA compounds in the presence of the Njoy solution matrix was determined by evaluating the strength of the signals produced in the spiked sample trial. These values were then used to calculate the LOD of each compound in the Njoy samples themselves. Table IV is a complete listing of all LOD levels:

Table IV - LOD of Liquid Extraction Method

Compound	LOD in solution with Njoy Matrix (ppb)	LOD in Sample – Calculated (ppb)
NNN	4.5	45 - 50
NAB	3.5	ca. 35
NAT	4.0	40 - 45
NNK	5.0	50 - 55

Vapor Capture Method Development

The sparging vapor capture method used by the FDA [B.J. Westenberger, CDER/OPS/OTR, Division of Pharmaceutical Analysis, FDA, May 4, 2009] for the capture of nicotine and related impurities was adapted with a number of modifications.

Procedure (Capture Method I, 'Pull')

1. All glassware was silanized to deactivate the glass surfaces.
2. The capture apparatus was set up, consisting of the e-cigarette, to in-line capture flasks, tubing and a 100 cc Drager hand pump, as displayed in Photo 1.

Photo 1 – Capture Apparatus Method I

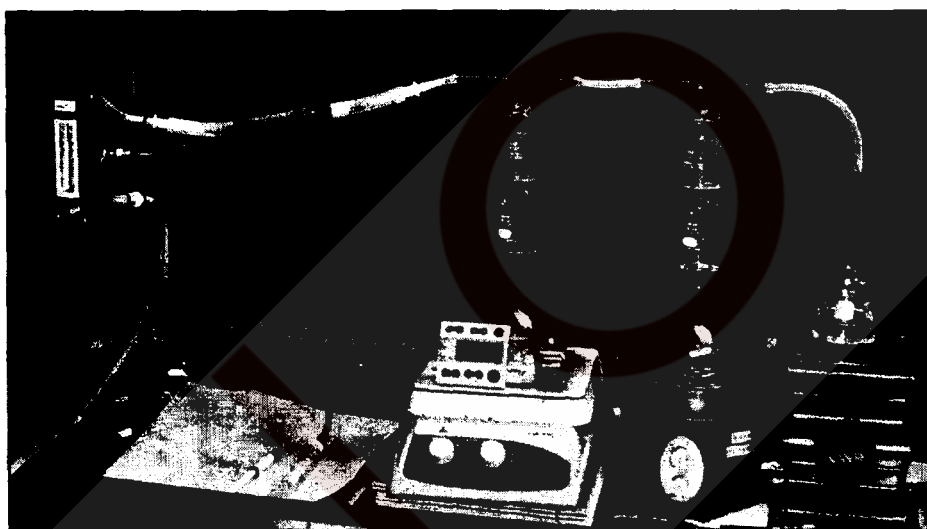


3. 75 ml of methylene chloride were placed in each capture flask. Multiple draws with the Drager hand pump were performed such that the filament of the e-cigarette was observed to activate 100 times. The theoretical corresponding volume of vapor produced should be 10 liters.
4. All glass surfaces were rinsed down with methylene chloride, flowing nitrogen gas was used to reduce the capture solvent to a residue.
5. A minimal amount (ca. 4 ml) of 100 mM ammonium acetate was used to reconstitute the remaining residue. The resulting solution was analyzed by LC-MSMS.

Several problems were discovered with this initial capture method; most notably the ability of the Drager pump to activate the filament of the Njoy unit. While this was not observed to be a limitation in earlier trial when 100 mM ammonium acetate was used as the capture solution, the presence of methylene chloride vapor in the system likely depresses the actual pressure drop across the filament assembly due to the high vapor pressure of the solvent. It was therefore determined that a reliable volume could not be measured by drawing vapor through the capture apparatus. A second capture method in which positive pressure was applied to the front end of the capture apparatus (e.g., 'push' method) was developed.

Procedure (Capture Method II – ‘Push’)

1. All glassware was silanized to deactivate the glass surfaces.
2. The capture apparatus was configured such that a tank of breathing quality compressed air was connected to a flow regulator. This regulator was set to deliver 3.5 SCFH (standard cubic feet per hour) and connected by silicone tubing to a Njoy E-cigarette. The tubing was attached to the front portion of the E-cigarette (battery end) and the compressed air was used to push air through the device. Silicone tubing was attached to the mouth piece and the vapor was directed into the series of capture flasks. Photo 2 illustrates the capture apparatus in this second configuration.

Photo 2 – Capture Apparatus Method II

3. 150 ml of methylene chloride were added to each capture flask. Additionally, a third capture flask was added at the end of the apparatus. This addition was made due to some observed TSNA breakthrough into the second flask when lab matrix spiked trials were run.
4. Vapor from the Njoy unit was generated in ‘puffs’ lasting for 3 seconds. The unit was then allowed to recover between puffs for approximately 15 – 25 seconds. The required number of puffs (61) to produce a total of 5 liters of vapor was performed.
5. All glass surfaces were rinsed down with methylene chloride, flowing nitrogen gas was used to reduce the capture solvent to a residue.
6. A minimal amount (ca. 4 ml) of 100 mM ammonium acetate was used to reconstitute the remaining residue. Internal standard solution (ca. 0.05 grams) was added and the resulting solution was analyzed by LC-MS/MS.

Evaporation Validation. In order to determine whether any of the target TSNA compounds are lost upon evaporation of the capture solvent, 75 ml of methylene chloride was spiked with a known amount of TNSA standards. This solvent was then reduced to a residue with flowing nitrogen at room temperature. The residue was reconstituted in ca. 4 ml of 100 mM ammonium acetate and analyzed by LC-MS/MS. The detected TSNA's were quantified with the results shown in Table V.

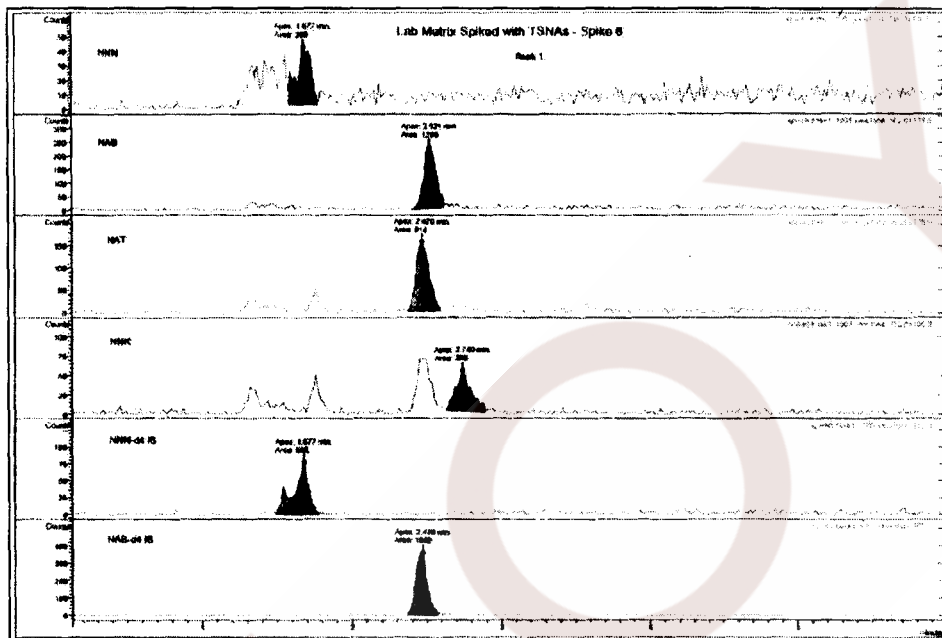
Table V – Evaporation Recoveries

Component ID	% Recovery
NNN	78
NAB	83
NAT	78
NNK	75

While some loss was observed, all recoveries were within 17-25% of the expected values. Losses may occur due to evaporation, but more likely occur during solvent transfer.

Lab Standard Control – Spiked with TSNA Compounds. Vapor from a lab standard solution was captured by the Capture Method II. A lab standard matrix was made up to simulate the Njoy solution matrix (30% PG, 10% Glycerol, 0.8 % nicotine and 60% water). This solution was then spiked with known amounts of each TSNA target compound. Figure 5a presents a stacked plot of the chromatograms produced by the analysis of the vapor solution from the lab standard solution spiked with TSNA's.

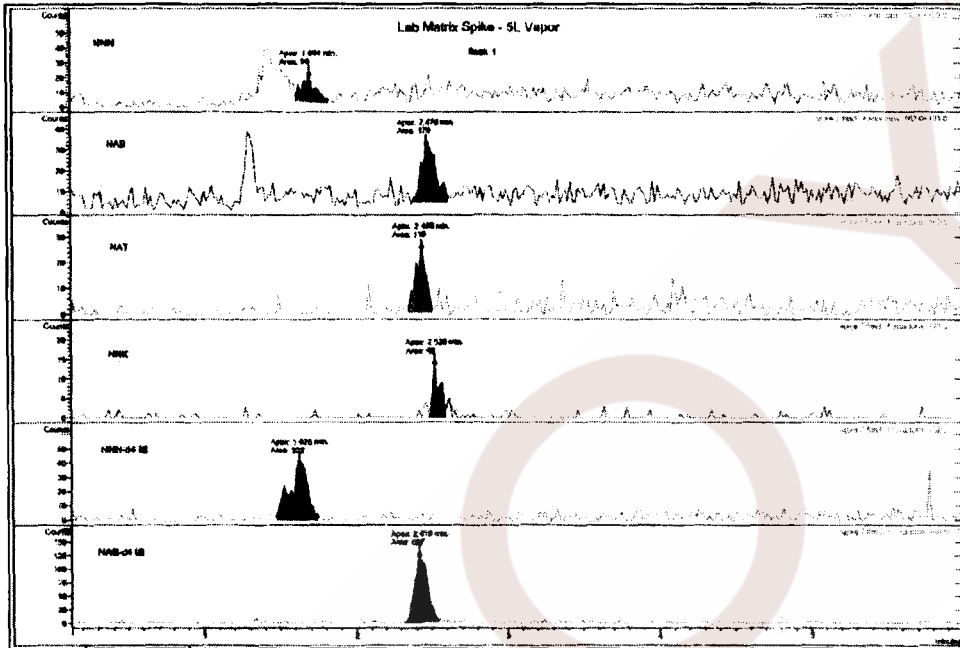
Figure 5a – Chromatograms from Lab Matrix Standard Spiked with Target TSNA Compounds



The detection of the target compounds in the residue from the capture flask confirms that these TSNA molecules can be transported from the liquid solution in the Njoy unit to a secondary location through the vapor.

A lower concentration spike solution was constructed to determine the LOD in solution by vapor capture method. Figure 5b is a stacked plot of all extracted ion chromatograms (EIC) for the target and internal standard compounds from this lower concentration lab matrix spike. Table VI contains the estimated lower concentration of the TSNA compounds in the sample solution that can be detected by the vapor capture method. The concentration of TSNA in the vapor (ng/ml) from this solution is also listed:

Figure 5b – Chromatogram from Lower TSNA Concentration Spiked Lab Matrix



**Table VI – LOD and TSNA Vapor Concentration Data
Vapor Capture Method II – Spiked Lab Matrix**

Target Compound Identity	Limit of Detection (ppb in Solution)	Corresponding Concentration in Vapor (ng/L)
NNN	25 - 30	approx. 1.5
NAB	15 - 20	approx. 1.2
NAT	20 - 25	approx. 1.5
NNK	20 - 25	approx. 1.4

It should be noted that these results were obtained from a simulated sample matrix, not from an actual Njoy solution. While these values are likely similar to that of the Njoy products, compositional variations between the simulated matrix and the Njoy product may give rise to differences in these concentrations.

SAMPLE ANALYSIS

Njoy Liquid Extraction Results. The liquid from each cartridge was measured by weighing the saturated fibrous plug prior to extraction, and then removing, drying and weighing the plug after extraction. In addition to this plug, the plastic inner casing was included in the extraction vial; a small amount (likely only several micro liters) of liquid was observed on the inner surface of some of these devices. All masses associated with these extractions are included in Table VII.

Table VII – Extract Mass Data

Sample Identity	Sample Solution (grams)	NNN-d4 Standard Solution	NAB-d4 Standard Solution	Total Mass Extract (w/ 100 mM NH ₄ OAc)
Traditional Light	1.0271	0.0655	0.0786	10.8536
Traditional Ultra Light	1.0370	0.0746	0.0655	11.0036
Menthol Regular	1.0546	0.0774	0.0678	10.7202
Menthol Light	1.0220	0.0833	0.0641	10.6786

No target TSNA compounds were detected in any of the analyzed sample solutions by the liquid extraction method. No peaks were detected at the expected analyte retention times in the chromatograms produced by the sample liquid extracts; representative chromatograms are included in the Liquid Extraction Appendix. All limits of detection are listed in the Summary and in text Table IV.

Njoy Vapor Capture Results. The cleanliness of all glassware was verified prior to sample trials. Additionally, a Njoy unit was sampled without the addition of a solution cartridge to insure that none of the target TSNA compounds were produced by the unit itself. Propylene glycol was used to wet the filament for this trial to create vapor. EIC stacks of both glassware blanks and the Njoy unit blank (without cartridge) are included in the Vapor Sample Analysis Appendix.

The four Njoy sample types (listed on the Title Page) were sampled in duplicate. Each sampling consisted of 61 'puffs' introduced into the vapor capture apparatus. Each puff had a three second duration (stopwatch timed) with a flow rate of 3.5 SCFH. The volume of captured vapor was calculated in Equation II.

Equation II

$$1 \text{ SCFH} = 7.86 \text{ ml/sec}$$

$$3.5 \text{ SCFH} \times 3 \text{ seconds} \times 61 \text{ 'puffs'} = 5034 \text{ ml} \approx 5\text{L}$$

A steady stream of vapor was observed to flow from the Njoy unit into the first capture flask. This vapor was observed to 'break through' the capture solvent in flask 1, but was not observed above the solvent in flask 2. No vapor was visually observed to travel to flask 3.

Due to residual pressure in the vapor capture apparatus tubing, it was necessary to detach the tubing between the Njoy unit and capture flask 1 between each puff. This prevented additional vapor beyond the desired volume from being captured by this study. *The volume of vapor collected should be considered an estimate* as each puff was performed manually by simultaneously turning the knob of the gas cylinder regulator and activating the stopwatch. A slight delay then occurred between closing the pressure regulator valve and disconnecting the tubing. Some variation in total vapor volume inevitably exists between samplings.

NAT was detected in the residue remaining from the vapor capture solutions in all four Njoy products. All sample EIC stacked chromatograms are included in the Vapor Sample Analysis Appendix. Table VIII contains the quantitative results achieved by LC-MSMS for the NAT concentration of the vapor produced by each of the four Njoy samples, calculated by Equation III.

Equation III

$$\text{Concentration of NAT in Solution} = \frac{\text{Area of Analyte} \times \text{Concentration of Internal Standard}}{\text{Area of Internal Standard} \times \text{NAT Response Factor}}$$

$$\text{Total ng NAT from Sample} = \text{Concentration NAT in Solution} \times \text{Total Mass Solution}$$

$$\text{Concentration NAT in Vapor (ng/L)} = \text{Total ng NAT from Sample} / 5 \text{ L}$$

Table VIII – NAT Concentration of Sample Vapors

Sample ID	NAT Concentration in Residue Solution (ppb)			Total NAT from Sample (ng)			Concentration NAT in Vapor (ng/L)		
	Trial 1	Trial 2	AVG	Trial 1	Trial 2	AVG	Trial 1	Trial 2	AVG
Traditional Light	3.9	2.5	3	13.7	11.6	13	2.7	2.3	2.5
Traditional Ultra Light	8.4	3.1	6	36.4	13.8	25	7.3	2.8	5
Menthol Regular	1.6	2.8	2	5.7	11.9	9	1.1	2.4	2
Menthol Light	4.6	2.8	4	20.2	12.1	16	4.0	2.4	3

The limits of detection for the other three TSNA compounds are listed in the Summary section and in text Table VI.

Analytical and Vapor Capture Method Considerations for the Njoy Samples

General

1. For the vapor capture from all Njoy samples, it was noted that the intensity of the signal from the deuterated internal standard compounds was considerably lower in capture flask 1 than in flasks 2 and 3. Likewise, several cases occurred when the NAT signal intensity was greater in flask 2 than in flask 1. These results strongly suggest that a suppression effect occurred in flask 1 which is likely due to the higher concentrations of other sample matrix components; e.g., propylene glycol, glycerol. However, the comparison of analyte signal to internal standard signal, which was also suppressed, should compensate for this effect.
2. For several of the sample trials, the NAT signal was detected in capture flask three. While the total intensity of this signal did not exceed 16% in all cases but one, there is a possibility that some NAT escaped from the vapor capture apparatus. This fact, combined with the 78% recovery of NAT in the evaporation study, necessitates the allowance of ca. 35% error for all NAT values.
3. The ability to concentrate the capture solution volume by allowing the volatile methylene chloride solvent to evaporate results in the low levels of TSNAs that can be detected in the vapor phase experiments. However, due to the 75 – 83% recoveries in the evaporation study for the other three TSNA target compounds, an adjustment of +17-25% for the TSNA LODs may be warranted.
4. At the conclusion of all sample analyses, the Njoy units were still able to generate visible vapor. Therefore, the sample cartridges were not exhausted. All TSNA concentrations were based on the initial five liters of vapor produced by the unit. It is unknown if the TSNA concentration in the vapor produced by the Njoy units is consistent over time or if the amount of TSNA entrained in the vapor changes as a function of usage.
5. The volume of vapor collected and used in all calculations should be considered an estimate. Each 'puff' was performed manually with some variation (perhaps $\pm 10-20\%$) in total vapor volume inevitably exists between samplings.

Traditional Light

1. Only one of the triplicate LC-MS/MS analyses of the flask 1 solution (trial 2) produced an NAT signal with sufficient intensity for integration. The results of this run was used to represent the recovery of NAT from this flask with the two injections with lower signal intensities were not included. This omission may skew the results upward, making the NAT concentration results for this trial slightly elevated.
2. While NAT was detected in flask 2 of trial 1, the signal strength was not sufficient for integration. The NAT contribution from flask 2 was therefore not included and the trial 1 results may be slightly skewed downward.

3. Breakthrough of NAT to flask three was not observed in either trial of the Traditional Light product.

Traditional Ultra Light

1. Breakthrough of NAT signal to capture flask three was observed in both Traditional Ultra Light trials. The total NAT concentration from flask 3 was 16% and 12% of the total NAT assayed. It is therefore possible that some NAT was lost during the analysis of this product.
2. One of the triplicate analyses of the flask 1 solutions from trial 2 did not produce NAT signal of sufficient intensity for quantitation. This run was not included in the data calculation. The NAT may be slightly over represented from this flask.

Menthol Regular

1. Signals associated with the presence of NAT were *only* quantitated in the extract from flask 3 in trial 1. A very low intensity signal may indicate the presence of NAT in flask 1 as well, but the intensity of this signal is well below the limits of integration. This result is somewhat unexpected and has no explanation at present.
2. Signals associated with NAT were detected in all three flasks in trial 2. The signal strength was only sufficient for quantitation in flask 2.

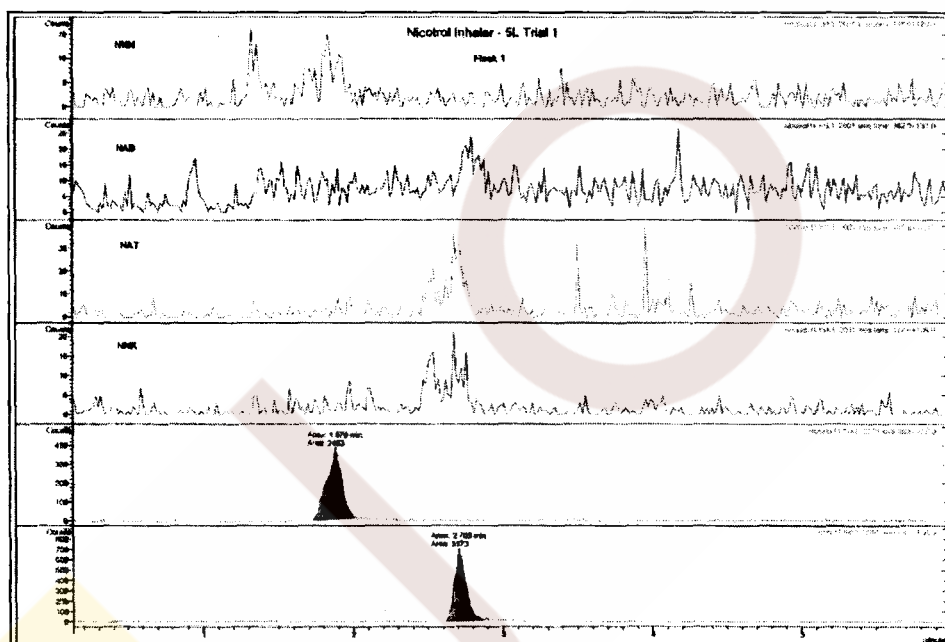
Menthol Light

NAT was detected in the extract from flasks 1 and 2 both analytical trials. No breakthrough was observed into flask 3 in trial 1. Two of the three flask 3 runs produced signal sufficient for integration in trial 2.

Nicotrol Inhaler. A Nicotrol[®] Inhaler unit with one cartridge insert was sampled by capture method II (e.g., push method). As was the case with the Njoy unit, approximately 5 L of air was passed through the Nicotrol[®] Inhaler unit. The air transported vapor bubbled through the three capture flasks containing methylene chloride.

Analysis of the residues produced by trial 1 sampling of the Nicotrol[®] Inhaler detected trace amounts of NNN, NAB and NAT. The signal strength from these analytes was extremely low, well below that required for integration. Figure 6a presents the stacked chromatograms from the residue in flask 1.

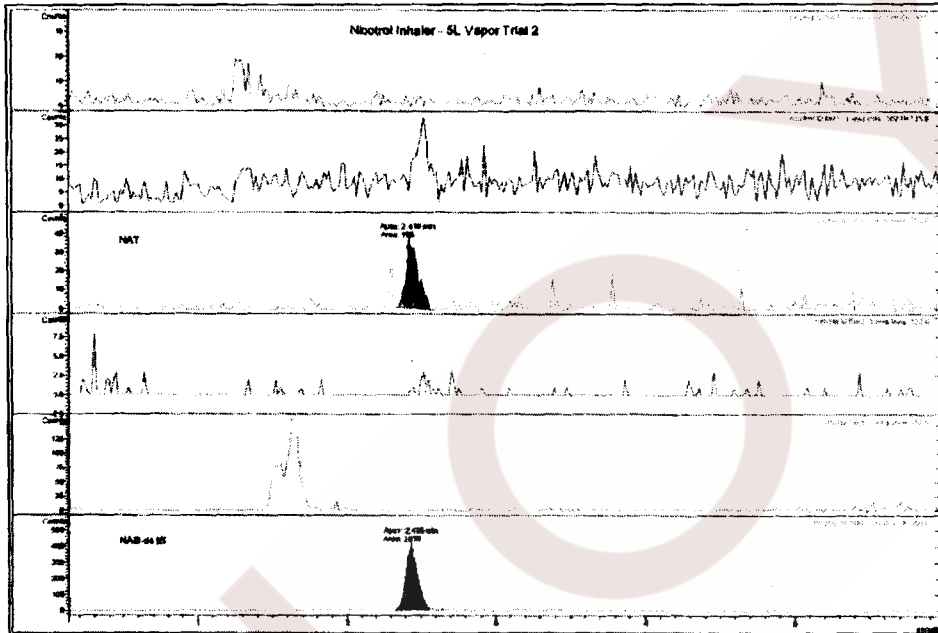
Figure 6a – Residue from Nicotrol[®] Inhaler – Trial 1 Flask 1



Note the very low intensity of peaks at ca. 1.8 min in the top EIC and 2.7 min in the second and third EIC. The peak at 2.7 min in the fourth EIC is likely associated with NAT and does not signify the presence of NNK (m/z 106 used for both analytes). No quantitation was possible from this trial.

A somewhat more intense signal associated with NAT was obtained in the second Nicotrol[®] Inhaler sampling trial from the residue in flask 1. NAB was again detected below the level of quantitation, NNN was not detected in this trial. Figure 6b is the stack EIC from flask 1, trial 2:

Figure 6b – Residue from Nicotrol[®] Inhaler – Trial 2 Flask 1



No TSNA signals were discernable from the background noise in residue from flasks 2 and 3 in either trial.

The peaks associated with NAT were integrated in the flask 1 chromatograms from trial 2 and used to calculate and estimated concentrations in the sample vapor are listed in Table IX.

Table IX - NAT Concentrations from Nicotrol[®] Inhaler Vapor

Sample ID	NAT Concentration in Residue Solution (ppb)			Total NAT from Sample (ng)			Concentration NAT in Vapor (ng/L)		
	Trial 1	Trial 2	AVG	Trial 1	Trial 2	AVG	Trial 1	Trial 2	AVG
Nicotrol [®] Inhaler	D	1.0	1	---	4.4	4	---	0.9	0.9

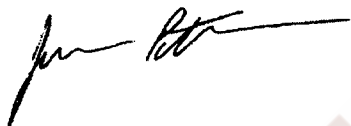
Due to differences in sample matrix, limits of detection for all TSNA compounds were not established from the Nicotrol[®] Inhaler unit vapor.

Considerations - Nicotrol® Inhaler

1. The Nicotrol® Inhaler unit does not produce a visible vapor as does the Njoy unit. It was therefore difficult to determine if the vapor capture apparatus was indeed activating the Nicotrol® Inhaler,
2. Due to the lack of visible vapor, it is unknown if the cartridge insert was exhausted prior to the completion of sampling.
3. No Nicotrol® Inhaler unit spike studies were performed to measure the efficiency of TSNA transfer by the sampling method. Further method development is required to validate the results from the Nicotrol® Inhaler.

As questions arise during your review of this report, please do not hesitate to call us.

ANALYZE Inc.



Jason Pattison
Consulting Chemist



Steven J. Valenty, Ph.D.
Consulting Chemist & President