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Customer:

Dr Michael Moynihan
22nd Century Limited LLC
8201 Main Street
Suite 6 Williamsville
New York
USA 14221

Sample Received: 14 March 2011

Date Testing Commenced: 16 March 2011

Date Reported: 01 April 2011

Samples Received: Magic Menthol

Testing Requirements: One thousand cigarettes in branded packets were submitted to the Scientific Services Laboratory of the Filtrona Technology Centre for testing for mainstream smoke yields.

Test Procedures: All tests have been carried out in accordance with documented in house test methods based on ISO standard methods when available. The reported data is only applicable to the sample received.

Results: The results are given on the page attached

Approved by:

(b) (6)

Laboratory Manager
Filtrona Technology Centre

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Mainstream Smoke Components			
Date	**01/04/2011**		
Brand	**Magic Menthol**		
Bar Code	**FSC 00953788**		
Component	**Yield Mean**		**Yield SD**
Tar	3.5	mg/cig	0.2
Nicotine	0.03	mg/cig	0.00
Carbon Monoxide	5.4	mg/cig	0.2
Benzo [a] Pyrene	0.9	ng/cig	0.1
Carbonyls			
Formaldehyde	5.3	µg/cig	1.5
Acetaldehyde	283.0	µg/cig	13.0
Acetone	*111.7*	*µg/cig*	*7.9*
Acrolein	12.0	µg/cig	0.9
Propionaldehyde	20.0	µg/cig	1.3
Crotonaldehdye	4.6	µg/cig	0.8
Methyl Ethyl Ketone	23.4	µg/cig	2.2
Butyraldehyde	10.4	µg/cig	1.1
Phenols			
Hydroquinone	15.5	µg/cig	1.0
Resorcinol	< 0.5	µg/cig	
Catechol	13.2	µg/cig	0.3
Phenol	5.1	µg/cig	0.3
meta & para-Cresol	3.9	µg/cig	0.2
orto-Cresol	1.5	µg/cig	0.1
Ammonia	24.8	µg/cig	2.0
Hydrogen Cyanide	44.0	µg/cig	4.6
Semi-volatile bases			
Pyridine	3.5	µg/cig	0.3
Quinoline	*0.04*	*µg/cig*	*0.01*
Miscellaneous Organics			
1,3 Butadiene	17.9	µg/cig	1.0
Isoprene	115.9	µg/cig	7.1
Acrylonitrile	5.6	µg/cig	0.2
Benzene	23.0	µg/cig	3.0
Toluene	31.9	µg/cig	4.6
Styrene	3.6	µg/cig	0.4
Nitrosamines			
N-Nitrosonornicotine NNN	39.1	ng/cig	0.2
N-Nitrosoanatabine NAT	5.7	ng/cig	0.4
N-Nitrosoanabasine NAB	1.3	ng/cig	0.1
4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone NNK	10.9	ng/cig	1.4
Aromatic Amines			
3-Aminobiphenyl	1.50	ng/cig	0.13
4-Aminobiphenyl	1.02	ng/cig	0.07
1-Aminonaphthalene	7.56	ng/cig	0.09
2-Aminonaphthalene	4.16	ng/cig	0.08
NOx Nitric Oxide	153.0	µg/cig	2.7
pH	7.04	pH units	0.01
Metals			
Beryllium	< 2	ng/cig	
Cobalt	< 2	ng/cig	
Nickel	< 10	ng/cig	
Lead	*< 5*	*ng/cig*	
Selenium	< 2	ng/cig	
Cadmium	13.8	ng/cig	1.3
Chromium	< 10	ng/cig	
Arsenic	< 1.5	ng/cig	
Menthol	0.34	mg/cig	0.01

Approved by:

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Customer:

Dr Michael Moynihan
22nd Century Limited LLC
8201 Main Street
Suite 6 Williamsville
New York
USA 14221

Sample Received: 14 March 2011

Date Testing Commenced: 16 March 2011

Date Reported: 01 April 2011

Samples Received: Magic

Testing Requirements: One thousand cigarettes in branded packets were submitted to the Scientific Services Laboratory of the Filtrona Technology Centre for testing for mainstream smoke yields.

Test Procedures: All tests have been carried out in accordance with documented in house test methods based on ISO standard methods when available. The reported data is only applicable to the sample received.

Results: The results are given on the page attached

Approved by:

(b) (6) [redacted]

Laboratory Manager
Filtrona Technology Centre

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Mainstream Smoke Components			
Date	**01/04/2011**		
Brand	**Magic**		
Bar Code	**FSC 00953771**		
Component	**Yield Mean**		**Yield SD**
Tar	3.7	mg/cig	0.2
Nicotine	0.03	mg/cig	0.00
Carbon Monoxide	6.3	mg/cig	0.4
Benzo [a] Pyrene	2.4	ng/cig	0.1
Carbonyls			
Formaldehyde	5.7	µg/cig	0.6
Acetaldehyde	352.6	µg/cig	40.6
Acetone	*138.1*	*µg/cig*	*19.0*
Acrolein	14.8	µg/cig	2.4
Propionaldehyde	25.3	µg/cig	3.0
Crotonaldehdye	7.5	µg/cig	0.8
Methyl Ethyl Ketone	28.4	µg/cig	2.4
Butyraldehyde	13.2	µg/cig	1.2
Phenols			
Hydroquinone	18.3	µg/cig	1.5
Resorcinol	< 0.5	µg/cig	
Catechol	14.1	µg/cig	1.0
Phenol	6.3	µg/cig	0.3
meta & para-Cresol	4.5	µg/cig	0.3
orto-Cresol	1.7	µg/cig	0.1
Ammonia	23.1	µg/cig	2.1
Hydrogen Cyanide	60.3	µg/cig	4.0
Semi-volatile bases			
Pyridine	6.0	µg/cig	0.4
Quinoline	*0.05*	*µg/cig*	*0.00*
Miscellaneous Organics			
1,3 Butadiene	21.9	µg/cig	2.8
Isoprene	136.1	µg/cig	24.2
Acrylonitrile	6.8	µg/cig	0.8
Benzene	27.6	µg/cig	3.4
Toluene	39.4	µg/cig	3.7
Styrene	4.8	µg/cig	0.1
Nitrosamines			
N-Nitrosonornicotine NNN	47.1	ng/cig	6.2
N-Nitrosoanatabine NAT	9.1	ng/cig	2.7
N-Nitrosoanabasine NAB	1.9	ng/cig	0.8
4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone NNK	17.6	ng/cig	4.0
Aromatic Amines			
3-Aminobiphenyl	1.55	ng/cig	0.09
4-Aminobiphenyl	0.89	ng/cig	0.09
1-Aminonaphthalene	8.30	ng/cig	0.20
2-Aminonaphthalene	4.38	ng/cig	0.11
NOx Nitric Oxide	200.4	µg/cig	6.7
pH	7.02	pH units	0.03
Metals			
Beryllium	< 2	ng/cig	
Cobalt	< 2	ng/cig	
Nickel	< 10	ng/cig	
Lead	*5.3*	*ng/cig*	*0.4*
Selenium	< 2	ng/cig	
Cadmium	16.8	ng/cig	0.4
Chromium	< 10	ng/cig	
Arsenic	< 1.5	ng/cig	
Menthol	Not Detected	mg/cig	

Approved by:

(b) (6)

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Customer:

Dr Michael Moynihan
22nd Century Limited LLC
8201 Main Street
Suite 6 Williamsville
New York
USA 14221

Sample Received: 14 March 2011

Date Testing Commenced: 16 March 2011

Date Reported: 01 April 2011

Samples Received: Newport Menthol Box

Testing Requirements: One thousand cigarettes in branded packets were submitted to the Scientific Services Laboratory of the Filtrona Technology Centre for testing for mainstream smoke yields.

Test Procedures: All tests have been carried out in accordance with documented in house test methods based on ISO standard methods when available. The reported data is only applicable to the sample received.

Results: The results are given on the page attached

Approved by:

(b) (6)

Laboratory Manager
Filtrona Technology Centre

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Mainstream Smoke Components			
Date	**01/04/2011**		
Brand	**Newport Menthol Box**		
Bar Code	**FSC 02657512**		
Component	**Yield Mean**		**Yield SD**
Tar	18.7	mg/cig	0.7
Nicotine	1.07	mg/cig	0.02
Carbon Monoxide	19.7	mg/cig	0.7
Benzo [a] Pyrene	9.3	ng/cig	0.1
Carbonyls			
Formaldehyde	33.0	µg/cig	1.0
Acetaldehyde	767.8	µg/cig	28.6
Acetone	*264.8*	*µg/cig*	*12.0*
Acrolein	75.2	µg/cig	1.2
Propionaldehyde	53.7	µg/cig	1.9
Crotonaldehdye	16.9	µg/cig	1.2
Methyl Ethyl Ketone	55.5	µg/cig	3.2
Butyraldehyde	39.9	µg/cig	4.3
Phenols			
Hydroquinone	64.4	µg/cig	2.1
Resorcinol	5.7	µg/cig	0.3
Catechol	59.0	µg/cig	0.8
Phenol	13.4	µg/cig	0.7
meta & para-Cresol	9.2	µg/cig	0.4
orto-Cresol	3.6	µg/cig	0.2
Ammonia	14.0	µg/cig	0.2
Hydrogen Cyanide	291.3	µg/cig	21.7
Semi-volatile bases			
Pyridine	18.0	µg/cig	1.2
Quinoline	*0.20*	*µg/cig*	*0.02*
Miscellaneous Organics			
1,3 Butadiene	60.7	µg/cig	1.9
Isoprene	493.5	µg/cig	23.1
Acrylonitrile	10.4	µg/cig	0.5
Benzene	52.8	µg/cig	1.8
Toluene	78.9	µg/cig	8.3
Styrene	24.6	µg/cig	0.9
Nitrosamines			
N-Nitrosonornicotine NNN	113.0	ng/cig	2.8
N-Nitrosoanatabine NAT	95.6	ng/cig	2.4
N-Nitrosoanabasine NAB	9.7	ng/cig	0.8
4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone NNK	57.6	ng/cig	4.7
Aromatic Amines			
3-Aminobiphenyl	0.83	ng/cig	0.09
4-Aminobiphenyl	0.61	ng/cig	0.10
1-Aminonaphthalene	10.48	ng/cig	0.40
2-Aminonaphthalene	2.32	ng/cig	0.12
NOx Nitric Oxide	163.4	µg/cig	11.9
pH	6.13	pH units	0.01
Metals			
Beryllium	< 2	ng/cig	
Cobalt	< 2	ng/cig	
Nickel	< 10	ng/cig	
Lead	*21.2*	*ng/cig*	*0.1*
Selenium	< 2	ng/cig	
Cadmium	63.3	ng/cig	1.4
Chromium	< 10	ng/cig	
Arsenic	4.3	ng/cig	0.3
Menthol	0.58	mg/cig	0.02

Approved by:

(b) (6) [redacted]

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Customer:

Dr Michael Moynihan
22nd Century Limited LLC
8201 Main Street
Suite 6 Williamsville
New York
USA 14221

Sample Received: 14 March 2011

Date Testing Commenced: 16 March 2011

Date Reported: 01 April 2011

Samples Received: Marlboro Gold Box King

Testing Requirements: One thousand cigarettes in branded packets were submitted to the Scientific Services Laboratory of the Filtrona Technology Centre for testing for mainstream smoke yields.

Test Procedures: All tests have been carried out in accordance with documented in house test methods based on ISO standard methods when available. The reported data is only applicable to the sample received.

Results: The results are given on the page attached

Approved by:

(b) (6)

Laboratory Manager
Filtrona Technology Centre

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Mainstream Smoke Components			
Date	**01/04/2011**		
Brand	**Marlboro Gold Box King**		
Bar Code	**FSC 02838423**		
Component	**Yield Mean**		**Yield SD**
Tar	10.1	mg/cig	0.3
Nicotine	0.72	mg/cig	0.02
Carbon Monoxide	10.5	mg/cig	0.2
Benzo [a] Pyrene	9.7	ng/cig	0.2
Carbonyls			
Formaldehyde	17.7	µg/cig	1.5
Acetaldehyde	473.8	µg/cig	11.2
Acetone	*167.3*	*µg/cig*	*3.2*
Acrolein	43.6	µg/cig	1.9
Propionaldehyde	34.7	µg/cig	0.7
Crotonaldehdye	8.9	µg/cig	0.5
Methyl Ethyl Ketone	35.7	µg/cig	1.1
Butyraldehyde	26.2	µg/cig	1.4
Phenols			
Hydroquinone	37.9	µg/cig	1.6
Resorcinol	3.2	µg/cig	0.1
Catechol	40.8	µg/cig	0.6
Phenol	10.6	µg/cig	0.2
meta & para-Cresol	7.0	µg/cig	0.2
orto-Cresol	2.8	µg/cig	0.2
Ammonia	9.9	µg/cig	2.0
Hydrogen Cyanide	137.0	µg/cig	1.7
Semi-volatile bases			
Pyridine	12.6	µg/cig	1.7
Quinoline	*0.16*	*µg/cig*	*0.02*
Miscellaneous Organics			
1,3 Butadiene	41.9	µg/cig	1.3
Isoprene	315.0	µg/cig	18.9
Acrylonitrile	7.5	µg/cig	0.2
Benzene	37.3	µg/cig	1.6
Toluene	53.2	µg/cig	5.5
Styrene	14.6	µg/cig	0.4
Nitrosamines			
N-Nitrosonornicotine NNN	102.8	ng/cig	2.3
N-Nitrosoanatabine NAT	98.9	ng/cig	3.8
N-Nitrosoanabasine NAB	8.4	ng/cig	1.4
4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone NNK	93.1	ng/cig	4.1
Aromatic Amines			
3-Aminobiphenyl	0.99	ng/cig	0.07
4-Aminobiphenyl	0.69	ng/cig	0.11
1-Aminonaphthalene	11.17	ng/cig	0.42
2-Aminonaphthalene	2.97	ng/cig	0.40
NOx Nitric Oxide	155.1	µg/cig	5.7
pH	6.47	pH units	0.02
Metals			
Beryllium	< 2	ng/cig	
Cobalt	< 2	ng/cig	
Nickel	< 10	ng/cig	
Lead	*14.3*	*ng/cig*	*0.3*
Selenium	< 2	ng/cig	
Cadmium	36.7	ng/cig	1.4
Chromium	< 10	ng/cig	
Arsenic	3.1	ng/cig	0.1
Menthol	Not Detected	mg/cig	

Approved by:

(b) (6) [redacted]

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Customer:

Dr Michael Moynihan
22nd Century Limited LLC
8201 Main Street
Suite 6 Williamsville
New York
USA 14221

Sample Received: 14 March 2011

Date Testing Commenced: 16 March 2011

Date Reported: 01 April 2011

Samples Received: Camel Blue Box King

Testing Requirements: One thousand cigarettes in branded packets were submitted to the Scientific Services Laboratory of the Filtrona Technology Centre for testing for mainstream smoke yields.

Test Procedures: All tests have been carried out in accordance with documented in house test methods based on ISO standard methods when available. The reported data is only applicable to the sample received.

Results: The results are given on the page attached

Approved by:

(b) (6) [redacted]

Laboratory Manager
Filtrona Technology Centre

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Mainstream Smoke Components			
Date	**01/04/2011**		
Brand	**Camel Blue Box King**		
Bar Code	**FSC 01230739**		
Component	**Yield Mean**		**Yield SD**
Tar	10.8	mg/cig	0.9
Nicotine	0.80	mg/cig	0.05
Carbon Monoxide	12.5	mg/cig	0.8
Benzo [a] Pyrene	6.7	ng/cig	0.7
Carbonyls			
Formaldehyde	19.3	µg/cig	2.5
Acetaldehyde	563.4	µg/cig	13.3
Acetone	*201.8*	*µg/cig*	*5.1*
Acrolein	48.9	µg/cig	4.3
Propionaldehyde	42.4	µg/cig	0.9
Crotonaldehdye	16.4	µg/cig	1.1
Methyl Ethyl Ketone	45.1	µg/cig	2.7
Butyraldehyde	30.3	µg/cig	0.3
Phenols			
Hydroquinone	38.5	µg/cig	3.1
Resorcinol	3.5	µg/cig	0.2
Catechol	41.3	µg/cig	2.2
Phenol	8.8	µg/cig	0.5
meta & para-Cresol	6.1	µg/cig	0.4
orto-Cresol	2.3	µg/cig	0.2
Ammonia	9.5	µg/cig	0.3
Hydrogen Cyanide	157.7	µg/cig	11.9
Semi-volatile bases			
Pyridine	11.5	µg/cig	1.6
Quinoline	*0.11*	*µg/cig*	*0.01*
Miscellaneous Organics			
1,3 Butadiene	46.9	µg/cig	1.5
Isoprene	400.3	µg/cig	7.9
Acrylonitrile	9.3	µg/cig	0.1
Benzene	45.7	µg/cig	4.0
Toluene	72.3	µg/cig	5.3
Styrene	16.9	µg/cig	0.9
Nitrosamines			
N-Nitrosonornicotine NNN	72.3	ng/cig	2.8
N-Nitrosoanatabine NAT	77.6	ng/cig	0.4
N-Nitrosoanabasine NAB	7.6	ng/cig	0.4
4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone NNK	66.8	ng/cig	2.1
Aromatic Amines			
3-Aminobiphenyl	0.91	ng/cig	0.01
4-Aminobiphenyl	0.55	ng/cig	0.08
1-Aminonaphthalene	9.85	ng/cig	0.37
2-Aminonaphthalene	2.70	ng/cig	0.24
NOx Nitric Oxide	156.0	µg/cig	5.4
pH	6.49	pH units	0.03
Metals			
Beryllium	< 2	ng/cig	
Cobalt	< 2	ng/cig	
Nickel	< 10	ng/cig	
Lead	*14.3*	*ng/cig*	*0.7*
Selenium	< 2	ng/cig	
Cadmium	40.8	ng/cig	1.4
Chromium	< 10	ng/cig	
Arsenic	2.8	ng/cig	0.3
Menthol	Not Detected	mg/cig	

Approved by:

(b) (6) [redacted]

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Alkaloid Reduced Tobacco (ART) Program

I. PRODUCT CONCEPT AND PROJECT OVERVIEW

A. Historical Perspective

The concept of de-nicotinized, or lowered nicotine, tobacco is not a new one. As far back as the early 1950's through the late 1960's, Philip Morris and other companies were experimenting with various means to reduce the nicotine level of tobacco.[1] Extraction of nicotine in tobacco with various organic solvents was attempted. Problems associated with this technology included a long extraction time to achieve the desired nicotine reduction, lack of selectivity for nicotine (other things were extracted), and residual solvent remaining in the tobacco. Ammonia treatment of the tobacco with subsequent steam-stripping was also investigated. Again, problems were substantial residual ammonia in the tobacco causing an off-taste, ~~and selectivity for nicotine.~~

B. Early Testing at General Foods

Philip Morris acquired General Foods in November 1985. The first official meeting of the two R&D Departments (GF and PM) occurred in early December, 1985.[2] The purpose of this meeting was for the two senior R&D staffs to become acquainted, discuss GF technology and review PM's technology. Information presented at this meeting by GF's staff included a discussion of supercritical CO_2 extraction of caffeine from coffee, among other things. General Foods had extensive experience extracting alkaloids (caffeine) from coffee. They found

[1]King Sano produced by Steffano Brothers in Philadelphia, Reynolds #1 Box, marketed in Germany/Switzerland, and Reemstma R6 marketed in Germany were all low nicotine cigarettes.
[2]K. Houghton, J. Charles, C. Lilly, B. Kuhn, D. Knudson, L. Meyer, M. Hauserman, and F. Resnick. General Foods R&D was represented by R. Guardia, T. Fassina and C. Cante.

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supercritical CO_2 to be highly selective for caffeine, the extraction was reasonably fast, and it did not leave any undesirable residue.

As a result, Philip Morris R&D decided to put a project team together headed by C. Lilly to evaluate supercritical CO_2 extraction of nicotine from tobacco. The PM Project Team[3] visited Maxwell House R&D in Hoboken, New Jersey to discuss, with Tom Fassina and others, the de-caffeination plant being built in Houston and the possibilities of extracting nicotine from tobacco. Fassina recommended that Philip Morris use the 14 liter extraction unit at GF's Tarrytown, NY R&D facility for initial trials with tobacco. Philip Morris representatives spent three weeks at Tarrytown extracting tobacco using carbon as an absorber (absorbents are materials used to receive the nicotine extracted from the tobacco). The 14 liter extractor was too small for further studies and Fassina recommended that future evaluations be conducted in Bremen, Germany at Kaffee Hag's pilot plant.

In April 1986, PM representatives visited the Bremen facility. A large cross-functional team was established consisting of members from PM R&D, PM Engineering, GF, FTR R&D, and the Bremen facility management. Extraction trials were conducted at Bremen during June and July 1986, again using carbon as the absorbent. Results of these trials were ~~disappointing~~ from a taste point of view. Apparently, too much non-nicotine materials were extracted and absorbed by the carbon.

The team returned to Bremen in September 1986 for additional trials. Various other materials were tried as absorbents for the extraction process. These materials included cellulose, cocoa shells, acidified paper, and tobacco stems.

[3]C. Lilly, H. Grubbs, D. Knudson and F. Utsch.

Various acidic materials, ~~including MAP (monoammonium phosphate)~~, were applied to the absorbents to increase the efficiency of extraction. Tobacco stems proved to be an excellent absorber because stems contain similar substances to those found in tobacco. That is, an equilibrium was established limiting the loss of many non-nicotine substances from tobacco. Based on the results of these trials, Philip Morris made the decision to build a supercritical CO_2 extraction pilot plant in November 1986.

The team made another trip to Bremen in January 1987. Only tobacco stems were used as an absorber during these trials. Various types of tobaccos were extracted to evaluate their taste characteristics after processing.

From April through July 1987 another set of trials were run in Bremen. The purpose of these trials was to extract sufficient amounts of tobacco to accumulate a stock of extracted tobacco for product development work.

C. Early Concept Testing

Concept studies were conducted in 1983, 1985, and 1987. These studies were carried out to help Product Development determine consumer interest in new or novel cigarette ideas. In each of these studies, concepts describing cigarettes with low levels of tar and/or nicotine were tested. In each case, they were rated among the top in consumer interest. ***All of these concepts also promised "good taste."***

In 1983 one of the top rated concepts was "an ultra-low tar version of a popular full-flavor brand." This cigarette was described as having the same basic flavor as the parent brand, but would be milder with reduced levels of tar and nicotine. In

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1985, the concept of "nicotine free" was added. This concept was revised in 1987 to be "95% nicotine removed." This cigarette was described as "made from special tobacco to give a good tasting smoke without nicotine". Also in 1987, the concept of a cigarette with equal nicotine and "95% of the tar removed" was tested. In all cases, promises of reduced tar and/or nicotine levels were rated highly by the consumer.

D. Decision to Commercialize

Based upon favorable subjective evaluations using stems as an absorber during the September 1986 trials in Bremen, the decision was made to evaluate the commercial feasibility of a supercritical CO_2 tobacco extraction facility. Engineering work began in 1986 on the design and construction of a commercial facility capable of producing alkaloid reduced filler to support a potential market of 12 billion cigarettes per year. Foot note → A 2% share of the US market.

The ART[4] Pilot Plant came on-line in August 1987. The first series of pilot plant trials focused on design issues for the commercial plant. Pilot plant priorities gradually ~~quickly~~ shifted toward supporting product development work to meet the program goals of a 97% nicotine extracted product with liking scores better than Doral.

Marketing began developing programs for a reduced nicotine cigarette to be launched in the first half of 1989. Product development work had advanced to a point where it was felt that an acceptable cigarette with 97% of the nicotine removed could be produced. Funds were approved in February 1988, to construct

[4] Note on terminology: "ART" was the original name for the program and was generally used to refer to the *process* for removing nicotine from tobacco. The term "De-Nic" was based on marketing plans and was generally used to refer to the *product* after extraction.

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a commercial facility at the Bermuda Hundred site adjacent to Park 500. This facility came on-line in mid-1989.

The 1988 Surgeon General's Report added a sense of urgency to the ART program. It equated nicotine to "hard drugs" with respect to nicotine's alleged addictive properties.

II. PRODUCT AND PROCESS DEVELOPMENT

Process and product development began in earnest when the ART Pilot Plant came on-line in August 1987. A large cross-functional product development team was established with members from Product Development, Process Development, Analytical Research and other areas in R&D and Manufacturing. The development objectives were:

- Develop a commercial supercritical CO_2 process to remove 97% of the extractable nicotine from filler.
- Cost optimize the commercial process to maximize capacity and minimize cost while achieving the desired product objectives
- Optimize the commercial process to obtain the best possible subjective character from cigarettes produced with this filler.
- Develop products with subjective character equal to or better than price-value brands, such as Doral, from filler which has had 97% of its nicotine extracted with supercritical CO_2.

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The ART process resulted in a dramatic change in the taste of the original tobacco. The type of tobacco chosen for extraction, the casing and flavors used, and cigarette construction also significantly affected the final subjective character of the cigarette. It was necessary for Process Development, Leaf, Flavor, and Cigarette Technology to work closely in making changes to any variable during the development cycle. A change to any one parameter could have substantial, and possibly adverse, effects on subjective character or process economics.

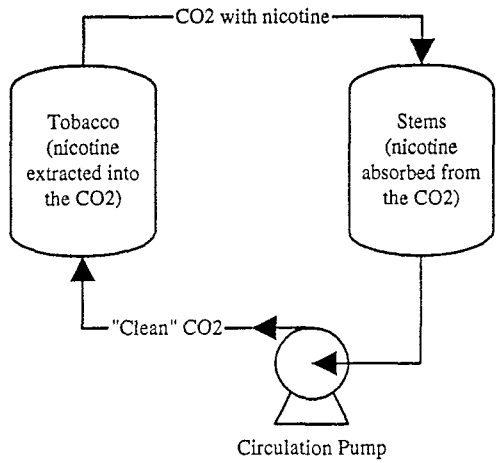
A. Process Development

The development program went through two distinct stages. In the first stage, the emphasis was on determining process parameters for achieving ~~achieving~~ the desired 97% nicotine extraction. This work resulted in a set of initial process specifications that was used as a design basis for the commercial facility. The second stage began after product testing indicated that the tobacco from this "best extraction" process had significant subjective deficiencies ~~problems~~. This latter work concentrated on modifying the extraction process in an attempt to improve the subjective character of the tobacco while still achieving the desired extraction.

1. Initial Process

The ART process works by recirculating supercritical CO_2 through a bed of tobacco. Nicotine is extracted from the tobacco into the CO_2. For the process to work, however, a second material, the absorbent, is needed to remove, or

ART Process



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"scrub", the nicotine ~~back out of the~~ from the CO_2 so that the CO_2 can pick up additional nicotine on its next pass through the tobacco bed. The key to the process is thus to enhance both the extraction of nicotine from the tobacco as well as the absorption of nicotine into the absorbent. The overall extraction efficiency of the process is influenced by both of these steps.

Ideally, only nicotine would be extracted from the tobacco; nothing else. Supercritical CO_2 approached that ideal process although some substances other than nicotine were extracted.[5] As a result, the process development not only had to concentrate on nicotine extraction, but also on the selectivity of the process for removing nicotine with minimal extraction of other tobacco components. It was felt that this would produce the best subjective product. Process testing determined that there were six process parameters that influenced the extraction.

Higher *extraction pressure* increases the rate of nicotine extraction from the tobacco. The Bremen trials had used 3,800 psi, and this was initially selected as the process specification. Higher *extraction temperature* also increases the nicotine extraction rate. While the Bremen trials had used 140°F, early testing indicated that temperatures as low as 130°F could still achieve extraction targets.

Although the process was very efficient at extracting nicotine from tobacco, it was apparent, even from the Bremen trials, that additives would be necessary to enhance the extraction efficiency. Increasing the *tobacco pH* increased the solubility of nicotine in CO_2. A pre-extraction casing was developed for the

[5]See Appendix 1.

tobacco that contained ammonium bicarbonate (AB), initially at an add-on rate of 3% of the tobacco weight on a dry weight basis. While the AB was successful in improving extraction, it was realized that the residual ammonia resulted in substantial off-taste problems. It was initially hoped that these problems could be rectified by further tobacco processing.

Just as higher pH enhanced the solubility of nicotine in CO_2, a lower *stem pH* helped to scrub the nicotine onto the absorbent. To lower the stem pH, monopotassium citrate (MPC), at an add-on rate of 12% of the stem weight on a dry weight basis, was initially used for this purpose.

Longer *extraction time* also increases the nicotine extraction. The length of extraction was defined not in time, but rather in the number of pounds of CO_2 that was circulated through the tobacco bed per pound of tobacco. This was referred to as "M/M" (M-over-M). While the other parameters were rigidly specified, the facility could vary M/M as needed to achieve the target 97% extraction of nicotine. At the conditions specified above, testing in the pilot plant showed that M/M as low as 150 could achieve this target. Obviously, lower M/M was desirable in order to increase the capacity of the plant.

A final parameter was the *stem-to-filler ratio.* The process required that there be enough stems to "hold" all the nicotine extracted from the tobacco. This establishes a minimum stem-to-filler ratio, which was found to be 1 pound of stem per pound of filler.

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In summary, the initial process conditions were 3,800 psi, 130°F, 3% AB add-on to the filler, 12% MPC add-on to the stems, about 125 M/M, and a 1 to 1 stem-to-filler ratio.

2. Process Modifications to Improve Subjectives

The conditions described above were developed primarily to optimize the process for nicotine extraction efficiency. However, evaluations of the pilot plant material showed significant subjective problems, which were also seen with the initial samples from the commercial facility. Process development activities shifted to a second stage in attempts to remedy these problems. As a result, all six of the process parameters listed above were changed.

The first major problem to be addressed was the off-taste in the product. Post-extraction treatments of the tobacco with various acidic materials, flavor materials, and steam were unsuccessful in eliminating this off-taste. Since the residual ammonia was a contributor to the off-taste, the AB add-on target was reduced from 3.0% to 2.5%. This resulted in some taste improvement, but the residual ammonia from the AB was always felt to be somewhat responsible for the off-taste of the De-Nic product. Reducing the AB add-on resulted in some loss of nicotine extraction efficiency.

The second major change was the reduction of the extraction pressure from 3,800 psi to 2,000 psi. Initial tests in the commercial facility showed that ~~we~~ it was ~~were~~ extracting more non-nicotine materials than previously thought. Subsequent tests showed that the reduction in extracting pressure improved the selectivity of the process to some extent. While the absolute extraction rate of

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nicotine was reduced, the rates for the non-nicotine materials were reduced to a greater extent. This change in extraction pressure resulted in some improvement in subjective character.

Both the AB reduction and extraction pressure reduction decreased the rate and efficiency of nicotine extraction, however. This required changes in the other process parameters to compensate and still achieve the 97% target. The extraction temperature was increased back to 140°F, and the M/M ratio was also increased to 175. Further, the extraction pressure reduction changed the density and flow properties of the CO_2 such that the recirculation rate had to be lower as well, which extended the absolute extraction time even further. All of these changes were undesirable from a cost and plant capacity viewpoint, but the severity of the subjective problems left no other alternatives.

A change was also made to the stem absorbent to improve its scrubbing efficiency. The MPC was replaced by citric acid (CA) to further reduce the stem pH. Because CA was more effective, the add-on rate was decreased to 8%, and the stem-to-filler ratio was reduced to 0.75 pounds stem per pound filler.

This second set of process conditions was developed over the latter half of 1989. In summary, these conditions were 2,000 psi, 140°F, 2.5% AB add-on to the filler, 8% CA add-on to the stems, 175 M/M, and a 0.75 stem-to-filler ratio. These specifications were officially adopted in early 1990 and used for all subsequent products.

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3. Later Process Development

If De-Nic sales volume ever approached plant capacity, we would not have been able to acquire enough stems to use as an absorbent. Consequently, process development activities in the pilot plant facility for the remainder of the program focused on developing a second generation extraction process, where a water and citric solution acid would be used as the absorbent, not stems. This process, while successful in eliminating the stems, had no effect on product subjectives and was not implemented in the commercial facility because sales volume did not justify the additional capital expenditure.

B. Product Development

Significant product development activities were associated with providing direction to the process development effort described in the previous section. In addition, there were other initiatives that did not directly affect the extraction process parameters.

1. Tobacco Blend

Extraction trials at the ART Pilot Plant were used to develop tobacco blends. Individual blend components, typically single grades of tobacco, were extracted and subjectively evaluated. Based on these subjective evaluations and availability of leaf, the DL Blend was developed.[6] The DL blend was used in the first four De-Nic test markets in mid-1989.[7]

[6]DL Blend: DL-Bright - 68.6%; DL-Burley - 19.6% (Bodied and thin); MT-Oriental - 11.8%, DL stands for Design Lamina

[7]1989 De-Nic Test Markets; NEXT: Omaha, Nebraska; Hartford, Connecticut; Toledo, Ohio; Merit De-Nic- Spokane, Washington.

In 1990, after the new low pressure extraction conditions were selected, blend work was re-initiated. Using low pressure extraction conditions, single grades of tobacco were again extracted and subjectively evaluated. Based on these results, various blends were developed, extracted and subjectively evaluated. As a result Blend 215 was chosen for the low pressure extractions.[8] Blend 215 was used for the Phoenix and Tampa test markets and the other existing test markets were converted to 215.

2. Flavors and Menthol

Flavors and casing systems are used to add pack aroma to the product, modify the taste direction of the product slightly and add the characteristic "Brand Signature". ***Flavors cannot make a bad product good or cover up major subjective deficiencies within a ~~product~~ cigarette.*** ~~The tobacco.~~

The key challenge in developing a flavor system for De-Nic products was overcoming the inherent off-taste in the product. Development efforts concentrated on masking or eliminating this taste and adding "tobacco-like" response to the product. Various post-extraction casing and flavor systems were evaluated for this program. Hundreds of individual flavors and flavors systems (including substantial work with Capsaisum) were subjectively evaluated on a laboratory, pilot and commercial scale. Unfortunately, particularly for the non-menthol version, we were never able to overcome the off-taste problem.

I'd like to include the reference to Capsaisum. It came up in the workmen hearings as a possible substitute for nicotine!

Development of the menthol prototypes achieved a higher ~~some~~ level of success. Menthol has the ability to "cover-up" some amount of off-taste. Menthol, like

[8]215-Blend; DL-Bright - 65%; 215-Burley - 25% (Bodied); MT-Oriental - 10%.

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nicotine, has a trigeminal sensorial response which may have contributed to higher liking scores by internal panels compared to the non-menthol version.

C. Consumer Testing in Support of Product Development

1. Internal Testing

All of the initial product testing of De-Nic was performed within R&D using PM smokers. This was done solely to prevent competitors from gaining knowledge of our program. We were fully aware of the limitations associated with testing products internally. Market acceptance of the products tested could not be ~~addressed~~ quantified with internal panels because of the small number of smokers and the variety of brands and deliveries that were being evaluated. Product improvements could, however, be assessed through measuring differences from controls.[9]

Sensory research on De-Nic began in 1986 with a test conducted to determine the overall sensory effects of nicotine extraction on various types of tobacco. It was found that extracted tobaccos were perceived by our internal smokers as lower in impact[10] and overall acceptability, and higher in off-taste. There were also fewer differences in taste attributes between types of tobacco after extraction. This implies that the extraction process was removing or modifying ~~taste~~ the inherent flavor components that were responsible for the individual character of the tobaccos.

[9]Controls refer to conventional cigarettes that have not undergone a nicotine extraction process.
[10]Impact can be defined as: Upon inhaling cigarette smoke, the primary sensation or response immediately felt in the back of the throat, typically short lived in duration and dissipates upon exhaling. Impact is followed by other sensations and flavor responses.

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From the end of 1986 through 1987, the Bremen tests were conducted to explore different extraction processes and the overall effects of processing on taste. The processing changes that were sensorially evaluated included carbon versus citrate on stems as the absorbent, and the use of ammonium bicarbonate as a processing aid to increase the rate of extraction. All De-Nic cigarettes, regardless of the extraction method used, were rated higher than conventional cigarettes in unusual flavor, off-taste, and mouthcoating.

The overall taste effects of processing were evaluated by a series of nicotine add-back studies. This testing indicated that adding back the extracted nicotine improved sensory ratings somewhat, however, overall acceptability was still lower than controls. These samples were evaluated by internal expert smokers only. Other important/inherent flavor components were also being lost or modified in the extraction process.

During this same time period (1986-1987), extended testing (over 5 packs) was conducted versus Doral Lights and Carlton. Doral Lights was chosen because it was considered to be one of the least acceptable cigarettes on the market, and Carlton was chosen because it was in the same nicotine range as De-Nic .

Testing of De-Nic showed a drop in acceptability from the first to the last cigarette in the first pack and leveling off thereafter to a similar acceptability rating to Carlton. Neither Carlton nor Doral Lights showed a similar drop over time. De-Nic was rated similar in acceptability to Carlton and lower than Doral Lights. Although De-Nic was rated higher in satisfaction than

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Carlton, it was also rated higher in off-taste, mouthcoating, and non-tobacco taste indicating that these deficiencies were not due to low nicotine content. One extended test, with internal expert smokers, showed that while an extracted cigarette with nicotine added back was less acceptable than a control, the acceptability did not drop over time. This may indicate that the presence of tar without nicotine might result in an initially more positive impression with increasing dissatisfaction with the cigarette over time when nicotine is missing.

During 1988 and early 1989, a series of tests were conducted to evaluate blends and flavors in an attempt to minimize off-notes. Mentholated cigarettes were also investigated. While slight directional improvements were seen, flavorings were unable to compensate for off-notes. An exception to this was menthol. Mentholated De-Nic products showed higher initial acceptability ~~as~~ compared ~~with~~ to non-menthol De-Nic cigarettes; however, the same drop in acceptability from first to last cigarette in a pack was noted.

Further extended testing among what? internal Smokers conducted during this time period concluded that a subset of people liked and would continue to smoke a De-Nic product. This group could not be defined in terms of socio-demographic variables. Why? Sample Size

Ok. Check with Int'l on this point

In 1990 sensory research was focused on different absorption processes and adding back non-nicotine materials that had been lost through processing. While some subjective improvements were seen, the product was still significantly less acceptable than conventional cigarettes.

George, Ok do you think this paragraph is redundant? Your call

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In 1990 and 1991, two internal studies were conducted to determine the effects of tar, nicotine, and menthol on the attributes of strength and menthol perception. All three variables were found to interact to affect these attributes:

- In non-menthol cigarettes, higher levels of tar and nicotine produced higher strength perceptions. The strength correlation was better for nicotine than tar. Tar was more tightly correlated with off-taste than was nicotine.

- In menthol cigarettes, menthol added to the overall strength perception (second to nicotine). The perception of menthol was not affected by nicotine.

This relationship was explored further using an external panel of smokers. In these tests, the tar and menthol were increased to a high enough level to compensate for the loss of strength in a reduced (one-half level) nicotine cigarette. The acceptability of these models was low, suggesting that an imbalance of tar and nicotine may lead to poor acceptability.

2. External POL Testing

During the end of 1989, large scale testing was begun on our external consumer panel. Each of these tests was conducted using over a thousand smokers representing the entire delivery range. The respondents were chosen from our established database (POL) composed of over 300,000 smokers across the United States. A series of monadic blind product tests were conducted on non-menthol cigarettes (85 and 100s) of different tar levels, extraction pressures, and with different blends and flavor systems.

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All non-menthol tests showed results that were significantly lower in liking than cigarettes of equivalent tar levels. The De-Nic cigarettes were also found to be less acceptable than conventional cigarettes in the same nicotine range (Carlton, Now) as evaluated by smokers of low nicotine cigarettes. This was in agreement with results found internally indicating that extracted cigarettes had been altered in taste character over and above the loss of nicotine.

III. MARKETING

A. Small Scale Concept and Trial Testing

During January 1988, three focus groups were held in New York City to gauge interest in a De-Nic Merit product. Since the product was being tested as a Merit entry, interviews were held among one group of Merit smokers and two groups of competitive low tar smokers. Trial interest was high, primarily because of the product's perceived health benefit. However, respondents indicated that repurchase of the De-Nic product would depend on its ability to deliver good taste. Some respondents felt that the De-Nic product fit with Merit's low tar image. However, the association of good taste with the Merit name was only believed by current Merit smokers and a few competitive smokers. For most competitive smokers, the Merit name conveyed an unacceptable taste perception, and therefore they would reject a new Merit entry.

A nicotine Concept Test was run during February 1988. A total of 750 interviews were conducted among 18-54 years olds. Menthol and non-menthol smokers in 10

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geographically dispersed markets were asked to assess four De-Nic concepts. The product concepts were:

- ◊ Nicotine Free
- ◊ Reduced Tar
- ◊ All Natural
- ◊ Less Visible Smoke

Of the four concepts tested, "Nicotine Free" and "Reduced Tar" appeared to have the greatest appeal to smokers, with one-third of the respondents expressing interest in each of these concepts. However, when those selecting "Nicotine Free" were told that there would be a taste sacrifice, interest in the product was reduced by more than half. The preference for "Nicotine Free" was based on the perceived health benefit. The disadvantage to this concept was that the taste would be compromised. Those smokers most interested in the "Nicotine Free" concept were: women, older, flavor low smokers, and ultra low non-menthol smokers. However, when smokers were informed there would be a taste sacrifice, there was less discrimination in appeal among smoker groups.

A series of focus groups and one-on-one interviews were conducted by Marketing Research in the fall of 1988 among flavor-low tar and ultra-low tar smokers.[11] These groups explored interest in the concept, initial reaction to the cigarette, and initial advertising direction. Consumers understood the concept and were generally interested in it. However, there was some degree of confusion about the

[11]Flavor-low tar smokers are those smokers in the Lights category, i.e. smoking brands in the 8 to 11 mg tar range. Ultra-low tar smokers are those smokers in the Ultra-Lights category, i.e. smoking brands in the 5 to 6 mg tar range.

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differences between low tar and low nicotine. Some respondents perceived low nicotine cigarettes as "healthier" and others considered them as a way to cut down on smoking. Although the taste was thought to be lighter than most people wanted, those that were interested in the concept expressed a willingness to adjust to it.

As part of the 1988 Continuous Tracking Study[12], two thousand smokers were asked a series of questions aimed at determining preference for either a nicotine free or tar reduced cigarette. Previous research evaluated four concepts and the results indicated that these two product concepts have the greatest appeal to consumers. Findings from this study were consistent with past findings. Specifically, the two concepts seemed to have equal consumer appeal with about half of all respondents preferring nicotine free and half preferring reduced tar. Interest dropped, however, when respondents were informed that they would have to make a taste compromise.

In January 1989, R&D conducted sixty-nine one-on-one interviews among flavor-low tar and ultra-low tar smokers. This research was designed to evaluate concept interest and initial cigarette acceptability in the context of a free-standing product named Next. Four and eight mg tar products were used in the study. Again, the concept was favorably received.

In the spring of 1989, concept/trial protocols were expanded to include more product usage. This research explored concept interest, single stick smoking (initial reaction to the product), and 2 pack placement with call-back followed by a

[12]Continuous Tracking Study: A telephone study conducted among 3,000 smokers per month to track current brand, alternate cigarette purchases, coupon usage and other brand information.

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product choice (carton of own or carton of De-Nic).[13] As in the internal studies, liking ratings dropped from single stick to 2 packs. In general the menthol product performed better than the non-menthol product. When offered a carton of own brand versus De-Nic, 25% of the non-menthol consumers chose De-Nic , versus 45% among menthol smokers.

B. Large Scale Consumer Use Testing

In July 1989, extended use testing was conducted by Marketing Research on a nationally representative sample of smokers (~4000). Consumers were sent up to three cartons, and were interviewed up to three times during the study. This research was conducted to determine long term appeal, to profile interested smokers, and to identify which packings had the greatest appeal. Sixty percent of the smokers initially contacted were interested in trying De-Nic. In long term smoking among consumers interested in the proposition, liking ratings improved over time. This indicates that interested consumers can adjust to the taste somewhat. At the end of the study, approximately 10% of the respondents rated De-Nic as excellent and expressed an interest to use it in place of their regular brand. This subgroup was identified as the intended audience. From this research the products with highest appeal were regular and menthol, flavor-low and ultra-low 100 mm.

Two POLs were conducted in March through June of 1989. These studies included blind product testing of three De-Nic models (at 4, 8 and 11 mg of tar),

[13]In this research each potential consumer (panelist) is shown a product concept statement and asked to rate their potential interest in that type of a product. Then the panelist is given a single cigarette to smoke and asked to rate it on liking. Next the panelist is given two packs of cigarettes to take with them and smoke at their leisure. The panelist is contact by phone several days later, asked to rate the two packs on liking, then asked a hypothetical product choice question: Would you like a carton of your own current brand or a carton of De-Nic?

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followed by telephone call-back. During this call-back the panelists were read a concept statement describing the test cigarettes they had smoked. The panelists were then offered a carton of De-Nic or their own brand. The panelists were then re-contacted and offered the carton choice again. Results showed that most consumers were interested in the De-Nic concept and that a segment (~17%) liked and continued to choose the De-Nic over their regular brand.

As previously found through internal and small scale concept/trial studies, both of these consumer studies identified a subgroup of smokers that liked and would continue to smoke a De-Nic product. These smokers were more likely to be female, over 30 years old, and consumers of 100mm ultra-low tar menthol cigarettes.

C. Ad-Pack Tests

1. Nicotine-Extracted Advertising/Single Cigarette Study - January 1989

   This study was conducted among 300 non-menthol smokers' to assess opinions and reactions to De-Nic products and advertising. The total sample was divided into three groups:

   Jimmy questioned the spelling. Use this on actual ad? Fix if necessary.

   - One third was shown a "N-ext" ad and given a control Merit cigarette to smoke.
   - One third was shown a "N-ext" ad and given a nicotine extracted product to smoke.
   - One third was shown a "Merit Free" ad and given a nicotine extracted product to smoke.

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Reactions to ads were assessed prior to respondents smoking the product. The respondents were given one stick to smoke and reactions were obtained. Key Findings of this study were:

- All of the ads effectively communicated the product proposition. Respondents shown the Merit Free ad more frequently mentioned lower nicotine while respondents in the group exposed to the N-ext ad and receiving De-Nic product were more likely to mention "Nicotine Extracted" more than respondents in the other two groups.

- Most respondents across the groups thought the product would be different than other major brands after viewing the ads and about half in each group thought this difference was extremely or very important. The primary reason attributed to this importance was the lower nicotine.

2. Ad/Pack Research - March 1989

An Ad/Pack was conducted among 1,778 flavor low and ultra low, non-menthol and menthol smokers to evaluate interest in two De-Nic concepts: Merit Free and Next.

Respondents were shown advertising and then given five packs to take home. Call backs were made after smoking two packs and then after smoking five packs. Respondents were then presented with a hypothetical situation of choosing a free carton of the De-Nic product or a free carton of their regular brand to further assess interest. Key findings of this study were:

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- Overall, prior to smoking the product, purchase interest based on the concept was low.[14] After smoking two packs of the De-Nic product, purchase interest decreased.

- No difference was found in purchase interest or opinion of taste after two packs between Merit Free and Next.

D. Test Markets

Below is a summary of the shipment and retail performance of De-Nic in it's various test markets and brand presentations. A brief recap of Merit Ultima's year 1 performance is also included for comparison.

1. Next De-Nic - Original Test Markets

Next De-Nic was tested in three different packing configurations, in three markets, beginning July 1989.[15] Retail shares reached highs of 1.3 to 2.7 share points in the first two months. Typically, 40 - 50% of volume consisted of '2 for the price of 1', and '5 for the price of 2' deals. In general, the higher the deal product offers, the higher the peak shares.

All markets peaked within two months and then showed consistent declines. In month three, shipment shares to our direct customers dropped below 0.1 to 0.2 share points, and, except for one brief surge to 0.35 share points in Toledo, remained there.

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[14]In this Ad/Pack test the respondents were rating purchase interest based on the Merit Free and Next ads. These ~~This~~ differ from previous concept research where the consumers were just given a concept without a name, pack, or imagery.

[15]Omaha, Nebraska - Ultra low product; Hartford, Connecticut - Flavor low product; Toledo, Ohio - Ultra and Flavor low products.

2. Merit De-Nic

Merit De-Nic's test market began in August 1989, one month after the original Next markets. Like the Next test markets, peak retail share of 1.6 share points was achieved in the first two months. Promotional offers were identical to the Next test markets. Also similar to the Next test markets, Merit De-Nic's shipments dropped to near 0.1 share of market after twelve weeks.

3. Next De-Nic -- Tampa Test Market

After poor showings in the original test markets, a modified product was introduced into the Tampa market in May 1990. Promotional retail support was extended longer than in the original markets, and was augmented by direct mail offers. Relatively higher retail shares were sustained into month three, with about one third of the brand's volume consisting of retail promotional product in the form of '2 for the price of 1' deals. Nevertheless, after about seven months, shipment shares had dropped to the 0.1 - 0.2 range. By the end of the year 1, they dropped to below 0.1 share points.

4. B&H De-Nic -- Arizona Test Market

The final De-Nic product tested at retail was B&H De-Nic, which began in October 1990. Retail shares built slower and peaked somewhat later than in other markets. Share built to 1.7 share points in months three and four. Except for the timing of the peak shares, ongoing tracking indicated that B&H De-Nic was not performing significantly differently from those earlier markets. Retail promotion product, in the form of '2 for the price of 1', comprised about

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30% of the brand's total retail volume during the first four months. By month eight, shipment shares had dropped to 0.2.

Can we add an introductory sent.?

E. Merit Ultima Performance in National Launch

Merit Ultima ~~was launched the first quarter of 1992~~. By the end of the year, national retail shares, as measured by PM's integrated Nielsen monthly panel, were in the 0.4-0.5 share point range. Shipment shares were averaging about 0.3 share points.

add a Sentence: Retailers & Wholesalers were complaining about the slow moving product & were asking to return product.

V. DECISION TO PULL THE PRODUCT

Shipment share in all the De-Nic test markets had dropped to a level of 0.1 to 0.2, and in the case of Tampa to below 0.1 share points. In addition, incidence of stores who used to, but no longer carry the product was increasing. This data, taken together, indicates that the demand for this product is dropping; the consumers are not pulling it through retail.

Does this work?

De-Nic Products were produced in a dedicated production facility. With Shipment Share at 0.1 to 0.2 level, this facility was idle most of the time. Facing decreasing share and high operating costs for an idle facility, the obvious business decision was made to shut the facility and pull the product from test market.

By way of comparison, Merit ultima 85's (1mg tar, 0.1mg nicotine) and 100's (2mg tar & 0.2mg nic) were launched to compete with Carlton & new in the first quarter of 1992

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~~Can we make a closing statement about work~~ Continuing until ~~35~~ on a new Dol version and export versions?

Appendix 1

What Else Besides Nicotine Was Extracted?

In addition to nicotine, the following compounds or compound classes have been reported as detected either in samples of the supercritical carbon dioxide used to extract the tobacco, from the liquid carbon dioxide that was stored in the working vessel, or from laboratory scale experiments. It is important to realize that the temperature and pressure used for the extraction changes the distribution of the compounds that are extracted. When possible, the comments below pertain to the extraction conditions (2200 psi) that were used near the close of the ART Program.

OILS AND WAXES: The generic terms of oils and waxes cover a number of compound classes and a larger number of specific compounds. These compounds are extracted by supercritical carbon dioxide. At the risk of over simplification, and with recognition of exceptions, these compounds (especially those of higher molecular weight) become more soluble in supercritical fluid carbon dioxide as the pressure and temperature increase. In a way analogous to essential oils in other plants such as citrus fruit, the presence and balance of these compounds in tobacco give it the characteristic flavor and aroma that is associated with tobacco. Removal of these compounds, or even a change in the concentration ratios, can destroy the characteristic flavor or aroma of the plant. As the ART Program progressed, movement from the ~~stringent~~ most effective extraction conditions to milder ones had a subjective impact.
positive

ISOPRENOIDS: Isoprenoids are a class of compounds that are acyclic terpenes derived from isoprene that are tobacco specific. Two of these are commonly associated with tobacco. They are solanesol and neophytadiene. Neophytadiene is normally the most

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abundant compound present in the mass spectrum of samples taken from the extraction fluid, and in laboratory scale extractions. Solanesol is of much larger molecular weight that neophytadiene. Although evidence of solanesol in the extraction fluid was found, it was not confirmed by standard analytical methods. The solubility of solanesol in the supercritical fluid would be significantly dependent on the temperature and pressure.

CARBOXYLIC ACIDS: Tobacco contains a number of carboxylic acids. The lower molecular weight acids are perhaps too polar to be soluble in supercritical carbon dioxide. However palmitic acid, oleic acid, and a number of C_{18}-fatty acids were found in samples taken from the extraction fluid. The impact of these compounds on flavor considerations is not well understood.

HYDROCARBONS: A series of hydrocarbons in the range $C_{27}H_{50}$ to $C_{33}H_{68}$ was found in the extraction fluid. A compound in this range that also contained aromatic as well as aliphatic character was detected but not identified. These compounds are of a nature to expect extraction by supercritical carbon dioxide. The impact of removal of these compounds on tobacco flavor and aroma is not known.

TOBACCO-SPECIFIC NITROSAMINES: Various fillers that had been extracted with supercritical carbon dioxide were analyzed for tobacco-specific nitrosamine (TSNA) content. TSNA levels of the extracted fillers were reduced relative to controls that had not been extracted.

OTHER COMPOUNDS: Other compounds that have been detected or suspected include cembranoids, stigmasterol. These compounds are known to be in tobacco and cembranoids have been reported to effect the "quality" of cigarette smoke.

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COMPOUNDS NOT EXTRACTED: Two classes of components not found in the extraction fluid are sugars and inorganic compounds. Although sugars are known to impact flavor, they alone are not sufficient to provide the necessary aroma and flavor for good subjective response.

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Advertising Claims

Appendix 2

1. Introducing De-Nicotined Tobacco With Rich Flavor.; Thanks to our patented FreePLUS process, it is now possible to naturally remove nicotine from fine tobaccos while leaving their rich flavor intact.; As a result, our new cigarette has less than 0.1 mg nicotine.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.

2. De-Nicotined Tobacco the Smooth Taste of a Light.; The only cigarette with less than 0.1 mg of nicotine made from de-nicotined tobacco.; It's a unique process that works much the way that caffeine is extracted naturally from coffee beans.; Nicotine extracted from fine tobaccos, creating a cigarette with a smooth, light taste and less than 0.1 mg nicotine.

3. De-Nicotined Tobacco the Smooth Taste of a Light; The only cigarette made from de-nicotined tobacco. So there's less than 0.1 mg of nicotine.

4. De-Nicotined Tobacco Retains Rich Flavor.; One-of-a-kind cigarette gives smokers unique option with less than 0.1 mg nicotine.; Our FreePLUS process now makes it possible to naturally remove nicotine from fine tobaccos while leaving their rich flavor intact.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.

5. De-Nicotined Tobacco.; Only one-of-a-kind NEXT offers smokers rich flavor with less than 0.1 mg nicotine.; That's because only NEXT is made with the patented FreePLUS process, which naturally extracts nicotine from tobacco, leaving rich flavor intact.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.

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6. De-Nicotined Tobacco the Smooth Taste of a Light.; The only one that removes nicotine directly from the tobacco. So there's less than 0.1 mg of nicotine.

7. Introducing De-nicotined Tobacco with Rich Flavor.; Smokers now have an important option that offers rich flavor and less than 0.1 mg nicotine.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.

8. De-nicotined Tobacco.; The only cigarette with less than 0.1 mg of nicotine made from de-nicotined tobacco.; It's a unique process that works much the way that caffeine is extracted naturally from coffee beans.

9. Introducing De-Nicotined Tobacco with Rich Flavor.; Thanks to our patented FreePLUS process, it is now possible to naturally remove nicotine from fine tobaccos while leaving their rich flavor intact.; As a result, our new cigarette has less than 0.1 mg nicotine.; De-nicotined tobacco and rich flavor in a cigarette you'll like to smoke.

10. De-Nicotined tobacco with rich flavor, or de-nicotined tobacco with menthol refreshment.; Naturally extracts nicotine from tobaccos leaving rich flavor and less than 0.1 mg nicotine.

11. Introducing De-Nicotined Tobacco with Rich Flavor.; Starting today, there's only one option for smokers who want rich flavor with less than 0.1 mg nicotine.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.; For the first time, you can now enjoy menthol freshness and less than 0.1 mg nicotine in a cigarette made with de-

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nicotined tobacco.; De-nicotined tobacco with menthol freshness in a cigarette you'll enjoy.

12. This Changes Everything.; New cigarette with de-nicotined tobacco and rich flavor gives smokers major reason to change.; NEXT is made possible by the patented FreePLUS process, which naturally extracts nicotine from fine tobaccos, leaving their rich flavor intact.; The result is a satisfying cigarette with less than 0.1 mg nicotine.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.

13. US Patent 4,153.063.; The patent is for the process. The FreePLUS process. The process that naturally extracts nicotine from tobacco, yet leaves rich flavor where it should be.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.

14. De-nicotined Tobacco and Rich Flavor are waiting for you right now.; With rich flavor and less than 0.1 mg nicotine.

15. The first cigarette with de-nicotined tobacco and rich flavor is coming.; Thanks to a patented Philip Morris process called FreePLUS it is now possible to naturally remove nicotine from tobacco without removing all the flavor.; It's because of this process that our rich de-nicotined tobaccos stay rich, even though less than 0.1 mg nicotine remains.

16. De-nicotined Menthol and Smooth Fresh Taste.; The result of a nicotine extraction process that remove nicotine directly from the tobacco itself. Much the way caffeine is naturally removed from coffee beans.; What you get is a smokeable menthol cigarette with smooth, fresh taste and less than 0.1 mg of nicotine.

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17. De-nicotined tobacco the smooth taste of a light.; The result of a nicotine extraction process that removes nicotine directly from the tobacco itself. Much the way caffeine is naturally removed from coffee beans. What you get is a smokeable cigarette with the smooth taste of a light and less than 0.1 mg of nicotine.

18. De-Nicotined Tobacco the Smooth Taste of a Light.; Benson & Hedges De-Nic is the only cigarette with less than 0.1 mg of nicotine made from de-nicotined tobacco.; The result of a unique process that works much the way caffeine is naturally extracted from coffee beans, for a smokeable cigarette, with the smooth taste of a light.

19. Less than 0.1 mg nicotine the smooth taste of a light.; Benson & Hedges De-Nic is the only cigarette with less than 0.1 mg of nicotine made from de-nicotined tobacco.; The result of a unique process that works much the way caffeine is naturally extracted from coffee beans.

20. Rich Tobacco De-Nicotined is still rich tobacco.; As a result of our patented FreePLUS process, there is only one option for smokers who want rich flavor and less than 0.1 mg nicotine.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.

21. Introducing De-Nicotined Tobacco with Rich Flavor.; Smokers now have important new option from Merit that offers rich flavor and less than 0.1 mg nicotine.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.

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22. Next De-Nic.; Smokers who enjoy rich flavor can take pleasure in a new cigarette with less than 0.1 mg nicotine.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.

23. Rich Tobacco De-Nicotined is still Rich Tobacco.; Breakthrough new cigarette with less than 0.1 mg nicotine keeps rich flavor intact.; Our patented FreePLUS process naturally extracts nicotine from fine tobaccos, without extracting flavor.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.

24. Menthol freshness stands out in NEXT De-Nic.; Our patented FreePLUS process new makes it possible to naturally remove nicotine from fine tobaccos.; As a result, our new cigarette has less than 0.1 mg nicotine.; De-nicotined tobacco with menthol freshness in a cigarette you'll enjoy.

25. NEXT De-Nic.; NEXT is made possible by the patented FreePLUS process, which naturally extracts nicotine from fine tobaccos, leaving their rich flavor intact.; The result is a satisfying cigarette with less than 0.1 mg nicotine.; De-nicotined tobacco and rich flavor in a cigarette you'll enjoy.

26. NEXT De-Nic.; The new is less than 0.1 mg nicotine. The first one made with de-nicotined tobacco and real menthol freshness.; Made possible by a patented process called FreePLUS that naturally extracts nicotine from tobacco.; De-nicotined tobacco with menthol freshness in a cigarette you'll enjoy.

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BIOTECHNOLOGY: A TOOL FOR REDUCED-RISK TOBACCO PRODUCTS - THE NICOTINE EXPERIENCE FROM TEST TUBE TO CIGARETTE PACK

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ABSTRACT

Biotechnology offers a means to "rapidly" alter tobacco plants to produce smoking products that deliver lower levels of known toxins and carcinogens. Although most products of biotechnology could be delivered in a traditional breeding program, biotechnology and genetic engineering offers a significantly more rapid and targeted technology to achieve the desired goal. At Vector Tobacco, we have targeted nicotine reduction as the first step towards using genetic engineering to modify tobacco such that it will deliver exceptionally low nicotine levels and have similar reductions in the associated tobacco-specific nitrosamines (TSNAs). Quinolinate phosphoribonucleotide transferase (QPTase) is one of the two key, rate-limiting enzymes in nicotine biosynthesis. Using transgenic technologies we have targeted QPTase as a means to reduce nicotine levels. The resulting plants accumulate approximately 1000 ppm nicotine (as opposed to the 25,000 ppm for the non-transformed controls) and have reductions of about twenty-fold in the TSNAs NNK and NNN. The resulting transgenic tobacco has been deregulated by the USDA and has been commercialized in Quest cigarettes. This presentation will summarize characterization of the tobacco gene encoding QPTase (NtQPT1), use of NtQPT1 to reduce nicotine levels, characterization of the field

performance of low-nicotine transgenic plants, and commercial production of low-nicotine cigarettes from genetically-modified tobacco.

INTRODUCTION

Vector 21-41, a transgenic tobacco producing very low nicotine levels, represents the first commercialized attempt to produce a tobacco product using tobacco modified by genetic engineering. Nicotine is widely believed to be the addictive component of tobacco. It also serves as the precursor for the production of tobacco specific nitrosamines (TSNAs), which are potent mutagens and potential carcinogens. Nicotine addiction has been studied extensively and researchers generally agree that smoking can be greatly reduced if nicotine delivery can be reduced below addictive levels. Attempts to reduce nicotine through traditional genetic manipulation have reduced nicotine levels by less than ten-fold. Nicotine removal by way of chemical processes resulted in a less than desirable product for processing. Reductions in nicotine delivery by filter ventilation is not effective, because of the concurrent reductions in tar delivery and compensation behaviors. Molecular biological technologies used to develop Vector 21-41 reduce nicotine levels 30- to 50-fold and produce a full-flavor product. This transgenic tobacco line resulted from a research program designed to understand the function of genes expressed exclusively in tobacco roots. Analysis of one root-specific cDNA clone (NtQPT1) revealed that it encoded quinolinic acid phosphoribosyltransferase (QPTase). QPTase is one of the key enzymes in nicotine biosynthesis. Nicotine is synthesized in the roots and is then transported to the leaves. Analysis of the NtQPT1 promoter revealed that it would direct transgene expression specifically in the root cortex. Analysis of the expression pattern of NtQPT1 revealed that it mimicked that of nicotine biosynthesis. Wounding and/or topping tobacco plants increased NtQPT1 mRNA levels and increased nicotine biosynthesis, whereas auxin treatment decreased NtQPT1 mRNA levels and reduced nicotine biosynthesis. In order to reduce nicotine levels, the NtQPT1 promoter was used to direct antisense expression of the NtQPT1 cDNA. Tobacco plants carrying this transgene were screened for reduced nicotine levels and the transgenic line designated Vector 21-41 exhibited the lowest levels. Vector 21-41 has nicotine levels less than 1000 parts per million (ppm) as opposed to normal tobacco's 20,000 to 40,000 ppm levels. Additionally, the levels of tobacco-specific nitrosamines (TSNAs) were drastically reduced in Vector 21-41 leaves and in smoke from cigarettes produced from Vector 21-41 tobacco. Tobacco products made from Vector 21-41 deliver nicotine below the addictive levels suggested by

Benowicz and Henningfield (1994). With these low nicotine levels, this product is targeted toward helping smokers control their use of tobacco. In limited, extended-use smoking panels, many participants believed the cigarettes made from Vector 21-41 tobacco have either helped them quit or believe they will eventually be able to quit. Additional, more extensive trials, conducted by Dr. Jed Rose of Duke University Medical School, are underway to confirm and quantify this observation.

NICOTINE BIOSYNTHESIS IN TOBACCO

Alkaloids are secondary metabolites that contain nitrogen in their cyclic organic structures. Alkaloids have been used by man as stimulants, drugs, narcotics, and poisons. Most alkaloids have unknown functions in the plants that produce them, although some alkaloids act as phytoalexins or are involved in plant-pest interactions. Nicotine is the predominant alkaloid found in tobacco. During the domestication of tobacco, cultivars producing high levels of nicotine were typically selected. Commercial cultivars typically produce nicotine at levels between 2% and 4% of dry weight (20,000 to 40,000 ppm). Nicotine has been found in other plant species and families including some non-solanaceous plants, however, the amount synthesized is much lower than that in N. tabacum (Leete, 1983).

Studies have been carried out to establish the nicotine biosynthetic pathway (Leete, 1983; Hashimoto and Yamada, 1994) by administering labeled compounds, studying tissue cultured plants, and most recently, using molecular techniques. Nicotine is composed of pyridine and N-methylpyrrolidine rings linked together and is produced by the condensation of two metabolites, nicotinic acid and N- methylpyrroline, that are synthesized by two separate pathways (Waller and Dermer, 1981) (Figure 1). Nicotinic acid is synthesized in the pyridine nucleotide cycle pathway (Wagner et al., 1986; Wagner and Wagner, 1985), whereas N-methylpyrroline is synthesized from ornithine or arginine via putrescine (Tiburcio and Galston, 1986).

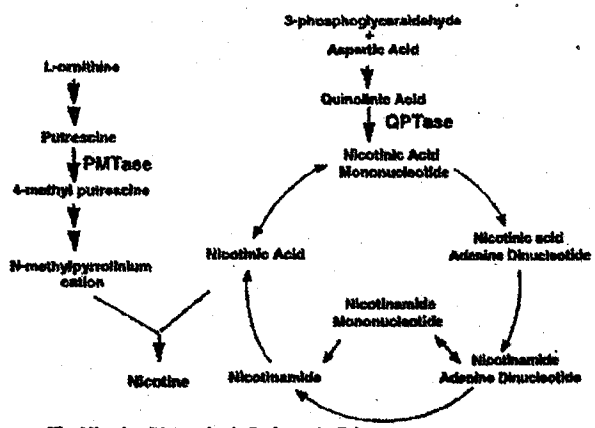


Figure 1: The Nicotine Biosynthetic Pathway in Tobacco

The synthesis of nicotine requires precursors from two different pathways. One precursor, N-methylpyrrolinium cation, is provided by the methylpyrroline pathway, while the other precursor, nicotinic acid, is the product of the pyridine nucleotide cycle. PMTase (putrescine methyl transferase) and QPTase (quinolinate phosphoribosyltransferase) are highlighted. They are the regulatory step enzymes in this pathway.

The enzymatic activities of the enzymes involved in nicotine biosynthesis have been assayed in different organs of the tobacco plant, in dedifferentiated and differentiated tobacco tissue, in tobacco root cultures, and in plants with different rates of nicotine production (Wagner et al., 1986). The regulatory step enzyme for the methylpyrroline pathway is PMTase, which controls the transition from primary to secondary metabolism. For the other pathway, the regulatory step enzyme is QPTase, which is the entry point for the pyridine-nucleotide cycle (Wagner et al., 1986). Several additional less stringent regulatory steps that help to complete the concerted regulatory system are observed.

Few enzymes in the nicotine biosynthetic pathway have been isolated and characterized. In particular, there is a lack of information regarding the enzyme responsible for the condensation of nicotinic acid and the N-methylpyrrolinium cation. A crude enzyme extract has been isolated from Nicotiana species that can catalyze the formation of nicotine from nicotinic acid and N-methylpyrrolinium chloride in the presence of oxygen (Friesen and Leete, 1990). Despite the importance of this enzyme activity, further characterization has not been reported.

THE PYRIDINE-NUCLEOTIDE CYCLE

Nicotinic acid is the source of the pyridine ring of nicotine (Dawson et al., 1956). The pyridine-nucleotide cycle has two possible pathways leading to nicotinic acid (Wagner and Wagner, 1985; Wagner et al., 1986). One enzymatic route takes advantage of the fact that nicotinamide adenine dinucleotide (NAD) is involved in a rather rapid turnover, as enhanced nicotine production stimulates the synthesis and degradation of NAD. The second route is a direct one, using one enzymatic step that transforms nicotinic acid mononucleotide (NAMN) to nicotinic acid. The study of nicotine biosynthesis had led to the discovery of a new pathway that exists in all higher plants for the formation of nicotinic acid. 3-phosphoglyceraldehyde, or its equivalent, condenses with aspartic acid or its equivalent to form quinolinic acid (Dalton, 1980). Quinolinic acid phosphoribosyltransferase (QPTase), which converts quinolinic acid to NAMN, serves as the entry-point enzyme into the pyridine nucleotide cycle that supplies nicotinic acid (Wagner and Wagner, 1985). The pyridine-nucleotide cycle provides NAD and nicotinic acid and is a part of primary metabolism, but also provides components for secondary metabolism, such as nicotine synthesis in the root of tobacco (Waller and Dermer, 1981).

THE METHYLPYRROLINE PATHWAY

The other precursor for nicotine is the N-methylpyrrolinium cation. In this part of the biosynthetic pathway, nicotine and tropane alkaloids share a common early pathway originating from ornithine and/or arginine by the action of ornithine decarboxylase (ODC) and arginine decarboxylase (ADC) and diverging at the N-methylpyrrolinium cation. The early pathway involves the symmetric diamine putrescine, that is found ubiquitously in plants and usually is metabolized to conjugated forms or to the polyamines, spermidine and spermine. Putrescine N- methyltransferase (PMTase) is the first committed enzyme in alkaloid biosynthesis and this step of the reaction is unique to alkaloid-producing plants. The PMT cDNA has been isolated from tobacco (Hibi et al., 1994). The PMT transcript can only be detected in tobacco roots. The deduced amino acid sequence of PMT cDNA encodes a 41 kD protein, PMTase. PMTase catalyzes the formation of N-methylputrescine, which is then oxidatively deaminated to 4-aminobutanol by diamine oxidase (DAO). 4-aminobutanol spontaneously cyclizes to the N-methylpyrrolinium cation, a direct precursor for nicotine (Feth et al., 1986).

GENETIC CONTROL OF NICOTINE BIOSYNTHESIS

Genetic control of total alkaloid production in tobacco plants has received considerable attention. Some studies indicate that differences in alkaloid levels among flue-cured cultivars are inherited quantitatively (Legg et al., 1965). Experiments and observations by Valleau (1949) showed that large differences in alkaloid content were controlled by a rather simple genetic system. This hypothesis has been supported further by the identification of the Nic1 and Nic2 loci. Hybrid lines were developed by incorporating genes from low alkaloid content Cuban cigar cultivars into Burley 21 cultivars by a series of 12 back-crosses (Legg et al., 1969). Detailed genetic analyses of parental, F1, F2, B1, and B2 generations of lines differing in alkaloid content were conducted. Legg and co-workers (1969) concluded that the low alkaloid lines differed from standard cultivars at two non-linked loci, Nic1 (formerly known as A) and Nic2 (formally known as B). Nic gene actions are semidominant and primarily additive. The genotypes of standard cultivars are Nic1/Nic1 Nic2/Nic2 and the low nicotine lines are nic1/nil nic2/nic2. Nic1/Nic1 nic2/nic2 lines are high intermediates and nic1/nic1 Nic2/Nic2 lines are low intermediates (Legg and Collins, 1971). The Nic1 locus has an approximately 2.4-fold stronger effect than the Nic2 locus. Doubled haploid tobacco breeding lines of these four alkaloid genotypes are established (Collins et al., 1974). These lines are not different in days to flower, number of leaves, leaf size, and plant height. However, the nic1/nic1 nic2/nic2 line is more susceptible to insect damage (Legg et al., 1970) and has longer maturity dates (Miller et al., 1982).

Different nicotine content among commercial cultivars of tobacco indicates that genes in addition to Nic1 and Nic2 may be involved in the inheritance of alkaloid production. However, the other genes have only minor effects in low nicotine plants (Legg et al., 1969). The enzymatic activities for the enzymes that participate in nicotine biosynthesis have been analyzed in four homozygous allelic Burley 21 combinations producing high, high intermediate, low intermediate, and low nicotine levels (Saunders and Bush, 1979). PMTase and QPTase activities in root tissues of these four genotypes are proportional to leaf nicotine level. No differences were observed for the QPTase activity in leaves of these four genotypes (Saunders and Bush, 1979). Both Nic1 and Nic2 affect PMTase and QPTase activities in roots, suggesting that these loci are involved in regulation of nicotine synthesis. A cDNA encoding PMT was isolated by subtraction hybridization of the wild-type line (Nic/Nic1 Nic2/Nic2) and the low nicotine mutant line

(nic1/nic1 nic2/nic2) (Hibi et al., 1994). PMT transcript levels in roots were correlated to nicotine levels in leaves in the nic mutants (Hibi et al., 1994). Similarly, NtQPT1 transcript levels correlate to nicotine levels in the nic mutants (Song, 1997).

SPATIAL AND TEMPORAL REGULATION OF NICOTINE BIOSYNTHESIS

As with many other metabolic pathways, the nicotine synthetic pathway is regulated spatially and temporally. Nicotine biosynthesis is also affected by internal and external stimuli such as plant hormones, light, nutrients, and stress. As demonstrated by the classical reciprocal grafting experiments between a nicotine producing plant, tobacco, and nonproducing plant, tomato (Dawson, 1941), nicotine is synthesized in the root and is translocated to the aerial parts of the plant. This is confirmed by observations that the enzymes involved in nicotine biosynthesis, PMTase, DAO, and QPTase have high enzyme activities in tobacco roots, but low enzyme activities in leaves. Accordingly, the transcripts of PMT and NtQPT1 are found exclusively in the root (Hibi et al., 1994; Song, 1997). The alkaloid-synthesizing function of plant roots shows remarkable specificity. Recent developments in plant organ culture technologies and characterization of several biosynthetic enzymes have confirmed the root-specific localization of alkaloid biosynthesis.

Nicotine biosynthesis can be detected immediately after germination of the seed. Nicotine formation is observed in connection with the protein metabolism of the aleurons in the radicle and the hypocotyl. The cotyledons remain free of nicotine. Later, when the hypocotyl elongates, nicotine levels decrease, while the roots continue to synthesize nicotine (Tso, 1972). Nicotine synthesis is associated with young, developing root tissue. The root-synthesized nicotine transports to leaves via the xylem (Tso, 1972). The leaves, however, not only accumulate the nicotine, but can also transform it into a wide array of products including nor-nicotine and TSNAs, all of which are derived from nicotine synthesized in the roots (Bush et al., 1993).

Every step in tobacco production that affects plant metabolism will influence the level of nicotine content in the leaves. Tobacco type, distribution in the plant, cultural practices, ripening, and fertilizer treatment are among some prominent factors (Tso, 1972). Experiments with cell suspension culture, callus culture and root culture of Nicotiana species have shown that light inhibits nicotine accumulation in the culture (Hobbs and Yeoman, 1991). Various environmental factors

and plant growth regulators, combined with the growth stage and growth conditions of tobacco, affect tobacco nicotine biosynthesis in roots.

Nicotine biosynthesis is regulated by topping, wounding, and plant growth regulators. Removal of flower heads and several young leaves (topping) is a common practice among tobacco growers. Topping eliminates apical dominance and increases nicotine content in tobacco leaves. Topping induces PMTase and QPTase activities in roots and thus increases nicotine synthesis levels (Saunders and Bush, 1979; Wagner et al., 1986). Topping rapidly induces the PMT transcript levels in roots. Exogenous supply of auxin down-regulates PMT transcript in cultured tobacco roots (Hibi et al., 1994). Auxin has long been known to down-regulate nicotine content in tobacco. In tobacco tissue culture, kinetin promotes nicotine production in the absence of auxin. Auxin strongly inhibits nicotine formation without affecting tissue growth (Tabata et al., 1971). The nicotine content of N. rustica root culture decreases in the presence of auxin (Rhodes et al., 1994).

Topping not only removes apical dominance, but also wounds the plant. Leaf damage has been shown to rapidly increase the concentration of different types of secondary metabolites normally found in undamaged leaves. Many of these damage-induced increases have proven defense-related roles. Baldwin (1991) reported large increases in the concentration of nicotine in N. sylvestris plants in response to leaf damage. These alkaloidal responses reduced the growth of a tobacco herbivore and protected alkaloid-induced tissues in laboratory feeding trials. They also demonstrated that the increased nicotine content was due to increased amounts of nicotine entering the xylem stream. This indicates that the wound-induced nicotine accumulation in leaves was due to the increased nicotine synthesis in the roots of the wounded plants.

IDENTIFICATION OF THE ROOT-SPECIFIC GENE, NtQPT1

NtQPT1 (originally called TobRD2) is a tobacco gene isolated as part of a screen for genes expressed specifically in roots of tobacco. Using a combination of differential screening and subtractive hybridization, we isolated a collection of genes expressed exclusively in tobacco roots and demonstrated that root-specific expression was regulated, at least in part, transcriptionally and that the genes were members of small gene families (Conkling et al., 1990). Comparisons of the NtQPT1 amino acid sequence with the GenBank database revealed limited similarity (15%) to a bacterial protein that encodes quinolinate phosphoribosyl trans-

ferase (QPTase). Quinolinate phosphoribosyl transferase is required for de novo NAD biosynthesis in both prokaryotes and eukaryotes. In tobacco, high levels of QPTase were detected in roots, but not in leaves. To determine unequivocally if NtQPT1 encodes QPTase, we sought to complement a bacterial strain (TH265) that is mutant in quinolinate phosphoribosyl transferase (nadC). This mutant cannot grow on medium lacking nicotinic acid. However, expression of the NtQPT1 protein in this bacterial strain conferred the NadC$^+$ phenotype, confirming that NtQPT1 encodes QPTase.

ANALYSIS OF TISSUE-SPECIFIC EXPRESSION OF THE NtQPT1 GENE

In situ hybridizations to root sections demonstrated that NtQPT1 is expressed in the root cortex. A genomic fragment encoding the NtQPT1 gene was isolated and approximately 5kb sequenced. The 5' and 3' untranslated sequences of the cDNA are identical to that of the genomic fragment, implying that this gene encodes the cDNA. Transcriptional fusions of the NtQPT1 promoter sequence were fused to GUS and transformed into tobacco via Agrobacterium-mediated transformation. Histochemical staining of transformants revealed root cortex-specific expression in a pattern mimicking that observed by in situ analysis of NtQPT1 transcripts. These data demonstrate that the NtQPT1 promoter contains all of the sequences necessary to direct transgene expression in the same tissues as the endogenous gene.

To characterize the NtQPT1 promoter, a 5'-nested deletion series containing approximately 2.0, 1.5, 1.4, 1.25, 0.9, 0.8, 0.7, 0.6, 0.5, 0.3, 0.2 and 0.13 kb of 5' flanking sequence were transformed into tobacco and analyzed for GUS expression (Fig. 2A and B). Promoters of 0.6 kb and greater directed root cortex-specific GUS expression at extremely high levels (about four-times that of the CaMV 35S promoter). Promoters of 0.13 kb directed root cortex-specific expression at lower levels.

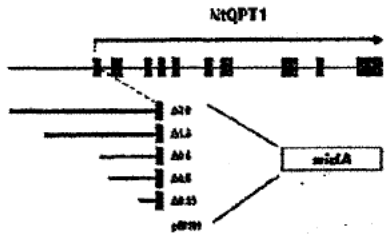
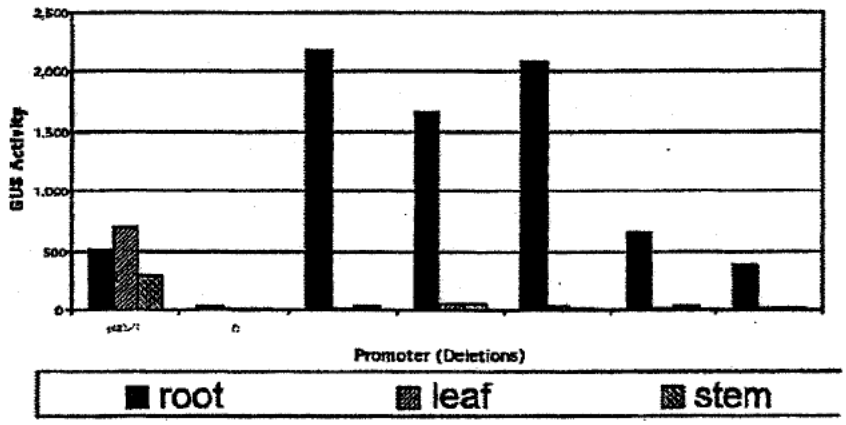


Figure 2: *NtQPT1* Promoter Analysis

A. Diagrammatic Representation of the *NtQPT1* Gene and the *NTQPT1* Chimeric Genes Used in this Study. The site of transcription initiation is indicated. Ten exons are presented as cross-hatched bars. The promoter deletion series is also shown and is fused to the gene encoding the GUS reporter gene *(uidA)*.



B. *NtQPT1* 5' Flanking Region Directed GUS Activities in Roots, Leaves, and Stems of Transgenic Tobacco. Average GUS activities from at least 20 independent transformants of each construct from roots (solid bars), leaves, (cross-hatched bars) and stems (shaded bars) for each deletion were plotted. pBI212 represents the cauliflower mosaic virus (CaMV) 35S promoter.

FUNCTIONAL CHARACTERIZATION OF NtQPT1

To determine if NtQPT1 is a key regulatory gene in the nicotine biosynthetic pathway, we examined the effects of Nic1 and Nic2 mutants, of topping, and of wounding on NtQPT1 steady-state mRNA levels. If NtQPT1 is, in fact, involved in nicotine biosynthesis, we would predict that NtQPT1 mRNA levels would be lower in nic1-/nic2- double mutants. NtQPT1 mRNA transcript levels in nic1-/nic2- double mutants were approximately 25% that of wild-type (Fig. 3). Further, there was a close correlation between nicotine levels and NtQPT1 transcript levels in these near isogenic lines.

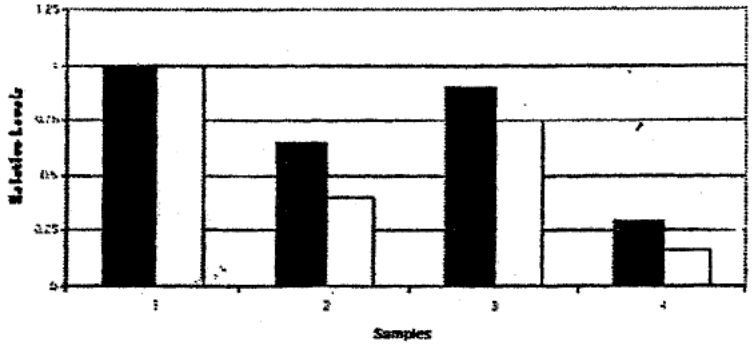


Figure 3: *NtQPT1* Transcript Levels are Regulated in the *nic* Mutant Lines

Total RNA was isolated from roots of near isogenic tobacco lines having the genotypes of:
1. *Nic1/Nic1 Nic2/Nic2*
2. *nic1/nic1 Nic2/Nic2*
3. *Nic1/Nic1 nic2/nic2*
4. *nic1/nic1 nic2/nic2*

NtQPT1 transcript levels were determined by RNA blotting and densitometric quantitation of the film. The relative levels of the NtQPT1 transcript in roots (solid bars) and relative nicotine content (open bars) is plotted. All values are normalized to those of the *Nic1/Nic1 Nic2/Nic2 line.*

If NtQPT1 is involved in nicotine biosynthesis, we would predict that NtQPT1 mRNA levels would increase following topping or wounding. NtQPT1 transcript levels in tobacco roots were quantified following topping and wounding. Within six hours of topping, the mRNA levels increased about eight-fold (Fig. 4). Within 24 hours of wounding (by crushing the leaf lamina with a hemostat), NtQPT1 transcript levels increased approximately 18-fold (Fig. 5).

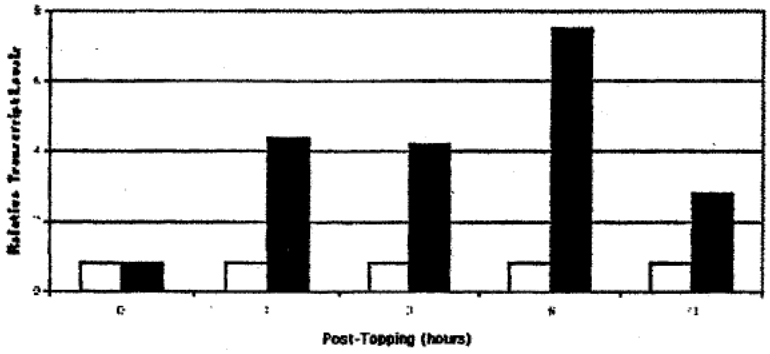


Figure 4: Topping Transiently Increases *NtQPT1* Transcript Levels

Tobacco plants were topped at flowering and roots were harvested at the time points indicated post-topping. Total RNA was isolated from roots and *NtQPT1* transcript levels determined by RNA blotting and densitometric quantitation of the film. The relative levels of the *NtQPT1* transcript were normalized to the 0 hour time point for each treatment. Topping-treated samples are presented as solid bars and control samples are presented as open bars.

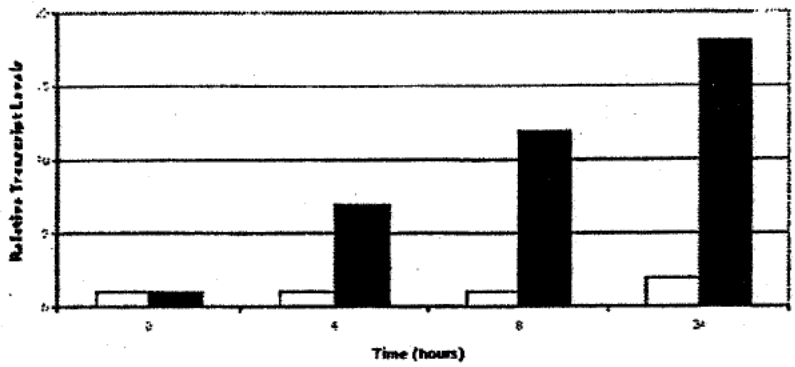


Figure 5: Wounding Increases NtQPT1 Transcript Levels

Four leaves around the second node of mature tobacco plants were wounded by crushing the leaf lamina adjacent to the mid rib using a surgical hemostat. A portion of the roots were harvested from each plant at the times indicated and pooled for RNA extraction. NtQPT1 transcript levels were determined by RNA blotting and densitometric quantitation of the film. The relative levels of the NtQPT1 transcript were normalized to the 0 hour time point for each treatment. Wounded samples are presented as solid bars and control samples are presented as open bars.

In summary, NtQPT1 is expressed in tobacco roots and encodes quinolinate phosphoribosyl transferase (QPTase). Expression of NtQPT1 is regulated by Nic1 and Nic2. Topping (removal of apical dominance) and wounding up-regulate expression of NtQPT1. Nicotine is synthesized in tobacco roots. QPTase is a key regulatory enzyme in nicotine biosynthesis. Saunders and Bush (1979) measured QPTase activities and nicotine levels in low nicotine tobacco lines (Nic1 and Nic2 mutants) and observed a strict correlation of QPTase activity and nicotine content. Nicotine biosynthesis is stimulated by topping and wounding. The expression and enzymatic function of the NtQPT1 gene is consistent with the hypothesis that it is a key regulatory gene in the nicotine biosynthetic pathway.

SUPPRESSION OF *NtQPT1* EXPRESSION

Because nicotine levels in tobacco leaves are directly proportional to the QPTase and PMTase enzyme activity levels and NtQPT1 encodes the QPTase responsible for nicotine biosynthesis, it stands to reason that nicotine levels could be reduced if expression of the NtQPT1 gene could be down-regulated. In order to reduce expression of the endogenous NtQPT1 gene, an antisense strategy was used. Antisense RNA is complementary to the mRNA transcribed by the endogenous gene. By a molecular mechanism that is not yet understood, expression of an antisense transcript in the same cell expressing sense transcripts of the endogenous gene can down-regulate the production of the gene product encoded by the

endogenous gene. Antisense suppression of gene expression requires a high degree of sequence complementarity between the antisense and sense transcripts. In order to reduce the expression of QPTase (and therefore, the levels of nicotine), the full-length NtQPT1 cDNA was cloned in the antisense orientation behind the 2.0 kb NtQPT1 promoter.

Transformation, Screening and Production of Homozygous Vector 21-41 Seeds

The low-nicotine tobacco cultivar, Vector 21-41, was developed by transforming Burley 21 with a binary Agrobacterium vector that carries the 2.0 kb NtQPT1 root-cortex specific promoter which drives the antisense expression of the NtQPT1 cDNA. Four hundred independent transformants (T0) were allowed to self. Progeny of the selfed-plants (T1) were screened for segregation of the transgene. Progeny of 268 of the 400 primary transformants segregated 3:1 (a single locus). The remaining 132 progeny segregated at ratios of 15:1 or higher (two or more loci). T1 progeny segregating 3:1 (resulting from transformation at a single locus) were subjected to further analysis.

Alkaloid levels of T1 progeny segregating 3:1 were measured qualitatively using a colorometric micro-assay. T1 progeny that had less than 10% of the nicotine levels of the Burley 21 parent were allowed to self to produce T2 progeny. Homozygous T2 progeny were identified by selecting populations in which 100% of the progeny carried the transgene (heterozygous progeny would segregate 3:1). Alkaloid levels in homozygous and heterozygous T2 progeny were measured qualitatively using the micro-assay and again showed levels less than 10% of the Burley 21 parent. Leaf samples of homozygous T2 progeny were sent to the Southern Research and Testing Laboratory in Wilson, NC for quantitative analysis of nicotine levels using Gas Chromatography/Flame Ionization Detection. Homozygous T2 progeny of transformant #41 had the lowest nicotine levels (~70 ppm) and were renamed Vector 21-41. Vector 21-41 plants were allowed to self (producing T3 progeny). T3 progeny were grown and nicotine levels assayed qualitatively and quantitatively. T3 progeny were allowed to self, producing T4 progeny. Samples of the bulked seeds of the T4 progeny were grown and nicotine levels tested. Table 1 describes the genetic analysis of Vector 21-41 and demonstrates that the low-nicotine phenotype co-segregates with the transgene.

Generation	Where Grown	Topped	Leaf Position Number	Zygosity	Nicotine (ppm)
T_1-1	Greenhouse	No	3	Homozygous	70
T_1-2	Greenhouse	No	3	Heterozygous	180
T_1-3	Greenhouse	No	3	Homozygous	100
T_1-4	Greenhouse	No	3	Heterozygous	200
T_1-5	Greenhouse	No	3	Nontransgenic	8,070
T_1-6	Greenhouse	No	3	Heterozygous	180
T_1-7	Greenhouse	No	3	Nontransgenic	8,020
T_1-8	Greenhouse	No	3	Heterozygous	250
T_1-9	Greenhouse	No	3	Heterozygous	170
T_1-10	Greenhouse	No	3	Nontransgenic	9,000
T_1-11	Greenhouse	No	3	Heterozygous	220
T_1-12	Greenhouse	No	3	Heterozygous	190
T_2-1	Greenhouse	No	3	Homozygous	100
T_2-3	Greenhouse	No	3	Homozygous	120
T_3	Greenhouse	No	3	Homozygous	190
T4	Greenhouse	No	3	Homozygous	190
T4	Field (NC)	Yes	Whole Plt	Homozygous	1,440
T4	Field (PA	Yes	Whole Plt	Homozygous	760
T4	Field (MS)	Yes	Whole Plt	Homozygous	1,100
T4	Field (LA)	Yes	1-8	Homozygous	1,490

Table 1. Nicotine levels in generations of greenhouse or field-grown Vector 21-41 tobacco. Zygosity represents whether the transgene is homozygous, heterozygous, or absent from the plants.

AGRONOMIC PERFORMANCE OF VECTOR 21-41

The agronomic performance of Vector 21-41 was determined in a field test conducted at the NCDA Central Crops Research Station (Clayton, NC). T4 progeny homozygous for the transgene were compared to the Burley 21 parent and a control transformant. The design was three treatments (Vector 21-41, a Burley 21 transformed line carrying only the NtQPT1 promoter [Promoter-Control], and untransformed Burley 21 [Wild-type]), 15 replicates, 10 plants per replicate. During the test, Orthene was applied two times at a rate of 1 lb/acre to control insects. No difference was observed in insect damage of the low nicotine line, Vector 21-41, and the Promoter Control and Wild-Type lines following this insect management protocol. A variety of agronomic and chemical traits (listed below) were assayed during the trial. The data are summarized in Table 2. The data

demonstrate that Vector 21-41 is substantially identical to its parent, Burley 21, with the exception of drastically lower nicotine levels and a two-fold increase in reducing sugars.

	TREATMENT		
	Vector 21-41	Promoter Control	Wild-type
Days from transplant to flower	57.1 ± 3.6	56.7 ± 3.4	57.6 ± 3.4
Height at flowering (cm)	118.6 ± 20.1	112.1 ± 21.4	110.8 ± 19.5
Leaf number at flowering	22.8 ±1.4	22.6 ± 4.9	23.4 ± 1.9
Leaf size (Length) (cm)			
5th leaf	37.1 ± 4.7	39.4 ± 4.0	38.3 ± 4.2
10th leaf	35.6 ± 3.6	38.3 ± 4.3	36.8 ± 3.3
15th leaf	30.7 ± 3.5	31.7 ± 2.5	32.8 ± 4.3
Leaf size (Width) (cm)			
5th leaf	15.6 ± 1.8	16.0 ± 2.7	17.0 ± 2.9
10th leaf	14.7 ± 2.3	15.8 ± 1.8	15.6 ± 1.6
15th leaf	13.7 ± 2.5	13.4 ± 2.0	13.2 ± 2.3
Internodes (mm)	66.0 ± 13.4	57.7 ± 12.5	54.6 ± 5.3
Yield (kg/ha)	890.3 ± 70.7	780 ± 68.5	809.2 ± 71.2
Nicotine (ppm)	1440 ± 660*	19,120 ± 8990	21,540 ± 9340
Nor-Nicotine (ppm)	400 ± 100*	1560 ± 220	1270 ± 520
Total Alkaloids (ppm)	2300 ± 700*	20,700 ± 9300	23,100 ± 940
% Total Nitrogen	2.53 ± 0.78	2.96 ± 0.42	2.64 ± 0.91
% Reducing Sugars	10.29 ± 0.89*	5.87 ± 2.04	5.51 ± 2.40

Data from 2000 field trial at Central Crops Research Station, Clayton, NC. Fifteen replicates of 10 plants per replicate. Data were analyzed using the F-test. *= Significant difference between Vector 21-41 and the Promoter Control and Wild-Type tobacco at the 1% level.

Table 2. Field performance of Vector 21-41: Growth characteristics of Vector 21-41 compared to the non-transformed parent (Wild-Type) and a control transgenic plant in which the *NtQPT1* promoter directs expression of the GUS gene (Promoter Control).

CIGARETTE PRODUCTION

Test cigarettes were produced using the air-cured Vector 21-41 tobacco from the 2000 field trial. Labstat International, Inc. quantified TSNA levels in mainstream smoke and compared them to TSNA levels of a standard, full-flavor cigarette. Table 3 presents those data. The TSNA levels of Vector 21-41 cigarettes were drastically lower than those of conventional cigarettes.

Brand No.	Wt. Of Cig. [mg/cig]	Puff Count (/cig)	TPM [mg/cig]	NNN [ng/cig]	NAT [ng/cig]	NAB [ng/cig]	NNK [ng/cig]
V-21	892	7.7	17.6	29.3	13.2	2.93	17.7
V-21	896	7.6	15.9	27.4	12.3	2.78	18.7
V-21	863	7.0	16.1	31.9	12.4	2.75	16.7
V-21	896	7.6	15.9	25.8	10.5	3.15	18.2
V-21	881	7.3	15.0	28.5	9.04	2.51	14.3
Average	885	7.4	16.1	28.5	NQ	NQ	NQ
Std Dev	14.1	0.3	0.9	2.3	NQ	NQ	NQ
Coeff Var	1.6	3.8	5.9	8.0	NA	NA	NA
Std	1024	7.5	21.5	435	(590)*	103	348
Std	1023	7.6	20.8	424	531	100	349
Std	1040	7.9	23.0	441	517	98.3	351
Std	1018	7.4	20.2	410	526	92.8	355
Std	1035	7.3	18.7	449	501	114	355
Average	1028	7.5	20.9	432	519	102	352
Std Dev	9.3	0.2	1.6	15.4	13.1	7.9	3.3
Coeff Var	0.9	2.7	7.6	3.6	2.5	7.8	1.0

( )* DENOTES AN OUTLIER
Note: A result of BDL (Below Method Detection Limit) indicates that no analyte was detected. A result of NQ indicates that the analyte was detected, but was below the practical limit of quantitation (LOQ).
NA = Not Applicable

Method Detection Limits

	Wt.of Cig. (mg/cig)	Puff Count (/cig)	TPM [mg/cig]	NNN [ng/cig]	NAT [ng/cig]	NAB [ng/cig]	NNK [ng/cig]
MDL	NA	NA	NA	3.11	3.79	2.30	5.79
LOQ	NA	NA	NA	10.4	12.6	7.67	19.3

V-21 = Vector 21-41
Std. = A full-flavor generic cigarette produced by Liggett.
TPM = Total Particulate Matter
NNN = N-Nitrosonornicotine
NAT = N-Nitrosoanatabine
NAB = N-Nitrosoanabasine
NNK = N=Nitrosonicotine keytone [4(Methylnitrosamino)-1-(3-pyridyl)-1-butanone]

Table 3. Tobacco specific nitrosamine levels under 'standard conditions' in cigarettes constructed from Vector 21-41 tobacco compared to a standard commercial cigarette with similar tar levels.

Vector Tobacco has used air-cured and flue-cured Vector 21-41 to manufacture the Quest brand of cigarettes. Table 4 lists the properties of this brand. Quest 1 delivers approximately 10 mg of tar and 0.6 mg of nicotine per cigarette; Quest 2 delivers approximately 10 mg of tar and 0.3 mg of nicotine per cigarette; and Quest 3 delivers approximately 10 mg of tar and less than 0.05 mg of nicotine per cigarette. Quest does not employ filter ventilation to obtain these levels.

	Quest 1 Low Nicotine	Quest 2 Extra Low Nicotine	Quest 3 Nicotine Free	******* Lights
Cigarette Wt.(mg)	895	911	991	925
Circumference (mm)	24.43	24.64	24.58	24.8
PD Vent Open (mm)	109	112	116	128
Air Dilution (%)	0.0	0.0	0.0	20.7
Hardness (%)	70.9	78.3	74.2	73.5
Smoking Date	8/19/02	8/19/02	6/25/02	10/25/01
CO (mg/cig)	10.8	13.2	11.7	10.6
Puff Count/Cig	7.8	8.0	8.0	7.6
Smk. Solids (mg/cig)	12.7	12.9	12.8	11.6
Nicotine (mg/cig)	0.59	0.28	0.04	0.79
Water (mg/cig)	1.7	1.9	2.0	0.9
NFDS (mg/cig)	10.4	10.6	10.7	9.9

Table 4. Characteristics of Quest 1, 2 and 3 compared to a popular king-sized light cigarette. Samples were smoked by the FTC method.

COMPATIBILITY OF GMO TOBACCO WITH NON-GMO TOBACCO PRODUCTION

An important consideration in the implementation of genetic engineering strategies in the development of new tobacco cultivars is the control of the GMO material once the modified tobacco reaches the marketplace. Some markets may not want GMO tobacco and, thus, the production of GMO tobacco should employ safeguards. At Vector Tobacco we have developed a number of measures to ensure that co-mingling is not an issue. Vector Tobacco contracts with growers for the production of Vector 21-41 and mandates that the grower produce no other tobacco types. Vector Tobacco provides growers with seedlings and accounts for all seedlings (excess seedlings are destroyed). Vector Tobacco contracts to buy 100% of the tobacco produced. Cured tobacco is processed at a collection point that is not used for non-GMO tobacco and is shipped to the manufacturing facility in dedicated trucks.

Among cultivated plants, tobacco is uniquely positioned to greatly decrease (or eliminate) the possibility of transfer of the new transgenes.

1. *Tobacco leaves, and not seeds, are the product. Thus, in the unlikely event of pollen transfer (see below), the new genes would not be present in the product.*
2. *A vast majority of tobacco producers plant certified seed and do not save seed back. Certified seed is produced in isolation from other tobacco cultivars.*
3. *Tobacco flowers are self-compatible and are tubular. This property greatly favors self-pollination.*
4. *Tobacco is not wind pollinated and pollinating insect vectors travel only short distances (<1320 feet).*
5. *During tobacco production, flowers are removed prior to anthesis, pollen dehiscence, and seed production (topping).*
6. *Tobacco is grown in regions of the U.S. in which wild, cross-compatible species are not found.*

To test the ability of transgenic tobacco to outcross during normal tobacco production, we performed the following experiment. Vector 21-41 was grown in an 80 meter X 80 meter plot. Non-transgenic Burley 21 tobacco was planted in border rows at distances of 50 meters, 100 meters, 200 meters, and 450 meters from the Vector 21-41 plot. The Vector 21-41 plants were topped as though it would be used in normal tobacco production. The non-transgenic Burley 21 border rows were allowed to flower and set seeds. Seeds were collected from each border row and approximately 100,000 seeds from each distance were screened for the presence of the transgene. No transgenic seedlings were detected from a total of 477,678 seedlings. These data demonstrate that if producers follow normal topping practices for transgenic crops, the chances of the transgene outcrossing are extremely small.

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DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 01–060–2]

Vector Tobacco; Availability of Determination of Nonregulated Status for Tobacco Genetically Engineered for Reduced Nicotine

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that the Vector Tobacco (USA) Ltd. tobacco designated as Vector 21–41, which has been genetically engineered for reduced nicotine, is no longer considered a regulated article under our regulations governing the introduction of certain genetically engineered organisms and products. Our determination is based on our evaluation of data submitted by Vector Tobacco (USA) Ltd. in its petition for a determination of nonregulated status, our analysis of other scientific data, and comments received from the public in response to a previous notice. This notice also announces the availability of our written determination document and our finding of no significant impact.

EFFECTIVE DATE: September 16, 2002.

ADDRESSES: You may read a copy of the determination, an environmental assessment and finding of no significant impact, the petition for a determination of nonregulated status submitted by Vector Tobacco (USA) Ltd., and all comments received on the petition and the environmental assessment in our reading room. The reading room is located in room 1141 of the USDA South Building, 14th Street and Independence Avenue SW., Washington, DC. Normal reading room hours are 8 a.m. to 4:30 p.m., Monday through Friday, except holidays. To be sure someone is there to help you, please call (202) 690–2817 before coming.

APHIS documents published in the **Federal Register**, and related information, including the names of organizations and individuals who have commented on APHIS dockets, are available on the Internet at *http://www.aphis.usda.gov/ppd/rad/webrepor.html*.

FOR FURTHER INFORMATION CONTACT: Dr. Susan Koehler, Biotechnology Regulatory Services, Suite 5B05, 4700 River Road Unit 147, Riverdale, MD 20737–1236; (301) 734–4886. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734–4885; e-mail: *Kay.Peterson@aphis.usda.gov*.

SUPPLEMENTARY INFORMATION:

Background

On May 1, 2001, the Animal and Plant Health Inspection (APHIS) received a petition (APHIS Petition No. 01–121–01p) from Vector Tobacco (USA) Ltd. (Vector) of Durham, NC, requesting a determination of nonregulated status under 7 CFR part 340 for tobacco (*Nicotiana tabacum* L.) designated as Vector 21–41, which has been genetically engineered to produce a very low level of nicotine. The Vector petition states that the subject tobacco should not be regulated by APHIS because it does not present a plant pest risk.

On February 12, 2002, APHIS published a notice in the **Federal Register** (67 FR 6480–6481, Docket No. 01–060–1) announcing that the Vector petition was available for public review. In the notice, APHIS solicited comments from the public on whether this tobacco presents a plant pest risk. APHIS also made available for public comment an environmental assessment for the proposed determination of nonregulated status. APHIS received 45 comments on the petition and the environmental assessment during the 60-day comment period which ended April 15, 2002. The comments were received from tobacco farmers, tobacco companies, State

farmers' organizations, State department of agriculture officials, private individuals, tobacco growers' cooperatives, tobacco marketing organizations, university cooperative extension centers, members of the U.S. Congress, a foundation seed producer, an agronomic consultant, a county chamber of commerce, and a consumer organization. Twenty-three of the comments were in favor of a determination of nonregulated status for Vector 21–41 tobacco, and 22 comments either opposed deregulation or recommended no action on the petition until certain concerns are addressed about the effects of Vector 21–41 deregulation on traditional tobacco growers and markets. A majority of commenters in favor of deregulation stressed that Vector 21–41 did not present a plant pest risk, displayed disease and insect susceptibilities and agronomic characteristics similar to conventionally bred tobacco, and noted the benefits to local economies from growing Vector 21–41. Those commenters not expressing support for deregulation of the subject tobacco at this time generally expressed the following concerns about Vector 21–41: Blue mold and insect susceptibilities, the limited number of years of field testing, the potential for gene transfer to conventional tobacco, and the potential for commingling with conventional tobacco in the absence of growing and processing guidelines. One commenter found the environmental assessment inadequate, alleging that it failed to address the impacts of Vector 21–41 on organic farmers and certain human health effects, including the impacts of an antibiotic resistance gene. We have provided a response to the comments as an attachment to our finding of no significant impact, which is available from the person listed under FOR FURTHER INFORMATION CONTACT.

Analysis

Vector 21–41 tobacco has been genetically engineered to express a quinolinic acid phosphoribosyltransferase (QPTase) in the reverse, or antisense position, which disrupts the normal expression of QPTase, a key enzyme in the biosynthetic pathway leading to the production of nicotine and related alkaloids. The effect of this genetic change is to reduce the nicotine levels of nicotine, nor-nicotine, and total alkaloids in the leaves of Vector 21–41 tobacco. The subject tobacco also contains the *nptII* marker gene derived from the bacterium *Escherichia coli*. The *nptII* gene encodes the enzyme neomycin phosphotransferase type II (NPTII) and is used as a selectable marker in the initial laboratory stages of plant cell selection. Expression of the added genes is controlled in part by gene sequences from the plant pathogen *Agrobacterium tumefaciens*. The *A. tumefaciens* method was used to transfer the added genes into the parental recipient Burley 21–LA tobacco variety.

The subject tobacco has been considered a regulated article under the regulations in 7 CFR part 340 because it contains gene sequences from a plant pathogen. Vector 21–41 tobacco has been field tested since 1999 in the United States under APHIS notifications. In the process of reviewing the notifications for field trials of this tobacco, APHIS determined that the vectors and other elements were disarmed and that the trials, which were conducted under conditions of reproductive and physical containment or isolation, would not present a risk of plant pest introduction or dissemination.

Determination

Based on its analysis of the data submitted by Vector, a review of other scientific data, field tests of the subject tobacco, and comments submitted by the public, APHIS has determined that Vector 21–41 tobacco: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than tobacco developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed; (4) will not cause damage to raw or processed agricultural commodities; and (5) will not harm threatened or endangered species or organisms, such as bees, that are beneficial to agriculture. Therefore, APHIS has concluded that the subject tobacco and any progeny derived from hybrid crosses with other nontransformed tobacco varieties will be as safe to grow as tobacco in traditional breeding programs that is not subject to regulation under 7 CFR part 340.

The effect of this determination is that Vector's 21–41 tobacco is no longer considered a regulated article under APHIS" regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated articles under those regulations no longer apply to the subject tobacco or its progeny. However, importation of Vector 21–41 tobacco or seeds capable of propagation are still subject to the restrictions found in APHIS" foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment has been prepared to examine the potential environmental impacts associated with this determination. The environmental assessment was prepared in accordance with (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. 4321 *et seq.*), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500–1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Based on that environmental assessment, APHIS has reached a finding of no significant impact with regard to its determination that Vector 21–41 tobacco and lines developed from it are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the petition and the environmental assessment and finding of no significant impact are available upon request from the individual listed under the FOR FURTHER INFORMATION CONTACT section of this notice.

Done in Washington, DC, this 26th day of November 2002 .

Peter Fernandez,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 02–30518 Filed 12–2–02; 8:45 am]

BILLING CODE 3410–34–P

Approval of Vector Tobacco (USA) Ltd. Petition (01-121-01p) Seeking a Determination of Non-regulated Status for Reduced-nicotine Tobacco Line Vector 21-41

Environmental Assessment and
Finding of No Significant Impact

September 2002

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA) has prepared an environmental assessment (EA) prior to approving a petition (APHIS number 01-121-01p) for a determination of nonregulated status received from Vector Tobacco (USA) Ltd. under APHIS regulations at 7 CFR Part 340. The subject of this petition, tobacco line Vector 21-41, is genetically engineered for reduced nicotine content by the insertion of an anti-sense version of the coding region of a tobacco gene encoding the enzyme quinolinic acid phosphoribosyltransferase, a key enzyme in the biosynthetic pathway for the synthesis of nicotine and related alkaloids. It is also genetically engineered to express a selectable marker, the enzyme neomycin phosphotransferase which confers antibiotic resistance. On February 12, 2002, APHIS published a notice in the Federal Register (67 FR 6480-6481, Docket no. 01-060-1) announcing the availability of the petition and EA for public review and comment. During the designated 60 day comment period, APHIS received 45 comments. APHIS' analysis of and response to these comments is included as an attachment to this finding. Based on the analysis in the EA and in our response to the comments, APHIS has reached a finding of no significant impact (FONSI) to the environment from its determination that tobacco line Vector 21-41, and progeny derived from it, shall no longer be considered regulated articles. This determination is attached to the EA as Appendix D.

Cindy Smith
Acting Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: SEP 16 2002

APHIS' Analysis and Response to Comments Received on Petition 01-121-01p and the EA.

APHIS received 45 comments on the petition and the EA during the 60-day comment period. The comments were received from tobacco farmers, tobacco companies, State farmers' organizations, State department of agriculture officials, private individuals, tobacco growers cooperatives, tobacco marketing organizations, university cooperative extension centers, members of the U.S. Congress, a foundation seed producer, an agronomic consultant, a county chamber of commerce, and a consumer organization. The comments in favor of a determination of nonregulated status for Vector 21-41 tobacco totaled 23, and 22 comments either opposed deregulation or recommended no action on the petition until certain concerns are addressed about the effects of Vector 21-41 deregulation on traditional tobacco growers and markets. A majority of commenters in favor of deregulation stressed that Vector 21-41 did not present a plant pest risk, that it displayed disease and insect susceptibilities and agronomic characteristics similarly to conventionally bred tobacco under typical management conditions, and that there were benefits to local economies from growing Vector 21-41. Those commenters not expressing support for deregulation of the subject tobacco at this time generally expressed concerns about its susceptibility to blue mold and insects, the limited number of years of field testing, the potential for gene transfer to and commingling with conventional tobacco and the subsequent effect on export markets. One commenter found the EA inadequate, stating that it failed to address the impacts of Vector 21-41 on human health, that it inadequately addressed the impact on organic farmers, and that an Environmental Impact Statement should have been prepared. Further characterization of and response to those comments in opposition are given below.

1. Susceptibility to blue mold and insects

Several commenters expressed concern over the susceptibility of Vector 21-41 to the blue mold fungal pathogen. Some indicated that this pathogen is highly adaptable and the potential for increased incidence of this pathogen in Vector 21-41 raises concerns regarding selection pressure on populations of this pathogen in the presence of genetic modifications in the plant. However, no rationale was provided for how the genetic modification in Vector 21-41 would enhance selection pressure for the blue-mold pathogen compared to many other tobacco varieties currently grown which are also susceptible to blue mold. It was further argued that it is irrelevant that the EA states that Vector 21-41 does not have increased susceptibility to diseases or insects when compared to the parental line Burley 21-LA since the parental line is known to exhibit increased susceptibility to blue mold and insect pests.

Relevance of the comparison of insect and disease susceptibility to Burley 21-LA

In response to these comments APHIS notes that the comparison of the reported disease and insect susceptibility of Vector 21-41 to the parental line Burley 21-LA, and Burley 21 (parent of Burley 21-LA) in Section V of the EA is relevant and appropriate to the determination of plant pest risk. The regulations at 7 CFR 340.6 (c) indicate that the required data and information to be submitted to support a determination of non-regulated status includes a description of known or potential differences from the unmodified recipient organism that would substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from

which it was derived, including disease and pest susceptibilities and agricultural or cultivation practices. The petitioner must also certify that the petition includes relevant data and information which are unfavorable to the petition. Because Burley 21-LA was not released as a commercial variety, APHIS included its parent, Burley 21 in the analysis. Burley 21 has not been grown for about 30 years, but is still used as a parent in breeding programs. APHIS also considered the availability and effectiveness of various cultural or chemical control methods to manage disease and insect pests to which Vector 21-41 tobacco is known or likely to be susceptible.

Characterization of the disease and insect susceptibility of Burley 21-LA and Burley 21
APHIS acknowledged in the EA that both Burley 21-LA and Burley 21 have low levels of disease resistance compared to newer Burley varieties and that Burley 21-LA is described as extremely susceptible to insect damage. In particular Burley 21-LA has been observed to be susceptible to blue mold as well as to two other fungal diseases: black shank (caused by *Phytophthora parasitica* var. *nicotinanae*), and black root rot (caused by *Thielaviopsis basicola)*. It is slightly more susceptible to blue mold than Burley 21. Burley 21 has no resistance to black shank, low resistance to black root rot, and high resistance to TMV and wild fire disease (Gary Palmer, Extension Tobacco Specialist, University of Kentucky (personal communication to Susan Koehler, November and December 11, 2001). Most Burley tobacco varieties today have resistance to black root rot and a few have some degree of resistance to black shank and blue mold (Gary Palmer, personal communication to Susan Koehler, and see also http://www.uky.edu/Ag/TobaccoProd/pubs/2001GuidetoBurleyVarieties.pdf). Burley 21-LA is also described as "extremely susceptible to insect damage" (Legg, et al., 1970). In particular, it is highly susceptible to grasshoppers and other leaf-feeding insects (Glenn Collins, University of Kentucky, personal communication to Susan Koehler, November, 2001). As indicated in the EA, this is not unexpected, since nicotine and some other alkaloids are known to have insecticidal activity.

Observed insect and disease susceptibility of Vector 21-41, and the ability of current agricultural practices to effectively manage pests in Vector 21-41
APHIS' analysis documented the fact that in field trials in North Carolina in 2000, both Vector 21-41 and Burley 21-LA were shown to be extremely susceptible to insect pests in the absence of systemic insecticides, but no differences in disease or insect susceptibility or leaf yield were observed between Vector 21-41 or Burley 21-LA plants that received two applications of 1 lb/acre Orthene. This insecticide treatment is one of the options recommended for control of several of the major foliar-feeding insect pests of tobacco (North Carolina State University, 2000). Topping was also conducted 60 days after transplant, prior to flowering, and suckers (leaf buds which develop in the leaf axils after topping occurs) were chemically controlled by treatment with maleic hydrazide. Early topping and good sucker control are recommended for control of budworms and hornworms, since moths of these pests are strongly attracted to flowers to lay their eggs. This practice is also reported to speed the decline of aphids, especially under hot, dry conditions (NC State Univ., 2000). Other biological and cultural control options are described.

Field tests of Vector 21-41 in Pennsylvania in 2001 indicate that it is susceptible to blue mold, a serious disease of tobacco caused by the fungus *Peronospora tabacina.* The North American Blue Mold Warning and Forecast System, a cooperative network of state coordinators, plant pathologists, and meteorologists, alerts growers about the threat of blue mold through their forecasting and tracking activities so that preventative measures can be implemented. In the petitioner's response of October 19, 2001 to APHIS' deficiency letter dated September 26, 2001, they noted that this System reported that blue mold occurred on other tobacco varieties in most of the tobacco production regions of North America during the 2001 growing season (see http://www.ces.ncsu.edu/depts/pp/bluemold/fcst2001/exec2001.htm.). Furthermore, in over 600 farm field trials of Vector 21-41 in Pennsylvania, Illinois, Louisiana, Mississippi, and Iowa, which were extensively monitored for disease and insect pests by farm owners, workers, Vector Tobacco agronomists, and contracted consultants, no other exceptional disease or pest susceptibility was observed. They claimed that the specific rates of insecticide, fungicide, and herbicide application for any one field depended on traditional agronomic factors, the geographic location, climate, and time of transplanting, but were always within approved label amounts. APHIS considered the fact that disease control in tobacco involves an integrated program of numerous practices, including crop rotation, root and stalk destruction, nitrogen management, soil pH management, plant spacing, varieties, and pesticides (see The University of Georgia College of Agricultural & Environmental Sciences Cooperative Extension Service web site on *Tobacco Disease Control* by Paul Bertrand at http://www.griffin.peachnet.edu/caes/tobacco/handbook/disease98.html). At least four of these practices are applicable to control or management of black root rot, black shank and blue mold. Chemicals recommended along with other practices by the University of Kentucky or in the 2002 North Carolina Agricultural Chemicals Manual for the control of these diseases include at least one or more of the following: the fumigant, Chloropicrin, carbamate, methyl bromide, Ridomil Gold EC or WSP, Ultra Flourish 2EC or 2E, Dithane Rainshield, Actigard, or Acrobat MZ (see *Current Blue Mold Controls* by http://www.uky.edu/Agriculture/kpn/kyblue/kyblu01/controls.htm and *Tobacco Disease Control Planning Should Be a Priority* at http://www.uky.edu/Agriculture/kpn/kyblue/kyblu01/related/rtd0001.htm, both by W. Nesmith, Extension Professor, University of Kentucky, and http://ipm.ncsu.edu/agchem/chptr6/605.pdf).

APHIS' analysis in the EA indicated that currently recommended and approved methods for controlling diseases and insects to which Vector 21-41 is susceptible should be effective and applicable for their control in Vector 21-41. These conclusions are supported by comments provided by growers of Vector 21-41 tobacco, and the tobacco company and crop consultant working with them to coordinate activities for planting, growing, and managing the crop.

2. Insufficient field data

Some of the same commenters also asserted there was insufficient field data to predict the long term impact under various and possible extreme weather conditions and wider range of individual farm production practices. APHIS notes that this concern appeared to be primarily aimed at the impacts from Vector 21-41 tobacco on traditional tobacco varieties as a result of out-crossing via pollen or commingling of seeds, plants, or leaf products and the impact on export markets. That concern is addressed below. APHIS notes that the field data or observations described above (and subsequently supported by field data reports received from the petitioner on June 5 and June 20, 2002 for field trials conducted in 2001 listed in Appendix A of the EA) regarding the disease and insect susceptibility of Vector 21-41 were made over 6 states, some representative of traditional tobacco growing areas, and others not. In addition, the petitioner noted in their letter of October 19, 2001 that no abnormal plant morphology or phenotypes due to the transgenes were observed. APHIS' analysis in the EA of data on cured-leaf nicotine levels from Vector 21-41 tobacco grown in four states indicated that it is consistently low as expected. Field data reports received June 5 and 20, 2002, which include analysis of additional samples and samples from two more states, indicate that the absolute average and median values for nicotine content from plants grown in 5 of the 6 states are still consistently lower than those reported for Burley 21 LA and normal wild-type tobacco varieties. Nicotine values from Vector 21-41 plants grown in a small (approximately 34 acre) experimental site in Hawaii, while higher than those from plants grown in the other states, were still below the 0.8% threshold required for the low-nicotine classification of Type 31-V or Type 73 tobacco defined by AMS, and were influenced by a combination of soil fertility, climatic conditions and cultural practices which are known to increase nicotine content. We believe that even though the field trials were conducted in only two years, the number and variety of locations should have presented a sufficiently broad range of weather conditions and individual production practices and pressures to predict with a reasonable degree of certainty that there will be no significant impacts to the environment.

3. Human health effects of tobacco products made with Vector 21-41

One commenter expressed concern that the commercialization of Vector 21-41 tobacco products would encourage smoking by giving a false sense of security. Along the same line, another commenter claimed that APHIS' EA was inadequate because it did not address human health effects (either direct; or later, indirect, reasonably-foreseeable effects) associated with the commercialization of a new tobacco product as required under NEPA and its implementing procedures. More specifically the commenter asserts that "the EA does not project human exposures rates of the tobacco, whether the products made with this tobacco will lead to increased smoking in the public, and many other health and disease impacts associated with smoking tobacco". The commenter also states that the EA does not address the human health impacts that may be caused when the public is exposed to the antibiotic resistance gene product through direct inhalation of smoke from this tobacco product.

In response to these comments APHIS notes that **addressing human health effects associated with the commerical use of the tobacco product is outside the scope of the EA.** The scope of the EA, as indicated in the Summary and under Alternative B, is to compare the environmental impacts of the introduction (importation, interstate movement and release) of Vector 21-41 tobacco and its progeny, without the safeguards afforded by 7 CFR Part 340, to impacts posed by the cultivation and distribution of tobacco not subject to these regulations. APHIS did consider the impacts of exposure to agricultural workers handling the tobacco plants.

Tobacco products, cigarettes, snuff etc., are made from tobacco leaves, which following detachment from the plant and curing, are no longer living. These products are therefore not subject to APHIS regulations at 7 CFR 340 because they do not meet the definitions provided therein of a regulated article or a plant pest. Impacts on human health from exposure to or use of these products, will therefore, not be determined by alternative actions identified in the EA. APHIS recognizes that use of some traditional tobacco products may have adverse consequences to human health, and did consider the potential human health effects from potential products made with Vector 21-41 tobacco, but this analysis was not included in the EA because it is believed to be outside the scope of impacts affected by the Agency's range of alternative actions. Vector 21-41 tobacco is a burley type of tobacco. Traditional burley tobacco is primarily used in cigarettes, but is also an important component of cigar, pipe, and chewing tobacco (Shew and Lucas, 1991). Tobacco is purchased and blended based on certain chemical criteria (including sugar and nicotine content) as well as appearance and smoke flavor. In addition, it is common practice to add sugars and other flavors to balance the smoke flavor, primarily by modifying the sensory impact of nicotine and other tobacco alkaloids (Leffingwell, 1999). It is difficult to predict what the composition of the final product might be, since as noted in the EA, data indicate that cured leaves of Vector 21-41 have significantly lower levels of nicotine and related alkaloids and higher levels of reducing sugars compared to a normal burley tobacco (Burley 21). The company is still working out the product formulation and currently, all the Vector 21-41 tobacco leaves harvested are still in the curing and aging process, which can typically last anywhere from 12 to 60 months (Scott Shore, Vector Tobacco (USA), Ltd., personal communication to Susan Koehler, May 3, 2002).

APHIS considered the information submitted by the petitioner regarding the content of nicotine and other alkaloids, total nitrogen, and reducing sugars in Vector 21-41 cured tobacco leaf, and TSNAs in smoke from cigarettes made from Vector 21-41 tobacco. We could find no reason why these differences would pose a greater human health threat than traditional burley tobacco if used in tobacco products, since the addictive component, nicotine, and carcinogenic TSNAs were reduced. The petition states that "Tobacco products made from Vector 21-41 deliver nicotine below the addictive levels suggested by Benowicz and Henningfield (1994)." Benowicz and Henningfield suggest that an absolute limit of 0.4 to 0.5 mg of nicotine per cigarette should be adequate to prevent or limit the development of addiction in most young people. This threshold

recognizes that nicotine uptake from smoke varies depending on how the cigarette is smoked and assumes a maximal bioavailability of 40%. As noted in the EA, the median value for nicotine content from samples taken from Vector 21-41 cured (oven-dried) leaves available at the time the EA was prepared was 0.05% (500 ppm or 0.5 mg/1000 mg), and assuming that 1000 mg of tobacco is used in the average cigarette, then it would be possible for cigarettes made from Vector 21-41 tobacco to meet this threshold. Additional nicotine data included in final field data reports submitted on June 5 and 20, 2002 do not change the conclusion that it would still be possible to meet this threshold. The effects of reduced nicotine in cigarette products on smoking behavior are debatable. But the stated intent of the petitioner is to develop a product which would help smokers reduce or control their smoking, and they indicate that trials are underway to confirm or quantify this. In as much as the product formulation is not finalized, any definitive conclusions regarding the human health effects of products made from Vector 21-41 tobacco would be speculative.

APHIS does not consider inhalation of the product of the antibiotic resistance gene in tobacco smoke to be an issue. The product of this gene is a protein, and proteins are broken down during curing, aging, and burning of the tobacco (Leffingwell, 1999). Breakdown products and derivatives of this protein would be similar to those derived from other proteins normally found in tobacco leaf.

Furthermore, APHIS identified within the EA the regulatory authority of other federal agencies or commissions relevant to the sale, use, packaging, labeling, and advertising of Vector 21-41 tobacco and its products. In particular, the FDA can assert jurisdiction over products made from Vector 21-41 tobacco if health claims are made regarding the product. The Federal Trade Commission oversees testing of "tar" and nicotine yields of cigarettes, which must be included in cigarette advertising and they would have the authority to address any unfair or deceptive cigarette advertising practices that might affect human health. APHIS' actions in no way prejudice or influence the regulatory authority or actions of these agencies with respect to Vector 21-41 tobacco products.

4) Impacts on organic farming were inadequately addressed in the EA.

One commenter stated that APHIS' EA contained an inadequate analysis of the potential impacts associated with the commercial use of genetically engineered tobacco on organic farming. They assert that we made an unsupported statement that nontransgenic tobacco seed would still be readily available for planting. APHIS checked several tobacco seed supplier websites, including domestic, international, private and public, and those marketing to organic growers (e.g. http://www.attra.org/attra-pub/tobacco.html), and there were plenty of traditional, non-genetically engineered tobacco varieties available, and there was no mention of these varieties not being available if genetically engineered tobacco seed was deregulated. There appears to be

an increasing interest among organic growers for indigenous and heirloom-variety types of tobacco and several possible sources of seed were listed.

The commenter further asserts that APHIS failed to analyze whether the marketplace and market-based standards will actually tolerate "adventitious presence" of genetically-engineered tobacco in organically produced tobacco products and the impact it will have on producers, processors and consumers of such products. APHIS has addressed the potential impacts of the Vector 21-41 tobacco on organic farmers in the EA. We made reference to the National Organic Program (NOP) administered by USDA's Agricultural Marketing Service, which considers that the presence of a detectable residue alone does not necessarily indicate use of a product of excluded methods that would constitute a violation of the standards. (Please refer to the preamble of the NOP final rule at residue testing, changes requested but not made, (3) Threshold for Genetic Contamination for a discussion of "adventitious presence" in relation to organic production at website: http://www.ams.usda.gov/nop/nop2000/Final%20Rule/preamble/pre-residues.htm.) Further, the NOP requires that organic production operations have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. The organic system plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. APHIS' analysis indicates that adventious presence of Vector 21-41 tobacco in organically-produced tobacco leaf of another type could only occur if the farmer planted seed that was commingled with or cross-pollinated by Vector 21-41 tobacco. Several reasons were provided in the EA for why this is extremely unlikely to occur.

Producers, processors, or consumers wishing to check for adventious presence of Vector 21-41 tobacco in their products could enlist the services of commerical laboratories (e.g. Genetic ID and Integrated Biomolecule Corporation) that offer testing for the detection of foreign genetic elements in commodities, including the nopaline synthase terminator used in both of the foreign gene constructs in Vector 21-41 tobacco. USDA's Agricultural Marketing Service and Grain Inspection, Packers and Stockyards Administration published an Advance Notice of Proposed Rulemaking on November 30, 2000 in the *Federal Register* (65 FR 71272- 71273) concerning the possible further development of additional testing and standardization for seeds and commodities designed to differentiate products such as non-biotechnology-derived commodities. Federal, State, private, and international groups involved in seed certification all allow for some level of accidental, incidental, or adventitious presence of off-types even in the purest seed categories, such as foundation and breeder seed.

5) APHIS should prepare an Environmental Impact Statement (EIS).

One commenter suggested that APHIS must prepare a complete environmental impact statement (EIS) to address the full range of impacts associated with the commercial planting of Vector 21-41 tobacco.

In response, APHIS notes that their NEPA Implementing Procedures (7 CFR 372) do not indicate that this type of action is of the class of actions normally requiring the preparing of an EIS. It is characteristic of the class of actions normally requiring an EA, but not necessarily an EIS. APHIS' analysis documented in the EA (and in the response to comments) does not indicate that a significant impact to the human environment is likely; therefore the preparation of an EIS is not necessary. This is the first petition for deregulation for a genetically engineered tobacco product that APHIS has received. APHIS does not believe a decision to deregulate Vector 21-41 tobacco, when combined with previous decisions to deregulate other genetically engineered crops with totally different traits, uses, and markets, will influence or cause a significant cumulative impact to the human environment which would warrant the preparation of an EIS.

6) Gene transfer to and commingling with conventional tobacco and market impacts. Many commenters expressed concern that out-crossing or commingling of Vector 21-41 tobacco with conventional varieties could lead to a loss of export sales estimated at $250 million because purchasers of U.S. tobacco, particularly those that focus on the large export market, have imposed a "GMO" (genetically-modified organism) tolerance level of zero. They request that the Vector 21-41 tobacco continue to be regulated under 7 CFR 340 to prevent cross-pollination and commingling until the Vector 21-41 tobacco is produced in a male-sterile form and/or a system is in place to identify the locations of its production and provide production guidelines or mandated programs that will prevent outcrossing or commingling. A minor concern was raised that if contamination occurs, it could affect the nicotine levels of conventional tobacco and raise concerns about the quality of the tobacco related to this trait.

In response, APHIS believes that such impacts on marketing, which are neither directly or indirectly related to disease, damage or injury to plant products, are not germane to APHIS' decision as to whether Vector 21-41 poses a plant pest risk. Nonetheless, APHIS did address the potential for outcrossing and/or commingling in the EA under the sections on potential impacts from gene introgression and potential impacts on organic farming (section V. C. and E., respectively).

Furthermore, as noted in Section II. C. of the EA, production and marketing of tobacco is subject to regulation by other agencies within the USDA. In particular, APHIS noted that the USDA, Agricultural Marketing Service (AMS) has established a classification and certification system for certain low nicotine types of tobacco (less than 0.8% nicotine oven dry weight in the cured leaf). This includes Type 31-V air-cured Burley tobacco with low nicotine and Type 73 flue-cured tobacco with low nicotine, as defined under 7 CFR 30.38 and 7 CFR 30.42, respectively. Restrictions and controls relating to the production and marketing of Type 31-V tobacco as a prerequisite for certification are described under 7 CFR 30.38 (c). In summary, these restrictions require that the tobacco must be grown under contract filed yearly with the AMS on a specified number of acres, and that it must be separated from other types of tobacco during cultivation,

housing, and handling. It can not be offered for sale, or be sold, marketed or disposed of unless it is clearly represented and identified as being low nicotine tobacco. In addition, the grower can not transfer seed, seedlings or plants furnished under the contract, nor can the grower harvest or save seed produced under the contract. AMS samples tobacco from each grower and assays it to determine whether it meets the low nicotine criteria to meet the classification. Similar restrictions are in place under 7 CFR 30.42 for Type 73 tobacco. Vector 21-41 tobacco is the first tobacco to have ever received this certification.

According to Ken Wall, Senior Marketing Specialist, AMS Tobacco Programs, (personal communication to Susan Koehler, November 14, 2001) Vector 21-41 received certification under this program as both Type 31-V and Type 73 last year. Bill Hawks, Under Secretary for USDA's Marketing and Regulatory Programs indicated in a letter dated April 26, 2002 to Mr. Bill Dickerson, Director of Plant Industry Division, North Carolina Department of Agriculture and Consumer Services, that destination audits were conducted for the 2001-2002 marketing year to confirm that there was no commingling of this tobacco with other types of tobacco, and that more than 7 million pounds of Vector 21-41 tobacco have been certified under this program to date. The petitioner has indicated that contracts have already been filed with AMS to certify as low-nicotine types all of the Vector 21-41 tobacco being grown during the 2002 growing season for marketing, and they intend to continue growing under the certification program in future years. All the Vector 21-41 tobacco previously grown, or scheduled to be grown in 2002, in the United States with the intent of marketing has been in non-quota areas or states, including Mississippi, Pennsylvania, Iowa, Illinois, and Louisiana, (Scott Shore, personal communication, May 21, 2002). (Hawaii, another non-quota state, is also being evaluated by the petitioner as a viable production site.) In the vicinity of where Vector 21-41 has been grown in LA, MS, IL, and IA, there are no other types of tobacco being grown. The petitioner purposefully chose these locations to avoid any concerns regarding commingling, and the grower contracts stipulate that the tobacco must be topped and suckers controlled (Scott Shore, personal communication to Susan Koehler, May 21, 2002).

Type 31-V and Type 73 tobacco, when classified and certified as such, are currently excluded from the quota system. They can be grown in non-quota areas and not be subject to penalties when marketed. If Vector 21-41 tobacco did <u>not</u> receive the Type 31-V or Type 73 certification, then it would just be considered Type 31 Burley (if air-cured) or Types 11-14 (if flue cured), which are subject to quotas. In that case a substantial penalty can be levied when it is marketed if it is grown in a non-quota area, and it could only be marketed without penalty in a quota area if an allotment is issued for the type. On May 15, 2002, the Farm Service Agency published a notice and request for public comment in the *Federal Register* (67 FR 34673) about whether to include Type 31-V or Type 73 in the definitions of tobaccos subject to quotas for the 2003 and subsequent crop years. The implications of a potential change in quota status of Type 31-V or

Type 73 on the potential for contamination of traditional types of tobacco via cross-pollination is discussed briefly in the notice.

Literature Cited (Includes only those references not already included in the EA.)

Leffingwell, J.C. 1999. Leaf Chemistry. Chapter 8 *in* Tobacco Production, Chemistry, and Technology, D.L. David and M.T. Nielsen eds. Blackwell Science Ltd., Oxford, England.

North Carolina State University. 2000. 2000 North Carolina Burley Tobacco Production Guide. Available at http://ipmwww.ncsu.edu/Production_Guides/Burley/chptr8.html) .

USDA/APHIS Decision on Vector Tobacco (USA) Ltd. Petition 01-121-01P Seeking a Determination of Nonregulated Status for Reduced-Nicotine Tobacco Line, Vector 21-41

Environmental Assessment

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) prior to making its determination on the regulated status of tobacco (*Nicotiana tabacum*) line Vector 21-41 that has been genetically engineered (transformed) to have reduced nicotine. APHIS received a petition (designated 01-121-01P) from Vector Tobacco (USA) Ltd. (Durham, North Carolina) for a determination that Vector 21-41 reduced-nicotine tobacco does not present a plant pest risk, and therefore should no longer be treated as a regulated article under APHIS regulations found at 7 CFR Part 340. The petition contains information in support of such a determination.

The Vector 21-41 reduced-nicotine tobacco has been developed with the intent of producing cigarettes or other tobacco products that will help smokers to reduce or quit their smoking. Nicotine is the major alkaloid in tobacco leaves and is widely believed to be the addictive component of tobacco. Nicotine levels in the leaves were reduced by using recombinant DNA techniques to insert an anti-sense version of the coding region (cDNA) of a tobacco gene encoding the enzyme quinolinic acid phosphoribosyltransferase (QPTase) into the genome of a Burley tobacco variety. The anti-sense gene construct disrupts the normal expression of QPTase. QPTase is one of the key enzymes in the biosynthetic pathway leading to the production of nicotine and related alkaloids. Because these alkaloids are also the precursors for the production of tobacco-specific nitroasmines (TSNAs), which are potent mutagens and potential carcinogens, Vector 21-41 tobacco also has reduced levels of TSNAs. Vector 21-41 tobacco has also been transformed to express the *nptII* selectable marker gene derived from the enteric bacterium *Escherichia coli* transposon Tn5. The *nptII* gene encodes the enzyme neomycin phosphotransferase that confers resistance to the aminoglycoside antibiotic kanamycin. This gene was inserted only as a tool to allow selection of tobacco tissue transformed to contain the new gene constructs. Vector 21-41 tobacco is a regulated article under APHIS regulations at 7 CFR Part 340 because the plant pest *Agrobacterium tumefaciens* was used as a donor organism for some DNA sequences used to regulate the expression of the new gene constructs and also as a vector to introduce the new gene constructs into the tobacco genome.

As a regulated article, the importation, interstate movement, or cultivation in the United States of Vector 21-41 tobacco has been conducted under authorizations from APHIS that require conditions of physical and reproductive confinement to preclude the regulated article from becoming mixed with nonregulated plants or products or persisting in the environment outside the field site. This EA has been prepared prior to issuing a determination of nonregulated status for Vector 21-41 tobacco in order to specifically addresses the potential for impact to the human environment that might be incurred through the cultivation and use in agriculture of this tobacco without the restrictions imposed by 7 CFR Part 340.

II. BACKGROUND

A. Development of Vector 21-41 tobacco.

The Vector 21-41 reduced-nicotine tobacco was developed at the North Carolina State University, Department of Genetics under a research grant to Dr. Mark Conkling from a private company called Alternative Cigarettes. Vector Group, the parent company of Vector Tobacco (USA) Ltd.[1] and the Liggett Group cigarette company, has sublicensed the technology with the intent of producing cigarettes or other tobacco products that will help smokers to reduce or quit their smoking. Nicotine, is the major alkaloid found in *N. tabacum* (Tso, 1972, see Chapter 23, Alkaloids), and is widely believed to be the addictive component of tobacco (Benowitz and Henningfield 1994). It is synthesized in the root and transported to the leaves (Tso, 1972). Figure 1 of the petition shows the biosynthetic pathway for nicotine in tobacco. Nicotine is synthesized from two metabolic precursors, nicotinic acid and N-methylpyrroline, each of which is synthesized by two separate pathways. Quinolinic acid phosphoribosyl-transferase (QPTase) is the key rate-limiting, regulatory enzyme in the biosynthetic pathway that leads to nicotinic acid. It converts quinolinic acid to nicotinic acid mononucleotide, which is then converted directly or indirectly into nicotinic acid. To develop a reduced nicotine tobacco, the petitioner states that they started with a non-commerical tobacco variety developed through traditional breeding to have reduced levels of alkaloids, Burley 21 LA, and genetically engineered it by inserting the coding region (cDNA) of the tobacco gene *NtQPT1,* which encodes the QPTase enzyme, in the anti-sense orientation behind its own root-specific promoter. The anti-sense gene construct was designed to disrupt the normal expression of QPTase in the root, thereby lowering the pool of nicotinic acid available for the production of nicotine, nor-nicotine, and other alkaloids derived from the pyridine ring of nicotinic acid. Nicotine, nornicotine, and other minor tobacco alkaloids give rise to the production of tobacco-specific N-nitroasmines (TSNAs) during tobacco processing and during smoking (Bush et al., 1993). Some TSNAs been demonstrated to be potent mutagens and potential carcinogens, and are thought to contribute to the increased risk of cancer of the upper digestive tract in tobacco chewers and for the increased risk of lung cancer in smokers (Hoffman et al., 1994). The petition includes data that demonstrates that Vector 21-41 tobacco has reduced levels of nicotine, nor-nicotine, and total alkaloids in the leaves, and reduced levels of specific TSNAs in smoke from cigarettes derived from it.

Vector 21-41 tobacco has also been transformed to constitutively express the *nptII* selectable marker gene derived from the *Eschericia coli* bacterial transposon Tn5. This gene encodes the enzyme neomycin phosphotransferase (NPTII) that confers resistance to the antibiotic kanamycin and allowed tissue that was transformed to express the new genes to be selected on medium that contains kanamycin. Regulatory DNA sequences derived from the nopaline synthase (*nos*) gene from the plant pathogenic bacterium, *Agrobacterium tumefaciens*, were fused to both of these gene constructs to facilitate their transcription in the transgenic plant. *A. tumefaciens* was also

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[1]In January 2001, Dr. Conkling left the University to work for Vector Tobacco (USA) Ltd., and on November 14, 2001, Vector Tobacco (USA) Ltd. was changed to Vector Tobacco, Inc.

used as a vector to introduce the anti-sense *NtQPT1* and *nptII* gene constructs into the genome of the recipient tobacco variety to create the transgenic line Vector 21-41. Because this plant pathogen was used as a vector and donor organism in the production of the genetically engineered Vector 21-41 tobacco, this tobacco is considered to be a regulated article under APHIS regulations at 7 CFR Part 340, and authorization for its importation, interstate movement and field testing in the United States and its territories is required.

Vector 21-41 tobacco has been field tested in a wide variety of locations, in at least 7 States under notifications from APHIS issued since 1999 that are listed in Appendix A. It has also been field tested in Argentina. This field testing was conducted, in part, to confirm that Vector 21-41 tobacco exhibits the desired agronomic and quality characteristics and does not pose a greater plant pest risk than the unmodified recipient from which it was derived. Although these field tests were conducted in agricultural settings, APHIS performance standards for notifications (7 CFR 340.3 (c) 1-6) stipulate that the regulated article not be planted with nonregulated plant material that is not part of the release, that it be contained or devitalized when no longer in use, and that the regulated article and its offspring must not persist in the environment after completion of the test. Therefore, measures were employed to ensure physical and reproductive confinement from other sexually compatible plants and to manage volunteers. Reports for those field tests completed under APHIS authorization that were due at the time of the petition submission, and other information contained in the petition, have been submitted to APHIS to support a determination that Vector 21-41 tobacco does not pose a plant pest risk (see Appendix C for a list of supporting data and information).

B. APHIS Regulatory Authority.

APHIS regulations under 7 CFR Part 340, which are promulgated pursuant to authority granted by the Plant Protection Act (Title IV, Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. 7701-7772) regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. Vector 21-41 tobacco has been considered a regulated article because a plant pathogen was used as a vector agent and as a donor organism for some noncoding DNA regulatory sequences.

Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it is derived, the Agency can grant the petition in whole or in part. Therefore, APHIS permits or notifications would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

C. Regulatory Authority of Other Federal Agencies Relevant to the Production and Use of Vector 21-41 Reduced-Nicotine Tobacco and Its Products.

Production and Marketing of Tobacco: Production and marketing of tobacco, including Vector 21-41 reduced-nicotine tobacco, is subject to regulation by other agencies within the USDA. The Agricultural Stabilization and Conservation Service and the Commodity Credit Corporation set production quotas and price levels for tobacco leaf under the federal tobacco support program ("No Net Cost Tobacco Program"). Under the quota system, limits are placed on the amount of different types of tobacco that can be grown. Under 7 CFR Part 30, the USDA's Agricultural Marketing Service (AMS) classifies tobacco based on distinctive characteristics determined by the variety, growth conditions, and methods of cultivation, harvesting, and curing. Two types of low nicotine tobacco (less than 0.8% nicotine oven dry weight in the cured leaf) are excluded from the quota system, when classified and certified as such by the AMS under their regulations at 7 CFR 30.38 and 7 CFR 30.42. Special restrictions and controls relating to the production and marketing of these types of tobacco are described under these regulations. Vector 21-41 is the first tobacco variety to have received this certification (Kenneth Wall, Senior Marketing Specialist, AMS Tobacco Programs and Mark Conkling, personal communication to Susan Koehler, 11/14/01).

Product Sale and Use: Currently, tobacco is not generally considered a food, feed, drug, or dietary supplement by the U.S. Food and Drug Administration. (FDA). But the FDA has asserted jurisdiction over cigarettes as a "drug" when health claims were made by vendors or manufacturers, and the courts have sustained the agency's assertions of jurisdiction. Should health claims be made on cigarettes made with Vector 21-41 tobacco, the FDA would have jurisdiction over them as a drug. In 1996 the FDA asserted jurisdiction over tobacco products under the Food, Drug, and Cosmetic Act (FFDCA) to regulate tobacco advertising and promotional campaigns as well as labeling and purchasing restrictions. But the legal authority of the FDA to regulate tobacco products in the absence of health claims was challenged by the tobacco industry and in June 2000, the United States Supreme Court ruled that Congress had not expressly given the FDA legal authority to regulate the tobacco industry.

Packaging, Labeling and Advertising of Tobacco Products: The Bureau of Alcohol, Tobacco and Firearms regulations cover packaging and require the disclosure of certain information on every tobacco product carton or package and prohibit certain promotional practices. The Federal Cigarette Labeling and Advertising Act bans cigarette advertising on television, radio, and other electronic media, and requires cigarette packages and advertising to carry specified health warnings, such as the Surgeon General's warning. The Department of Justice, the Federal Communications Commission and the Federal Trade Commission (FTC) ensure compliance with this Act. The FTC oversees testing of "tar" and nicotine yields of cigarettes, which must be included in cigarette advertising, and they have the authority to address unfair or deceptive cigarette advertising practices under the Federal Trade Commission Act.

III. PURPOSE AND NEED

In compliance with the National Environmental Policy Act (NEPA) of 1969 (42 U.S.C. 4321 *et seq.*) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372), APHIS has prepared this EA before making a determination on the status of Vector 21-41 tobacco as a regulated article under APHIS regulations. Vector Tobacco (USA) Ltd., (Durham, North Carolina) submitted a petition requesting a determination from APHIS that Vector 21-41 reduced-nicotine tobacco no longer be considered a regulated article under 7 CFR Part 340.

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the "no action" alternative, APHIS would come to a determination that Vector 21-41 tobacco and its progeny should continue to be regulated under 7 CFR Part 340. Permits or acknowledgment of notifications from APHIS would still be required for their introduction. APHIS would choose this alternative if there were insufficient evidence to demonstrate lack of plant pest risk from the cultivation or movement of Vector 21-41 and its progeny without the containment restrictions that would otherwise be imposed under 7 CFR Part 340.

B. Proposed Action: Determination of Nonregulated Status

Under this alternative, Vector 21-41 tobacco and its progeny would no longer be considered regulated articles under 7 CFR Part 340. Permits or notifications to APHIS would no longer be required for introductions in the United States and its territories of Vector 21-41 tobacco or its progeny. A basis for this determination would be established, which would result in a "Finding of No Significant Impact (FONSI) under NEPA. Unrestricted cultivation and movement of this tobacco would be permitted by APHIS. Such a determination, however, does not preclude any restriction which might be placed on the cultivation, distribution, or sale of this tobacco by other regulatory agencies also having authority over this tobacco, e.g. those restriction imposed by the USDA, AMS as described in section II, C above. Importation of Vector 21-41 tobacco seeds and other propagative material would still continue to be regulated and inspected for pests and noxious weeds under APHIS regulations at 7 CFR 319.37 and under the Federal Seed Act at 7 CFR 201.

C. Determination of Nonregulated Status, in Part

The regulations at 7 CFR Part 340.6 (d) (3) (i) state that APHIS may "approve the petition in whole or in part." There are two ways in which a petition might be approved in part:

Approval of only some of the lines requested in the petition. Some petitions request de-regulation of lines derived from more that one independent transformation event. In those cases, supporting data must be supplied for each line; and APHIS could approve certain lines requested in the petition, but not others.

Approval of the petition with geographic restrictions. APHIS might determine that the regulated article poses no significant risk in certain geographic areas, but may pose a significant risk in others. In this case, APHIS may choose to approve the petition with a geographic limitation stipulating that the approved lines could only be grown in certain geographic areas.

V. POTENTIAL ENVIRONMENTAL IMPACTS

The potential environmental impact of each of the alternatives cited in IV. A.- C. will be presented.

Alternative A.

In a decision to choose alternative A., no action, these plants would still require APHIS authorization to be planted, imported, or moved interstate. Such introductions could continue under APHIS authorized notifications. In this case the applicant must certify that they will meet the performance standards that are designed to ensure physical and reproductive confinement of Vector 21-41 tobacco and any progeny derived from it. Thus far, no adverse effects to the environment or human health have been reported as a result of field tests of Vector 21-41 tobacco grown under APHIS authorization.

Growers could choose to grow other tobacco varieties if they choose not to grow Vector 21-41 under contract with the developer under APHIS authorization. However, there are currently no other tobacco varieties that have been commercialized that meet the AMS definition of "low nicotine" tobacco (Kenneth Wall, AMS and Dr. Gary Palmer, Extension Tobacco Specialist, University of Kentucky, personal communication to Susan Koehler, November 2001).

Alternative B.

A decision to choose alternative B, deregulation of Vector 21-41 reduced-nicotine tobacco, is addressed below. The environmental impacts of the introduction of Vector 21-41 tobacco and its progeny into the United States and it territories without the safeguards afforded by 7 CFR Part 340 are compared to any impacts posed by the cultivation and distribution of tobacco not subject to these regulations.

A. Plant pathogenic properties

APHIS considered the potential for the transformation vector or process, the introduced DNA sequences, or their expression products to cause or aggravate disease symptoms in Vector 21-41 tobacco or in other plants, or to cause the production of plant pathogens. We also considered whether data indicate that unanticipated plant pest effects would arise from cultivation of Vector 21-41 tobacco.

The petition states that a disarmed binary vector system based on the tumor-inducing Ti-plasmid of *Agrobacterium tumefaciens* was used to introduce the new genes into Burley 21 LA tobacco (see Petition, pg. 16). The system is considered "disarmed" because (1) the plant hormone genes necessary for the formation of crown gall tumors, which are normally produced upon infection of plants with *A. tumefaciens*, are removed from the T-DNA, that portion of the Ti-plasmid that is normally transferred to plant cells upon *A. tumefaciens* infection; and (2) the virulence functions are provided on a separate Ti-plasmid that lacks the T-DNA. Therefore, the transformation process should not lead to crown gall disease in Vector 21-41 tobacco.

APHIS analyzed molecular genetic and inheritance data provided in the petition that characterize the new genetic material in the transgenic line selected from a single transformation event (T_0 #41) and designated as Vector 21-41(see Appendix C). Data demonstrate that Vector 21-41 tobacco contains within a single locus: (1) two complete copies of the T-DNA insert from the disarmed Ti-plasmid pYTY32 in reverse orientation with respect to each other, each of which contains (a) the *nptII* gene encoding neomycin phosphotransferase, originally derived from *E. coli* Tn5, whose transcription is initiated and terminated by the promoter and termination/polyadenylation sequences, respectively, derived from the nopaline synthase (*nos*) gene from *A. tumefaciens*, and (b) the anti-sense quinolinic acid phosphoribosyl-transferase (QPTase) *Nt-QPT1* cDNA derived from tobacco whose transcription is initiated by its own promoter and is terminated by the *nos* terminator as described above; (2) a small portion of the backbone sequence located outside of the left T-DNA border in pYTY32 that includes truncated versions of the *Pseudomonas aeruginosa* derived *tetA* gene and the *E. coli* plasmid pRK2 derived *trfA* gene; and (3) the T-DNA left and right border sequences from *A. tumefaciens* bordering each T-DNA insert.

Data provided in the petition indicate that both the *nptII* and anti-sense *Nt-QPTI* genetic constructs are expressed in Vector 21-41 and produce the expected traits. Full length copies of the bacterial *tetA* and *trfa* genes would encode proteins that confer resistance to the antibiotic tetracycline and plasmid DNA transfer and conjugations functions respectively, as expressed in their donor organisms. However since only half of these genes are present in Vector 21-41, and they lack regulatory sequences necessary to initiate expression in plants, they would not be expressed in Vector 21-41 tobacco. Molecular genetic data were provided that indicate that no other complete protein coding sequences present outside of the T-DNA border in pYTY32 were inserted into Vector 21-41.

Although some of the donor organisms of the introduced sequences are described as plant or human pathogens, the introduced sequences are not known to cause or aggravate diseases in plants, nor in animals or humans. *A. tumefaciens* is a plant pathogen, but the non-coding regions of the *nos* gene derived from it are commonly used to regulate transgene expression in plants. Neither these sequences nor the T-DNA borders will cause or aggravate disease symptoms in plants. *E. coli* is not a plant pathogen. It is a coliform bacterium found commonly in the guts of humans and animals, and is not generally considered to be a human, animal, or plant pathogen (American Biological Safety Association, 1998; CAB Intl., 2001) . The *nptII* gene has a safe history of use as a selectable marker for plant transformations. *P. aeruginosa* is not a plant pathogen, but is listed as a human pathogen (ABSA, 1998) and as a natural enemy of certain insects (CAB, Intl., 2001). Regardless, of its status as a pathogen, the truncated *tetA* gene derived from it will not be expressed in Vector 21-41 tobacco.

Molecular genetic and inheritance data provided in the petition indicate that the new *nptII* and anti-sense *NtQPT1* genetic constructs are expressed and inherited as dominant, tightly linked traits, consistent with insertion of both transgenes at a single locus. Early generations of transformant #T_0-41 from which Vector 21-41 was selected all exhibit kanamycin resistance, and Vector 21-41 plants were shown to express NPTII protein in the leaves, roots, and stems. These plants also had nicotine levels in their leaves that were consistently lower than those determined for progeny of the original transformant #T_0-41 that lost the transgenes through segregation and for those reported for "wild-type" Burley 21-LA and "promoter control" plants that were grown in North Carolina in 2000. ("Promoter control" plants were reported to be Burley 21-LA transformed to carry only the *NtQPT1* promoter fused to a marker gene.) In the North Carolina experiment, Vector 21-41 leaves also had significantly lower levels of nor-nicotine and total alkaloids compared to both the "wild-type" and "promoter control" Burley 21-LA plants. APHIS notes, however, that the nicotine and total alkaloid levels of leaves derived from both the "promoter control" and the "wild-type" Burley 21-LA plants in the 2000 North Carolina field experiment and the nicotine content derived from the leaves taken from null segregants in the greenhouse are all higher than those reported previously for Burley 21-LA (Legg et al., 1970; and Paul Legg and Lowell Bush, personal communication to Susan Koehler, December 6, 2001). The source of this discrepancy remains unresolved, but could possibly be due to cultural or environmental effects which are known to effect nicotine content (Mark Conkling, letter to Susan Koehler, January 11, 2002.). The nicotine levels measured by AMS from at least 830 leaf samples from Vector 21-41 tobacco grown during the 2001 growing season in over 100 locations from four states (Louisiana, Mississippi, Illinois, and Pennsylvania) did vary slightly between states, with both the average and median values ranging from approximately 0.04 to 0.10 % oven dry weight (400 - 1000 ppm). The average and median values for all samples combined were 0.079% and 0.05%, respectively, and the maximum value reported was 0.40%. The average nicotine content reported for Burley 21-LA and Burley 21 (the normal alkaloid progenitor of Burley 21-LA) is 0.2 to 0.25% and 3.5%, respectively (Legg et al., 1969 and 1970). Therefore the data indicate that the average nicotine content for cured Vector 21-41 tobacco leaves grown over a variety of locations is approximately 2.5- and 44- fold lower, respectively, than the

average previously reported for Burley 21-LA and Burley 21, and only 0.02% of Vector 21-41 samples exceeded the average reported for Burley 21-LA.

Data also indicate that specific TSNAs, which are derived from nicotine and other alkaloids, were 15- to 39-fold lower in smoke from cigarettes made from Vector 21-41 tobacco when compared to standard, full-flavor generic cigarettes. In particular, the levels of N-nitrosonornicotine (NNN) and N-nitrosonicotine keytone (NNK) from Vector 21-41 cigarette smoke are approximately 15- and 18-fold lower, respectively, than those derived from the standard cigarette, and are approximately 12- and 2.7-fold lower, respectively, than those reported from cured Burley 21-LA tobacco leaf (Lowell Bush, Professor of Agronomy, University of Kentucky, personal communication to Susan Koehler, December 6, 2001). Both of these TSNAs are known carcinogens (Hoffman et al., 1994). This data taken together indicate that the anti-sense *NtQPT1* gene construct in Vector 21-41 is functioning as expected to lower the pool of nicotinic acid available in the root for the synthesis of nicotine, nor-nicotine and other alkaloids derived from the pyridine ring of nicotinic acid. However, in addition to reduced levels of nicotine and other alkaloids, Vector 21-41 was also found to have levels of reducing sugars in the leaves that were about twice those of the "wild-type" and "promoter control" Burley 21-LA plants, but which are well within the range of reducing sugars observed among different tobacco varieties. APHIS knows of no information which would lead one to believe that this would cause a plant pest risk.

APHIS analysis of agronomic data and observations provided on field tests conducted in 2000 in Clayton, North Carolina, and in 2001 in Pennsylvania, Illinois, Louisiana, Mississippi, and Iowa using a typical insect and disease management program indicate that Vector 21-41 does not have increased susceptibility to diseases or insects when compared to the parental line, or to that reported previously for Burley 21-LA (Legg et al., 1970) or Burley 21 (Gary Palmer, Extension Tobacco Specialist, and Glenn Collins, University of Kentucky, personal communication to Susan Koehler, November and December, 2001). Both Burley 21 and Burley 21-LA have low levels of disease resistance compared to newer Burley varieties and Burley 21-LA is described as extremely susceptible to insect damage. The petitioner notes that a field experiment was conducted in Clayton, North Carolina in 2000 to compare Vector 21-41 tobacco with the "wild-type" and "promoter control" plants grown in the absence of systemic insecticides, and all plants were destroyed by insects, primarily tobacco horn worms and tobacco bud worms. No difference in insect predation was observed between Vector 21-41 and the controls.

B. Potential impacts based on the relative weediness of Vector 21-41 tobacco compared to currently cultivated tobacco varieties.

APHIS evaluated whether Vector 21-41 tobacco is any more likely to become a weed than the parent tobacco line, or other tobacco currently cultivated. The assessment considers the weediness status and any weediness characteristics of tobacco and the unique characteristics of

Vector 21-41 tobacco. APHIS also evaluated whether Vector 21-41 tobacco is any more likely to transmit weedy characteristics to other cultivated tobacco.

Tobacco (*N. tabacum*) has been grown for commercial purposes in at least 97 countries around the world (Shew and Lucas, 1991). In a search of the major weed references (Holm et al., 1977 and 1979; Muenscher, 1980; Reed, 1977, Weed Science Society of America, 1989), it is listed as a common weed in Hawaii by Holm et al.(1979), but it is not listed as a weed anywhere else in the world, nor is it present on the lists of noxious weed species distributed by the Federal Government (7 CFR Part 360) or on the list of weeds regulated by the states (see http://www.aphis.usda.gov/npb/statenw.html). Its status as a weed in Hawaii is questionable, as the only *Nicotiana* species listed in a recent reference of Hawaiian weeds is *N. glauca* Graham, commonly referred to as tree tobacco (Haselwood and Motter, 1983). APHIS' analysis of the characteristics of tobacco reveals that it possesses few of the characteristics described by Baker (1965) that are thought to contribute to the weediness potential of plants that are notably successful weeds. The only ones identified are self-compatibility and very high seed output in favorable conditions (up to 3,000 seeds per flower) (Shew and Lucas, 1991). One characteristic of weeds not mentioned by Baker, but which is in part responsible for the classification of some Solanaceous plants as weeds, including *N. glauca* and *N. suaveolens*, is the production of alkaloids, including nicotine, which can cause adverse health effects in humans and livestock (Holm et al., 1979; James et al., 1992). Cultivated tobacco is unlikely to become a weed under most agricultural situations because the common practice of topping prevents seed formation. Even if seed were produced, the seeds are very small (0.5 mm in diameter) and usually require germination in a carefully prepared sterile seedbed or in a greenhouse to avoid devastating seedling pathogens, most important of which is blue mold (Shew and Lucas, 1991). Tobacco volunteers are not common, but if found, can be easily controlled by herbicides or mechanical means.

Vector 21-41 tobacco exhibits no characteristics that would cause it to be more weedy than the non-transgenic parent tobacco line or other cultivated tobacco, and therefore is unlikely to transmit weedy characteristics to other tobacco. In particular, as noted above, Vector 21-41 is significantly lower in nicotine and other alkaloids than Burley 21-LA and Burley 21; it is susceptible to blue mold, and is equally susceptible to insects as Burley 21-LA. In addition, Burley tobacco requires higher rates of nitrogen fertilizer than other types of tobacco (Shew and Lucas, 1991). APHIS evaluated data collected from the 2000 field trial in Clayton, North Carolina that show that no significant differences were observed between Vector 21-41 and the non-transgenic parent tobacco line in other agronomic characteristics which might influence the plant's ability to compete as a weed. These characteristics include days from transplant to flowering, height at flowering, leaf number at flowering, leaf size (width and length) and yield. In addition, data were provided that demonstrate that the average and range in the number of seeds produced per pod from greenhouse grown plants of Vector 21-41 tobacco is similar to that

of Burley 21-LA and Tn90, a currently cultivated Burley type of tobacco, and is slightly less than that for two other flue-cured tobacco varieties, Ti121 and Ti57.

C. Potential impacts from gene introgression from Vector 21-41 tobacco into its sexually-compatible relatives.

APHIS evaluated the potential for genes from Vector 21-41 tobacco to introgress into sexually compatible wild relatives and considered whether such introgression would result in increased weediness or other adverse environmental effects. Only six *Nicotiana* species are described as native to the United States, and none of these are listed as threatened or endangered by the U.S. Fish and Wildlife Service. The potential for genes from *N. tabacum* to naturally introgress into these species is extremely low for several reasons (see Appendix B for details). First, all but one of these do not occur in proximity to areas of cultivation or seed production of tobacco in the United States. Second, the species are either not cross compatible with *N. tabacum* or their progeny exhibit sterility or genetic instability. Vector 21-41 is not reported to have flowering characteristics different from those observed for other *N. tabacum,* and there is no reason to believe that it would have a greater ability to produce fertile hybrids with these species.

Several other reasons exist for why gene introgression from Vector 21-41 is extremely unlikely to occur in these indigenous species, or other species of *Nicotiana* that either occur as naturalized weeds in the United States or that are used for ornamental, medicinal or commercial purposes, e.g. *N. alata, N. glauca, N. forgetiana, N. langsdorffii, N. longiflora, N. rustica, N. sauveolens, N. sylvestris, N. sanderae,* and *N. tomentosa* (Shew and Lucas, 1991; see taxonomic information for *Nicotiana* species in the GRIN database at http://www.ars-grin.gov/cgi-bin/npgs/html). (1) As stated in the petition (pg. 4), Vector Tobacco intends to produce all seed in greenhouses that will house only Vector 21-41 plants; and the vents of these greenhouses will be screened and managed in such a way as to prevent the entry or exit of pollen-carrying insects. (2) Restrictions placed on the cultivation of low-nicotine varieties by AMS regulations preclude the harvesting or transfer of seed produced from these plants under contract. (3) Topping and sucker control methods, which are common practice for production of commercial tobacco and Vector 21-41, will reduce or eliminate the availability of Vector 21-41 pollen for cross-hybridization. (4) Even if some flowers were produced, tobacco is primarily self-pollinating (95%) and fertilization generally occurs before the flower opens. It is not wind pollinated and any outcrossing relies on insect pollinators. McMurtrey et al., (1960) reported that outcrossing rates of adjacent plantings of untopped tobacco (including a Burley variety) measured over 3 years in Maryland ranged from 2-11 percent, and outcrossing rates declined with distance, and were usually less than 0.3% at 1/4 mile (1,320 feet, the isolation distance for certified seed of tobacco of different varieties). Since Vector 21-41 tobacco does not exhibit characteristics that might be expected to cause it to be any more competitive or weedy than other cultivated tobacco, or to adversely effect nontarget organisms (see Section D), any impact from gene introgression from Vector 21-41 into wild relatives is not expected to be greatly different from that of other varieties of cultivated tobacco.

D. Potential impact on nontarget organisms, including beneficial organisms and threatened or endangered species.

APHIS evaluated the potential for Vector 21-41 tobacco and its products to have damaging or toxic effects on nontarget organisms representative of the exposed agricultural environment, including those that are recognized as beneficial to agriculture or as threatened or endangered in the United States.

The expression of NPTII in tobacco plants is not expected to have deleterious effects or significant impacts on nontarget organisms, including beneficial organisms and threatened and endangered species, based on information provided in the petition (pg. 18) and APHIS analyses of previously deregulated transgenic plants that express NPTII. The protein is not toxic and it shares no homology with proteins known to be toxic or allergenic (U.S. FDA 1998).

The reduced levels of nicotine and total alkaloids in Vector 21-41 are expected to make this tobacco less toxic to insects or other animals that might feed on tobacco. In general, tobacco is not used as a feed plant because of its vertebrate toxicity. Teratogenic problems resulting from tobacco alkaloids have occurred in the United States when pregnant swine were allowed to forage on tobacco stalks (Crowe, 1969). Nicotine is a naturally occurring insecticide and feeding deterrent in *Nicotiana* species, in particular *N. tabacum* and *N. rustica* (Bush and Crowe, 1989). However as noted earlier, many pests of tobacco have adapted to the alkaloids in tobacco or have ways to avoid it (Tso, 1972, Chapter 23, Alkaloids). APHIS' evaluation of the many insects that feed on tobacco indicate that none of these insects feed exclusively on tobacco or other *Nicotiana* species, and therefore they are unlikely to have a specific requirement for nicotine or its metabolites. Reducing sugars, which were reported to be about two fold higher in Vector 21-41 leaves compared to non-transgenic parent line, are not included among the types of defense compounds produced by plants that have toxic or anti-feeding properties.

APHIS can not identify any threatened or endangered species that specifically feed or live on tobacco, and no *Nicotiana* species are listed as threatened or endangered. Vector 21-41 tobacco does not exhibit traits that would expand or change the range in habitats in which tobacco is normally grown, so its cultivation is not expected to displace any plant species. For these reasons, no effect on species recognized or proposed as threatened or endangered in the United States by the U.S. Fish and Wildlife Service is expected.

E. Potential impacts on agricultural and cultivation practices

APHIS considered potential impacts associated with the cultivation of Vector 21-41 tobacco on current agricultural practices, including potential impacts on organic farming. Potential impacts on minorities, low income populations, and children were also considered.

Potential impacts of Vector 21-41 on pest control and other agricultural practices

As noted in Section V.A. above, Vector 21-41 does not have increased susceptibility to diseases or insects when compared to the parent line or to that reported previously for Burley 21-LA or Burley 21 when using a typical disease and insect management program. Various cultural and chemical control methods have been described by agricultural extension services for the management of disease and insect pests of tobacco to which Vector 21-41 is known or likely to be susceptible. There is no reason to believe that these methods would not also be implemented for Vector 21-41 tobacco. The petitioner notes that "The experience from the over 600 farm field trials conducted in five states this year [2001] indicate that existing approved amounts of pesticides labeled for use on tobacco can control insect, disease, and weed problems.". Pesticide use on U.S. tobacco has been restricted and monitored for many years by the USDA under The Food Security Act of 1985. Growers must certify to the Farm Service Agency that any pesticides used in tobacco production have been approved by the EPA for use on tobacco and were applied in accordance with labeled directions. Failure to comply with requirements can result in loss of price support, fines, or imprisonment (USDA, ERS, 2001).

Potential impacts on organic farming

It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic tobacco leaf , will be significantly impacted if a determination for non-regulated status were to be granted for Vector 21-41 tobacco for the following reasons. (1) Nontransgenic tobacco will still be sold and will be readily available to those who wish to plant it. (2) USDA's National Organic Program requires that organic farmers plant certified (nonengineered) seed (http://www.ams.usda.gov/nop/nop2000/Final%20Rule/nopfinal.pdf), and if a grower can verify that this was the case, then any adventitious presence of transgenic material in organically grown products will not keep that product from being certified as organic. (3) According to AMS regulations, the reduced-nicotine Vector 21-41 tobacco would need to be grown under contract with the company, therefore growers will know it is transgenic. (4) Vector Tobacco intends to take several steps to ensure identity preservation of its seed stock of Vector 21-41 tobacco (see Petition, pg. 4.). (5) Cross pollination of other tobacco in the field by Vector 21-41 tobacco is highly unlikely (for the reasons described in Section V. Alternative B. Paragraph C. above). (6) Even if cross-pollination of non-transgenic plants were to occur, only an extremely small percentage of transgenic seed would likely be produced, but the leaves harvested from the cross-pollinated plants to be marketed would not be transgenic.

Potential impacts on humans, including minorities, low income populations, and children

Under Executive Order 13045, we attempted to identify and assess environmental health or safety risks that might disproportionately affect children. We also considered any possible adverse impacts on minorities and low income populations as specified under Executive Order 12898. As noted above, APHIS does not anticipate that there will be a significant change in pest control or cultural practices if Vector 21-41 were deregulated, and pesticide use in tobacco is

closely monitored by the USDA. Because Vector 21-41 tobacco has significantly reduced levels of nicotine compared to traditional tobacco, APHIS does expect there to be some benefit to farm workers or their children who might come into contact with tobacco in the field, particularly during topping of the tobacco plants. A green tobacco syndrome is caused by adsorption of nicotine from skin contact in workers who handle wet tobacco plants. Symptoms, including headache, dizziness, and vomiting, have been reported in up to 50% of tobacco handlers. Fortunately they usually resolve within a few hours after tobacco plant contact ceases and contaminated areas of the body are washed (see *Risks From Chemical Use on the Farm* by Frederick W. Oehme DVM, PhD, Comparative Toxicology Laboratories, Kansas State University, at http://www.vet.ksu.edu/links/agromed/oehmemorning.htm.).

F. Potential impacts on raw or processed agricultural commodities.

APHIS analysis of information and data provided in the petition regarding: (1) the disease and insect susceptibility of Vector 21-41 tobacco; (2) the analysis of air-cured leaves for percent nicotine, nor-nicotine, total alkaloids, total nitrogen and reducing sugars; and (3) the analysis of smoke from cigarettes derived from Vector 21-41 tobacco leaves for TSNAs, reveal no differences between either the reported non-transgenic recipient Burley 21-LA or other tobacco that could have a direct or indirect plant pest effect on any raw or processed plant commodity.

G. Potential environmental impacts outside the United States associated with a determination of nonregulated status as requested by Vector Tobacco (USA) Ltd.

APHIS has also considered potential environmental impacts outside the United States and its territories associated with a determination of nonregulated status for Vector 21-41 tobacco. Any international trade in tobacco subject to this determination would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that are signatory to the Convention (116 countries as of June, 2001). In addition, issues that may relate to commercialization and transboundary movement of particular agricultural biotechnology products are being addressed in IPPC and other international fora. APHIS also continues to play a role in working toward harmonization of biosafety and biotechnology guidelines and regulations included within our regional plant protection organization, the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection. APHIS also participates regularly in biotechnology policy discussions at fora sponsored by the European Union and the Organization for Economic Cooperation and Development. APHIS periodically holds bilateral or quadrilateral discussion on biotechnology regulatory issues with other countries, most often with Canada, Mexico, Japan, and Argentina. We also have participated in numerous conferences intended to enhance international cooperation on safety in biotechnology and sponsored several workshops on safeguards for

planned introductions of transgenic crops (crucifers, maize, wheat, potatoes, rice, tomatoes) most of which have included consideration of international biosafety issues. Of particular relevance to crops with their centers of origin in South and Central America, we sponsored a Latin-American Biosafety workshop on field testing and commercialization of transgenic plants. Mexico and many of the countries in South America where most of the wild relatives of tobacco exist (e.g. Brazil, Argentina, and Uruguay) have procedures in place that require a full evaluation of transgenic plants before they can be introduced into their environment.

Alternative C, Approval of the Petition in Part.

Approval of only some of the lines requested in the petition. The petition received from Vector Tobacco (USA) Ltd. requested a determination of nonregulated status for only a single line, Vector 21-41. Data demonstrate that this line was derived from a single transformation event, transformant #41. Therefore, APHIS can consider only that one line for approval.

Approval of the petition with geographic restrictions. APHIS could identify no impacts to the human environment that are specific to a particular geographical region of the United States or its territories. Even if Vector 21-41 tobacco were grown in regions of the United States where it's wild relatives occur, there are several factors described in Section V, Alternative B, Paragraph C, that make gene introgression unlikely, and any impact from gene introgression into wild relatives is expected to be no worse than that from other cultivated tobacco. APHIS could not identify any risks to non-target organisms or to threatened and endangered species. Therefore, APHIS finds no reason to place geographic restrictions on the cultivation or movement of Vector 21-41 tobacco.

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Appendix A

USDA-APHIS approved field tests of Vector 21-41 tobacco.

APHIS Notification Number	States	Effective Date[1]	Acreage Requested
99-180–05N[2]	NC	7/29/99	5.0
01-024-26N	PA	2/23/01	1838.0
01-061-01N	PA	4/01/01	1061.5
01-081-03N	TX	NOT PLANTED	
01-094-07N	MS	5/04/01	100.0
01-102-01N	MS	5/12/01	210.0
01-102-03N	LA	5/12/01	367.0
01-102-04N	IL	5/12/01	1030.0
01-102-05N	PA	5/12/01	424.0
01-114-02N	LA	5/24/01	81.0
01-116-01N	MS	5/26/01	21.0
01-117-03N	IA	5/27/01	6.0
01-122-01N	PA	6/01/01	5.0
01-143-07N	HI	6/22/01	40.0
01-158-01N	IL	7/07/01	20.0

[1]Plantings must take place within one year of the effective date.

[2]Field data report submitted, and included in the petition. Field data reports are due within 6 months of termination of the field test.

Appendix B. Potential for introgression from *Nicotiana tabacum* to its sexually compatible relatives.

N. tabacum has a somatic chromosome number of 2N = 48 (i.e., 24 pairs of chromosomes) and originated in South America as an amphidiploid species resulting from a hybridization of two progenitor species believed to be *N. sylvestris* (2N=24) and *N. tomentosiformis* (2N=24) (Smith, 1979). The USDA, Agricultural Research Service, Germplasm Resources Information Network (GRIN) database recognizes 68 different taxa at the species level within the genus *Nicotiana,* which is subdivided into three subgenera and 14 sections (Smith 1979). Of these species, 45 species are native to North and/or South America, 17 are native to Australia, one is native to Polynesia, Tonga, and S. Melanesia, and one is native to Africa. Others are listed as cultivars. Only six *Nicotiana* species are listed as native to the United States. Their distribution within the United States (as referenced in the GRIN database and references therein), their somatic chromosome number (Smith, 1979), and their reported ability to produce viable, fertile offspring with *N. tabacum* (as described in the petition, pg. 2; and in Chaplan and Burk, 1979; and confirmed in discussion with Dr. David Zaitlin, tobacco breeder, University of Kentucky, Tobacco and Health Research Instititue, personal communication to Susan Koehler, 12/9/01) are summarized in the table below. The potential for genes from *N. tabacum* to naturally introgress into its sexually compatible relatives in the United States is extremely low for several reasons. First, with the exception of *N. plumbaginofolia*, none of these occur in proximity to major areas of cultivation or seed production of tobacco. In the United States, tobacco is typically grown in the midwest and eastern states. Statistics show that for the years 1997-1999, that the largest acreage occurred in the following states in descending order: North Carolina, Kentucky, Tennessee, South Carolina, Virginia, Georgia, Ohio, Indiana, Pennsylvania, Maryland, Florida, Missouri, Connecticut, Wisconsin, West Virginia, and Massachusettes (USDA, NASS, 2000). Most farmers do not produce their own tobacco seed, but use certified seed produced under conditions of reproduction isolation as specified under 7 CFR 201.76, most of which is grown in the same areas where commerical production of tobacco occurs. Second, the species are either not reported to be cross compatible with *N. tabacum* or their progeny exhibit sterility or genetic instability which would further reduce the potential for gene introgression back into the wild population.

Naturally occurring intergeneric crosses with tobacco as one parent (e.g., tobacco x tomato) have not been reported. Intergeneric hybrid plants using tobacco as one parent have been produced under laboratory conditions by fusion of protoplasts (cells lacking cell walls) and subsequent regeneration of whole plants. Most of these intergeneric hybrids are either sterile or produce nonviable seeds (Steward and Krikorian 1979, and Glen Collins, personal communication to Susan Koehler, Nov. 2001).

Nicotiana species native to the United States	Somatic chromosome number	Distribution within the U.S.	Cross-compatible with *N. tabacum*	Fertility of hybrids
N. attenuata Torr. ex S. Watson	2N=24	Western U.S., Mexico to Canada	No	
N. obtusifolia M. Martens & Galeotti (Syn = *N. palmeri* A. Gray and *N. trigonophylla* Dunal)	2N=24	Texas, New Mexico, Arizona, California, Nevada, Utah	Only in laboratory experiments	
N. repanda Willd. ex Lehm.	2N=48	Texas (gulf coast)	Yes	Male sterile
N. quadrivalvis Pursh (Syn = *N. bigelovii* (Torr.) S. Watson)	2N=48	California, Nevada, Oregon	Yes	Male sterile
N. clevelandii A. Gray	2N=48	California, Arizona, New Mexico	No	
N. plumbaginifolia Viv.	2N=20	Florida	Only under controlled conditions.	Male and female sterile: genetic instability, loss of *N. plumb.* genome

References:
See Literature Cited, Section VI.

Appendix C. Data submitted with the petition or in the October 19, 2001 response to the deficiency letter dated September 21, 2001 (RTDL) in support of nonregulated status for Vector 21-41 Tobacco.

Molecular Genetic Characterization and Inheritance Data
NtQPT1 gene is expressed in the roots: Petition Fig. 2., pg. 11. In situ localization of *NtQPT1* mRNA in parraffin sections of tobacco roots.
NtQPT1 promoter is capable of directing transgene expression in a root-specific manner: Petition Fig. 3, pg. 11. Histochemical localization of GUS activity in trangenic roots; Petition Fig. 4., pg. 12. GUS activities in roots, leaves, and stems of transgenic tobacco plants carrying the CaMV 35S promoter, the promoterless GUS, and 5' nested deletions of the *NtQPT1* promoter fused to GUS.
NtQPT1 gene encodes a functional QPTase: Petition Fig. 5., pg. 13. Complementation of *E. coli* Strain TH266 (*nadC*) by expression of the *NtQPT1* cDNA.
Vector 21-41 contains two copies of the *nptII* gene: Petition Fig. 9., pg. 23. Genomic DNA Blot of Vector 21-41 (probed for *nptII*).
Vector 21-41 contains full length copies of the *nptII* gene and *NtQPT1* promoter in the expected orientation: Petition Fig.10., pg. 24. PCR Analysis of the Transgene in Vector 21-41.
Vector 21-41 contains the full length copies of the anti-sense *NtQPT1* cDNA with its *NtQPT1* promoter and *nos* terminator in the correct orientation: Petition Fig. 11., pg. 26. PCR Analysis of the Transgene in Vector 21-41.
Vector 21-41 contains partial *tetA* and *trfA* sequences flanking the left T-DNA border located between the left T-DNA border of the two inverted repeat copies of the T-DNA inserts. Petition Fig. 12., pg. 28. PCR Analysis of the Transgene in Vector 21-41 and Petition Fig. 13., pg. 30. Amplification Between the Two Tandem Inverted T-DNA Repeats.
Vector 21-41 contains no complete protein coding sequences present outside of the T-DNA border in pYTY32: RTDL, Fig. 1, pp. 6-8.
Progeny from transformant T_0 #41 segregated 3:1 for kanamycin resistance: Petition Table 2., pg. 19. T_0 Segregation Analysis of Tranformant #41.
T_2 seeds from plant T_1-1 derived from T_0 #41 are homozygous for kanamycin resistance: Petition Table 3., pg. 20. Segregation Analysis of T_1 Generation of Transformant #41 (for kan^r).
T_1-1, T_2-1, and later generations (T_3 and T_4) designated Vector 21-41 have low nicotine levels in their leaves compared to null segregants. Petition. Table 4. Pg. 21. Nicotine levels in Generations of Vector 21-41 Tobacco.

Phenotypic Characterization: Intended and Unintended Effects
NPTII expression in Vector 21-41 T_4 plants in leaves, roots, and stems as measured by enzyme-linked immunosorbent assays (ELISAs). Petition pg. 31 (See also Table 2 mentioned above for kanamycin resistance.)
Agronomic traits and chemical traits (nicotine, nor-nicotine, total alkaloids, nitrogen and reducing sugars in cured leaves) of Vector 21-41 tobacco grown in Clayton, NC in 2000 compared to promoter control and wild type (Burley 21-LA) plants. Petition Table. 5., pg. 32. (See also Table 4 mentioned above for nicotine content in NC and other locations, and also RTDL, pg. 3, and pg. 13 for additional agronomic data for 53 field trials in PA in 2001.) (AMS also provided nicotine data to APHIS for Vector 21-41 tobacco grown in 2001 at the request of the petitioner.)
Seed production per pod from greenhouse grown plants of Vector 21-41 tobacco compared to Burley 21-LA and four other tobacco varieties. RTDL, Table 1, pg. 12. (Note: Number of pods in that Table 1 refers to the number of pods from which the data was gathered. The number of pods was limited to 50/plant, M. Conkling personal communication 10/24/01.)
Disease and insect susceptibility of Vector 21-41 tobacco. Petition, pg. 31, & RTDL, pp 10-11.
Yields of Tobacco-Specific Nitrosamines in Mainstream Tobacco Smoke: "Standard" Conditions. (Vector 21-41 vs. a "full-flavor" cigarette) Petition Table 6, pg. 34

Appendix D. Determination of non-regulated status for Vector 21-41 tobacco.

In response to a petition (designated 01-121-01p) from Vector Tobacco (USA) Ltd., APHIS has determined that genetically engineered tobacco line Vector 21-41 and progeny derived from it will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits or acknowledged notifications that were previously required for environmental release, importation, or interstate movement under those regulations will no longer be required for Vector 21-41 tobacco and its progeny. Importation of seeds and other propagative material would still be subject to APHIS foreign quarantine notices at 7 CFR Part 319 and the Federal Seed Act regulations at 7 CFR Part 201. This determination is based on APHIS' analysis of field, greenhouse, and laboratory data and references provided in the petition and other relevant information as described in this environmental assessment that indicate that Vector 21-41 will not pose a plant pest risk for the following reasons. (1) It exhibits no plant pathogenic properties - although a plant pathogen was used in the development of this tobacco, these plants are not infected by this organism, nor do they contain genetic material from this pathogen that can cause plant disease. (2) It exhibits no characteristics that would cause it to be more weedy than the non-transgenic parent tobacco line or other cultivated tobacco. (3) Gene introgression from Vector 21-41 tobacco to native, introduced, or naturalized species of *Nicotiana* in the United States is extremely unlikely, and it is not likely to increase the weediness potential of any resulting progeny nor adversely effect genetic diversity any more than would introgression from other cultivated tobacco. (4) Given current tobacco pest management practices and grading and certification systems for tobacco leaf, the disease and insect susceptibility of Vector 21-41 tobacco and the quality characteristics of its cured leaves should not cause a direct or indirect plant pest effect on raw or processed plant commodities, nor should it reduce the ability to control pests in tobacco or other crops, compared to the non-transgenic parent or other tobacco. (5) It has no potential to have a greater damaging, harmful, or toxic effect on organisms beneficial to agriculture than does other cultivated tobacco. In addition to our finding of no plant pest risk, there will be no affect on threatened or endangered species resulting from a determination of non-regulated status for Vector 21-41 tobacco and its progeny.

APHIS also has concluded that there may be new varieties bred from Vector 21-41 tobacco; however, they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for tobacco descended from Vector 21-41 tobacco, or those observed for other tobacco varieties not considered regulated articles under 7 CFR Part 340.

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Date: SEP 16 2002

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A randomized trial of nicotine replacement therapy in combination with reduced-nicotine cigarettes for smoking cessation

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A randomized double-blind, active controlled, parallel group, multi-center phase II clinical trial was conducted to evaluate the efficacy of reduced-nicotine cigarettes as a novel smoking cessation treatment (under Investigational Device Exemption 69,185). The concept for a reduced-nicotine cigarette designed to progressively wean smokers from the smoking habit is based on research demonstrating that successful smoking cessation is not only dependent on withdrawal of nicotine, but also on weaning from the habitual sensory and behavioral reinforcement of smoking. Treatment consisted of Quest brand of cigarettes (Quest 1, 2, and 3), which respectively deliver 0.59 ± 0.06, 0.3 ± 0.05, and less than 0.05 mg nicotine, either alone or in combination with nicotine replacement therapy (NRT). The primary endpoint was 4 weeks of continuous abstinence (Weeks 7–10), with additional follow-up at 3 and 6 months. Adult men and women smokers (*N*=346), motivated to quit, were randomized to one of three treatment groups: Quest plus NRT (NRT pretreatment 2 weeks before, and NRT after the quit date), Quest plus placebo patch, or active control plus NRT (conventional cigarette, followed by NRT after quit date). Results showed that Quest plus NRT was more effective than active control plus NRT in achieving 4 weeks of continuous abstinence (32.8% vs. 21.9%). Quest plus placebo patch yielded an abstinence rate similar to that of the active control plus NRT (16.4% vs. 21.9%). No serious adverse events were attributable to the investigational product. Quest plus NRT offers promise as a new smoking cessation treatment.

Introduction

Cigarette smoking is a complex behavior that is now understood to be sustained by the pharmacological properties of nicotine and the reinforcing behavioral and sensory cues associated with the act of smoking (Benowitz, 1999; Monchuk, Rousu, Shogren, Nonnemaker, & Kosa, 2007; Rose & Behm, 2004a). These pharmacological and behavioral components are thought to be mediated by distinct neural and psychological processes (Naqvi & Bechara, 2005). Currently many smoking cessation products and methodologies are available to smokers, including nicotine replacement therapies (NRT) such as the nicotine skin patch, and medications available by prescription, such as bupropion and varenicline. In general, these approaches fall short of helping the majority of smokers to achieve long-term abstinence. NRT in combination with counseling and education remains effective in only a minority of patients, with success rates increasing by 1.5 2.0-fold compared with placebo regardless of the type of NRT (Silagy, Lancaster, Stead, Mant, & Fowler, 2004). Relapse is common following NRT use, and it has been estimated that 75% to 80% of smokers who receive treatment are unable to achieve long-term abstinence at 6 or 12 months (Fiore, Smith, Jorenby, & Baker, 1994; Hajek et al., 1999). Similar relapse rates are observed even with the use of prescription medications such as bupropion or varenicline (Fiore et al., 1994; Hughes et al., 2003; Hurt et al., 2002; Jorenby et al., 2006; Litten & Allen, 1999; Peters & Morgan, 2002; West, 2003).

Current treatment approaches may have limited efficacy because they do not adequately address the

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behavioral and sensory components of cigarette addiction, which have been shown to be important in the reinforcement of smoking. For example, blocking or altering airway sensory stimulation using local anesthesia or menthol (Rose & Behm, 2004a) leads to reduced smoking satisfaction; this result suggests that sensory stimulation remains an important mechanism to satisfy craving for cigarettes. Indeed, promising results have been reported in clinical trials using citric acid inhalation to substitute for some components of airway sensations sought by smokers (Behm, Schur, Levin, Tashkin, & Rose, 1993; Westman, Behm, & Rose, 1995). The use of nicotine-free cigarettes, in the absence of concomitant nicotine delivery, consistently produce satisfaction, reward, and craving reduction in smokers (Brauer et al., 2001; Breland, Buchhalter, Evans, & Eissenberg, 2002; Buchhalter, Schrinel, & Eissenberg, 2001; Butschky, Bailey, Henningfield, & Pickworth, 1995; Dallery, Houtsmuller, Pickworth, & Stitzer, 2003; Gross, Lee, & Stitzer, 1997; Pickworth, Fant, Nelson, Rohrer, & Henningfield, 1999; Rose, Behn, Westman, & Johnson, 2000; Westman, Behm, & Rose, 1996). In addition, the use of denicotinized cigarettes elicits higher subjective ratings than those elicited by intravenous nicotine administration (Rose et al., 2000; Westman et al., 1996). These studies suggest that sustaining smoking-related sensory cues may be an effective behavioral weaning tool to help reduce relapse (Rose & Behm, 2004b).

Moreover, use of denicotinized cigarettes may promote rapid extinction of the reinforcing value of smoking-related behavioral and sensory cues. Recent studies in both laboratory and outpatient-type research settings have demonstrated that use of denicotinized cigarettes over a period of 1 2 weeks weakens the reinforcing effects of smoking (Donny, Houtsmuller, & Stitzer, 2007; Rose & Behm, 2004a). Denicotinized cigarettes may reduce smoking of conventional cigarettes through two mechanisms: first, by providing a temporary behavioral substitute for conventional cigarettes to acutely relieve craving; second, by removing the primary reinforcing effects of nicotine, less craving is generated on a continuing basis.

A complementary approach to weaken the primary reinforcing effects of nicotine is pretreatment with NRT prior to the quit date. Pretreatment with NRT has been shown to reduce the reinforcing effects of smoking (Rose, Behm, Westman, & Kikovich, 2006), and to increase abstinence rates regardless of whether conventional, low nicotine, or denicotinized cigarettes are smoked prior to the quit date (Rose et al., 2006; Schuurmans, Diacon, van Biljon, & Bolliger, 2004). However, in such a context, the use of denicotinized cigarettes in combination with NRT obviates concerns about any potential increased toxicity related to nicotine associated with concurrent use of NRT plus conventional cigarettes (Rose, Behm, Westman, Bates, & Salley, 2003). Currently marketed low tar and nicotine cigarettes achieve their Federal Trade Commission (FTC) ratings through ventilation holes in the filter, despite containing tobacco with significant quantities of nicotine (Benowitz et al., 1983). The high nicotine content of the tobacco raises concerns that the compensatory smoking behavior often seen in ventilated-filter cigarette smokers may further increase toxicity (Kozlowski, Pope, & Lux, 1988; Rose & Behm, 2004b).

No optimal way remains to convert dependent smokers into permanent nonsmokers. A broad range of treatment options may be necessary to improve upon current success rates and to achieve long-term abstinence. Denicotinized cigarettes, such as Quest, may improve upon existing smoking cessation approaches by substituting for the sensory and behavioral cues associated with cigarette use while helping to maximally dissociate these cues from the contingent delivery of nicotine reinforcement, thus providing an effective exit strategy from cigarette addiction.

To test this novel concept for a smoking cessation aid, we conducted a randomized, double-blind, active controlled, parallel group, multicenter phase II clinical trial in healthy smokers to compare (a) conventional cigarettes plus NRT treatment with (b) progressive use of cigarettes with decreasing nicotine content (Quest 1, 2 and 3) alone or in combination with NRT, in achieving smoking cessation.

Method

Participants

The study population consisted of 346 generally healthy smokers, 21 65 years of age, recruited from five centers (Central Kentucky Research, Lexington, KY; University Clinical Research, Inc., Pembroke Pines, Florida; Triangle Medical Research Associates, Raleigh, North Carolina; Clinical Research Associates Inc., Nashville, Tennessee; and Rochester Clinical Research, Inc., Rochester, New York). Subjects were required to have smoked an average of 15 or more cigarettes per day for at least 1 year prior to randomization, be motivated to quit smoking, and be able to return for scheduled follow-up examinations for a total of 8 months. A carbon monoxide (CO) measurement >15 ppm corrected for ambient levels at baseline was required for entry into the study (the corrected CO level was determined by taking the subject's measured CO reading and subtracting the CO reading of the ambient air prior to testing, as per the instructions for use of the CO

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monitor.) Subjects who qualified as capable of comprehending the nature of the study and were willing to provide informed consent were entered into the study. Exclusion criteria included current use of NRT or other tobacco-based product (e.g., chew, snuff, or other); usual brand consisting of menthol variety of cigarettes; smoking more than 3 packs of cigarettes per day; serious pathophysiology that might impose a risk to the subject (at the discretion of the physician); or use of illegal drugs. Subjects were also excluded if they were pregnant, were planning to become pregnant, or were lactating; were consuming an average of three or more drinks of alcohol per day; or were hypertensive (systolic blood pressure higher than 140 mmHg and/or diastolic blood pressure higher than 90 mmHg).

Study materials

Four types of test cigarettes were used in this study: Quest 1, 2 and 3 cigarettes, and active control cigarettes (the active control was a conventional, American blended cigarette with a nicotine yield of 0.80 ± 0.10 mg/cigarette and a tar yield of 10.2 ± 0.5 mg/cigarette). Quest cigarettes differ from traditional cigarettes by the inclusion of genetically modified tobacco with reduced nicotine content. As measured by the Federal Trade Commission (FTC) regimen, Quest 1 delivers 0.59 ± 0.06 mg nicotine (9.0 ± 1.0 mg tar) per cigarette; Quest 2 delivers 0.30 ± 0.05 mg nicotine (8.0 ± 1.25 mg tar) per cigarette; and Quest 3 delivers less than 0.05 mg nicotine (8.5 ± 1.0 mg tar) per cigarette. However, the actual tobacco rods of Quest cigarettes contain the following total amounts of nicotine: 8.9 mg (Quest 1), 5.1 mg (Quest 2), and 0.48 mg nicotine (Quest 3).

The product is designed to provide smokers who wish to quit with the sensory and behavioral reinforcement of a conventional cigarette while withdrawing inhaled nicotine in a step-wise manner. The transdermal nicotine patch used in this study was a generic over-the-counter product (1-800-PATCHES), in 21-, 14-, and 7-mg doses. All patches were 24-hr applications to be reapplied daily. Placebo patches were manufactured to match the size, appearance, and texture of an active NRT patch to preserve blinding.

To blind the cigarette assignment, Quest 1, Quest 2, Quest 3, and active control cigarettes were labeled in accordance with their study visit period, and were packaged 20 cigarettes per pack and 10 packs per carton. All investigational products were indistinguishable from one another. The 21-, 14-, 7-mg, and placebo NRT patches were repackaged in an identical manner in accordance with the randomization scheme of the study. Twenty patches were packaged in each box. Each study kit contained the appropriate number of cigarette cartons and patches for one study subject.

Procedure

The study was conducted in compliance with Good Clinical Practices and under an Investigational New Drug Application (IND) from the Food and Drug Administration. Healthy male and female smokers interested in quitting smoking were recruited from the community and screened for eligibility as described above. Once written consent was provided, the subject underwent a complete screening procedure, consisting of relevant medical history, physical examination, urine pregnancy test in women of childbearing potential, drug screen, exhaled CO, saliva cotinine, and completion of study questionnaires (Minnesota Smoking Withdrawal Questionnaire, Cigarette Evaluation Scale Questionnaire, Sensory Questionnaire, and Fagerström Test for Nicotine Dependence [FTND]).

Following screening, eligible subjects were randomized in a 1:1:1 ratio scheme to one of three treatment arms, as illustrated in Figure 1. Subjects were examined and evaluated every 2 weeks after randomization until the end of Week 18 (the 3-month quit date) and then 3 months later at the end of Week 31 (6-month quit date), in accordance with a predetermined visit schedule of 12 expected clinic visits.

During the first 6 weeks of the study, subjects receiving Quest plus NRT and those receiving Quest plus placebo patch transitioned from their usual brand of cigarettes to Quest cigarettes. Subjects in each of the two groups smoked ad libitum for 2 weeks at each Quest nicotine level beginning with Quest 1, then proceeding to Quest 2, and finally to Quest 3. Two weeks before the quit date, at the beginning of Week 5, Quest use in the Quest plus NRT group was supplemented with the addition of a transdermal nicotine patch (21 mg), consistent with the study protocol specified pretreatment in this group, while subjects in the Quest plus placebo patch group added a placebo patch.

The end of Week 6 was considered the quit date, at which time subjects were instructed to quit all smoking. Subjects in the Quest plus NRT group then received a 21-mg transdermal nicotine patch for an additional 4 weeks, followed by a 14-mg patch for 2 weeks, a 7-mg patch for 2 weeks, and a placebo patch for 2 weeks. Subjects in the Quest plus placebo patch group received a placebo patch starting at Week 5 which was continued to the end of Week 16, the end of all treatment.

Subjects in the active control plus NRT group were provided (the same) active control cigarettes

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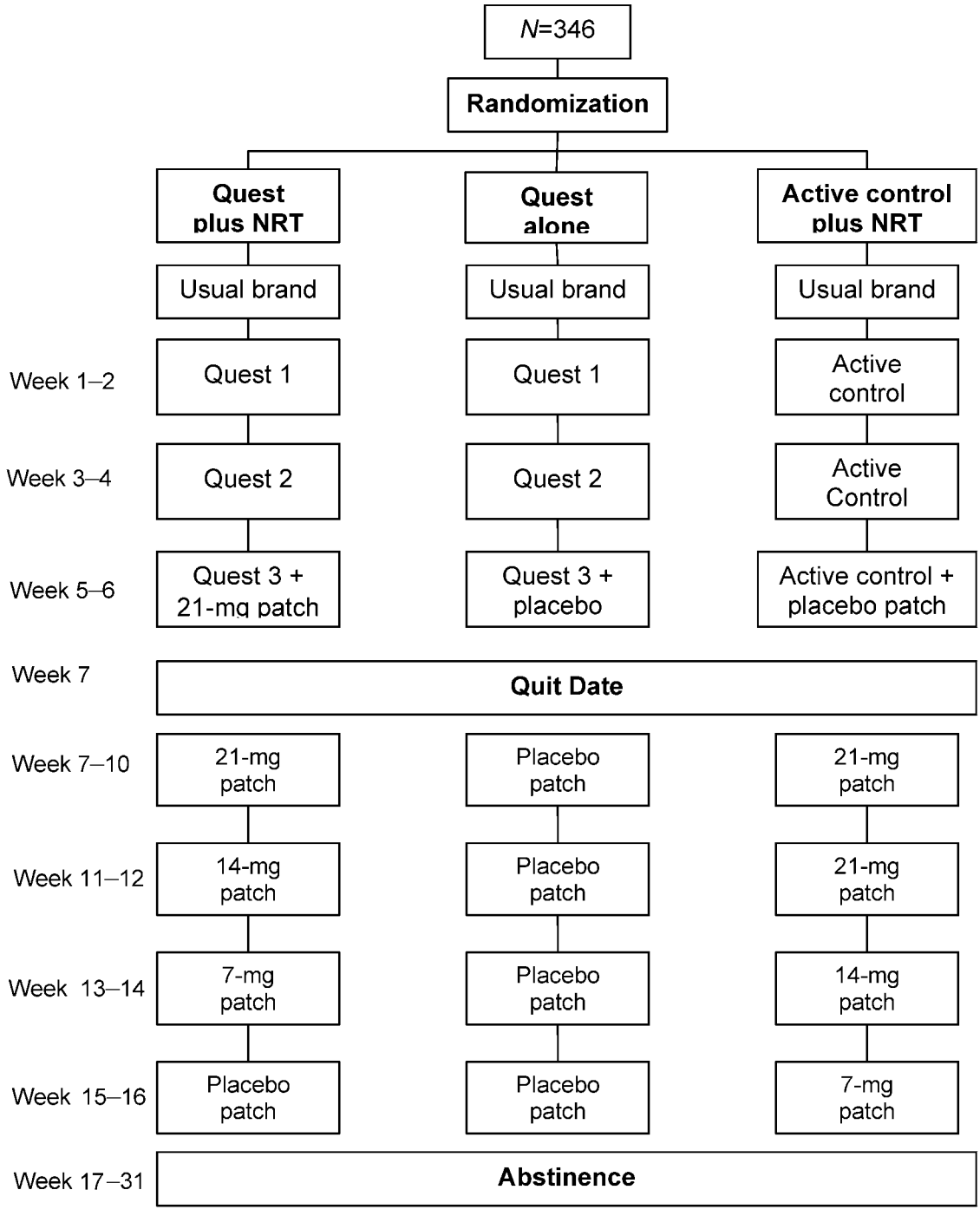


Figure 1. Clinical treatment arms.

as described above. These cigarettes were sham-faded, every 2 weeks, in parallel with the nicotine-fading provided in the Quest plus NRT and Quest plus placebo patch groups. To maintain blinding of patch assignment, the active control plus NRT received placebo patches during the 2 weeks immediately preceding their quit date. Subsequently, NRT treatment in this group complied with current labeling requirements for OTC patches, indicating treatment with active NRT following the quit date, or in this study at the beginning of Week 7. Furthermore, careful consideration was made to limit active patch exposure to 10 weeks, with 6 weeks of exposure to the highest dosage form (21 mg) and no more than 2 weeks exposure at lower weaning doses of 14 and 7 mg, respectively. To provide appropriate controls to the Quest plus NRT and Quest plus placebo patch groups, subjects in the active control plus NRT group received 6 weeks of a 21-mg patch, followed by 2 weeks of a 14-mg patch, and 2 weeks of a 7-mg patch. From Weeks 17 to 31, subjects in

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the three groups were expected to be abstinent. After the initial 4-week abstinence period, abstinence was again measured 3 months after the quit date, and at 6 months after the quit date. All subjects received behavioral support with printed materials and a 10-min individual counseling session at the first clinic visit by a certified smoking cessation counselor.

Study endpoints

The primary endpoint of this study was 4 weeks of continuous abstinence measured from Weeks 7 to 10 of the study. Abstinence was determined by self report and verified by exhaled CO$<$10 ppm for each subject. Subjects were considered to have completed the study if they had completed all follow-up examinations through Week 31. Secondary endpoints included: quit rates at 3 and 6 months; evaluation of preference and satisfaction of Quest over usual brand; severity of withdrawal symptoms; and compensatory smoking behavior. Safety was assessed by reports of adverse events.

Analyses

The study was designed to test two null hypotheses:

- H_{01}: Probability of quitting in the Quest plus NRT group=probability of quitting in the active control plus NRT group;
- H_{02}: Probability of quitting in the Quest plus placebo patch group=probability of quitting in the active control plus NRT group.

For both hypotheses the alternatives were one-tailed (p=.025) with probability in the active control plus NRT group being less than in the Quest groups. The rationale for a one-tailed test was based on previous research (Rose et al., 2006; Schuurmans et al., 2004) indicating a potential benefit in smoking cessation. Two-tailed probabilities are also presented.

These hypotheses were tested using the intent-to-treat (ITT) population of all randomized subjects and the Fisher exact test. Adjustment for multiplicity was made using the Bonferroni correction (Kleinbaum, 1988). Descriptive statistics included mean, standard deviation, median, and range for continuous variables, and frequency and proportion for discrete variables. Group differences were tested using the chi-square statistic for categorical data and analysis of variance for continuous variables. If subjects without a valid Week 10 assessment or abstinence were dropped out because of lack of efficacy and his/her abstinence status could not be determined, the subject was considered a treatment failure. Logistic regression was used to identify prognostic factors and to conduct an adjusted analysis of the primary outcome. All statistical tests report two-tailed p values with no adjustment for multiplicity, except where noted.

Results

Table 1 shows subject disposition for each of the three treatment groups at the end of Week 10, the predetermined date for evaluation of the primary effectiveness endpoint of the study. Two of the 346 subjects enrolled (0.6%) did not meet all eligibility criteria. All 346 subjects received study treatment. A total of 262 (75.7%) subjects met the criteria for assessment of 4 weeks of continuous abstinence. Eighty-four (24.3%) subjects were unable to be assessed at Week 10. The most common reasons for the lack of assessment was lost to follow-up (9%) and subjects withdrawing consent (8.1%). At Week 10, discontinuation rates were 24.1%, 31.9% and 16.7%, for Quest plus NRT, Quest plus placebo patch, and active control plus NRT, respectively. Quest plus NRT versus active control plus NRT was p=.19, and between the p-value for Quest plus placebo patch versus active control plus NRT was p=.028.

Demographic characteristics by treatment group are summarized in Table 2. Of the 346 subjects enrolled, 53.8% were male and 46.2% were female. The percent of males was significantly different between the groups (p=.0029). The mean age was 45.9 years. The majority of subjects were White (92.2%); 3.5% of the study population was Hispanic, 3.2% was Black, and 0.6% was Asian or Pacific Islander.

Table 3 summarizes the baseline characteristics of the study population. Baseline characteristics were comparable between groups. Most subjects reported smoking light cigarettes (48.6%), followed by full

Table 1. Subject disposition.

Measure	Quest plus NRT (n 116), n (%)	Quest plus placebo patch (n 116), n (%)	Active control plus NRT (n 114), n (%)	Total (N 346), n (%)
All eligibility criteria met	115 (99.1%)	115 (99.1%)	114 (100.0%)	344 (99.4%)
Received study treatment	116 (100.0%)	116 (100.0%)	114 (100.0%)	346 (100.0%)
Completed 4 weeks (week 10)	88 (75.9%)	79 (68.1%)	95 (83.3%)	262 (75.7%)
Discontinued	28 (24.1%)	37 (31.9%)	19 (16.7%)	84 (24.3%)
Completed study* (week 31)	32 (27.6%)	20 (17.2%)	33 (28.9%)	85 (24.6%)

Note. n, number of randomized subjects; % $n/N \times 100\%$. *Subject disposition data from CRF Study Completion/Early Withdrawal from the question, "Did the patient complete study?"

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Table 2. Summary of subject demographics.

	Quest plus NRT (*n* 116)	Quest plus placebo patch (*n* 116)	Active control plus NRT (*n* 114)	Total (*N* 346)
Sex, *n* (%)				
Men	41 (35.3%)	67 (57.8%)	52 (45.6%)	160 (46.2%)
Women	75 (64.7%)	49 (42.2%)	62 (54.4%)	186 (53.8%)
Age at randomization (years)				
Number of available subjects	116	116	114	346
Mean (*SD*)	45.5 (11.1)	46.1 (10.5)	46.3 (11.0)	45.9 (10.9)
Range	21 66	23 65	24 66	21 66
Race, *n* (%)				
Asian or Pacific Islander	0	1 (0.9%)	1 (0.9%)	2 (0.6%)
Black, not of Hispanic origin	4 (3.4%)	4 (3.4%)	3 (2.6%)	11 (3.2%)
Caucasian, not of Hispanic origin	109 (94.0%)	107 (92.2%)	103 (90.4%)	319 (92.2%)
Hispanic	2 (1.7%)	3 (2.6%)	7 (6.1%)	12 (3.5%)
Other, specify	1 (0.9%)	1 (0.9%)	0	2 (0.6%)

Table 3. Summary of baseline characteristics.

	Quest plus NRT (*n* 116)	Quest plus placebo patch (*n* 116)	Active control plus NRT (*n* 114)	Total (*N* 346)
Current number of cigarettes per day				
Number of available subjects	116	116	114	346
Mean (*SD*)	27.0 (9.5)	25.4 (8.6)	25.9 (8.5)	26.1 (8.9)
Range	15 60	15 60	15 50	15 60
First attempt to quit, *n* (%)				
No	104 (89.7%)	109 (94.0%)	102 (89.5%)	315 (91.0%)
Yes	12 (10.3%)	7 (6.0%)	12 (10.5%)	31 (9.0%)
Number of previous quit attempts				
Number of available subjects	116	115	114	345
Mean (*SD*)	4.4 (5.0)	3.5 (3.8)	4.2 (7.1)	4.0 (5.5)
Years of smoking				
Number of available subjects	116	116	114	346
Mean (*SD*)	26.3 (11.4)	26.4 (11.2)	27.8 (11.4)	26.8 (11.3)
Range	4 51	1 53	5 50	1 53
Corrected CO measurement (ppm)				
Number of available subjects	116	116	114	346
Mean (*SD*)	28.7 (13.3)	26.6 (8.6)	27.3 (9.7)	27.5 (10.7)
Range	15 82	15 56	14 60	14 82

flavor cigarettes (23.4%), and ultra-light cigarettes (21.1%) (data not presented). More than 90% of subjects indicated that this was not their first attempt to quit smoking. The number of previous attempts ranged from 0 to 50; however, most subjects reported between 1 and 4 previous quit attempts (M=4.0). The mean duration of smoking reported was 26.8 years. At baseline, the mean CO measurement was 27.5 ppm.

Results for the primary endpoint, 4 weeks of continuous abstinence following the quit date, are provided in Table 4. A global chi-square test was performed to test overall level of significance in a 2×3 table. This test showed statistical significance (p=.01). Pair-wise comparisons were then performed between the Quest plus NRT and the active control plus NRT, and the Quest plus placebo patch and the active control plus NRT as specified by the study design.

The proportion of subjects abstinent at 4 weeks was 32.8% for Quest plus NRT, 16.4% for Quest plus placebo patch, and 21.9% for active control plus

Table 4. Proportion of subjects achieving 4-week continuous abstinence.

	Quest plus NRT (*n* 116)	Quest plus placebo patch (*n* 116)	Active control plus NRT (*n* 114)
4-week abstinence, *n* (%)	38 (32.8%)	19 (16.4%)	25 (21.9%)
95% *CI* for 4-week abstinence rate	(24.3%, 42.1%)	(10.2%, 24.4%)	(14.7%, 30.6%)
p value compared to active control plus NRT*	0.04	0.89	

Note. Subjects whose corrected CO<10 ppm, number of cigarettes smoked since last visit, or 4-week abstinence could not be determined were treated as not meeting 4-week continuous abstinence. *n*, number of randomized subjects; % $n/N \times 100\%$. Exact 95% *CI* are given; *One-sided Fisher's exact test for 4-week abstinence proportions compared with active control plus NRT.

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NRT. The one-tailed p value for the comparison of 4-week abstinence proportions in the Quest plus NRT group compared with the active control plus NRT was .04 (two-tailed $p=.08$). The abstinence proportions between the Quest plus placebo patch and active control plus NRT was not statistically significant ($p=.89$).

As noted in Table 2, there was an imbalance in the proportion of men and women across groups. For this reason, a logistic regression analysis was conducted on the primary outcome measure, adjusting for sex. The adjusted odds ratio and p value for the comparison of Quest plus NRT versus active control plus NRT group were similar to the value without this adjustment ($p=.04$, one-tailed); the p value for the comparison of Quest plus placebo patch versus active control plus NRT group remained nonsignificant ($p=.32$, one-tailed).

An exploratory logistic regression analysis was conducted that included terms for sex, treatment group, and a sex × treatment interaction. Separate analyses were conducted for the Quest plus NRT and active control plus NRT groups, and the QUEST alone and placebo plus NRT groups. The results suggested an interaction between sex and treatment group: for the Quest plus NRT and active control plus NRT groups ($p=.08$), and for the Quest plus placebo patch and active control plus NRT groups ($p=.11$). Logistic regression analyses were then conducted separately for men and women. The results of these analyses indicated that women in the Quest plus NRT group had a significantly higher 4-week continuous abstinence rate than women in the active control plus NRT group: 27% versus 15%, $p=.02$. For men, however, there was no difference: 31% versus 27%, $p=.68$. The abstinence rate in the Quest plus placebo patch group did not differ from that of the active control plus NRT for women (19% vs. 15%, $p=.28$); there was, however, a trend for men to have a lower probability of abstinence in the Quest plus placebo patch group than in the active control plus NRT group (12% vs. 27%, $p=.06$).

Logistic regression, using treatment failure imputation and univariate predictor variables, was used to identify factors predicting treatment outcome. The model identified number of cigarettes smoked per day at baseline as significantly correlated with 4-week abstinence rates ($p=.02$); lighter smokers had a higher abstinence rate. Additionally, just prior to the quit date (Week 6), cigarettes smoked per day strongly predicted outcome ($p=.003$); again, smoking fewer cigarettes was associated with subsequent abstinence. Abstinence was not significantly correlated with baseline FTND score; however, abstinence was strongly predicted by FTND score at Week 6 ($p=.0001$). Subjects showing lower FTND scores at that time point had a higher probability of achieving abstinence. An additional predictor of abstinence at Week 6 was compliance with smoking only the Quest 3 cigarettes during the previous 2 weeks ($p=.005$). In the Quest plus NRT and Quest plus placebo patch groups, subjects reporting no smoking of regular cigarettes had a significantly higher abstinence rate (44%) than those reporting any smoking of regular cigarettes (28%).

Some 103 subjects (29.8%) had missing 6-month abstinence status, which included 89 subjects (25.7%) whose abstinence status was also missing at 3 months. These subjects were assumed failures at the respective follow-up times. No statistically significant differences in abstinence were observed at 3 months between Quest plus NRT and active control plus NRT groups ($p=.38$) or between Quest plus placebo patch and active control plus NRT groups ($p=.89$) or at 6 months ($p=.43$, $p=.77$, respectively).

Subjects found the experience of using Quest cigarettes less satisfying than regular cigarettes. This was demonstrated by responses to the Cigarette Evaluation Scale Questionnaire on which mean satisfaction ratings were higher for cigarettes in the Active control plus NRT group ($M=3.1$, $SD=1.30$) than for Quest 3 cigarettes in the Quest plus NRT group ($M=2.2$, $SD=1.05$; $F[1,200]=27.20$, $p=.0001$) or Quest plus placebo patch group ($M=2.5$, $SD=1.21$, $F[1,194]=10.26$, $p=.002$).

The number of Quest 1 cigarettes consumed increased relative to the control cigarettes. From Week 1 to Week 3, the number of control cigarettes smoked increased slightly (M change=2.0 cigarettes/day, $SD=6.51$), whereas there was a significantly greater increase of 3.8 Quest 1 cigarettes/day ($SD=6.30$) in the Quest plus NRT group ($F[1,219]=4.29$, $p=.04$) and 4.6 Quest 1 cigarettes/day ($SD=6.66$) in the Quest plus placebo patch group ($F[1,220]=9.01$, p=.003). Similarly, in Weeks 4 5 the number of Quest 2 cigarettes smoked was greater than the corresponding number in the active control plus NRT group. The increase relative to Week 1 in the active control plus NRT group was 3.9 cigarettes/day ($SD=7.62$), whereas the number of Quest 2 cigarettes smoked increased by 7.1 cigarettes/day ($SD=8.33$) in the Quest plus NRT group ($F[1,12]=9.06$, $p=.003$) and by 6.5 cigarettes/day ($SD=8.42$) in the Quest plus placebo patch group (F[1,212]=5.88, $p=.02$). In contrast, the number of Quest 3 cigarettes smoked showed no greater change relative to Week 1 than did the control cigarettes: an increase of 2.3 control cigarettes/day ($SD=8.69$) in the active control plus NRT group versus 0.3 Quest 3 cigarettes/day ($SD=11.36$) in the Quest plus NRT group ($F[1,200]=1.82$, $p=.18$) and 3.2 Quest 3 cigarettes/day ($SD=11.50$) in the Quest plus placebo patch group ($F[1,198]=0.44$, $p=.51$).

Exhaled CO levels also showed a slightly greater increase after switching to Quest 1 or Quest 2 cigarettes

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than after switching to the control cigarettes. While CO levels remained constant from Week 1 to Week 3 in the active control plus NRT group (*M* change= 0.2 ppm, *SD*=10.25), CO levels increased nonsignificantly by 1.5 ppm (*SD*=11.27) with Quest 1 cigarettes in the Quest plus NRT group ($F[1,220]=1.46$, $p=.22$) and by 2.5 ppm (*SD*=10.69) in the Quest plus placebo patch group ($F[1,221]=3.64$, $p=.06$). By Week 5, CO levels remained constant in the active control plus NRT group (*M* change vs. Week 1 of 0.2 ppm, *SD*=10.59). There was, however, a trend for CO levels to increase upon smoking Quest 2 cigarettes, by 2.9 ppm (*SD*=11.25) in the Quest plus NRT group ($F[1,213]=3.26$, $p=.07$) and by 2.9 ppm (*SD*=10.82) in the Quest plus placebo patch group ($F[1,215]=3.39$, $p=.07$). No differential increase in CO levels was seen with Quest 3 cigarettes; by Week 7 the change in CO level was 2.5 ppm (*SD*=11.26) in the active control plus NRT group versus 5.4 ppm (*SD*=14.43) in the Quest plus NRT group ($F[1,203]=2.46$, $p=.12$) and 3.0 ppm (*SD*=12.54) in the Quest plus placebo patch group ($F[1,200]=0.09$, $p=.76$).

There were three serious adverse events, none of which was deemed to be treatment related. Two events were a case of bladder carcinoma, and a case of fibula and tibia fractures. The third event was a subject in the Quest plus NRT with reported chest discomfort and increased blood pressure. Because of a history of cardiovascular disease (myocardial infarction, angioplasty, hypertension), the subject was discontinued. Three subjects developed dermatological reactions while using the NRT patch.

Discussion

Results of this study support the potential usefulness of a progressively denicotinized cigarette plus NRT for promoting smoking cessation. In this study, Quest plus NRT showed a higher abstinence rate than standard-of-care NRT. Although not meeting the strict Bonferroni criterion for statistical significance, the results are nonetheless suggestive that Quest plus NRT treatment increased the abstinence rate beyond standard-of-care NRT. The magnitude of the cessation effect in the Quest plus NRT group (32.8%) versus active control plus NRT group (21.9%) is a clinically relevant improvement in 4-week quit rates. Treatment success was also predicted by cigarettes smoked per day. Heavier smokers had less success quitting. It is reasonable to theorize that the benefit of Quest plus NRT may be understated given that use of an active control plus NRT cigarette in a sham-fading procedure in and of itself may have an effect on abstinence.

As hypothesized based on the literature, the benefit of precessation NRT was consistent with the higher success rate in the group of subjects pretreated with NRT 2 weeks before quitting, relative to the Quest without NRT and standard NRT groups. In a previous study, precessation NRT was found to increase quit rates, and, when used in conjunction with denicotinized cigarettes, also resulted in higher compliance with use of denicotinized cigarettes (Rose et al., 2006). Compliance, in turn, was shown to predict abstinence in this study and in previous unpublished studies with Quest cigarettes. Concurrent NRT may thus serve two important functions in a cigarette-weaning program. First, as just noted, concurrent NRT may facilitate compliance with the denicotinized cigarette-weaning regimen. In fact, discontinuation rates were higher with Quest plus placebo patch than with Quest plus NRT. Second, concurrent NRT separates the source of nicotine from the act of smoking; this dissociation of smoking behavior from nicotine reinforcement should facilitate behavioral extinction.

In the context of precessation NRT, the use of denicotinized cigarettes in combination with NRT may also help decrease concerns about the toxicity potential of nicotine. Smokers frequently express concerns about the safety of smoking conventional cigarettes concurrently with NRT. Although no clear basis for such concern has been established, it is conceivable that sensitive subpopulations might receive a dose of nicotine when smoking conventional cigarettes concurrently with NRT that may not be well tolerated. This suggests that the use of denicotinized cigarettes may offer assurance of safety or absence of nicotine toxicity during the weaning period. Importantly, there were no significant safety issues identified in smoke chemistry and toxicology studies of Quest cigarettes, which found Quest cigarettes to be consistent with conventional cigarettes with the exception of reduced nicotine levels.

This study demonstrated a clinically important increase in abstinence for the Quest 1, 2, 3 plus NRT group. However, the abstinence rate in the Quest plus placebo patch group (16.4%) was similar to that of conventional treatment with NRT (21.9%), which does not rule out the possibility that Quest plus placebo patch may be similarly as effective.

The results also suggest that the Quest plus NRT treatment may have had differential efficacy in females versus males. A substantial increase in 4-week abstinence was seen in the Quest plus NRT group versus active control plus NRT group for women. This observation of greater efficacy of the Quest plus NRT treatment relative to NRT alone in women is consistent with previous literature showing that NRT alone appears to be less efficacious in women than in men (Perkins, 2001). Moreover, the sensory aspects of smoking may be more important for female smokers (Perkins et al., 2001). To the extent that treatments using Quest 3 cigarettes

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diminish the rewarding value of conditioned sensory cues *via* extinction, it is reasonable to speculate that this effect may be greater in women. Conversely, men may benefit more from the NRT component of treatment (with or without Quest), as suggested by the trend for a lower success rate using Quest plus placebo patch. It will be important to determine whether the differential efficacy of the Quest plus NRT treatment in women is borne out in future studies. If so, it may be a particularly useful therapeutic approach for women who are less likely to respond to conventional NRT.

A deficiency of this study was that long-term abstinence (at 3 and 6 months) could not be adequately examined. Attrition over the follow-up period was significant and showed that better methods should be instituted in long-term studies of smoking cessation to sustain adherence over the observation period. Thus, the durability of the response seen in this study at 4 weeks needs to be further explored. Nevertheless, based on the results at 4 weeks, a confirmatory phase III study is warranted.

Some degree of compensatory smoking was noted in the early stages of weaning using the Quest 1 and Quest 2 cigarettes, based on the number of cigarettes smoked per day and exhaled CO measurements. The extent of compensation was modest and resulted in a relative increase in these indices of smoking of about 10 15%. Short-term exposure to these slightly higher levels of smoking during the nicotine-weaning regimen was not associated with adverse effects. In contrast to Quest 1 and Quest 2, use of Quest 3 was not associated with an increase in cigarettes per day or CO levels over conventional cigarettes. We interpret these results to mean that smokers may attempt to compensate for reduced cigarette nicotine content when it is above a certain threshold, but when the nicotine content is sufficiently reduced, compensation does not occur. Any initial compensatory increase in smoking would be expected to extinguish because increased smoking would fail to achieve a perceptible increase in nicotine reward. Previous studies have also suggested little or no compensation with denicotinized cigarettes (Rose & Behm, 2004b). In a recently published paper, Strasser, Lerman, Sanborn, Pickworth, and Feldman (2007) reported a modest degree of compensation when smokers smoked a single cigarette of Quest 1, 2 or 3 in a laboratory setting. However, none of the CO boosts exceeded that of subjects' usual brands (Strasser et al., 2007). The results with Quest 3 differed from those of the present study, which found no increase in CO relative to Quest 1 and 2. This could be related to the more prolonged exposure in a real-world, as opposed to a laboratory, setting during which subjects adjusted to the reduced nicotine delivery.

In conclusion, this study demonstrated that the use of cigarettes with progressive reductions in nicotine content is a promising adjunct to current smoking cessation strategies that warrants further study in additional controlled trials. Since current strategies often focus on pharmacological treatments while neglecting the strong conditioned reinforcing aspects of the sensory and behavioral components, cigarettes with reduced nicotine content offer a tool to promote extinction of these habit components. By developing this and related approaches, the armamentarium of efficacious smoking cessation treatments may be significantly expanded.

Acknowledgments

The following people are acknowledged for their contribution to this research: Ryung Suh, MD, MPP, MBA, MPH; Rosanne B. McTyre, PhD; T. Jeffrey Clark, PhD, MBA; and Jeffrey Baetz.

Declaration of interest: This study was funded by Vector Tobacco Inc. Dr. Anthony P. Albino is an employee of Vector Tobacco. Dr. Karen M. Becker is a paid consultant to Vector Tobacco. Dr. Jed E. Rose received no financial or other compensation for his contribution to this research. The authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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October 13, 2006

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RE: IND 69,185; Serial Number: 0008
General Correspondence
Quest Smoking Cessation Product
Vector Tobacco, Inc.

Dear [redacted]

Submitted in triplicate, please find an amendment to the above-referenced IND which includes non-material and editorial revisions to the Phase II Clinical Study Report, originally submitted June 12, 2006 (serial number: 0006).

Should you have any questions, I can be reached directly at:

[redacted]

This submission contains confidential, commercial and trade secret information, and Vector Tobacco Inc. hereby claims the legal protection of such confidential material under the applicable provisions of 18 USC 1905, 21 USC 331(j) and 5 USC 522(b).

Respectfully submitted,

[redacted]

For: Vector Tobacco Inc.

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12. **CONTENTS OF APPLICATION**

This application contains the following items: *(Check all that apply)*

- [x] 1. Form FDA 1571 *[21 CFR 312.23(a)(1)]*
- [x] 2. Table of Contents *[21 CFR 312.23(a)(2)]*
- [x] 3. Introductory statement *[21 CFR 312.23(a)(3)]*
- [x] 4. General investigational plan *[21 CFR 312.23(a)(3)]*
- [x] 5. Investigator's brochure *[21 CFR 312.23(a)(5)]*
- [ ] 6. Protocol(s) *[21 CFR 312.23(a)(6)]*
  - [x] a. Study protocol(s) *[21 CFR 312.23(a)(6)]*
  - [x] b. Investigator data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
  - [x] c. Facilities data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
  - [x] d. Institutional Review Board data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
- [ ] 7. Chemistry, manufacturing, and control data *[21 CFR 312.23(a)(7)]*
  - [x] Environmental assessment or claim for exclusion *[21 CFR 312.23(a)(7)(iv)(e)]*
- [x] 8. Pharmacology and toxicology data *[21 CFR 312.23(a)(8)]*
- [ ] 9. Previous human experience *[21 CFR 312.23(a)(9)]*
- [ ] 10. Additional information *[21 CFR 312.23(a)(10)]*

13. IS ANY PART OF THE CLINICAL STUDY TO BE CONDUCTED BY A CONTRACT RESEARCH ORGANIZATION? [x] YES [x] NO

IF YES, WILL ANY SPONSOR OBLIGATIONS BE TRANSFERRED TO THE CONTRACT RESEARCH ORGANIZATION? [x] YES [x] NO

IF YES, ATTACH A STATEMENT CONTAINING THE NAME AND ADDRESS OF THE CONTRACT RESEARCH ORGANIZATION, IDENTIFICATION OF THE CLINICAL STUDY, AND A LISTING OF THE OBLIGATIONS TRANSFERRED.

14. NAME AND TITLE OF THE PERSON RESPONSIBLE FOR MONITORING THE CONDUCT AND PROGRESS OF THE CLINICAL INVESTIGATIONS

[redacted]

15. NAME(S) AND TITLE(S) OF THE PERSON(S) RESPONSIBLE FOR REVIEW AND EVALUATION OF INFORMATION RELEVANT TO THE SAFETY OF THE DRUG

[redacted]

I agree not to begin clinical investigations until 30 days after FDA's receipt of the IND unless I receive earlier notification by FDA that the studies may begin. I also agree not to begin or continue clinical investigations covered by the IND if those studies are placed on clinical hold. I agree that an Institutional Review Board (IRB) that complies with the requirements set fourth in 21 CFR Part 56 will be responsible for initial and continuing review and approval of each of the studies in the proposed clinical investigation. I agree to conduct the investigation in accordance with all other applicable regulatory requirements.

16. NAME OF SPONSOR OR SPONSOR'S AUTHORIZED	17. SIGNATURE OF SPONSOR OR SPONSOR'S AUTHORIZED
[redacted]	[redacted]

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A PROSPECTIVE, DOUBLE-BLIND, RANDOMIZED, ACTIVE CONTROLLED, PARALLEL GROUP, MULTICENTER PHASE II CLINICAL TRIAL TO EVALUATE THE EFFECTIVENESS OF QUEST ALONE AND IN COMBINATION WITH NICOTINE REPLACEMENT THERAPY AS A SMOKING CESSATION AID

Protocol Number: Vector-201

Revision 1.0

October 13, 2006

Approved by:	Title	Date	Signature
[redacted]			

Revision History[1]

REV.	BY	DATE	CHANGE
1.0	[redacted]	10/13/06	Non-material editorial corrections.

[1] Detailed revision history located in **Appendix C.**

A PROSPECTIVE, DOUBLE-BLIND, RANDOMIZED, ACTIVE CONTROLLED, PARALLEL GROUP, MULTICENTER PHASE II CLINICAL TRIAL TO EVALUATE THE EFFECTIVENESS OF QUEST ALONE AND IN COMBINATION WITH NICOTINE REPLACEMENT THERAPY AS A SMOKING CESSATION AID

Protocol Number: Vector-201

CLINICAL REPORT v1.0

October 13, 2006

Vector Tobacco Inc.

One Park Drive, Suite 150

Research Triangle Park, NC 27709

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A PROSPECTIVE, DOUBLE-BLIND, RANDOMIZED, ACTIVE CONTROLLED, PARALLEL GROUP, MULTICENTER PHASE II CLINICAL TRIAL TO EVALUATE THE EFFECTIVENESS OF QUEST ALONE AND IN COMBINATION WITH NICOTINE REPLACEMENT THERAPY AS A SMOKING CESSATION AID

Protocol Number: Vector-201

CLINICAL REPORT v1.0

October 13, 2006

Vector Tobacco Inc.

One Park Drive, Suite 150

Research Triangle Park, NC 27709

Reviewed/Approved by:

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Vector Tobacco Inc.

A PROSPECTIVE, DOUBLE-BLIND, RANDOMIZED, ACTIVE CONTROLLED, PARALLEL GROUP, MULTICENTER PHASE II CLINICAL TRIAL TO EVALUATE THE EFFECTIVENESS OF QUEST ALONE AND IN COMBINATION WITH NICOTINE REPLACEMENT THERAPY AS A SMOKING CESSATION AID

Protocol Number: Vector-201

CLINICAL STUDY REPORT

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ABBREVIATIONS

AE	Adverse event
AHRQ	Agency for Healthcare Research and Quality
ALA	American Lung Association
ANOVA	Analysis of variance
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CO	Carbon monoxide
CRF	Case report form
CRO	Contract research organization
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
FTC	Federal Trade Commission
FTND	Fagerström Test for Nicotine Dependence
GEE	Generalized estimating equation
ID	Identification
IOM	Institute of Medicine
ITT	Intent-to-treat
IUD	Intrauterine device
NRT	Nicotine replacement therapy
OTC	Over-the-counter
PHS	Public Health Service
QD	Quit date
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard deviation
UB	Usual brand
US	United States

A PROSPECTIVE, DOUBLE-BLIND, RANDOMIZED, ACTIVE CONTROLLED, PARALLEL GROUP, MULTICENTER PHASE II CLINICAL TRIAL TO EVALUATE THE EFFECTIVENESS OF QUEST ALONE AND IN COMBINATION WITH NICOTINE REPLACEMENT THERAPY AS A SMOKING CESSATION AID

Protocol Number: Vector-201

EXECUTIVE SUMMARY

The primary objective of this prospective, double-blind, randomized, active controlled, parallel group, multicenter Phase II clinical trial was to assess the efficacy and tolerability of a reduced nicotine and nicotine-free cigarette product (Quest 1, Quest 2, and Quest 3) alone and in combination with nicotine replacement therapy (NRT) as a smoking cessation aid through a diminishing amount of nicotine in a step-down approach, i.e., Quest 1 to Quest 2 to Quest 3.

A total of 346 healthy smokers (≥ 15 cigarettes per day) who were motivated to quit smoking were enrolled in the trial and followed over 8 months at five clinical study sites. Following screening, eligible subjects were randomized in a 1:1:1 ratio to one of three treatment arms:

- Group 1: Quest 1, Quest 2 and Quest 3 plus NRT (patch)
- Group 2: Quest 1, Quest 2 and Quest 3 plus placebo patch
- Group 3: Active Control cigarettes (conventional) plus NRT (patch)

Subjects were scheduled to attend twelve (12) pre-determined visits over a period of 32 weeks, during which treatment was administered and/or subjects were evaluated regarding safety and/or efficacy. For one week prior to treatment distribution, all groups were allowed to smoke their usual brand (UB) of cigarettes. Over the following six weeks, Groups 1 and 2 were transitioned to Quest 1 for two weeks, Quest 2 for two

weeks, and Quest 3 for two weeks. During this period, Group 3 received a "sham-faded" Active Control (i.e., conventional American-blended cigarettes). At Week 5, NRT was dispensed in all groups and was provided over a duration of 12 weeks, in the following distribution schedule:

Schedule of NRT Distribution By Treatment Group

Group 1 Quest 1, 2, 3	21 mg patch	14 mg patch	7 mg patch	Placebo patch
Group 2 Quest 1, 2, 3	Placebo patch			
Group 3 Conventional cigarettes	Placebo patch	21 mg patch	14 mg patch	7 mg patch

The Quest 1, 2, 3 product concept includes use of NRT starting two weeks before the quit date because preliminary data suggest this enhances efficacy.

The beginning of Week 7 was defined as the "quit date" (i.e., quit all smoking). In addition to NRT, all subjects received behavioral support through a 10-minute individual counseling session by a certified smoking cessation counselor and printed materials.

The primary endpoint, 4 weeks of continuous abstinence, was assessed four weeks following the quit date. Abstinence was verified by self report and exhaled carbon monoxide (CO) < 10 ppm. Secondary endpoints included: 1) quit rates at 3 and 6 months following treatment termination; 2) corrected CO levels; 3) saliva cotinine concentration levels; and 4) various measures of preference for and satisfaction with Quest over UB.

Three study populations were defined: 1) an intent-to-treat (ITT) population, consisting of all randomized subjects; 2) a per-protocol population, consisting of all subjects for

whom 4-week abstinence status could be determined and who were not protocol violators (those who discontinued before week 7 due to failure to quit smoking and whose 4-week abstinence status was missing were considered failures); and 3) a safety population, consisting of all randomized subjects who received at least one study cigarette.

Analysis of the primary endpoint, 4 weeks of continuous abstinence, included a series of sensitivity tests, employing a range of imputation methods for missing data, to determine whether the 4-week abstinence rate was statistically different between Groups 1 and 3, and Groups 2 and 3. Analysis of the primary endpoint was an ITT analysis using treatment failure imputation. Subsequently, the sensitivity of this analysis was examined using statistical methodology to impute missing values. The additional sensitivity analyses on the ITT population were based on multiple imputation (Rubin 1987), all available data, and treatment failure for subjects who discontinued due to lack of efficacy; and, finally, a treatment failure analysis on the per-protocol analysis was conducted. The results of these analyses are summarized in the table below and in the figure on the following page.

Four-Week Abstinence Proportions by Method of Imputation

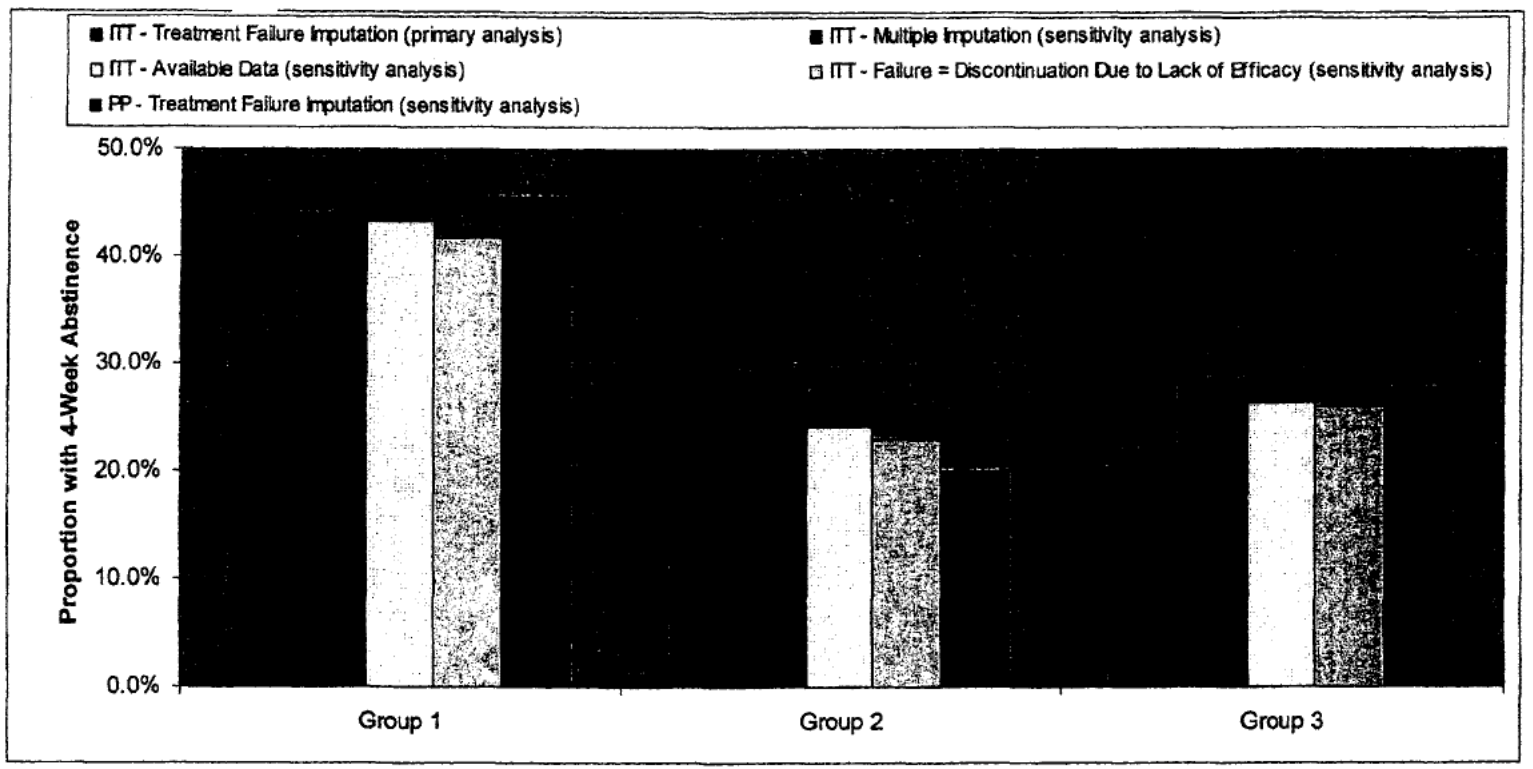
Primary Analysis			
Using Treatment Failure (ITT Population)	32.8% (0.0449)	16.4% (0.8922)	21.9%
Additional Sensitivity Analyses			
Using Multiple Imputation (ITT Population)	44.0% (0.0297)	29.3% (0.4567)	28.6%
Based on Available Data (ITT Population)**	43.2%	24.1%	26.3%

Four-Week Abstinence Proportions by Method of Imputation

Using Treatment Failure for Subjects Who Discontinued Due to Lack of Efficacy (Excluding Subjects Whose Abstinence Status Could Not be Determined)	41.8% (0.0169)	22.9% (0.7455)	26.0%
Using Treatment Failure (Per-Protocol Population)	45.5% (0.0213)	20.0% (0.9017)	27.8%

* One-sided Fisher's exact test for four-week abstinence proportions compared with Group 3, at the 0.05 and 0.025 levels using Bonferroni adjustment.
** For the available population (ITT), statistical significance was not computed due to the diminished sample size.

FOUR-WEEK ABSTINENCE PROPORTIONS[2]



* = statistically significant at p = 0.05 level (Group 1 compared with Group 3)
** = statistically significant at p = 0.025 level (Group 1 compared with Group 3)

[2] For the available population (ITT), statistical significance was not computed due to the diminished sample size.

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This study demonstrated that Quest with NRT was more effective than standard of care NRT treatment in achieving abstinence at four weeks and suggests that Quest alone may be as effective as standard of care NRT. The treatment effects that could be statistically tested (ITT with treatment failure imputation, ITT with multiple imputation, ITT with discontinuation due to lack of efficacy, and per-protocol analysis with treatment failure imputation) were significant at the 0.05 significance level. Statistical significance was maintained upon adjustment in the ITT treatment failure analysis for subjects who discontinued due to lack of efficacy and in the per-protocol analysis with treatment failure imputation. Irrespective of the analysis, no significant differences were noted when comparing Group 2 with Group 3.

No statistically significant differences were observed in three- or six-month quit rates, although at the time of determination of abstinence, the proportion of available subjects was approximately 40% in Groups 1 and 3 and 30% in Group 2 at 3 months, and decreased to approximately 30% and 20% respectively at 6 months. There were no serious adverse events attributable to the study product. Three instances of patients developing dermatologic reactions while using the NRT patch were noted to be likely due to irritation. In general, as expected, subjects found the experience of using Quest cigarettes less satisfying than traditional cigarettes.

The results of this study indicate that Quest used in combination with NRT is more effective than the standard of care NRT regimen in achieving abstinence at four weeks, and that Quest alone may be as effective as standard of care NRT.

Vector Tobacco Inc.

A PROSPECTIVE, DOUBLE-BLIND, RANDOMIZED, ACTIVE CONTROLLED, PARALLEL GROUP, MULTICENTER PHASE II CLINICAL TRIAL TO EVALUATE THE EFFECTIVENESS OF QUEST ALONE AND IN COMBINATION WITH NICOTINE REPLACEMENT THERAPY AS A SMOKING CESSATION AID

Protocol Number: Vector-201

CLINICAL REPORT

1.0 INTRODUCTION

Tobacco use is a risk factor for a variety of diseases including lung cancer, heart disease, chronic obstructive pulmonary disease, stroke and peripheral vascular disease and is the leading cause of preventable death in the United States (PHS 2000). An estimated 23% of American adults are current smokers, and, although a considerable number of smokers quit each year, the prevalence of smoking is largely unchanged due to new smokers and relapse among those who quit (Benowitz 1999; CDC 2002).

Tobacco dependence is currently viewed as a chronic disease condition often requiring multiple quit attempts before successful abstinence can be achieved. The Centers for Disease Control and Prevention (CDC) reports that, of an estimated 46.5 million adult smokers in the United States (US) in 2000, 70% wanted to quit and had made at least one prior attempt to quit during the preceding 12 months. Most initial attempts at abstinence fail, and relapse continues to be common among former smokers. For example, among the 15.7 million adults who attempted cessation in the year 2000 for a day or more, only approximately 4.7% remained abstinent 3 to 12 months later (CDC 2002).

The current standard of care for smoking cessation calls for education, counseling, the support of health care providers, and pharmacologic interventions (e.g., nicotine replacement therapies; buproprion; varenicline) (FDA 2006; PHS 2000). Success rates (4 week continuous abstinence or "quit rates") in controlled clinical trials with optimal treatment have demonstrated 1.5 to 2.0 times improvement compared to placebo (Silagy et al. 2004). This is only an effective quit rate of approximately 15%, defined as 4 weeks continuous abstinence. Additionally, of those smokers who receive treatment and initially quit, even fewer achieve long-term abstinence at 6 and 12 months (Fiore et al. 1994; Hajek et al. 1999).

Based on these data, there is presently no single optimal way to convert smokers to permanent non-smokers. A broader range of treatment options are needed to improve upon the current limited success rates for abstinence, and to avoid relapse (Fiore and Baker 1995; IOM 2001; PHS 2000).

1.1 Rationale for Product Design

Cigarette smoking is a complex behavior that is sustained by the pharmacological properties of nicotine and reinforcing behavioral and sensory cues (Benowitz 1999; Rose and Behm 2004). Smokers report reduced satisfaction when sensory signals are blocked or altered using methods such as local airway anesthesia (Rose 2003) and addition or elimination of menthol (Rose and Behm 2004). Nicotine delivered intravenously in the absence of the sensory and behavioral aspects of cigarette smoking is rated a less satisfying experience than delivery via inhalation from a cigarette. Nicotine-free cigarettes, in the absence of concomitant nicotine delivery, consistently produce satisfaction, reward, and craving reduction in smokers (Brauer et al. 2001; Breland et al. 2002; Buchhalter et al. 2001; Butschky et al. 1995; Dallery et al. 2003; Gross et al. 1997; Pickworth et al. 1999; Rose et al. 2000; Westman et al. 1996).

Currently available pharmacological approaches for smoking cessation in combination with counseling and education (i.e., maximal intervention) are effective in a minority of patients, with the probability of success with all types of nicotine replacement therapy (NRT) increasing by 1.5 to 2.0 fold compared to placebo (Silagy et al. 2004). Relapse is common following NRT use, and it has been estimated that 75 – 80% of smokers who receive treatment are unable to achieve long-term abstinence at 6 or 12 months (Fiore et al. 1994; Hajek et al. 1999). There is an important public health need to improve the standard of care by providing more effective smoking cessation treatments, and alternative treatments for smokers who may not succeed with, or do not have access to, treatment that includes NRT.

The data from studies of the behavioral and pharmacodynamic properties of nicotine-free and denicotinized cigarettes indicate that the efficacy of current pharmacologic interventions for smoking cessation that rely on nicotine delivery could be improved by a device or product that: a) delivers the sensory and behavioral cues associated with cigarette use, in addition to NRT; and/or b) provides the sensory and behavioral cues alone. In either case, the treatment breaks the association of those cues with the contingent delivery of nicotine reinforcement and promotes extinction of smoke cues.

Vector Tobacco Inc. (Vector Tobacco) has developed a reduced nicotine and nicotine-free cigarette product as a potential weaning tool to help smokers quit smoking. Based on findings from studies of nicotine-free cigarettes, including Quest, the product is designed to reduce dependence on nicotine by separating the behavioral and sensory reinforcement of smoking from the delivery of nicotine.

1.2 Quest Smoking Cessation Product

Quest Smoking Cessation Product (or "Quest") is a tobacco-based (botanical) medical product in the form of cigarettes, manufactured to provide reduced and nicotine-free cigarettes to the following specifications.[3]

QUEST CHEMICAL AND PHYSICAL SPECIFICATIONS

	Quest 1	Quest 2	Quest 3
Chemical:			
Tar (mg/cigt)	9.0 ± 1.0	8.0 ± 1.25	8.5 ± 1.0
Nicotine (mg/cigt)	0.59 ± 0.06	0.30 ± 0.05	< 0.05
Moisture	12.4 % ± 1.0 %	12.4 % ± 1.0 %	12.4 % ± 1.0 %
Carbon Monoxide (mg/cigt)	12.5 max	12.5 max	12.5 max
Physical:			
Total Weight (mg/cigt)	984 ± 70	984 ± 70	943 ± 70
Circumference (mm)	24.6 ± 0.1	24.6 ± 0.1	24.6 ± 0.1
Pressure Drop (mm H_2O)	115 ± 10	115 ± 10	123 ± 10
Hardness (%), unequlilibrated	74 ± 3	74 ± 3	74 ± 3

Quest 1 cigarettes have a low nicotine level of 0.59 ± 0.06 mg, Quest 2 cigarettes have an extra low nicotine level of 0.30 ± 0.05 mg, and Quest 3 cigarettes are considered nicotine-free (nicotine level < 0.05 mg). The only difference from conventional cigarette manufacturing is the inclusion of genetically modified nicotine-free tobacco.

1.3 Previous Clinical Experience with Investigational Product

Besides an economic study that examined smokers' potential preferences for Quest products (Monchuk et al. 2003), Quest cigarettes have been evaluated in two clinical studies to date.

[3] Vector Tobacco Inc. currently sells Quest brand cigarettes as low, extra low, and nicotine-free cigarettes. These commercial products are cigarettes as customarily marketed and do not bear smoking cessation or other medical claims.

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1.3.1 Smoking Cessation Study of Quest (Rose, unpublished data)

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[4] Nonetheless, previous studies indicate that success rates would be higher for the combination if the patch is continued beyond the quit smoking date (Brauer et al. 2001).

1.3.2 Effects of Low Nicotine Content Cigarettes on Smoke Intake (Rose and Behm 2004)

Rose and Behm (2004) conducted a blinded, randomized, within-subjects study designed to determine if compensatory smoking behaviors differ depending on whether highly ventilated filtered or reduced-nicotine filtered cigarettes are being consumed by the same subjects. Sixteen smokers participated in two separate 8-hour sessions where they randomly smoked either low-nicotine filtered (Quest brand, 0.02 mg/cigarette smoke yield) or highly ventilated filter cigarettes (0.2 mg/cigarette smoke yield) *ad libitum* during each session, with a counterbalanced order of smoking conditions presented across subjects. Endpoints evaluated included expired-air carbon monoxide (CO), puff volume and number of puffs.

The number of cigarettes smoked during each 8-hour session was significantly higher when subjects consumed the highly ventilated filter cigarettes versus the Quest cigarettes (mean = 11.9 and 10.4, respectively). Cumulative puff volume taken from the last cigarette of the session was also significantly higher for the highly ventilated filtered cigarettes (800 cc) than for the low-nicotine filtered cigarettes (500 cc). Expired-air CO, to assess compensation, was measured by dividing the CO values at the end of each session by the Federal Trade Commission (FTC) standardized values for CO delivery for each type of cigarette. Higher ratios would indicate a compensatory increase in smoking behavior. The compensation index was significantly higher for the highly ventilated filter cigarettes (index = 11.1) than for the low-nicotine filtered cigarettes (index = 2.4).[5] Thus, the authors concluded that compensatory smoking behaviors

[5] The compensation index was graphed incorrectly in the paper and an erratum reflecting the corrected values was submitted.

increased significantly during the highly ventilated filter cigarette sessions, relative to the Quest low-nicotine filtered cigarettes.

2.0 STUDY OBJECTIVE

The objective of the Phase II study was to assess the effectiveness and tolerability of Quest 1, Quest 2, and Quest 3 alone and in combination with NRT compared to conventional pharmacologic therapy (i.e., the NRT patch alone) as a smoking cessation aid. The results of this study will serve to optimize the design of the pivotal trial (Phase III) and provide objective data to support a decision regarding the development of one or both product concepts.

3.0 STUDY DESIGN

This study was a prospective, randomized, multicenter, double-blind, parallel group, active controlled Phase II trial in which a total of 346 healthy smokers who were motivated to quit smoking were enrolled and followed over 8 months at five clinical sites.

Healthy smokers with a desire to quit smoking were screened for eligibility. This screening included evaluation of relevant medical / smoking history, including documentation of current usual brand, physical examination, urine pregnancy test (in women of child-bearing potential), drug screen, exhaled carbon monoxide (CO), saliva cotinine level, and completion of four questionnaires. Following screening, eligible subjects were randomized in a 1:1:1 ratio to one of the three treatment arms:

- Group 1: Quest 1, Quest 2 and Quest 3 plus NRT (patch)
- Group 2: Quest 1, Quest 2 and Quest 3 plus placebo patch
- Group 3: Active Control cigarettes (conventional) plus NRT (patch)

Figure A (below) outlines the study schematic for the Phase II study.

Figure A
DOUBLE-BLIND, RANDOMIZED, ACTIVE CONTROLLED, PARALLEL GROUP PHASE II TRIAL OF QUEST ALONE, AND WITH NRT

[redacted]

		V1	V2		V3		V4		V5		V6				V7		V8		V9		V10		V11				V12
GROUP	Condition		-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	31
1	QUEST 1,2,3 NIC PATCH		UB 100%	QUEST 1		QUEST 2		QUEST 3 21 mg Patch		21 mg patch				14 mg patch		7 mg patch		Plac Patch		Abstinence							
2	QUEST 1,2,3 ALONE		UB 100%	QUEST 1		QUEST 2		QUEST 3 Plac Patch		Placebo Patch				Plac Patch		Plac Patch		Plac Patch		Abstinence							
3	ACTIVE CONTROL 1,2,3[1] CONTROL		UB 100%	CONTROL 1		CONTROL 2		CONTROL 3 Plac Patch		21 mg patch						14 mg patch		7 mg patch		Abstinence							
	Weeks		-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	31

[redacted]

QD - Quit All Smoking
[1] Active Control Cigarettes: Conventional, American blended and Sham-faded
V1 = visit 1; V2 = visit 2; V3 = visit 3;... V12 = visit 12

Subjects in Groups 1 and 2 transitioned from their usual brand (UB) cigarettes to Quest cigarettes. During the first 6 weeks (Week 1 – Week 6), subjects in Groups 1 and 2 smoked *ad libitum* for two weeks at each Quest nicotine level beginning with Quest 1, then proceeding to Quest 2, and finally to Quest 3. At Week 5, Quest use was supplemented in the first treatment group with the addition of a transdermal nicotine patch (21 mg), while subjects in Group 2 added a placebo patch. Visit 5 was considered the "quit date" (QD; i.e., quit all smoking).

Subjects in Group 1 received a 21 mg transdermal nicotine patch for an additional 4 weeks (total period Week 5 – Week 10), followed by a 14 mg transdermal nicotine patch for 2 weeks (Week 11 and 12), a 7 mg transdermal nicotine patch for 2 weeks (Week 13 and 14), and a placebo patch for 2 weeks (Week 15 and 16). Subjects in Group 2 received only a placebo patch starting at Week 5 through Week 16.

Group 3 was a blinded control for the use of Quest 1, Quest 2, and Quest 3 in Group 1, whereby subjects were provided with an Active Control cigarette (nicotine dose comparable to a conventional, American-blended cigarette). These Active Control cigarettes were "sham-faded" every 2 weeks in parallel with the fading provided by Quest 1, Quest 2, and Quest 3 in Group 1. Sham fading consisted of providing a fresh supply of identical Active Control cigarettes at the same time during the study when Group 1 was changing from Quest 1 to Quest 2 or from Quest 2 to Quest 3. The NRT treatment for Group 3 was the current standard of care, indicating the addition of a placebo patch during Week 5 and 6 preceding the "quit date" at Week 7 (Visit 5). Subjects in Group 3 used a 21 mg transdermal nicotine patch for 6 weeks (Week 7 – Week 12), followed by a 14 mg transdermal nicotine patch for 2 weeks (Week 13 and 14) and a 7 mg transdermal nicotine patch for 2 weeks (Week 15 and 16).

According to instructions given to subjects, all patches, both placebo and NRT, were 24-hour applications to be re-applied daily. Additionally, all subjects received behavioral support through a 10 minute individual counseling session at Visit 1 by a certified

smoking cessation counselor and printed materials (*You Can Quit Smoking*) (AHRQ 2005).

3.1 Rationale for Study Design

As described above, Quest 3 was combined in the first treatment arm with a transdermal nicotine patch (21 mg) at the beginning of Week 5. The application of the patch 2 weeks prior to the quit date was intentional and was included because this regimen was associated with improved durability of long-term abstinence rates, as suggested by several recent studies described below. Following the quit date, subjects remained on a traditional patch schedule until the end of Week 14. The current design incorporated 2 weeks of placebo patch treatment following standard NRT treatment in order to promote consistency when assigning abstinence rates, maintain blinding, and avoid within-trial study bias.

Schuurmans and colleagues (2004) found that nicotine patch pre-treatment before cessation increased sustained abstinence rates at 6 months from 12% in the placebo group to 22% in the experimental group. Historically, one would begin NRT on his / her quit day, confronting the subject not only with a sudden behavioral change, but also with a new route of receiving nicotine. Pre-treatment with NRT may weaken the association between nicotine intake (rewarding effect) and the behavior of smoking, thereby aiding in a disassociation of the conditioned behavior pattern. Secondly, pre-patch treatment may reduce the need for inhaled nicotine. Finally, pre-patch treatment may ease the transition from inhaled nicotine to transdermal nicotine replacement by familiarization with the mode of delivery. Similar support for patch pre-treatment has been suggested by Rose et al. (2006), who found that pre-treatment with a nicotine patch two weeks before the quit date resulted in 50% abstinence rates at 4 weeks.

Patch pre-treatment was incorporated into the current study design as a means to further test the maximum potential benefit of the Quest product. Smoking Quest 3 cigarettes may hold a particular advantage when paired with patch pre-treatment since Quest 3 contains no nicotine. Thus, smoking nicotine-free cigarettes during pre-treatment may allow for an even easier transition to the patch, with subjects acclimating to their new nicotine source while still afforded the behavioral and sensory aspects provided by the cigarette.

The control group, Group 3, complied with current labeling requirements for over-the-counter (OTC) patches, indicating that treatment with active NRT start following the quit date. Furthermore, careful consideration was made to limit active patch exposure to 10 weeks, with no more than 6 weeks of exposure to the highest dosage form (21 mg) and no more than 2 weeks exposure at lower weaning dosages of 14 mg and 7 mg, respectively.

4.0 ENDPOINTS

4.1 Primary Endpoint

- **Four weeks of continuous abstinence**
  The primary endpoint, i.e., 4 weeks of continuous abstinence (Weeks 7 – 10), was assessed after treatment termination, thus at Week 11 (Visit 7). Abstinence was determined by self report and verified by exhaled CO < 10 ppm for each subject.

4.2 Secondary Endpoints

- **Quit rates at 3 and 6 months following treatment termination**
  Quit rates at 3 and 6 months (at Visits 11 and 12, respectively) following treatment termination were verified by self report and exhaled CO < 10 ppm.

- **Evaluation of preference and satisfaction of Quest over usual brand**
  Preference and satisfaction of Quest over usual brand was determined by subjective questionnaires.

- **Evaluation of the severity of withdrawal symptoms**
  The severity of withdrawal symptoms including negative mood, urges to smoke, and difficulty concentrating was determined by subjective questionnaires.

- **Evaluation of compensatory smoking behavior**
  Compensatory smoking was determined by the number of cigarettes smoked and was chemically verified by CO levels.

5.0 SUBJECT POPULATION

Three hundred forty-six (346) smokers who 1) provided informed consent for participation in this trial; 2) were capable of comprehending the nature of the study; and 3) were likely to comply with the visit schedule were entered into the study, provided they also conformed to the following criteria.

5.1 Inclusion Criteria

1. Subjects considered by the investigator to be in general good health and who were between 21 and 65 years of age.
2. Subjects were required to have a history of smoking an average of 15 cigarettes or more per day for at least one year.
3. Subjects were required to be motivated to quit smoking.
4. Subjects were required to have a corrected CO measurement of greater than 15 ppm at baseline.
5. Subjects were required to be willing and able to return for scheduled follow-up examinations for a total of 8 months.
6. Subjects were required to read, understand, complete, and sign the informed consent form. Subjects were provided with a copy of this form.
7. Subjects were required to be able to read, understand, and complete the questionnaires independently.

5.2 Exclusion Criteria

1. Subjects who currently used any form of nicotine replacement therapy (NRT) or other tobacco-based product (e.g., chew, snuff, etc.).

2. Subjects whose usual brand consisted of a menthol variety.
3. Subjects with known serious pathophysiology, or topical or systemic disorders of any kind that would confound the results of the study.
4. Subjects who were using illegal drugs at baseline.
5. Subjects who were pregnant or lactating, or who planned to become pregnant or lactating during the course of the study.
6. Subjects participating in any other clinical trial of an investigational drug or device during the time of this clinical investigation or within 30 days prior to the screening visit.
7. Subjects taking anti-depressants, anti-psychotics, Clonidine, Zyban, or any of the classes of drugs listed in the Accutest 10-Multidrug screen.
8. Subjects with a positive Accutest 10-Multidrug screen.
9. Subjects with systolic blood pressure over 140 mmHg and/or diastolic blood pressure over 90 mmHg.
10. Subjects consuming an average of three or more drinks of alcohol per day.
11. Subjects consuming greater than an average of three packs of cigarettes per day.

6.0 STUDY MATERIALS AND METHODS

6.1 Description of Investigational Product

Quest Smoking Cessation Product (or "Quest") is a tobacco-based (botanical) medical product in cigarette form. The product is prepared using traditional cigarette manufacturing protocols. The only difference from traditional cigarette manufacturing is the inclusion of genetically-modified tobacco to reduce nicotine levels. As a 'finished good,' the product has been developed to meet specifications similar to conventional cigarettes in both design and use, as illustrated by the following product specifications.

QUEST CHEMICAL AND PHYSICAL SPECIFICATIONS

	Quest 1	Quest 2	Quest 3
Chemical:			
Tar (mg/cigt)	9.0 ± 1.0	8.0 ± 1.25	8.5 ± 1.0
Nicotine (mg/cigt)	0.59 ± 0.06	0.30 ± 0.05	< 0.05
Moisture	12.4 % ± 1.0 %	12.4 % ± 1.0 %	12.4 % ± 1.0 %
Carbon Monoxide (mg/cigt)	12.5 max	12.5 max	12.5 max
Physical:			
Total Weight (mg/cigt)	984 ± 70	984 ± 70	943 ± 70
Circumference (mm)	24.6 ± 0.1	24.6 ± 0.1	24.6 ± 0.1
Pressure Drop (mm H_2O)	115 ± 10	115 ± 10	123 ± 10
Hardness (%), unequlilibrated	74 ± 3	74 ± 3	74 ± 3

The genetically modified plant used to produce the low nicotine yielding tobacco product has been designated Vector 21-41. This line is a transgenic tobacco that produces very low nicotine levels by disrupting the normal expression of a quinolinic acid phosphoribosyltransferase, a key enzyme in the biosynthetic pathway leading to the production of nicotine. Vector 21-41 tobacco is a comparable plant to traditional wild-type tobacco and more significantly, its non-transgenic parent, Burley 21LA. Vector 21-41 does not express any novel agronomic trait such as insect resistance or herbicide tolerance.

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[redacted]

The nicotine patch is an over-the-counter (OTC) product; brand names include Nicoderm CQ and Nicotrol. Generic products are available as well. The protocol specifies compliance with current labeling for OTC patches. Careful consideration was made to limit *active* patch exposure to 10 weeks, with no more than 6 weeks of exposure to the highest dosage form (21 mg) and no more than 2 weeks exposure at lower weaning dosages of 14 mg and 7 mg, respectively. The patch was to be replaced daily (every 24 hours) as directed. In order to ensure blinding, a placebo patch was utilized in this study as well.

The Active Control cigarette used by Group 3 was a conventional, American-blended cigarette with a tar content of 10.2 ± 0.5 mg/cigarette and a nicotine content of 0.80 ± 0.10 mg/cigarette; however, the cigarettes were "sham-faded" as described above every two weeks in parallel with the fading provided by Quest 1, Quest 2, and Quest 3 in Groups 1 and 2.

The objective of the Active Control cigarette was to deliver a constant nicotine concentration with the same appearance and taste as the investigational product. This matching was aimed to reduce confounding and/or bias in the trial outcome that: 1) may have been introduced by other features of the device that differ from the Active Control; or 2) may have led to breaking of the blinding.

All participating subjects smoked for 6 weeks (either Quest 1, Quest 2, Quest 3, or conventional "faded" cigarettes) and received 12 weeks of application of the

nicotine and/or placebo patch with no subjects receiving more than 10 weeks of active NRT. The last 2 weeks of smoking and first 2 weeks of patch use overlapped (Weeks 5 and 6).

6.2 Packaging of Investigational Product

All study products were labeled as follows: "Caution: New Drug – Limited by Federal Law to investigational use by qualified investigators only. Keep out of the reach of children. For clinical trial use only." Additionally, cigarette packs communicated the following Surgeon General's warning:

> "SURGEON GENERAL'S WARNING: Quitting Smoking Now Greatly Reduces Serious Risks to Your Health."

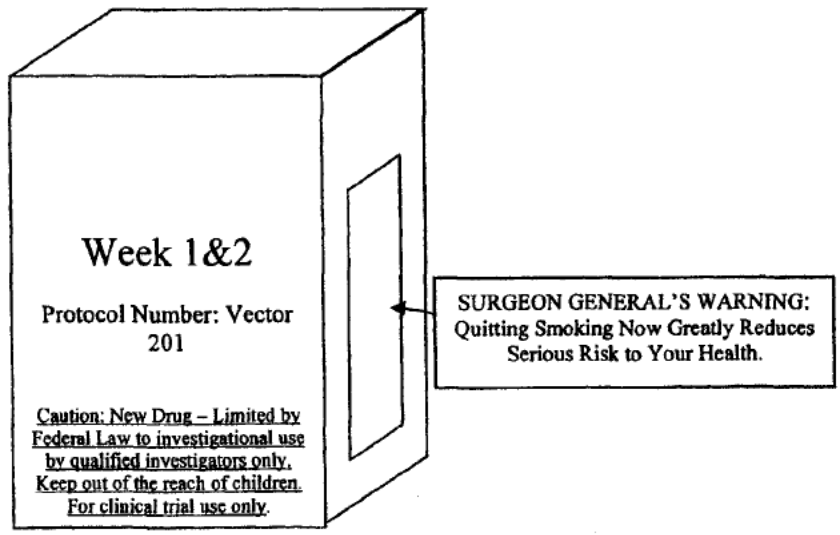
Quest 1, Quest 2, Quest 3, and the Active Control were packaged as 20 cigarettes per pack and 10 packs per carton, and were labeled in accordance with their study visit period:

- Week 1 & 2
- Week 3 & 4
- Week 5 & 6

Quest 1, Quest 2, Quest 3, and the Active Control were packaged in identical packages, such that there were no visible markings, and they were indistinguishable from conventional cigarettes. An example of a cigarette pack is provided in **Figure B**, below.

Figure B

EXAMPLE OF CIGARETTE PACK



Each carton of cigarettes had a label on the box (**Figure C**). The label contained the protocol number, study period, the three-digit subject identification number (preprinted), and the new drug caution.

Figure C

EXAMPLE OF CIGARETTE CARTON LABEL

Caution: New Drug – Limited by Federal Law to investigational use by qualified investigators only. Keep out of the reach of children. For clinical trial use only.

Protocol Number: Vector-201

Week 1 & 2

Subject ID: ___ ___ ___ (Preprinted)

A date-coding stamp was embossed into the bottom of each cigarette pack and carton, which distinguished each Quest product from one another and from the

Active Control. The date-coding system consisted of an arbitrary number to avoid bias and was traceable to the lot number for each production run of investigational drug product.

Labeling of the NRT patch and placebo patch were similar to the cigarettes. NRT patches in 21 mg, 14 mg, and 7 mg were repackaged. Placebo patches were manufactured to match the size, appearance, and texture of an active NRT patch.

NRT patches in 21 mg, 14 mg, and 7 mg, and placebo patches were repackaged in an identical manner and in accordance with the randomization scheme, as described above for the cigarette packaging (**Figure D**). A total of 20 patches were packaged in one box.

- The placebo patch box was labeled with a subject identification number and one of the following time periods: Week 5 & 6, Week 7 & 8, Week 9 & 10, Week 11 & 12, Week 13 & 14, or Week 15 & 16.

- The 21 mg NRT patch box was labeled with a subject identification number and one of the following time periods: Week 5 & 6, Week 7 & 8, Week 9 & 10, or Week 11 & 12.

- The 14 mg NRT patch box was labeled with a subject identification number and one of the following time periods: Week 11 & 12, or Week 13 & 14.

- The 7 mg NRT patch box was labeled with a subject identification number and one of the following time periods: Week 13 & 14, or Week 15 & 16.

Figure D

EXAMPLE OF PATCH BOX LABEL

Caution: New Drug – Limited by Federal Law to investigational use by qualified investigators only. Keep out of the reach of children. For clinical trial use only.

Protocol Number: Vector-201

Week 9 & 10

Subject ID: ___ ___ ___ (Preprinted)

Re-apply daily (every 24 hours).

Each study kit contained the appropriate number of cigarette cartons and patches for one study subject (**Figure E**). The kit was labeled with the protocol number, subject identification number (preprinted), subject initials, and the new drug caution.

Figure E

EXAMPLE OF STUDY KIT LABEL

Caution: New Drug – Limited by Federal Law to investigational use by qualified investigators only. Keep out of the reach of children. For clinical trial use only.

Protocol Number: Vector-201

Subject ID: ___ ___ ___ (Preprinted)

Subject Initials: _____

6.3 Subject Entry

Healthy male and female smokers interested in quitting smoking were recruited from the community.

The investigative staff explained the study purpose, procedures, and subject responsibilities to the potential participant. The staff also determined a subject's willingness and ability to meet the follow-up requirements. When it was established that the subject was likely to be eligible for participation, written informed consent was obtained. The subject signed and dated the informed consent form, and the person explaining the consent also signed and dated the form. One copy of the informed consent form was retained with the subject records and a signed copy was provided to the subject.

Once the written consent was provided, the subject underwent complete screening procedures, consisting of relevant medical history, physical examination, urine pregnancy test (in women of child-bearing potential),[6] drug screen, exhaled CO, saliva cotinine, and the completion of four questionnaires.

Subject identification (subject ID) numbers were randomly assigned a treatment group (Group 1, 2, or 3) in a 1:1:1 ratio by an independent statistician using a global randomization scheme. The size of the randomization block was six, except for the last block, which was three. Six subjects were assigned per block, except for the last block (where only three subjects were assigned). The total number of blocks was 63. Each center was allowed to enroll up to 100 subjects until a total of 345 subjects were enrolled. The randomization table had sets of

[6] Sexually active women were required to use appropriate contraceptive measures for the duration of the study. Medically acceptable contraceptives included: (1) surgical sterilization, (2) approved hormonal contraceptives (such as birth control pills, Depo-Provera, or Lupron Depot), (3) barrier methods (such as a condom or diaphragm) used with a spermicide, (4) abstinence, or (5) an intrauterine device (IUD).

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six sequential rows with two assignments for each treatment group. The table was sorted by row number from 001 to 375. The randomization table was maintained by [redacted] the Contract Research Organization (CRO) for the study, after drug packaging was complete, and the drug packaging team used the randomization table to create patient kits labeled 001 to 375. Clinical sites or centers were each assigned a number from 01 to 05. A subject was randomized by assigning him or her to the next sequential drug kit from among the kits available at a center. The two-digit center number was added to the three-digit kit number to become the subject ID number. The study was double-blinded, indicating that the investigator and subject were both blinded to the treatment. Only the statistician responsible for developing the randomization key and those in charge of manufacturing and packaging the test articles viewed the randomization scheme. The kits were pre-packaged, displayed a subject ID number, and contained all cigarettes and patches to be dispensed over the course of the trial. The maximum number of subjects enrolled at any one site was 100.

Once subject eligibility was confirmed, the subject was scheduled to return to the site in 7 days. At that time they were enrolled in the study, and the next sequential subject ID number was assigned. This number consisted of 5 digits in the following format: XX-XXX, the first 2 digits representing the pre-assigned site number and the final three digits taken from the next sequential kit available. For example, the first patient enrolled at site "02", which had been allocated the next available kit number 180, would be assigned the subject ID number, 02-180.

A subject who was screened, but not entered into the study (i.e., a screen failure), was recorded on a screen log.

The subject ID number and subject initials were recorded on all study documents and linked the study treatment and the study documents to the subject's name and medical record. To maintain confidentiality, neither the subject's name nor any

other personal identifiers were recorded on any study document other than the informed consent form.

6.4 Examination Schedule

Subjects were examined and evaluated according to the following schedule of visits:

Visit 1: Screening / Baseline (Day -7 through Day 0)
Visit 2: Randomization (Day 0) ± 2 days post screening
Visit 3: 2 weeks ± 3 days post randomization
Visit 4: 4 weeks ± 3 days post randomization
Visit 5: 6 weeks ± 3 days post randomization ("quit date")
Visit 6: 8 weeks ± 3 days post randomization
Visit 7: 10 weeks ± 3 days post randomization ("4-week abstinence rate")
Visit 8: 12 weeks ± 3 days post randomization
Visit 9: 14 weeks ± 3 days post randomization
Visit 10: 16 weeks ± 3 days post randomization
Visit 11: 18 weeks ± 7 days post randomization ("3-month quit rate")
Visit 12: 31 weeks ± 7 days post randomization ("6-months quit rate")

6.5 Clinical Parameters

The clinical parameters to be evaluated were:

- Self report diaries (Visits 1 through 5)
- Saliva cotinine concentration (all Visits)
- Exhaled, background, and corrected CO (all Visits)
- Blood pressure and heart rate (all Visits)
- Minnesota Smoking Withdrawal Questionnaire (all Visits)

- Cigarette Evaluation Scale Questionnaire (Visits 1 through 5)
- Sensory Questionnaire (Visits 1 through 5)
- Fagerström Test for Nicotine Dependence (Visits 1 through 5)
- DNA (deoxyribonucleic acid) blood analysis[7]

6.6 Data Reporting

A case report form (CRF) booklet was provided by Vector Tobacco or its designee for each subject enrolled in the study. The appropriate CRF was completed at each visit. The investigator signed each subject's completed CRF once he / she had reviewed all data contained within. All CRFs were completed in a legible manner in black ink. Any corrections were made by drawing a single line through the incorrect entry, entering the correct information, and initialing and dating the change.

All clinical data generated in the study were submitted to Vector Tobacco or its designee for quality assurance review, data entry, and statistical analysis. All forms were reviewed for completeness and evident recording errors were rectified by contact with the appropriate clinical site.

6.7 Study Completion Procedures

6.7.1 Subject Completion

Subjects were considered to have completed the study if they completed all follow-up examinations through Visit 12.

[7] DNA was collected for future analysis of genetic determination of smoking behavior.

6.7.2 Subject Termination

Subjects were terminated from the study at the discretion of the investigator only for reasons related to the study treatment regimen that would jeopardize the subjects' health and/or welfare if they were to continue in the study. Terminated subjects were considered to have completed the study and were not replaced. However, every effort was made to follow terminated subjects (due to an adverse event) for safety reasons using the appropriate CRFs until the planned end of the study period. Notification of a subject termination due to an adverse event was made immediately to Vector Tobacco or its designee.

6.7.3 Subject Discontinuation

Subjects were discontinued from the study for non-treatment-related reasons only when no other option was possible. Reasons for discontinuation included, but were not necessarily limited to, 1) voluntary withdrawal from the study by the subject; 2) subject moved from the area or was determined to be lost to follow-up; and 3) subject was unwilling or unable to cooperate with study requirements (e.g., assigned treatment, follow-up visits, etc.). The reason for discontinuation was recorded on the appropriate CRF. Discontinued subjects were not replaced in the study. All such subjects were counted as failures in the treatment failure imputation and as missing values in the multiple imputation approach.

Subjects who did not quit smoking after the Visit 5 quit date or who started smoking again after the Visit 5 quit date and prior to Visit 10 were discontinued from the study. All such subjects were counted as failures due to lack of efficacy. All assessments for that specific visit were performed including a physical and pregnancy test, if applicable.

Prior to discontinuing a subject, every effort was made to contact the subject to either encourage the subject to maintain compliance with the protocol or to obtain as much follow-up data as possible regarding the subject's current status.

Efforts to contact the subject consisted of documentation of at least three attempts to contact them by phone, followed by at least two certified letters with return receipt.

A study completion form was required to be completed for all subjects who completed, discontinued, or were terminated from the study.

7.0 STATISTICAL METHODS

7.1 Sample Size Calculation

The sample size calculation for this study was based on the following criteria.

- The primary endpoint of the study was continuous abstinence from the beginning of Week 7 to the end of Week 10 (i.e., four weeks continuous abstinence). It was based on self report and verified by exhaled CO < 10 ppm.

- The statistical hypotheses for the study were:

  H_{01}: $P_{G1} = P_{G3}$ versus H_{A1}: $P_{G1} > P_{G3}$

  and

  H_{02}: $P_{G2} = P_{G3}$ versus H_{A2}: $P_{G2} > P_{G3}$.

  P_{G1}: Proportion of smokers abstinent at 4 weeks for Group 1 (Quest 1, 2, and 3 plus NRT)

  P_{G2}: Proportion of smokers abstinent at 4 weeks for Group 2 (Quest 1, 2, and 3 plus placebo patch)

  P_{G3}: Proportion of smokers abstinent at 4 weeks for Group 3 (Active Control cigarettes plus NRT)

- The proportion of smokers abstinent at 4 weeks were assumed to be 0.25, 0.25 and 0.1 for Groups 1, 2 and 3, respectively.

- Significance level (α) = 0.05 (one-sided) and statistical power (β) = 80%.

- Since the study was testing two primary hypotheses, it was decided that the tests for each of those hypotheses would be conducted

using a level of significance of 0.05, as well as a lower threshold to consider multiple hypothesis testing. This lower threshold provided a reduced risk of rejecting the null hypothesis (i.e., no difference between groups) when it was true, to adjust for the higher chance of making a Type I error in at least one of the tests. This adjustment was accomplished using the Bonferroni method. The adjusted threshold of significance was equal to 0.025.

- Fisher's exact test was used in calculating the sample size.

Based on the above criteria, a sample size of 115 for each group was determined to be sufficient for hypothesis testing. Therefore, the total sample size was estimated to be 345 subjects. Since early dropouts (or discontinuations) were all assumed to be failures, no sample size adjustment was deemed necessary to compensate for the dropout rate (or discontinuation rate).

7.2 Study Endpoint and Covariates

7.2.1 Primary Efficacy Endpoint

The primary endpoint was continuous abstinence for four weeks (Weeks 7 – 10), which was assessed after treatment termination at Week 10 (Visit 7). A subject met the criteria for success if both of the following criteria were met:

1. No cigarettes were smoked between the beginning of Week 7 to the end of Week 10.
2. The corrected exhaled CO was < 10 ppm at Visits 6 and 7 (Weeks 8 and 10).

If at least one of these two conditions were not met, the subject failed to achieve 4-week abstinence.

7.2.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints for the study were:

- Proportion of smokers abstinent at three months and six months (Visits 11 and 12) following treatment termination. A subject was considered as having achieved abstinence at 3 months (or 6 months) if the following two conditions were met:
  1. No cigarettes were smoked from Week 7 to Month 3 (or Month 6).
  2. The corrected exhaled CO was < 10 ppm for all visits between Visit 6 and Visit 11 (or Visit 12).
- Corrected exhaled CO level and change from baseline
- Saliva cotinine concentration level
- Minnesota Smoking Withdrawal Questionnaire and change from baseline
- Cigarette Evaluation Scale Questionnaire and change from baseline
- Sensory Questionnaire and change from baseline
- Fagerström Test for Nicotine Dependence and change from baseline

7.2.3 Safety Endpoints

The safety endpoints included any treatment-emergent adverse events, treatment-related adverse events, treatment-emergent serious adverse

events, treatment-related serious adverse events, adverse events leading to study discontinuation, and adverse events by severity, concomitant medications, and vital signs (i.e., heart rate and blood pressure).

7.2.4 Baseline Covariates

The following potential prognostic factors were collected at baseline:

Categorical Factors

- Gender (male, female)
- Race (Caucasian, non-Caucasian)
- Reproductive status / birth control methods (Female subjects: post-menopausal for 1 year, surgically sterilized, contraceptive measures [hormonal contraceptive, barrier method with spermicide, IUD, abstinence, other]. Male subjects: not applicable)
- Medical history by body category (normal, abnormal)
- Physical examination by body category (normal, abnormal)
- Type of usual brand of cigarettes (full flavor, light, ultra-light, king size, 100 cigarette, other)
- First attempt to quit smoking (yes, no)
- Most frequent smoking location (home, at work – anywhere, at work – designated area, bar, restaurant, other)
- Saliva cotinine level (1-10, 10-30, 30-100, 100-200, 200-500, 500-2000, >2000 ng/mL)

Continuous Factors

- Age (years)
- Weight (pounds, or lbs)
- Height (inches, or in)

- Heart rate (bpm)
- Systolic and diastolic blood pressure (mmHg)
- Years of smoking
- Current number of cigarettes per day
- Corrected CO measurement (ppm)
- Minnesota Smoking Withdrawal Questionnaire
- Cigarette Evaluation Scale Questionnaire
- Sensory Questionnaire
- Fagerström Test for Nicotine Dependence

7.3 Overall Statistical Analysis Plan

For the primary outcome, a one-sided significance level of 0.05, as well as an adjusted level of 0.025, were used for each of the two pairwise comparisons: Group 1 versus Group 3 and Group 2 versus Group 3. For all other statistical tests, a significance level of 0.05 was used, except for the assessment of interaction effects. The latter was considered in analysis of variance (ANOVA), logistic regression analyses, and generalized estimating equation (GEE) models, whether they were pre-specified in the analysis plan or were considered exploratory. Interaction effects were tested at the 0.10 significance level.

Descriptive statistics (mean, standard deviation, median and range for continuous variables, and frequency and proportion for discrete variables) and 95% confidence intervals (CIs), if appropriate, are presented.

7.4 Analysis Populations

- The primary efficacy population was the intent-to-treat (ITT) population, which consisted of all randomized subjects, regardless of whether a subject received study treatment.

- Per-protocol population was also defined as a subset of the ITT population which satisfied the following criteria:

  1. The 4-week abstinence status could be determined. Subjects who discontinued due to failure to quit smoking and whose 4-week abstinence status was missing were considered failures. All other subjects whose 4-week abstinence status was missing were excluded.
  2. No major protocol violations including the entry criteria were reported. The Medical Monitor provided the subject IDs that had major protocol violations prior to the database lock and unblinding of treatment groups. The major protocol violations are summarized in **Section 13.0**.

The safety population consisted of all randomized subjects who received study treatment. Safety data are summarized by the randomized treatment group.

7.5 Subject Accountability, Demographics, and Baseline Characteristics

The number of subjects in each treatment group is presented for each analysis population described in **Section 7.4**. The reason for subject exclusion from any analysis population was summarized. The number and percentage of subjects who discontinued study treatment and the reasons of discontinuation were tabulated by treatment group. The number and percentage of subjects who had major protocol violations were also summarized.

The demographic and baseline characteristics were summarized using descriptive statistics by treatment group. Inferential analyses comparing the baseline characteristics between the treatment groups were not performed.

7.6 Efficacy Analyses

7.6.1 Handling of Missing Data

- Missing 4-week abstinence status

  A method utilizing both the treatment failure imputation and multiple imputation was implemented. If a subject dropped out due to lack of efficacy and his / her abstinence status could not be determined, this subject was considered a treatment failure. Lack of efficacy was defined as the reason for early study termination stated as failure to quit smoking.

  For all other subjects whose abstinence status could not be determined, including those who dropped out due to reasons other than failure to quit smoking, their abstinence status was imputed using the following multiple imputation method (Rubin 1987):

  1. A logistic regression for the probability of achieving abstinence was fitted for all subjects who had non-missing abstinence status and for the subjects for whom treatment failure imputation was used. The logistic regression included the treatment group, the study site, and the baseline covariates (age, gender, race, first attempt to quit smoking, cigarette brand type, saliva cotinine level, years of smoking, current number of cigarettes per day, and corrected CO measurement). If fewer than 5.0% of subjects had missing values for each covariate

listed above, the missing covariate value was imputed by the sample mean or the mode of the observed values for continuous covariate and categorical covariate, respectively. If a categorical covariate had multiple modes, a randomly chosen mode was assigned to a subject with a missing value. If at least 5.0% of subjects had missing values for a covariate, this covariate was excluded from the logistic regression. Sample statistics of this covariate were reported.

2. $\hat{\beta}$ was the vector of the estimated regression parameters with covariance matrix V from Step 1. New regression parameters were then generated from the posterior predictive distribution of the parameters as:

$$\beta^* = \hat{\beta} + V_h Z,$$

where V_h is the upper triangular matrix in the Cholesky decomposition, $V = V_h' V_h$, and Z is a vector of independent random normal variates.

3. For each subject with missing abstinence status whose reason of early termination was not lack of efficacy, his / her probability of achieving abstinence was estimated by the logistic regression with the parameters generated in Step 2.

4. A random variate was drawn from a uniform distribution between 0 and 1. If this random variate was less than the probability in Step 3, then the

abstinence status was imputed as a success. Otherwise, the abstinence status was imputed as a failure.

5. Steps 2 to 4 were repeated 5 times to obtain 5 complete data sets.

The 5 complete data sets were then analyzed. The main assumption of this multiple imputation method was that the missingness was non-informative.

- Other study endpoints

  Treatment-failure imputation was used for the 3-month and 6-month abstinence rates. No imputation was used for other endpoints.

7.6.2 Primary Endpoint

For each complete data set generated by the imputation method described in **Section 7.6.1** (Rubin 1987), the difference of the sample abstinence rates for each treatment comparison was obtained ($P_{G1} - P_{G3}$ and $P_{G2} - P_{G3}$). The *p-value* was calculated by combining the rate differences using the formula outlined in Rubin and Schenker (1991). Specifically, D_k and V_k, where $k = 1, ..., M$ were the rate differences and their associated variances were calculated from the M complete data sets for a treatment comparison. The multiple imputation estimate of the rate difference was the average of the M complete-data estimates:

$$\overline{D} = \sum D_k / M$$

The variance of $\bar{D}$ was given by:

$$V = \sum V_k / M + (1 + M^{-1}) \sum (D_k - \bar{D})^2 / (M - 1)$$

Under the null hypothesis of no treatment effect, $\bar{D} V^{-1/2}$ follows a t-distribution with the degree of freedom *df*, where:

$$df = (M-1)[1 + \frac{M-1}{M+1} \frac{\sum V_k}{\sum (D_k - \bar{D})^2}]^2$$

The abstinence rates were estimated by the average of the sample rates in the complete data sets. The standard errors were estimated by a similar formula as above. p_k was the sample abstinence rate with variance v_k for data set *k*. The variance of the combined abstinence rate was given by:

$$v = \bar{v} + (1 + M^{-1})B,$$

where $\bar{v}$ is the average of the variances of $p_1, ..., p_M$, and B is the sample variance of $p_1, ..., p_M$.

Secondary analyses of the 4-week abstinence rates included:

1. For each complete data set generated by the imputation method described in **Section 7.6.1**, a logistic regression for the abstinence status with a covariates treatment group, study site, and treatment-by-site interaction was performed for each treatment comparison. The interaction effect was estimated by using the formula in Rubin and Schenker (1991). If it was significant at the 2-sided 0.1 significance level, then the treatment groups were compared separately for each study site.

2. Subjects with missing abstinence status whose reason for early termination was not lack of efficacy were excluded. Subjects with missing abstinence status who dropped out due to lack of efficacy

were imputed as treatment failures. Treatment comparisons were performed by Fisher's exact test.

3. Logistic regression was used to identify prognostic factors and compare treatments by adjusting for baseline covariates. A backward elimination procedure was utilized to select baseline covariates at a significance level of 0.1. The initial model included the baseline covariates listed in **Section 7.2.4**. The treatment group was always included in the model. Treatment failure imputation was used in this analysis.

4. All subjects with missing abstinence status in the ITT population were imputed as treatment failures. Treatment comparisons were performed by Fisher's exact test.

5. Fisher's exact test was performed on the per-protocol population.

7.6.3 Secondary Endpoints

- **Quit rates at three months and six months (Visits 11 and 12, respectively) following treatment termination**: The quit rates at three and six months were analyzed separately using the analysis methods 2, 3, 4 and 5 for the 4-week abstinence rate (**Section 7.6.2**).

- **Other secondary endpoints**: The analyses of the rest of the secondary endpoints were descriptive. Summary statistics at each visit were reported. The *p-values* were reported for descriptive purposes only. Missing data were not imputed.

- **Corrected exhaled CO level and change from baseline:** The Wilcoxon rank-sum test was used to compare the distribution of the corrected exhaled CO level in Group 1 versus Group 3 and in Group 2 versus Group 3 at each study visit.

- **Saliva cotinine concentration level:** The Jonckheere-Terpstra test was used to compare the distribution of the saliva cotinine concentration level in Group 1 versus Group 3 and in Group 2 versus Group 3 at each study visit.

- **Minnesota Smoking Withdrawal Questionnaire, Cigarette Evaluation Scale Questionnaire, Sensory Questionnaire, and Fagerström Test for Nicotine Dependence**: Each question was analyzed individually at each visit by the Jonchkheere-Terpstra test and Chi-square test for ordinal response and binary response, respectively. Change from baseline in each question was analyzed by the Wilcoxon rank-sum test.

7.6.4 Exploratory Analyses

Exploratory analyses were performed for four-week abstinence proportions using treatment failure imputation stratified by baseline covariates. A summary of these results is provided in **Table S2** in **Appendix A.**

7.7 Safety Analyses

7.7.1 Exposure to Investigational Treatment

Summary statistics for the number of study cigarettes, the number of regular cigarettes, and the number of patches used by visit are provided. The number of study cigarettes per day, the number of regular cigarettes per day, and the total number of cigarettes per day are also summarized by visit.

7.7.2 Adverse Events

All adverse events (AEs) reported following the initiation of the study treatment were included in the safety analysis. AEs were coded using the MedDRA dictionary. Subject risk (i.e., the percent of subjects reporting an event) of treatment-emergent AEs were tabulated by system organ class and preferred term. Subject risk of serious adverse events (SAEs), AEs leading to withdrawal, AEs related to study treatment, and SAEs related to study treatment were also provided. In addition, since subjects started using patches after Visit 4, AEs that occurred after Visit 4 were summarized to evaluate the safety profile of the patch.

7.7.3 Concomitant Medications

Baseline and on-study concomitant medications were summarized by WHODrug ACT and drug class. Baseline concomitant medications are medications that were used on or before the date of randomization. On-study concomitant medications are medications that were used on or after the date of randomization.

7.7.4 Vital Signs

Descriptive statistics for blood pressure and heart rate were tabulated at each visit.

8.0 ADVERSE EXPERIENCE REPORTING

8.1 Adverse Event Definitions

An Adverse Event (AE) is defined as:
Any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of a pharmaceutical product whether or not considered related to the pharmaceutical product. An untoward medical occurrence in a patient or subject administered a pharmaceutical product does not necessarily have to have a causal relationship with the treatment under investigation.

A Serious Adverse Event (SAE) is defined as:
An AE, occurring at any dose, that results in any of the following outcomes:

- Death
- Is life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability / incapacity
- Is a congenital anomaly or birth defect

8.2 Adverse Event Assessment

All AEs, including the following, were assessed by the investigator, and recorded in the subject's study chart and on the appropriate CRF pages.

- Observed or self-reported problems
- Complaints
- Physical signs and symptoms

- Medical condition which occurred during the study, but which was absent at baseline
- Medical condition present at baseline, but which appeared to worsen during the study

The need to capture AEs was not dependent upon whether or not the clinical event was associated with the use of the investigational product.

Each AE was described as follows: date of onset, date of resolution, severity, frequency of the event (single episode, intermittent, continuous), action taken with study treatment (treatment held, treatment discontinued), relationship to investigational treatment, outcome, and seriousness criteria. Each AE was recorded separately.

AE severity was assessed using the following definitions:

Mild	Subject was aware of sign or symptom, but it was easily tolerated
Moderate	Discomfort was sufficient to cause interference with subject's usual activity
Severe	Event was incapacitating to subject, with inability to work or do usual activity
Life Threatening	Event posed an immediate risk of death to subject unless medical intervention was obtained

The relationship to investigational treatment was assessed by the investigator as defined below.

RELATIONSHIP TO THE INVESTIGATIONAL TREATMENT

Not Related	Evidence exists that the adverse event definitely has a cause other than the investigational product (e.g., pre-existing condition or underlying disease, intercurrent illness, or concomitant medication) and does not meet any other criteria listed.
Possibly Related	A temporal relationship exists between the event onset and administration of investigational product. Although the adverse event appears unlikely to be related to the investigational treatment, it can not be ruled out with certainty; and/or the event can not be readily explained by the subject's clinical state or concomitant therapies.
Probably Related	A temporal relationship exists between the event onset and administration of investigational product, and appears with some degree of certainty to be related based on known therapeutic and pharmacologic actions of the investigational treatment. It can not be readily explained by the subject's clinical state or concomitant therapies.
Definitely Related	Strong evidence exists that the investigational treatment caused the adverse event. There is a temporal relationship between the event onset and administration of the investigational product. There is strong therapeutic and pharmacologic evidence that the event was caused by the investigational product. The subject's clinical state and concomitant therapies are ruled out as a cause.

Outcome was assessed using the following categories: recovered / resolved, not recovered / not resolved, recovered / resolved with sequelae, fatal, or unknown.

8.3 Adverse Event Reporting

All subjects who were exposed to the investigational treatment were evaluated for AEs. All AEs were evaluated beginning with onset, and evaluation continued until the last day of the study, until recovery was noted, or until the investigator determined the subject's condition was stable, whichever occurred first.

The investigator took all appropriate and necessary therapeutic measures required for resolution of an AE. Any medication necessary for the treatment of an AE

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was recorded on the concomitant medication CRF. If more than one distinct AE occurred, each event was recorded separately.

8.4 Serious Adverse Event Reporting

All serious adverse events (SAEs) that occurred during the study, including death, ***were required to be reported within 24 hours of knowledge of the event*** by telephone to [redacted]. All SAEs were to be followed up immediately in writing, by faxing the appropriate SAE form to [redacted]. All faxed reports were followed by telephone contact to assure receipt of the fax. Study SAEs were reported from Visit 1 (Day -7) until 30 days after the last study related procedure.

9.0 STUDY MONITORING

Vector Tobacco or its designee monitored all clinical studies in a manner consistent with applicable health authority regulations and the clinical research standards adopted by Vector Tobacco. Study monitoring involved the following elements:

1. Vector Tobacco or its designee met with the investigator(s) prior to the initiation of the study in order to review the adequacy of the subject population, facilities, and equipment with respect to the needs of the study, and to familiarize the investigator(s) with the study protocol.

2. Vector Tobacco or its designee met with the investigator(s) at the time study subjects began to be enrolled in order to ensure that subjects were being properly selected and that study data were being correctly recorded.

3. Vector Tobacco or its designee visited the clinical site during the study to review and/or collect CRFs.

4. Interim monitoring visits and telephone consultation occurred as necessary during the course of the study to ensure the proper progress and documentation of the study findings.

10.0 STUDY RESULTS

A total of five clinical sites participated in this randomized, multicenter, double-blind, parallel group, active controlled Phase II trial. The database for this clinical report was locked on March 3, 2006. The database lock occurred after all subjects had completed the study, after the statistical analysis plan (SAP) had been completed, and after all protocol deviations / violations that could affect either the safety and/or efficacy outcomes had been identified.

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11.0 ENROLLMENT AND ACCOUNTABILITY

Subject enrollment by site is summarized in **Table 1**, below. A total of 346 subjects were enrolled at 5 participating US centers. A total of 116 subjects were randomized to Group 1 (Quest 1, Quest 2, Quest 3 plus NRT), 116 subjects were randomized to Group 2 (Quest 1, Quest 2, Quest 3 plus placebo patch), and 114 subjects were randomized to Group 3 (Active Control cigarettes plus NRT).

Table 1 Subject Enrollment

[redacted]				
01	26 (32.5%)	28 (35.0%)	26 (32.5%)	80
02	16 (30.8%)	19 (36.5%)	17 (32.7%)	52
03	25 (36.8%)	20 (29.4%)	23 (33.8%)	68
04	19 (31.1%)	22 (36.1%)	20 (32.8%)	61
05	30 (35.3%)	27 (31.8%)	28 (32.9%)	85
	116	116	114	

Group 1: Quest 1, Quest 2 and Quest 3 plus NRT (patch)
Group 2: Quest 1, Quest 2 and Quest 3 plus placebo patch
Group 3: Active Control cigarettes (conventional) plus NRT (patch)
N = number of randomized subjects
n = number of subjects randomized in each site and treatment group
% = n / site total x 100%

[redacted]

Table 2 summarizes subject disposition. Only two of the 346 subjects enrolled (0.6%) did not meet all eligibility criteria. All subjects (n = 346, 100.0%) received study treatment. A total of eighty-five subjects (24.6%) completed the study through Visit 12.

Reasons for early discontinuation are also presented in **Table 2**. The most common reason for discontinuation was failure to quit smoking (n = 133, 38.4%), followed by lost to follow-up (n = 59, 17.1%) and discontinuation at the subject's request / withdrawal of consent (n = 46, 13.3%).

Table 2 Subject Disposition

All Eligibility Criteria Met	115 (99.1%)	115 (99.1%)	114 (100.0%)	344 (99.4%)
Received Study Treatment	116 (100.0%)	116 (100.0%)	114 (100.0%)	346 (100.0%)
Completed Study*	32 (27.6%)	20 (17.2%)	33 (28.9%)	85 (24.6%)
Reason for early discontinuation				
Adverse Event	3 (2.6%)	2 (1.7%)	3 (2.6%)	8 (2.3%)
Lost to Follow-Up	16 (13.8%)	22 (19.0%)	21 (18.4%)	59 (17.1%)
Non-Compliance	3 (2.6%)	5 (4.3%)	4 (3.5%)	12 (3.5%)
Other, specify	1 (0.9%)	0	1 (0.9%)	2 (0.6%)
Sponsor's Request	0	0	1 (0.9%)	1 (0.3%)
Subject failed to quit smoking	44 (37.9%)	49 (42.2%)	40 (35.1%)	133 (38.4%)
Subject's Request / Withdrawal of consent	17 (14.7%)	18 (15.5%)	11 (9.6%)	46 (13.3%)

N = number of randomized subjects
n = number of subjects
% = n / N x 100%
*Subject disposition data from CRF Study Completion/Early Withdrawal from the question, "Did the patient complete study?"

Table 3 summarizes subject disposition by the analysis methodology used.

Table 3 Subject Disposition by Analysis Methodology

Primary Analysis			
Treatment Failure			
Imputed as failure	28 (24.1%)	37 (31.9%)	19 (16.7%)
Not imputed (success)	38 (32.8%)	19 (16.4%)	25 (21.9%)
Not imputed (failure)	50 (43.1%)	60 (51.7%)	70 (61.4%)
Additional Sensitivity Analyses			
Imputed by Multiple Imputation	25 (21.6%)	33 (28.4%)	18 (15.8%)
Imputed as failure	3 (2.6%)	4 (3.4%)	1 (0.9%)
Not imputed (success)	38 (32.8%)	19 (16.4%)	25 (21.9%)
Not imputed (failure)	50 (43.1%)	60 (51.7%)	70 (61.4%)
Based on Available Data			
Not imputed (success)	38 (32.8%)	19 (16.4%)	25 (21.9%)
Not imputed (failure)	50 (43.1%)	60 (51.7%)	70 (61.4%)
Excluded	28 (24.1%)	37 (31.9%)	19 (16.7%)
Treatment Failure due to Lack of Efficacy			
Imputed as failure	3 (2.6%)	4 (3.4%)	1 (0.9%)
Not imputed (success)	38 (32.8%)	19 (16.4%)	25 (21.9%)
Not imputed (failure)	50 (43.1%)	60 (51.7%)	70 (61.4%)
Excluded	25 (21.6%)	33 (28.4%)	18 (15.8%)

Per-protocol			
Imputed as failure	1 (0.9%)	0	0
Not imputed (success)	30 (25.9%)	13 (11.2%)	22 (19.3%)
Not imputed (failure)	36 (31.0%)	52 (44.8%)	57 (50.0%)
Excluded	49 (42.2%)	51 (44.0%)	35 (30.7%)

Figure F illustrates the time to dropout. The median time to dropout was 12.8 weeks for Group 1, 10.5 weeks for Group 2, and 14.2 weeks for Group 3.

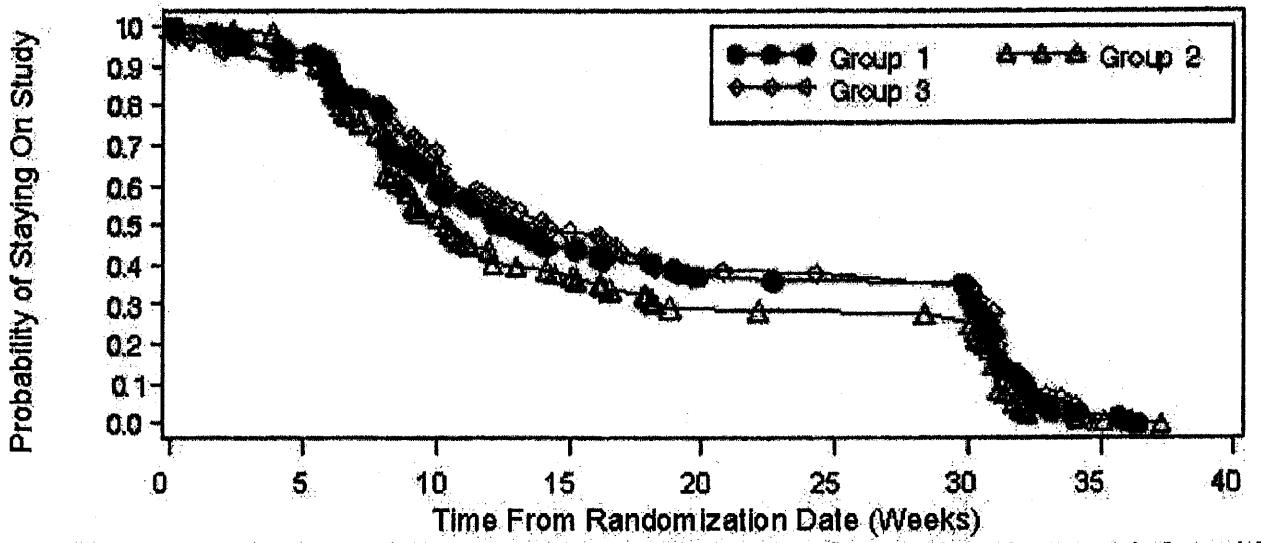
Tables 4.1 and **4.2** provide subject accountability by visit, Visits 1 to 7, and Visits 8 to 12, respectively. At Visit 7, determination of 4-week abstinence, the proportion of subjects with clinical data available was 60.3% (70/116) in Group 1, 49.1% (57/116) in Group 2, and 61.4% (70/114) in Group 3.

At Visit 11, determination of 3-month quit rates, the proportion of subjects with clinical data available was 38.8% (45/116) in Group 1, 31.9% (37/116) in Group 2, and 40.4% (46/114) in Group 3.

The number of available subjects further decreased at Visit 12. At this visit, determination of 6-month quit rates, the proportion of subjects with clinical data available was 29.3% (34/116) in Group 1, 20.7% (24/116) in Group 2, and 29.8% (34/114) in Group 3.

Figure F

KAPLAN-MEIER PLOTS FOR TIME TO DROPOUT*



Median time to drop out is 12.79, 10.50 and 14.21 Weeks for treatment groups 1, 2, and 3 respectively

*36 subjects exceeded the 32 week cutoff in order complete Visit 12 at a later week

Table 4.1 Subject Accountability By Visit
(Visits 1 to 7)

Group 1 (N = 116)							
Available	116 (100.0%)	116 (100.0%)	114 (98.3%)	108 (93.1%)	102 (87.9%)	87 (75.0%)	70 (60.3%)
Discontinued	0	0	2 (1.7%)	5 (4.3%)	5 (4.3%)	13 (11.2%)	18 (15.5%)
Missing	0	0	0	1 (0.9%)	2 (1.7%)	4 (3.4%)	3 (2.6%)
Group 2 (N = 116)							
Available	116 (100.0%)	116 (100.0%)	114 (98.3%)	110 (94.8%)	98 (84.5%)	81 (69.8%)	57 (49.1%)
Discontinued	0	0	2 (1.7%)	4 (3.4%)	11 (9.5%)	17 (14.7%)	23 (19.8%)
Missing	0	0	0	0	1 (0.9%)	1 (0.9%)	2 (1.7%)
Group 3 (N = 114)							
Available	114 (100.0%)	114 (100.0%)	109 (95.6%)	107 (93.9%)	104 (91.2%)	96 (84.2%)	70 (61.4%)
Discontinued	0	0	5 (4.4%)	2 (1.8%)	3 (2.6%)	8 (7.0%)	25 (21.9%)
Missing	0	0	0	0	0	0	1 (0.9%)

N = number of randomized subjects
% = n / N x 100%
Available = number of subjects with any clinical data
Discontinued = number of subjects who discontinued at each visit (not cumulative)
Missing = number of subjects with no clinical data

Table 4.2 Subject Accountability By Visit
(Visits 8 to 12)

Group 1 (N = 116)					
Available	60 (51.7%)	54 (46.6%)	52 (44.8%)	45 (38.8%)	34 (29.3%)
Discontinued	9 (7.8%)	7 (6.0%)	3 (2.6%)	6 (5.2%)	14 (12.1%)
Missing	4 (3.4%)	3 (2.6%)	2 (1.7%)	3 (2.6%)	0
Group 2 (N = 116)					
Available	45 (38.8%)	44 (37.9%)	39 (33.6%)	37 (31.9%)	24 (20.7%)
Discontinued	10 (8.6%)	3 (2.6%)	5 (4.3%)	2 (1.7%)	15 (12.9%)
Missing	4 (3.4%)	2 (1.7%)	2 (1.7%)	2 (1.7%)	0
Group 3 (N = 114)					
Available	62 (54.4%)	57 (50.0%)	53 (46.5%)	46 (40.4%)	34 (29.8%)
Discontinued	9 (7.9%)	4 (3.5%)	5 (4.4%)	7 (6.1%)	12 (10.5%)
Missing	0	1 (0.9%)	0	0	0

N = number of randomized subjects
% = n / N x 100%
Available = number of subjects with any clinical data
Discontinued = number of subjects who discontinued at each visit (not cumulative)
Missing = number of subjects with no clinical data

12.0 DEMOGRAPHICS, BASELINE AND SMOKING CHARACTERISTICS

12.1 Demographics

Demographic information for the study population is provided in **Table 5**, below. Of the 346 subjects enrolled, 186 (53.8%) were male and 160 (46.2%) were female. The mean age was 45.9 years (standard deviation, or SD, 10.9; range 21 – 66 years). The majority of subjects were Caucasian (319/346, or 92.2%); 3.5% of the study population were Hispanic, 3.2% were Black, and 0.6% were Asian or Pacific Islander. Mean weight was 183.6 pounds (SD 45.2; range 87 – 312 lbs), and mean height was 67.6 inches (SD 4.3; range 58 – 83 inches).

Subject demographics are summarized by site in **Tables S1.1** through **S1.5** in **Appendix A**.

Table 5 Summary of Subject Demographics

Gender – n (%)					0.0029
Female	41 (35.3%)	67 (57.8%)	52 (45.6%)	160 (46.2%)	
Male	75 (64.7%)	49 (42.2%)	62 (54.4%)	186 (53.8%)	
Race – n (%)					0.5677
Asian or Pacific Islander	0	1 (0.9%)	1 (0.9%)	2 (0.6%)	
Black, not of Hispanic Origin	4 (3.4%)	4 (3.4%)	3 (2.6%)	11 (3.2%)	
Caucasian, not of Hispanic Origin	109 (94.0%)	107 (92.2%)	103 (90.4%)	319 (92.2%)	
Hispanic	2 (1.7%)	3 (2.6%)	7 (6.1%)	12 (3.5%)	
Native American or Alaska Native	0	1 (0.9%)	0	1 (0.3%)	
Other, specify	1 (0.9%)	0	0	1 (0.3%)	
Age at Randomization					0.8450
Number of available subjects	116	116	114	346	
Mean (SD)	45.5 (11.1)	46.1 (10.5)	46.3 (11.0)	45.9 (10.9)	
Median	45.9	45.1	46.1	45.7	
Minimum, maximum	21, 66	23, 65	24, 66	21, 66	

Table 5 Summary of Subject Demographics

Weight (lbs)					0.7139
Number of available subjects	116	116	114	346	
Mean (SD)	186.4 (43.5)	181.8 (48.8)	182.6 (43.3)	183.6 (45.2)	
Median	184.5	178.2	184.2	181.4	
Minimum, maximum	100, 312	105, 311	87, 312	87, 312	
Height (in)					0.0936
Number of available subjects	115	116	114	345	
Mean (SD)	68.3 (4.5)	67.0 (4.1)	67.4 (4.2)	67.6 (4.3)	
Median	69.0	66.5	67.5	67.8	
Minimum, maximum	59, 83	59, 77	58, 80	58, 83	

N = number of randomized subjects
Percentage is based on the number of available subjects.
* Fisher's Exact Test for categorical variables and F-test for continuous variables.

Among treatment groups, the only statistically significant difference in subject demographics was gender. However, a subgroup analysis of 4-week abstinence using treatment failure imputation showed that while the odds ratios of 4-week abstinence were different between males and females, there was no interaction effect (**Table S2** in **Appendix A**).

12.2 Baseline Characteristics

Table 6 summarizes the baseline characteristics for the study population. Most subjects reported smoking light cigarettes (168/346, or 48.6%), followed by full flavor cigarettes (81/346, or 23.4%), and ultra-light cigarettes (73/346, or 21.1%). As anticipated, over 90% of subjects indicated this was not their first attempt to quit smoking. The number of previous quit attempts ranged from 0 to 50; however, most subjects reported between 1 and 4 previous quit attempts. The mean period of smoking reported was 26.8 years (SD 11.3; range 1 – 53 years).

At baseline, the mean corrected CO measurement was 27.5 ppm (SD 10.7; range 14 – 82 ppm). Saliva cotinine levels were > 2000 ng/mL in 56.6% of subjects (196/346), and between 500 to 2000 ng/mL in 38.2% of subjects (132/346). Subjects reported smoking most frequently at home (189/346, or 54.6%) or at work (100/346, or 28.9%).

For female subjects, the reproductive status / birth control methods reported were use of contraceptive measures (17.9%), surgical sterilization (16.2%), and post-menopausal for one year (12.1%).

Baseline characteristics are summarized by site in **Tables S3.1** through **S3.5** in **Appendix A**.

Table 6 Summary of Baseline Characteristics

Usual Cigarette Brand – n (%)				
100 Cigarette	7 (6.0%)	7 (6.0%)	8 (7.0%)	22 (6.4%)
Full Flavor	30 (25.9%)	24 (20.7%)	27 (23.7%)	81 (23.4%)
King Size	1 (0.9%)	0	1 (0.9%)	2 (0.6%)
Light	57 (49.1%)	61 (52.6%)	50 (43.9%)	168 (48.6%)
Ultra-Light	21 (18.1%)	24 (20.7%)	28 (24.6%)	73 (21.1%)
First Attempt to Quit – n (%)				
No	104 (89.7%)	109 (94.0%)	102 (89.5%)	315 (91.0%)
Yes	12 (10.3%)	7 (6.0%)	12 (10.5%)	31 (9.0%)
Number of Previous Quit Attempts – n (%)				
0	12 (10.3%)	7 (6.0%)	12 (10.5%)	31 (9.0%)
1	17 (14.7%)	25 (21.6%)	22 (19.3%)	64 (18.5%)
2	15 (12.9%)	15 (12.9%)	23 (20.2%)	53 (15.3%)
3	24 (20.7%)	25 (21.6%)	20 (17.5%)	69 (19.9%)
4	13 (11.2%)	22 (19.0%)	10 (8.8%)	45 (13.0%)
5	10 (8.6%)	8 (6.9%)	7 (6.1%)	25 (7.2%)
6	6 (5.2%)	3 (2.6%)	6 (5.3%)	15 (4.3%)
7	2 (1.7%)	1 (0.9%)	1 (0.9%)	4 (1.2%)
8	1 (0.9%)	0	2 (1.8%)	3 (0.9%)
10	9 (7.8%)	6 (5.2%)	6 (5.3%)	21 (6.1%)
12	2 (1.7%)	1 (0.9%)	1 (0.9%)	4 (1.2%)
15	1 (0.9%)	0	0	1 (0.3%)
20	2 (1.7%)	1 (0.9%)	1 (0.9%)	4 (1.2%)
24	0	0	1 (0.9%)	1 (0.3%)
30	2 (1.7%)	1 (0.9%)	0	3 (0.9%)

Table 6 Summary of Baseline Characteristics

50	0	0	2 (1.8%)	2 (0.6%)
Unknown	0	1 (0.9%)	0	1 (0.3%)
Number of Previous Quit Attempts				
Number of available subjects	116	115	114	345
Mean (SD)	4.4 (5.0)	3.5 (3.8)	4.2 (7.1)	4.0 (5.5)
Median	3.0	3.0	2.5	3.0
Minimum, maximum	0, 30	0, 30	0, 50	0, 50
Years of Smoking				
Number of available subjects	116	116	114	346
Mean (SD)	26.3 (11.4)	26.4 (11.2)	27.8 (11.4)	26.8 (11.3)
Median	25.0	26.2	26.0	25.8
Minimum, maximum	4, 51	1, 53	5, 50	1, 53
Current Number of Cigarettes per Day				
Number of available subjects	116	116	114	346
Mean (SD)	27.0 (9.5)	25.4 (8.6)	25.9 (8.5)	26.1 (8.9)
Median	25.0	20.0	20.0	20.0
Minimum, maximum	15, 60	15, 60	15, 50	15, 60
Corrected CO Measurement (ppm)				
Number of available subjects	116	116	114	346
Mean (SD)	28.7 (13.3)	26.6 (8.6)	27.3 (9.7)	27.5 (10.7)
Median	25.5	25.0	25.5	25.0
Minimum, maximum	15, 82	15, 56	14, 60	14, 82

Table 6 Summary of Baseline Characteristics

Saliva Cotinine Level (ng/mL) – n (%)				
30 – 100	0	0	1 (0.9%)	1 (0.3%)
100 – 200	0	1 (0.9%)	4 (3.5%)	5 (1.4%)
200 – 500	3 (2.6%)	5 (4.3%)	4 (3.5%)	12 (3.5%)
500 – 2000	36 (31.0%)	54 (46.6%)	42 (36.8%)	132 (38.2%)
> 2000	77 (66.4%)	56 (48.3%)	63 (55.3%)	196 (56.6%)
Most Often Smoking Location – n (%)				
At Work (anywhere)	29 (25.0%)	18 (15.5%)	31 (27.2%)	78 (22.5%)
At Work (in designated area)	9 (7.8%)	9 (7.8%)	4 (3.5%)	22 (6.4%)
Bar	1 (0.9%)	1 (0.9%)	4 (3.5%)	6 (1.7%)
Home	57 (49.1%)	72 (62.1%)	60 (52.6%)	189 (54.6%)
Other, specify	18 (15.5%)	15 (12.9%)	15 (13.2%)	48 (13.9%)
Restaurant	2 (1.7%)	0	0	2 (0.6%)
Unknown	0	1 (0.9%)	0	1 (0.3%)
Reproductive Status / Birth Control Method – n (%)				
Post-menopausal for one year	11 (9.5%)	19 (16.4%)	12 (10.5%)	42 (12.1%)
Surgically sterilized	15 (12.9%)	22 (19.0%)	19 (16.7%)	56 (16.2%)
Contraceptive measures	15 (12.9%)	26 (22.4%)	21 (18.4%)	62 (17.9%)

Table 6 Summary of Baseline Characteristics

Hormonal Contraceptive	3 (2.6%)	6 (5.2%)	5 (4.4%)	14 (4.0%)
Barrier Method with Spermicide	9 (7.8%)	15 (12.9%)	12 (10.5%)	36 (10.4%)
Intra-Uterine Device	0	1 (0.9%)	1 (0.9%)	2 (0.6%)
Abstinence	3 (2.6%)	4 (3.4%)	3 (2.6%)	10 (2.9%)
Not applicable	75 (64.7%)	49 (42.2%)	62 (54.4%)	186 (53.8%)

N = number of randomized subjects
Percentage is based on the number of available subjects.

12.3 Study and Regular Cigarettes Smoked

Table 7 (below) summarizes the mean, median, minimum and maximum number of regular and study cigarettes smoked per day by visit and by treatment group. Several subjects reportedly smoked regular cigarettes beyond the second visit either because they had an insufficient supply of study cigarettes or due to non-compliance with the study treatment.

Table S4 in **Appendix A** shows the summary of study and regular cigarettes smoked in total, by visit and by treatment group; and **Table S5** in **Appendix A** presents the summary of change from Visit 2 in total cigarettes smoked per day.

Table 7 Summary of Study and Regular Cigarettes Smoked Per Day (Safety Population)

Visit 2			
Number of regular cigarettes smoked per day			
Number of available subjects	116	116	114
Mean (SD)	26.2 (9.8)	25.3 (9.8)	26.2 (9.0)
Median	23.7	22.0	22.4
Minimum, maximum	9, 61	8, 59	13, 58
Visit 3			
Number of study cigarettes smoked per day			
Number of available subjects	112	113	109
Mean (SD)	28.3 (10.5)	29.0 (11.3)	27.5 (10.5)
Median	26.0	26.7	26.1
Minimum, maximum	1, 57	10, 59	4, 56
Number of regular cigarettes smoked per day			
Number of available subjects	112	114	109
Mean (SD)	1.3 (3.6)	0.9 (2.4)	0.8 (2.6)
Median	0.1	0.0	0.0
Minimum, maximum	0, 29	0, 17	0, 22
Visit 4			
Number of study cigarettes smoked per day			
Number of available subjects	107	107	107
Mean (SD)	32.1 (11.9)	31.1 (12.2)	29.9 (11.8)
Median	31.0	30.4	26.2

Table 7 Summary of Study and Regular Cigarettes Smoked Per Day (Safety Population)

Minimum, maximum	0, 62	1, 58	10, 62
Number of regular cigarettes smoked per day			
Number of available subjects	108	107	107
Mean (SD)	0.8 (4.6)	0.5 (2.0)	0.2 (1.0)
Median	0.0	0.0	0.0
Minimum, maximum	0, 41	0, 16	0, 8
Visit 5			
Number of study cigarettes smoked per day			
Number of available subjects	99	97	103
Mean (SD)	25.1 (14.0)	26.5 (14.1)	28.3 (11.8)
Median	23.7	25.0	26.6
Minimum, maximum	1, 57	0, 57	5, 57
Number of regular cigarettes smoked per day			
Number of available subjects	99	97	104
Mean (SD)	0.5 (1.5)	1.2 (3.5)	0.1 (0.9)
Median	0.0	0.0	0.0
Minimum, maximum	0, 10	0, 17	0, 8

N = number of randomized subjects who received study treatment
Number of cigarettes per day = number of cigarettes smoked / number of days in the last visit

12.4 Daily Patch Use

Table 8 provides a summary of daily patch use by visit. The proportion of subjects that reportedly used the patch daily varied from 66.0% (Group 2 at Visit 5) to 84.9% (Group 3 at Visit 10).

Table 8 Summary of Daily Patch Use (Safety Population)

Visit 5	72 /101 (71.3%)	64 / 97 (66.0%)	75 /104 (72.1%)
Visit 6	69 / 87 (79.3%)	63 / 79 (79.7%)	77 / 95 (81.1%)
Visit 7	58 / 70 (82.9%)	44 / 56 (78.6%)	52 / 70 (74.3%)
Visit 8	46 / 60 (76.7%)	36 / 45 (80.0%)	50 / 61 (82.0%)
Visit 9	43 / 54 (79.6%)	35 / 43 (81.4%)	47 / 57 (82.5%)
Visit 10	38 / 49 (77.6%)	30 / 39 (76.9%)	45 / 53 (84.9%)

N = number of randomized subjects who received study treatment
% was based on the number of available subjects in the corresponding visit

13.0 PROTOCOL VIOLATIONS AND ANALYSIS POPULATION

Major protocol violations, defined as issues of eligibility and adherence to the study protocol, are summarized in **Table 9**. A total of 106 protocol violations in 90 subjects were reviewed and classified prior to breaking the blind. The majority of protocol violations involved compliance with the trial regimen, specifically NRT compliance less than 75% (n = 55, 15.9%) and study cigarette compliance less than 95% (n = 43, 12.4%). In addition, four subjects had CO measurements of less than 15 ppm at Visit 2, two subjects did not meet the eligibility criteria, and two subjects ran out of investigational cigarettes prior to their next visit.

Table 9 Summary of Major Protocol Violations

Overall	35 (30.2%)	35 (30.2%)	20 (17.5%)	90 (26.0%)
CO less than 15 ppm at Visit 2	1 (0.9%)	1 (0.9%)	2 (1.8%)	4 (1.2%)
Ran out of investigational product (cigarettes) before next visit	0	1 (0.9%)	1 (0.9%)	2 (0.6%)
NRT compliance < 75%	25 (21.6%)	19 (16.4%)	11 (9.6%)	55 (15.9%)
Study cigarette compliance < 95%	15 (12.9%)	21 (18.1%)	7 (6.1%)	43 (12.4%)
Failed to meet eligibility criteria	1 (0.9%)	1 (0.9%)	0	2 (0.6%)

N = number of randomized subjects
n = number of subjects
% = n / N x 100%

Table 10 summarizes the analysis populations. The intent-to-treat (ITT) population consisted of all randomized subjects (n = 346), regardless of whether the subject received study treatment. The per-protocol population, a subset of the ITT population, consisted

of 211 subjects, who satisfied the following criteria:

- The 4-week abstinence status could be determined. Subjects who discontinued before week 7 due to failure to quit smoking and whose 4-week abstinence status was missing were considered failures.

- Absence of major protocol violations including the entry criteria.

The safety population consisted of all randomized subjects who received at least one study cigarette (n = 346).

Table 10 Analysis Populations

Intent-to-Treat Population	116 (100.0%)	116 (100.0%)	114 (100.0%)	346 (100.0%)
Per-Protocol Population	67 (57.8%)	65 (56.0%)	79 (69.3%)	211 (61.0%)
Safety Population	116 (100.0%)	116 (100.0%)	114 (100.0%)	346 (100.0%)

N = number of randomized subjects
n = number of subjects
% = n / N x 100%

The reasons for missing the 4-week abstinence determination are provided in **Table 11.** For 84 subjects, the 4-week abstinence status could not be determined. The most common reasons for missing 4-week abstinence were either lost to follow-up (n = 31, 36.9%) or discontinuation at the subject's request / withdrawal of consent (n = 28, 33.3%). More subjects in Group 2 (n = 28) than in Group 1 (n = 17) or Group 3 (n = 14) had missing 4-week abstinence due to either lost to follow-up or discontinuation at the subject's request / withdrawal of consent. Seven subjects failed to quit smoking and were considered failures in the per-protocol population. Six patients (two in each study group) were discontinued on or before Visit 7 due to an adverse event.

Table 11 Reasons for Missing Four-Week Abstinence Status

Discontinued on or before Visit 7	25 (89.3%)	36 (97.3%)	19 (100.0%)	80 (95.2%)
Adverse Event	2 (7.1%)	2 (5.4%)	2 (10.5%)	6 (7.1%)
Lost to Follow-Up	8 (28.6%)	14 (37.8%)	9 (47.4%)	31 (36.9%)
Non-Compliance	2 (7.1%)	3 (8.1%)	1 (5.3%)	6 (7.1%)
Other, specify	1 (3.6%)	0	0	1 (1.2%)
Sponsor's Request	0	0	1 (5.3%)	1 (1.2%)
Subject failed to quit smoking	3 (10.7%)	3 (8.1%)	1 (5.3%)	7 (8.3%)
Subject's Request / Withdrawal of consent	9 (32.1%)	14 (37.8%)	5 (26.3%)	28 (33.3%)
Discontinued after Visit 7	3 (10.7%)	1 (2.7%)	0	4 (4.8%)
Missing CO or smoking status at Visits 6 or 7	3 (10.7%)	1 (2.7%)	0	4 (4.8%)

N = number of randomized subjects whose 4-week abstinence status is missing
n = number of subjects
% = n / N x 100%

14.0 PRIMARY EFFICACY ENDPOINT – FOUR WEEKS OF CONTINUOUS ABSTINENCE

Results for analysis of the primary endpoint, 4 weeks of continuous abstinence are provided in **Table 12.** As specified, analysis included a series of sensitivity tests, employing a range of imputation methods for missing data, to determine whether the 4-week abstinence rate was statistically different between Groups 1 and 3, and Groups 2 and 3. Analysis of the primary endpoint was an ITT analysis using treatment failure imputation. Subsequently, the sensitivity of this analysis was examined using statistical methodology to impute missing values. The additional sensitivity analyses on the ITT population were based on multiple imputation, all available data, and treatment failure for subjects who discontinued due to lack of efficacy; and, finally, a treatment failure analysis on the per-protocol analysis was conducted. The results of these analyses are described in detail below and are summarized in **Table 12.**

Table 12 Four-Week Abstinence Proportions by Method of Imputation

Primary Analysis			
Using Treatment Failure (ITT Population)	32.8% (0.0449)	16.4% (0.8922)	21.9%
Additional Sensitivity Analyses			
Using Multiple Imputation (ITT Population)	44.0% (0.0297)	29.3% (0.4567)	28.6%
Based on Available Data (ITT Population)**	43.2%	24.1%	26.3%

Table 12 Four-Week Abstinence Proportions by Method of Imputation

Using Treatment Failure for Subjects Who Discontinued Due to Lack of Efficacy (ITT Population Excluding Subjects Whose Abstinence Status Could Not be Determined)	41.8% (0.0169)	22.9% (0.7455)	26.0%
Using Treatment Failure (Per-Protocol Population)	45.5% (0.0213)	20.0% (0.9017)	27.8%

* One-sided Fisher's exact test for four-week abstinence proportions compared with Group 3, at the 0.05 and 0.025 levels using Bonferroni adjustment.
** For the available population (ITT), statistical significance was not computed due to the diminished sample size.

14.1 Primary Analysis: Four Weeks of Continuous Abstinence – Treatment Failure, ITT Population

The primary endpoint of this study was 4 weeks of continuous abstinence. A treatment failure imputation was performed specifically for subjects who dropped out due to lack of efficacy and/or whose abstinence status could not be determined and, thus, were considered treatment failures. **Table 13** summarizes the proportion of subjects abstinent at 4 weeks using treatment failure imputation for the ITT population, with a one-sided significance level including a conservative adjustment for multiple comparisons. The calculated proportion of subjects abstinent at 4 weeks was 32.8% for Group 1, 16.4% for Group 2, and 21.9% for Group 3. The *p-value* for the comparison of 4-week abstinence proportions in Group 1 compared to Group 3 was 0.0449, statistically significant at the 0.05 level. A comparison of 4-week abstinence proportions between Group 2 and Group 3 did not reveal a statistically significant difference (p = 0.8922) under either threshold.

Table 13 Proportion of Subjects Abstinent at Four Weeks (Visits 6 and 7) Using Treatment Failure Imputation (ITT Population)

Corrected exhaled CO < 10ppm – n (%)	43 (37.1%)	31 (26.7%)	38 (33.3%)
No cigarettes smoked since last visit – n (%)	49 (42.2%)	29 (25.0%)	34 (29.8%)
Four-week abstinence – n (%)	38 (32.8%)	19 (16.4%)	25 (21.9%)
95% CI for 4-week abstinence rate	(24.3%, 42.1%)	(10.2%, 24.4%)	(14.7%, 30.6%)

Table 13 Proportion of Subjects Abstinent at Four Weeks (Visits 6 and 7) Using Treatment Failure Imputation (ITT Population)

p-value compared with Group 3*	0.0449	0.8922	

Subjects whose corrected CO < 10ppm, number of cigarettes smoked since last visit, or four-week abstinence cannot be determined were treated as not meeting that criterion.
N = number of randomized subjects
% = n / N x 100%
Exact 95% CI are given
* One-sided Fisher's exact test for four-week abstinence proportions compared with Group 3, at the 0.05 and 0.025 levels using Bonferroni adjustment.

Table 14 summarizes the proportion of subjects abstinent at 4 weeks using treatment failure imputation for the ITT population, stratified by study site. Although no significant treatment-by-site interactions were observed (p = 0.9361), a significant site main effect was noted (p = 0.0002). One or more aspects of executing the protocol at Site 2, and possibly Site 5, may have affected the proportion of smokers who achieved 4-week abstinence at those sites.

Table 14 Proportion of Subjects Abstinent at Four Weeks (Visits 6 and 7) Using Treatment Failure Imputation, Stratified by Study Site (ITT Population)

Site 1			
Corrected exhaled CO < 10ppm – n (%)	14 / 26 (53.8%)	11 / 28 (39.3%)	12 / 26 (46.2%)
No cigarettes smoked since last visit – n (%)	14 / 26 (53.8%)	8 / 28 (28.6%)	7 / 26 (26.9%)
Four-week abstinence – n (%)	14 / 26 (53.8%)	8 / 28 (28.6%)	7 / 26 (26.9%)
95% CI for 4-week abstinence rate	(33.4%, 73.4%)	(13.2%, 48.7%)	(11.6%, 47.8%)

Table 14 Proportion of Subjects Abstinent at Four Weeks (Visits 6 and 7) Using Treatment Failure Imputation, Stratified by Study Site (ITT Population)

Site 2			
Corrected exhaled CO < 10ppm – n (%)	0 / 16 (0.0%)	1 / 19 (5.3%)	0 / 17 (0.0%)
No cigarettes smoked since last visit – n (%)	10 / 16 (62.5%)	9 / 19 (47.4%)	8 / 17 (47.1%)
Four-week abstinence – n (%)	0 / 16 (0.0%)	1 / 19 (5.3%)	0 / 17 (0.0%)
95% CI for 4-week abstinence rate	(0.0%, 20.6%)	(0.1%, 26.0%)	(0.0%, 19.5%)
Site 3			
Corrected exhaled CO < 10ppm – n (%)	11 / 25 (44.0%)	9 / 20 (45.0%)	13 / 23 (56.5%)
No cigarettes smoked since last visit – n (%)	11 / 25 (44.0%)	6 / 20 (30.0%)	8 / 23 (34.8%)
Four-week abstinence – n (%)	11 / 25 (44.0%)	5 / 20 (25.0%)	8 / 23 (34.8%)
95% CI for 4-week abstinence rate	(24.4%, 65.1%)	(8.7%, 49.1%)	(16.4%, 57.3%)
Site 4			
Corrected exhaled CO < 10ppm – n (%)	4 / 19 (21.1%)	1 / 22 (4.5%)	2 / 20 (10.0%)
No cigarettes smoked since last visit – n (%)	3 / 19 (15.8%)	1 / 22 (4.5%)	1 / 20 (5.0%)
Four-week abstinence – n (%)	3 / 19 (15.8%)	1 / 22 (4.5%)	1 / 20 (5.0%)
95% CI for 4-week abstinence rate	(3.4%, 39.6%)	(0.1%, 22.8%)	(0.1%, 24.9%)
Site 5			
Corrected exhaled CO < 10ppm – n (%)	14 / 30 (46.7%)	9 / 27 (33.3%)	11 / 28 (39.3%)

Table 14 Proportion of Subjects Abstinent at Four Weeks (Visits 6 and 7) Using Treatment Failure Imputation, Stratified by Study Site (ITT Population)

No cigarettes smoked since last visit – n (%)	11 / 30 (36.7%)	5 / 27 (18.5%)	10 / 28 (35.7%)
Four-week abstinence – n (%)	10 / 30 (33.3%)	4 / 27 (14.8%)	9 / 28 (32.1%)
95% CI for 4-week abstinence rate	(17.3%, 52.8%)	(4.2%, 33.7%)	(15.9%, 52.4%)
Site Main Effect*			0.0002
Treatment-by-site interactions*			0.9361

Subjects whose criterion cannot be determined were treated as not meeting the criterion
N = number of randomized subjects
Exact 95% CI are given
* *p-values* were based on a logistic regression with the covariates treatment group, site, and their interactions.

Logistic regression was used to identify prognostic factors and compare treatments by adjusting for baseline covariates. A backward elimination procedure was utilized to select baseline covariates at a significance level of 0.10. The treatment group was included in the model. Using treatment failure imputation, the significant baseline covariates are shown in **Table 15**. The model identified baseline saliva cotinine level ($p = 0.0026$), number of cigarettes smoked per day ($p = 0.0200$), years of smoking ($p = 0.0888$), and treatment group ($p = 0.0320$) as significantly correlated with 4-week abstinence rates.

Table 15 Significant Baseline Covariates in Logistic Regression Model for Four-Week Abstinence Rates Using Treatment Failure Imputation (ITT Population)

Baseline Saliva Cotinine Level (1 – 500 vs > 2000 ng/mL)	0.0026	0.490	0.133	1.803
Baseline Saliva Cotinine Level (500 – 2000 vs > 2000 ng/mL)		0.355	0.195	0.645
Cigarettes per day	0.0200	0.962	0.931	0.994
Years of Smoking	0.0888	0.980	0.958	1.003
Treatment Group (Group 1 vs Group 3)	0.0320	1.648	0.892	3.043
Treatment Group (Group 2 vs Group 3)		0.703	0.354	1.396

Covariates selected by a backward elimination procedure at a two-sided significance level of 0.1. Treatment group was always included in the model.

14.2 Additional Sensitivity Analyses

14.2.1 Four Weeks of Continuous Abstinence – Multiple Imputation, ITT Population

Table 16 summarizes the proportion of abstinent subjects at 4 weeks using multiple imputation for the ITT population with the one-sided significance level, including a conservative adjustment for multiple comparisons. The calculated proportion of subjects abstinent at 4 weeks was 44.0% for Group 1, 29.3% for Group 2, and 28.6% for Group 3. The *p-value* for 4-week abstinence proportions in Group 1 compared to Group 3 was 0.0297, which was statistically significant at the 0.05 level. The *p-value* for 4-week abstinence proportions in Group 2 compared to Group 3 was 0.4567, which was not statistically significant under any threshold.

Table 16 Proportion of Subjects Abstinent at Four Weeks (Visits 6 and 7) Using Multiple Imputation (ITT Population)

Four-week abstinence – (%)	44.0%	29.3%	28.6%
95% CI for 4-week abstinence rate	(28.7%, 59.2%)	(17.2%, 41.4%)	(19.7%, 37.5%)
p-value compared with Group 3*	0.0297	0.4567	

N = number of randomized subjects
% = average % of the complete-data estimates by multiple imputation
95% CI use normal approximation
* One-sided t-test for four-week abstinence at 0.05 and 0.025 level using Bonferroni adjustment.

Table 17 summarizes the proportion of subjects abstinent at 4 weeks using multiple imputation for the ITT population, stratified by study site. Site

main effects (p = 0.2279) and treatment-by-site interactions (p = 0.7458) were not significant.

Table 17 Proportion of Subjects Abstinent at Four Weeks (Visits 6 and 7) Using Multiple Imputation, Stratified by Study Site (ITT Population)

Site 1			
Four-week abstinence – (%)	66.2%	37.1%	30.8%
95% CI for 4-week abstinence rate	(30.7%, 100.0%)	(8.5%, 65.8%)	(11.1%, 50.5%)
Site 2			
Four-week abstinence – (%)	7.5%	13.7%	3.5%
95% CI for 4-week abstinence rate	(0.0%, 21.8%)	(0.0%, 38.9%)	(0.0%, 15.1%)
Site 3			
Four-week abstinence – (%)	62.4%	48.0%	42.6%
95% CI for 4-week abstinence rate	(27.1%, 97.7%)	(22.6%, 73.4%)	(20.9%, 64.3%)
Site 4			
Four-week abstinence – (%)	27.4%	25.5%	14.0%
95% CI for 4-week abstinence rate	(0.0%, 56.4%)	(0.0%, 76.4%)	(0.0%, 39.7%)
Site 5			
Four-week abstinence – (%)	39.3%	21.5%	40.7%
95% CI for 4-week abstinence rate	(17.7%, 61.0%)	(0.2%, 42.8%)	(22.0%, 59.4%)

Table 17 Proportion of Subjects Abstinent at Four Weeks (Visits 6 and 7) Using Multiple Imputation, Stratified by Study Site (ITT Population)

Site main effect*			0.2279
Treatment-by-site interactions*			0.7458

N = number of randomized subjects
% = average percent of the complete-data estimates by multiple imputation
95% CI use normal approximation
* *p-values* were based on a logistic regression with the covariates treatment group, site, and their interactions.

14.2.2 Four Weeks of Continuous Abstinence – Available Data, ITT Population

The protocol required subjects to quit smoking at Visit 5 (Week 7), and 4-week abstinence was determined at Visit 7 (Week 11). Abstinence was determined by self report and verified by exhaled CO less than 10 ppm. If at least one of these two conditions was not met, the subject was classified as having failed to meet the primary endpoint (i.e., 4 weeks of continuous abstinence).

Table 18 summarizes the proportion of subjects abstinent at 4 weeks based on all available data for the ITT population. The proportion of smokers abstinent at 4 weeks according to the primary endpoint criteria was 43.2% (38/88) for Group 1, 24.1% (19/79) for Group 2, and 26.3% (25/95) for Group 3.

According to subjects' self report, the proportion who did not smoke any cigarettes during the 4-week interval was 56.3% for Group 1, 36.7% in Group 2, and 36.2% in Group 3. Exhaled CO less than 10 ppm was observed in 43 subjects (48.9%) in Group 1, 31 subjects (40.8%) in Group 2, and 38 subjects (40.4%) in Group 3.

Table 18 Proportion of Subjects Abstinent at Four Weeks (Visits 6 and 7) Based on Available Data (ITT Population)

Corrected exhaled CO < 10ppm – n (%)	43 / 88 (48.9%)	31 / 76 (40.8%)	38 / 94 (40.4%)
No cigarettes smoked since last visit – n (%)	49 / 87 (56.3%)	29 / 79 (36.7%)	34 / 94 (36.2%)

Table 18 Proportion of Subjects Abstinent at Four Weeks (Visits 6 and 7) Based on Available Data (ITT Population)

Four-week abstinence – n (%)*	38 / 88 (43.2%)	19 / 79 (24.1%)	25 / 95 (26.3%)

Percentage is based on the number of available subjects.
For the available population (ITT), statistical significance was not computed due to the diminished sample size.
* There are 1, 3, and 2 subjects in Groups 1, 2, and 3, respectively, who have 1 missing component of the 4-week abstinence variable and a negative response to the other component. This results in a negative response for the 4-week abstinence rate and different numbers of subjects in the summary.

14.2.3 Four Weeks of Continuous Abstinence – Treatment Failure for Subjects Who Discontinued Due to Lack of Efficacy, ITT Population Excluding Subjects Whose Abstinence Status Could Not be Determined

Table 19 summarizes 4-week abstinence proportions using treatment failure imputation for subjects who were discontinued due to lack of efficacy only; subjects whose abstinence status could not be determined were excluded from this analysis. In other words, only subjects with an evaluable endpoint were included. This approach decreased the sample size to n = 91 in Group 1, n = 83 in Group 2, and n = 96 in Group 3, and resulted in 4-week abstinence proportions of 41.8% for Group 1, 22.9% for Group 2, and 26.0% for Group 3. Comparisons among treatment groups revealed a significantly higher proportion of abstinence in Group 1 compared to Group 3 (p = 0.0169), and no significant differences between Groups 2 and 3 (p = 0.7455).

Table 19 Four-Week (Visits 6 and 7) Abstinence Proportions Using Treatment Failure Imputation for Subjects Who Discontinued Due to Lack of Efficacy Only (ITT Population Excluding Subjects Whose Abstinence Status Could Not Be Determined)

Corrected exhaled CO < 10ppm – n (%)	43 / 91 (47.3%)	31 / 83 (37.3%)	38 / 96 (39.6%)
No cigarettes smoked since last visit – n (%)	49 / 90 (54.4%)	29 / 83 (34.9%)	34 / 95 (35.8%)
Four-week abstinence – n (%)	38 / 91 (41.8%)	19 / 83 (22.9%)	25 / 96 (26.0%)
95% CI for 4-week abstinence rate	(31.5%, 52.6%)	(14.4%, 33.4%)	(17.6%, 36.0%)

Table 19 Four-Week (Visits 6 and 7) Abstinence Proportions Using Treatment Failure Imputation for Subjects Who Discontinued Due to Lack of Efficacy Only (ITT Population Excluding Subjects Whose Abstinence Status Could Not Be Determined)

p-value compared with Group 3*	0.0169	0.7455	

Subjects whose criterion cannot be determined were treated as not meeting the criterion
N = number of randomized subjects
% was based on number of randomized subjects who had non-missing data or who discontinued study due to lack of efficacy
Exact 95% CI are given
* One-sided Fisher's exact test for four-week abstinence proportions compared with Group 3, at the 0.05 and 0.025 levels using Bonferroni adjustment.

Subsequent stratification by site using this approach is provided in **Table 20**. Again, a significant site main effect was noted ($p < 0.0001$).

Table 20 Four-Week (Visits 6 and 7) Abstinence Proportions Using Treatment Failure Imputation for Subjects Who Discontinued Due to Lack of Efficacy Only (ITT Population Excluding Subjects Whose Abstinence Status Could Not Be Determined), Stratified by Study Site

Site 1			
Corrected exhaled CO < 10ppm – n (%)	14 / 20 (70.0%)	11 / 23 (47.8%)	12 / 24 (50.0%)
No cigarettes smoked since last visit – n (%)	14 / 20 (70.0%)	8 / 23 (34.8%)	7 / 24 (29.2%)
Four-week abstinence – n (%)	14 / 20 (70.0%)	8 / 23 (34.8%)	7 / 24 (29.2%)
95% CI for 4-week abstinence rate	(45.7%, 88.1%)	(16.4%, 57.3%)	(12.6%, 51.1%)
Site 2			
Corrected exhaled CO < 10ppm – n (%)	0 / 13 (0.0%)	1 / 13 (7.7%)	0 / 14 (0.0%)
No cigarettes smoked since last visit – n (%)	10 / 13 (76.9%)	9 / 13 (69.2%)	8 / 13 (61.5%)
Four-week abstinence – n (%)	0 / 13 (0.0%)	1 / 13 (7.7%)	0 / 14 (0.0%)
95% CI for 4-week abstinence rate	(0.0%, 24.7%)	(0.2%, 36.0%)	(0.0%, 23.2%)
Site 3			
Corrected exhaled CO < 10ppm – n (%)	11 / 17 (64.7%)	9 / 12 (75.0%)	13 / 20 (65.0%)
No cigarettes smoked since last visit – n (%)	11 / 17 (64.7%)	6 / 12 (50.0%)	8 / 20 (40.0%)
Four-week abstinence – n (%)	11 / 17 (64.7%)	5 / 12 (41.7%)	8 / 20 (40.0%)
95% CI for 4-week abstinence rate	(38.3%, 85.8%)	(15.2%, 72.3%)	(19.1%, 63.9%)

Table 20 Four-Week (Visits 6 and 7) Abstinence Proportions Using Treatment Failure Imputation for Subjects Who Discontinued Due to Lack of Efficacy Only (ITT Population Excluding Subjects Whose Abstinence Status Could Not Be Determined), Stratified by Study Site

Site 4			
Corrected exhaled CO < 10ppm – n (%)	4 / 14 (28.6%)	1 / 11 (9.1%)	2 / 15 (13.3%)
No cigarettes smoked since last visit – n (%)	3 / 13 (23.1%)	1 / 11 (9.1%)	1 / 15 (6.7%)
Four-week abstinence – n (%)	3 / 14 (21.4%)	1 / 11 (9.1%)	1 / 15 (6.7%)
95% CI for 4-week abstinence rate	(4.7%, 50.8%)	(0.2%, 41.3%)	(0.2%, 31.9%)
Site 5			
Corrected exhaled CO < 10ppm – n (%)	14 / 27 (51.9%)	9 / 24 (37.5%)	11 / 23 (47.8%)
No cigarettes smoked since last visit – n (%)	11 / 27 (40.7%)	5 / 24 (20.8%)	10 / 23 (43.5%)
Four-week abstinence – n (%)	10 / 27 (37.0%)	4 / 24 (16.7%)	9 / 23 (39.1%)
95% CI for 4-week abstinence rate	(19.4%, 57.6%)	(4.7%, 37.4%)	(19.7%, 61.5%)
Site Main Effect*			< .0001
Treatment-by-site interactions*			0.6710

Subjects whose criterion cannot be determined were treated as not meeting the criterion
N = number of randomized subjects
% was based on number of randomized subjects who had non-missing data or who discontinued study due to lack of efficacy
Exact 95% CI are given
* *p-values* were based on a logistic regression with the covariates treatment group, site, and their interactions.

14.2.4 Four Weeks of Continuous Abstinence – Per-Protocol Population

Table 21 summarizes 4-week abstinence proportions using the treatment failure imputation for the per-protocol population. Four-week abstinence proportions were 45.5% for Group 1, 20.0% for Group 2, and 27.8% for Group 3. Group 1 had a higher proportion of subjects who abstained than Group 3 (p = 0.0213), while Group 2 had a lower proportion of subjects who abstained compared to Group 3 (p = 0.9017).

Table 21 Four-Week (Visits 6 and 7) Abstinence Proportions, Using Treatment Failure Imputation (Per-Protocol Population)

Corrected exhaled CO < 10ppm – n (%)	35 / 66 (53.0%)	23 / 62 (37.1%)	34 / 78 (43.6%)
No cigarettes smoked since last visit – n (%)	41 / 65 (63.1%)	20 / 65 (30.8%)	29 / 78 (37.2%)
Four-week abstinence – n (%)*	30 / 66 (45.5%)	13 / 65 (20.0%)	22 / 79 (27.8%)
95% CI for 4-week abstinence rate	(33.1%, 58.2%)	(11.1%, 31.8%)	(18.3%, 39.1%)
p-value compared with Group 3**	0.0213	0.9017	

N = number of randomized subjects in the per-protocol population
Exact 95% CI are given
*There are 1, 3 and 2 subjects in Groups 1, 2 and 3, respectively, having 1 missing component of the 4-week abstinence and a negative response of the other component. This results in a negative response for the 4-week abstinence and different numbers of subjects in the summary.
** One-sided Fisher's exact test for four-week abstinence proportions compared with Group 3, at the 0.05 and 0.025 levels using Bonferroni adjustment.

Table 22 summarizes 4-week abstinence proportions by site using the treatment failure imputation for the per-protocol population. A significant site main effect was observed ($p = 0.0054$) for the 4-week abstinence proportions using treatment failure imputation for the per-protocol population.

Table 22 Four-Week (Visits 6 and 7) Abstinence Proportions Using Treatment Failure Imputation, Stratified by Study Site (Per-Protocol Population)

Site 1			
Corrected exhaled CO < 10ppm – n (%)	12 / 15 (80.0%)	6 / 16 (37.5%)	9 / 20 (45.0%)
No cigarettes smoked since last visit – n (%)	12 / 15 (80.0%)	4 / 16 (25.0%)	5 / 20 (25.0%)
Four-week abstinence – n (%)	12 / 15 (80.0%)	4 / 16 (25.0%)	5 / 20 (25.0%)
95% CI for 4-week abstinence rate	(51.9%, 95.7%)	(7.3%, 52.4%)	(8.7%, 49.1%)
Site 2			
Corrected exhaled CO < 10ppm – n (%)	0 / 12 (0.0%)	1 / 9 (11.1%)	0 / 11 (0.0%)
No cigarettes smoked since last visit – n (%)	10 / 12 (83.3%)	6 / 9 (66.7%)	6 / 10 (60.0%)
Four-week abstinence – n (%)*	0 / 12 (0.0%)	1 / 9 (11.1%)	0 / 11 (0.0%)
95% CI for 4-week abstinence rate	(0.0%, 26.5%)	(0.3%, 48.2%)	(0.0%, 28.5%)
Site 3			
Corrected exhaled CO < 10ppm – n (%)	7 / 11 (63.6%)	8 / 11 (72.7%)	12 / 17 (70.6%)
No cigarettes smoked since last visit – n (%)	7 / 11 (63.6%)	5 / 11 (45.5%)	7 / 17 (41.2%)

Table 22 Four-Week (Visits 6 and 7) Abstinence Proportions Using Treatment Failure Imputation, Stratified by Study Site (Per-Protocol Population)

Four-week abstinence – n (%)	7 / 11 (63.6%)	4 / 11 (36.4%)	7 / 17 (41.2%)
95% CI for 4-week abstinence rate	(30.8%, 89.1%)	(10.9%, 69.2%)	(18.4%, 67.1%)
Site 4			
Corrected exhaled CO < 10ppm – n (%)	4 / 10 (40.0%)	0 / 7 (0.0%)	2 / 11 (18.2%)
No cigarettes smoked since last visit – n (%)	3 / 9 (33.3%)	0 / 8 (0.0%)	1 / 12 (8.3%)
Four-week abstinence – n (%)*	3 / 10 (30.0%)	0 / 8 (0.0%)	1 / 12 (8.3%)
95% CI for 4-week abstinence rate	(6.7%, 65.2%)	(0.0%, 36.9%)	(0.2%, 38.5%)
Site 5			
Corrected exhaled CO < 10ppm – n (%)	12 / 18 (66.7%)	8 / 19 (42.1%)	11 / 19 (57.9%)
No cigarettes smoked since last visit – n (%)	9 / 18 (50.0%)	5 / 21 (23.8%)	10 / 19 (52.6%)
Four-week abstinence – n (%)*	8 / 18 (44.4%)	4 / 21 (19.0%)	9 / 19 (47.4%)
95% CI for 4-week abstinence rate	(21.5%, 69.2%)	(5.4%, 41.9%)	(24.4%, 71.1%)

Table 22 Four-Week (Visits 6 and 7) Abstinence Proportions Using Treatment Failure Imputation, Stratified by Study Site (Per-Protocol Population)

Site Main Effect**			0.0054
Treatment-by-site interactions**			0.4072

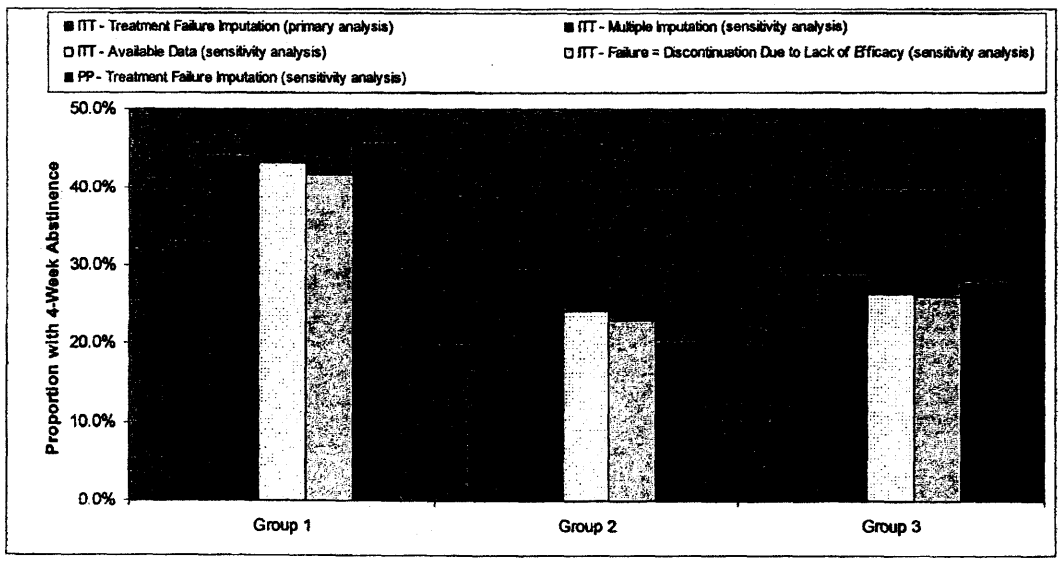
N = number of randomized subjects in the per-protocol population
% was based on number of randomized subjects in the per-protocol population who had non-missing data
Exact 95% CI are given
*There are 1, 3 and 2 subjects in Groups 1, 2 and 3, respectively, having 1 missing component of the 4-week abstinence and a negative response of the other component. This results in a negative response for the 4-week abstinence and different numbers of subjects in the summary.
** *p-values* were based on a logistic regression with the covariates treatment group, site, and their interactions.

14.3 Summary

In summary, **Figure G** (below) illustrates the 4-week abstinence proportions using the various analysis cohorts described above. The 4-week abstinence proportions observed in Group 1 (Quest 1, 2, and 3 with NRT) were significantly higher than the 4-week abstinence proportions observed in Group 3 (Active Control cigarettes with NRT) for the primary analysis, treatment failure imputation in the ITT population ($p < 0.05$), as well as for the additional sensitivity analyses: for multiple imputation in the ITT population ($p < 0.05$), for treatment failure imputation for subjects who discontinued due to lack of efficacy in the ITT population ($p < 0.025$), and for treatment failure imputation in the per-protocol population ($p < 0.025$). No significant differences were noted between Groups 2 and 3.

Figure G

FOUR-WEEK ABSTINENCE PROPORTIONS[8]



* = statistically significant at p = 0.05 level (Group 1 compared with Group 3)
** = statistically significant at p = 0.025 level (Group 1 compared with Group 3)

[8] For the available population (ITT), statistical significance was not computed due to the diminished sample size.

Using all statistical analysis methods, this study showed that Group 1 was statistically different from Group 3, under a 0.05 significance level. Using the Bonferroni adjusted value (i.e., 0.025), statistical significance was maintained in the ITT treatment failure analysis for subjects who discontinued due to lack of efficacy and in the per-protocol analysis with treatment failure imputation. Irrespective of the analysis, no significant differences were noted when comparing Group 2 with Group 3. However, the data show that Quest alone may be as effective as standard of care NRT.

15.0 SECONDARY EFFICACY ENDPOINTS

15.1 Quit Rates at 3 and 6 Months Following Treatment Termination

Abstinence at 3 Months (or 6 Months) was defined as:

- No cigarettes smoked from Week 7 to Month 3 (or Month 6)
- Corrected exhaled CO < 10 ppm from Visit 6 to Visit 11 (or Visit 12)

Tables S6 and **S7** in **Appendix A** list the reasons for missing data at the 3-month and 6-month visits, respectively. One hundred and three (29.8%) subjects had missing 6-month abstinence status, which included the 89 (25.7%) subjects whose abstinence status was missing at 3 months. The most common reason for missing 6-month abstinence status was lost-to-follow-up (n = 38), followed by discontinuation at the subject's request / withdrawal of consent (n = 33) and failure to quit smoking (n = 15).

Three-month and six-month abstinence proportions where those subjects missing abstinence status were counted as failures are summarized in **Table S8** (**Appendix A**). Three-month abstinence proportions were 17.2% in Group 1, 10.3% in Group 2, and 14.9% in Group 3. Six-month abstinence proportions were 11.2% in Group 1, 7.8% in Group 2, and 9.6% in Group 3. No statistically significant differences in abstinence were observed between Groups 1 and 3 or between Groups 2 and 3, for either 3-month or 6-month abstinence.

Three-month and six-month abstinence proportions using the failure imputation for the ITT population stratified by site are provided in **Tables S9** and **S10**,

respectively (**Appendix A**). Only 3-month abstinence proportions showed a significant site main effect ($p = 0.0242$) using the treatment failure imputation.

Baseline covariates were evaluated in a logistic regression model for 3-month and 6-month abstinence rates (**Tables S11** and **S12** in **Appendix A**). As shown in **Table S11**, baseline saliva cotinine levels were significantly correlated with 3-month abstinence rates.

Three-month and six-month abstinence proportions were also determined where subjects who were discontinued due to lack of efficacy were included as failures. Subjects whose abstinence status could not be determined were excluded. Using this approach (**Table S13** in **Appendix A**), 3-month abstinence proportions were 22.5% for Group 1, 14.8% for Group 2, and 18.5% for Group 3; and 6-month abstinence proportions were 14.8% for Group 1, 11.3% for Group 2, and 12.2% for Group 3. No significant differences were observed when comparing either Group 1 or Group 2 to Group 3, with respect to 3-month and 6-month abstinence. Site stratification revealed a significant site main effect for 3-month abstinence ($p = 0.041$) only (**Table S14** in **Appendix A**). There were no significant observations for 6-month abstinence stratified by sites (**Table S15** in **Appendix A**).

Table S16 in **Appendix A** summarizes 3-month and 6-month abstinence proportions for the per-protocol population. For the per-protocol population, 3-month abstinence proportions were 26.2% for Group 1, 12.7% for Group 2, and 21.1% for Group 3. No significant differences were observed when comparing either Group 1 or Group 2 to Group 3.

For the per-protocol population, 6-month abstinence proportions were 16.7% for Group 1, 8.1% for Group 2, and 13.9% for Group 3. Again, no significant differences were observed when comparing either Group 1 or Group 2 to Group 3.

As shown in **Tables S17** and **S18 (Appendix A)**, respectively, site stratification of 3-month and 6-month abstinence determined for the per-protocol population did not reveal any significant site main effects or treatment-by-site interactions.

Tables 23 and **24** summarize 3-month and 6-month abstinence proportions by method of imputation, respectively.

Table 23 3-Month Abstinence Proportions by Method of Imputation

Treatment Failure (ITT Population)	20 / 116 (17.2%)	12 / 116 (10.3%)	17 / 114 (14.9%)
Treatment Failure Due to Lack of Efficacy (Excluding Subjects Whose Abstinence Status Could Not Be Determined)	20 / 89 (22.5%)	12 / 81 (14.8%)	17 / 92 (18.5%)
Treatment Failure (Per-Protocol Population)	17 / 65 (26.2%)	8 / 63 (12.7%)	16 / 76 (21.1%)

Table 24 6-Month Abstinence Proportions by Method of Imputation

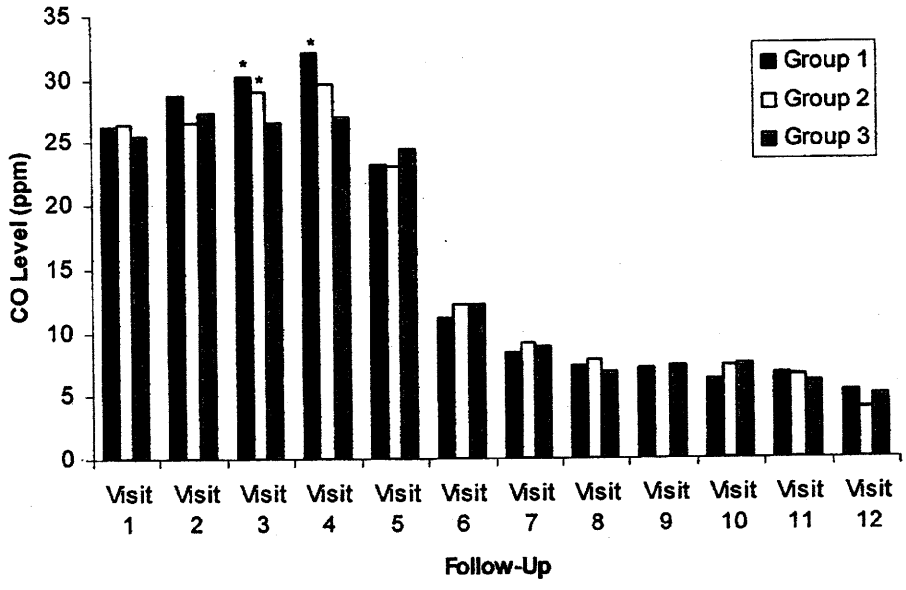
Treatment Failure (ITT Population)	13 /116 (11.2%)	9 / 116 (7.8%)	11 / 114 (9.6%)
Treatment Failure Due to Lack of Efficacy (Excluding Subjects Whose Abstinence Status Could Not Be Determined)	13 / 88 (14.8%)	9 / 80 (11.3%)	11 / 90 (12.2%)
Treatment Failure (Per-Protocol Population)	10 / 60 (16.7%)	5 / 62 (8.1%)	10 / 72 (13.9%)

15.2 Corrected Exhaled CO Levels and Change from Baseline

A summary of the corrected exhaled CO levels for the ITT population is provided in **Table S19** in **Appendix A.**

Figure H (below) illustrates the corrected CO for each treatment group by visit. There were significant differences ($p \leq 0.05$) when comparing Group 1 to Group 3 at Visit 3 and Visit 4, and when comparing Group 2 to Group 3 at Visit 3. While there appeared to be an initial trend in CO levels between groups at Visit 3 and Visit 4, this trend was not sustained over the remaining visits.

Figure H
CORRECTED EXHALED CO LEVELS



* *p-value* ≤ 0.05 when compared with Group 3.

Table S20 in **Appendix A** summarizes the change from baseline in corrected exhaled CO levels. At Visit 5, the mean decrease from baseline in exhaled CO

level of -5.4 (SD 14.4) for Group 1 was significantly greater than the mean decrease of -2.5 (SD 11.2) in Group 3. Given one out of 20 comparisons (i.e., 5.0%), this is likely an isolated finding due to chance alone.

Saliva cotinine concentration levels were measured using Accutest NicAlert. The results were categorized as follows: 1-10 ng/mL, 10 – 30 ng/mL, 30 – 100 ng/mL, 100 – 200 ng/mL, 200 – 500 ng/mL, 500 – 2000 ng/mL, and > 2000 ng/mL. Saliva cotinine concentration levels are summarized in **Table S21** in **Appendix A**. Significant differences involving saliva cotinine concentration levels were noted when comparing Group 1 to Group 3 at Visit 2, and when comparing Group 2 to Group 3 at Visits 5 and 6.

15.3 Minnesota Nicotine Withdrawal Questionnaire

Subjects were asked to complete the Minnesota Nicotine Withdrawal Questionnaire at each visit, including the screening visit. Subjects were asked to rate the following categories "for the period of today": 1) depressed mood / sad; 2) insomnia / sleep problems / awakening at night; 3) angry / irritable / frustrated; 4) anxious / nervous; 5) difficulty concentrating; 6) restless / impatient; 7) increased appetite / hungry / weight problem; and 8) desire or craving to smoke. The rating scale consisted of none, slight, mild, moderate, or severe. The Minnesota Nicotine Withdrawal Questionnaire does not have a composite scoring system.

Each question was analyzed individually by the two-sided Jonckheere-Terpstra test and the resulting data are presented in **Appendix A, Tables S22.1** through **S22.8**. Furthermore, the change from baseline was analyzed using the Wilcoxon rank-sum test (**Appendix A, Tables S23.1** through **S23.8**).

Given the number of statistical tests required and the infrequency with which results were found to be statistically significant, it is likely that some of the following results were due to chance. These results may, however, be informative in designing future studies.

- Depressed mood / sad: Beyond the screening visit, only subjects in Group 1 selected slight or mild depressed mood / sad more frequently than subjects in Group 3 at Visit 5 ($p = 0.0223$; **Table S22.1** in **Appendix A**).

- Insomnia / sleep problems / awakening at night: A higher percentage of subjects in Group 1 indicated sleep problems compared with subjects in Group 3 at Visit 5 ($p = 0.0002$; **Table S22.2** in **Appendix A**). Statistical significance was also reflected in the change from baseline involving insomnia / sleep problems / awakening at night. The mean change in Group 1 was 0.5 ± 1.4, while the mean change in Group 3 was -0.1 ± 1.1 at Visit 5 ($p = 0.0004$) (**Table S23.2** in **Appendix A**).

- Angry / irritable / frustrated: A significantly higher proportion ($p = 0.004$) of subjects in Group 1 reported being angry / irritable / frustrated than Group 3 subjects at Visit 5 (**Table S22.3** in **Appendix A**). As presented in **Table S23.3** in **Appendix A**, the mean change at Visit 5 from baseline involving being angry / irritable / frustrated was 0.5 ± 1.3 in Group 1 compared to 0.1 ± 1.1 in Group 3 ($p = 0.0198$). In addition, the mean change at Visit 4 from baseline involving being angry / irritable / frustrated was 0.5 ± 1.2 in Group 2 compared to 0.1 ± 1.2 in Group 3 ($p = 0.0214$).

- Anxious / nervous: A higher percentage of being anxious / nervous was reported by subjects in Group 1 compared to those in Group 3 at Visit 5 ($p = 0.005$; **Table S22.4** in **Appendix A**).

- Difficulty concentrating: A significantly higher proportion of subjects in Group 1 reported difficulty concentrating at Visit 4 ($p = 0.0189$), Visit 5 ($p = 0.0044$), and Visit 6 ($p = 0.0372$) compared to subjects in Group 3 (**Table S22.5** in **Appendix A**).

- Increased appetite / hunger / weight problems: Subjects in Group 1 indicated a higher rate of increased appetite / hunger / weight problems than subjects in Group 3 at Visit 5 ($p = 0.0332$; **Table S22.7** in **Appendix A**). This statistically significant finding was also reflected in the change from baseline involving increased appetite / hunger / weight problems. The mean change in Group 1 was 0.6 ± 1.1, while the mean change in Group 3 was 0.3 ± 1.2 at Visit 5 ($p = 0.0472$) (**Table S23.7** in **Appendix A**). Furthermore, significant differences in change from baseline involving increased appetite / hunger / weight problems were noted when comparing Group 2 to Group 3 at Visit 9 ($p = 0.0328$) and at Visit 10 ($p = 0.0176$).

- Desire or craving to smoke: Interestingly, at Visit 3, significantly more subjects in Groups 1 and 2 experienced desire or craving to smoke or a significantly more intense desire or craving to smoke than subjects in Group 3, following 2 weeks of using Quest 1 cigarettes. This phenomenon was not observed following 2 weeks of using Quest 2 at Visit 4 or using Quest 3 with or without the NRT patch at Visit 5 (**Table S22.8** in **Appendix A**). Subjects in Group 1 indicated a higher rate of desire or craving to smoke than subjects in Group 3 at Visit 12 ($p = 0.0466$; **Table S22.8** in **Appendix A**). This statistically significant finding was also observed in the change at Visit 12 from baseline involving desire or craving to smoke when comparing Group 1 and Group 3 (**Table S23.8** in **Appendix A**).

15.4 Cigarette Evaluation Scale Questionnaire

Subjects were asked to complete the Cigarette Evaluation Scale at Visits 1 through 5. A total of 11 questions were asked, which were categorized into 5 component scales (satisfaction, psychological reward, nausea / dizziness, enjoy sensation, and reduce craving), and where subjects were asked to rate the last cigarette smoked using the following rating scheme:

- not at all
- very little
- a little
- moderately
- a lot
- quite a lot
- extremely

Replies to each of the 11 questions were tabulated and analyzed using the Jonckheere-Terpstra test (see **Appendix A, Tables S24.6** through **S24.17**). In addition, the change from baseline was calculated and analyzed using the Wilcoxon rank-sum test for all questions (**Appendix A, Tables S25.6** through **S25.17**). The results from the 5 compent scales and the change in baseline from the 5 component scales are described below:

- Satisfaction: Subjects in Group 1 were less satisfied than subjects in Group 3 for both Visit 4 ($p = 0.0454$) and Visit 5 ($p < 0.0001$). Subjects in Group 2 were less satisfied than subjects in Group 3 for Visit 5 ($p = 0.0006$), as seen in **Appendix A, Table S24.1.** Regarding changes from baseline, both Group 1 and Group 2 were less satisfied than Group 3 ($p = 0.0004$ and $p = 0.0018$, respectively; see **Appendix A, Table S25.1**).

- Psychological reward: There were no statistically significant differences between Group 1 or Group 2 compared to Group 3 (see **Appendix A, Table 24.2**); and there were no significant differences from baseline between either Group 1 or Group 2 compared to Group 3 (see **Appendix A, Table 25.2**)

- Nausea / dizziness: Both Group 1 and Group 2 experienced less nausea / dizziness than Group 3 at Visit 3 ($p = 0.0272$ and $p = 0.0048$, respectively), suggesting that the gradual transition to a lower nicotine level from Quest helped ease nicotine withdrawal symptoms (see **Appendix A, Table 24.3**). Group 2 also experienced less nausea / dizziness at Visit 4 ($p = 0.0062$) compared to Group 3. Regarding changes from baseline, Group 2 experienced less nausea / dizziness at Visit 3 ($p = 0.0332$) compared to Group 3 (see **Appendix A, Table 25.3**).

- Enjoy sensation: Both Group 1 and Group 2 experienced lower enjoyment of the cigarette sensation than Group 3 at Visit 5 ($p = 0.0025$ and $p = 0.0078$, respectively), as seen in **Appendix A, Table 24.4**. Regarding changes from baseline, Group 2 experienced lower enjoyment of the cigarette sensation than Group 3 at Visit 5 ($p = 0.0340$; see **Appendix A, Table 25.4**).

- Reduced craving: Both Group 1 and Group 2 reported higher reductions in craving than Group 3 at Visit 2 ($p = 0.0005$ and $p = 0.0012$, respectively), although both groups reported lower reductions in craving compared to Group 3 at Visit 5 ($p = 0.0096$ and $p = 0.0647$, respectively), as seen in **Appendix A, Table 24.5**. Regarding changes from baseline, Group 1 and Group 2 reported lower reductions in craving compared to Group 3 at Visits 3, 4, and

5 ($p = 0.0016$ and 0.0006 at Visit 3; $p = 0.0016$ and $p = 0.0010$ at Visit 4; $p < 0.0001$ and $p < 0.0001$ at Visit 5; see **Appendix A, Table 25.5**)

These results suggest that earlier studies noting decreased satisfaction and enjoyment of the cigarette sensation of Quest 2 and Quest 3 were accurate, especially with regards to Quest 3. The tobacco mix for higher denicotinized content was associated with a "bad" taste (**Table S25.7** in **Appendix A**).

15.5 Sensory Questionnaire

Subjects were asked to complete the Sensory Questionnaire at Visits 1 through 5. A total of nine questions were asked regarding the sensations subjects experienced from puffs of the last study cigarette smoked (e.g., how satisfying were the puffs from your last cigarette?). The following rating scheme was used in response to each question:

- not at all
- very little
- a little
- moderately
- a lot
- quite a lot
- extremely

Replies were tabulated and analyzed using the Jonckheere-Terpstra test (see **Appendix A, Tables S26.1** through **S26.9**). In addition, the change from baseline was calculated and analyzed using the Wilcoxon rank-sum test (**Appendix A, . Tables S27.1** through **S27.9**).

As shown in **Tables S26.1** and **S26.2**, subjects smoking Quest 3 cigarettes liked the puffs of their last cigarette considerably less and indicated that the puffs were significantly less satisfying than subjects smoking conventional cigarettes.

As illustrated in **Table S26.3**, subjects smoking either Quest 1 (Visit 3), Quest 2 (Visit 4), or Quest 3 (Visit 5) rated the nicotine level of the study cigarette significantly lower than subjects smoking conventional cigarettes.

Table S26.4 summarizes how similar the puffs of the last study cigarette were rated compared to the subject's own brand. Subjects rated the puffs of Quest 2 (Visit 4) and Quest 3 (Visit 5) as considerably more different than their usual brand compared to subjects using conventional cigarettes.

In general, regarding the strength of puffs on the tongue, in the back of the mouth and throat, windpipe, and chest, only subjects in Group 1 reported a reduced strength while using Quest 1, 2, and 3 compared to Group 3, while no significant differences were observed when comparing Group 2 with Group 3 (**Tables S26.5, S26.7** through **26.9**).

15.6 Fagerström Test for Nicotine Dependence

The Fagerström Test for Nicotine Dependence (FTND) was completed at Visits 1 through 5. Subjects were asked to answer 6 questions in order to calculate their level of nicotine dependence (see **Table S28**). **Table S29** summarizes the change in baseline for FTND score.

In addition to the calculated level of dependence, replies to all 6 questions were tabulated and analyzed using the Jonckheere-Terpstra test (see **Appendix A, Tables S30.1** through **S30.6**). In addition, the change from baseline was

calculated and analyzed using the Wilcoxon rank-sum test (**Appendix A, Tables S31.1** through **S31.6**).

No significant differences involving the individual questions were observed when comparing Group 1 with Group 3 or Group 2 with Group 3.

16.0 SAFETY ENDPOINTS

16.1 Adverse Events

A summary of adverse events (AEs) experienced by study subjects is provided in **Table 25**. The proportion of treatment-emergent AEs was 61.2% (n = 71) in Group 1, 52.6% (n = 61) in Group 2, and 60.5% (n = 69) in Group 3. The proportion of treatment-related AEs was 44.0% (n = 51) in Group 1, 27.6% (n = 32) in Group 2, and 36.8% (n = 42) in Group 3.

Table 25 Summary of Adverse Events (Safety Population)

Treatment-emergent AEs	71 (61.2%)	61 (52.6%)	69 (60.5%)
Treatment-related AEs	51 (44.0%)	32 (27.6%)	42 (36.8%)
Possibly Related	38 (32.8%)	24 (20.7%)	34 (29.8%)
Probably Related	10 (8.6%)	7 (6.0%)	8 (7.0%)
Definitely Related	12 (10.3%)	5 (4.3%)	9 (7.9%)
Serious AEs	2 (1.7%)	0	1 (0.9%)
Treatment-related serious AEs	0	0	0
AEs leading to study product stopped	4 (3.4%)	1 (0.9%)	4 (3.5%)
AEs leading to study discontinuation	3 (2.6%)	2 (1.7%)	3 (2.6%)

N = number of randomized subjects who received study treatment
% = n/N x 100%

A total of eleven (11) subjects experienced an AE that was either directly or indirectly related to discontinuation from the study (**Table 26**). Of these 11 subjects, nine stopped the study product due to an AE. However, three of these nine subjects discontinued the study for reasons other than an AE (e.g., withdrew informed consent, failure to quit smoking.). The additional two subjects were discontinued from the study due to the occurrence of an AE during the course of treatment. Summaries of the subjects who were discontinued from the study due to product being stopped or an AE are provided in **Appendix B.**

Table 26 Subjects Who Experienced Adverse Events Directly or Indirectly Leading to Study Discontinuation

01295	1	Nausea	Yes	Yes
03051	1	Nausea / vivid dreams	Yes	No
03067	1	Rash, itchiness under patch site	Yes	Yes
04015	1	Chest urticaria	Yes	No
03060	2	Itchiness on chest and legs / redness under patch site	Yes	Yes
01311	3	Headache and cough	Yes	Yes
02043	3	Contact dermatitis	Yes	Yes
05110	3	Localized muscle aches under patch site	Yes	Yes
05245	3	Rash under patch site	Yes	No
03064	1	Chest pressure	No	Yes
04003	2	Shortness of breath / chest pain / insomnia	No	Yes

16.2 Serious Adverse Events

SAEs were reported for three subjects: two subjects in Group 1 and one subject in Group 3. Chest discomfort and increased blood pressure were reported for subject 03064, fibula and tibia fractures were reported for subject 05121, and bladder carcinoma was reported for subject 01084. Individual summaries involving these SAEs are provided in **Appendix B**. None of these three reported SAEs were considered treatment-related (see **Table S32**).

Table 27 summarizes SAEs by system organ class and preferred term.

Table 27 Summary of Serious Adverse Events by System Organ Class and Preferred Term (Safety Population)

Subjects with Any Serious Adverse Events	2 (2%)	0	1 (<1%)
General disorders and administration site conditions	1 (<1%)	0	0
Chest discomfort	1 (<1%)	0	0
Injury, poisoning and procedural complications	0	0	1 (<1%)
Fibula fracture	0	0	1 (<1%)
Tibia fracture	0	0	1 (<1%)
Investigations	1 (<1%)	0	0
Blood pressure increased	1 (<1%)	0	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (<1%)	0	0
Bladder neoplasm	1 (<1%)	0	0

N = number of randomized subjects who received study treatment
% = n/N x 100%

As indicated previously, nine subjects stopped taking the study product due to an AE and subsequently discontinued from the study. **Table 28** provides a summary of these nine subjects, categorized by system organ class and preferred term. An additional two subjects discontinued the study due to an AE alone.

Table 28 Summary of Adverse Events Leading Directly to Study Discontinuation by System Organ Class and Preferred Term (Safety Population)

Subjects with Any Adverse Events Leading to Study Product Stopped	4 (3%)	1 (<1%)	4 (4%)
Gastroinestinal disorderse	2 (2%)	0	0
Nausea	2 (2%)	0	0
General disorders and administration site conditions	1 (<1%)	1 (<1%)	2 (2%)
Application site dermatitus	1 (<1%)	0	1 (<1%)
Application site discomfort	0	0	1 (<1%)
Application site erythema	0	1 (<1%)	0
Application site pruritus	1 (<1%)	0	0
Nervous system disorders	0	0	1 (<1%)
Headache	0	0	1 (<1%)
Psychiatric disorders	1 (<1%)	0	0
Abnormal dreams	1 (<1%)	0	0
Skin and subcutaneous tissue disorders	1 (<1%)	1 (<1%)	1 (<1%)
Dermatitus contact	0	0	1 (<1%)
Pruritus	0	1 (<1%)	0
Urticaria	1 (<1%)	0	0

N = number of randomized subjects who received study treatment
% = n/N x 100%

Table S33 in **Appendix A** provides a summary of treatment-emergent AEs by system organ class and preferred term.

Table S34 in **Appendix A** provides a summary of treatment-related AEs by system organ class and preferred term. The most common treatment-related AE consisted of psychiatric disorders. The most commonly reported psychiatric disorders were insomnia, abnormal dreams, irritability, and anxiety.

Other frequently reported treatment-related AEs were nervous system disorders, and general disorders and administration site conditions. Headache was the most predominantly reported nervous system disorder. Application site disorders related to the NRT / placebo patch application included pruritus, dermatitis, discomfort, irritation, discoloration, and erythema.

Table S35 in **Appendix A** provides a summary of treatment-emergent AEs by maximum severity, system organ class, and preferred term. Since subjects began using the patch at Visit 4, **Table S36** in **Appendix A** provides a summary of AEs experienced after Visit 4 by system organ class and preferred term. **Table S37** in **Appendix A** provides a summary of severe or life threatening AEs by system organ class and preferred term.

16.3 Vital Signs

Table S38 in **Appendix A** provides a summary of vital signs (heart rate and blood pressure by visit and treatment group). Clinically significant changes in heart rate or blood pressure were reported as an AE. Otherwise, there were no clinically significant changes in vital sign parameters noted.

16.4 Concomitant Medications

Concomitant medication usage was collected at baseline and throughout the study, and the results are tabulated in **Tables S39** and **S40,** respectively **(Appendix A).**

16.5 Medical History and Physical Examination

Table S41 summarizes the medical history by body category. Medical history obtained showed that the highest proportion of reported disorders involved the musculoskeletal system, gastrointestinal tract, and genitourinary tract.

Tables S42.1 through **S42.3** summarize the findings at physical examination for baseline, Visit 10, and the end of the study, respectively.

17.0 SUMMARY

A total of 346 subjects were enrolled at 5 participating centers in the US. Two of the 346 subjects (0.6%) did not meet all eligibility criteria. All enrolled subjects received study treatment: 116 subjects were randomized to Group 1 (i.e., Quest 1, Quest 2, Quest 3 plus NRT), 116 subjects were randomized to Group 2 (i.e., Quest 1, Quest 2, Quest 3 plus placebo patch), and 114 subjects were randomized to Group 3 (i.e., active control cigarettes plus NRT). Eighty-five subjects (24.6%) completed the study through Visit 12. The most common reasons for early study discontinuation were failure to quit smoking (38.4%), lost to follow-up (17.1%), and discontinuation at the subject's request / withdrawal of consent (13.3%). The median time to drop out was 12.8 weeks for Group 1, 10.5 weeks for Group 2, and 14.2 weeks for Group 3.

Of the 346 subjects enrolled, 53.8% were male and 46.2% were female. The mean age was 45.9 years (SD 10.9; range 21 – 66 years). The majority of subjects were Caucasian (92.2%). The vast majority of subjects (44.8%) reported smoking light cigarettes, followed by full flavor cigarettes (21.7%), and ultra-light cigarettes (19.1%). As anticipated, over 90% of subjects indicated that this was not their first attempt to quit smoking; most subjects reported between one through four previous quit attempts. The mean period of smoking reported was 26.8 years (SD 11.3; range 1 – 53 years). At baseline, the mean corrected CO measurement was 27.5 ppm (SD 10.7; range 14 – 82 ppm). Saliva cotinine levels were > 2000 ng/mL in 56.6% of subjects, and between 500 to 2000 ng/mL in 38.2% of subjects. Subjects reported smoking most frequently either at home (54.6%) or at work (28.9%).

A total of 109 major protocol violations, defined *a priori* as issues of eligibility and adherence to the study protocol, were identified in 90 subjects. The majority of the protocol violations involved compliance with the trial regimen, specifically NRT compliance less than 75% (n = 55, 15.9%) and study cigarette compliance less than 95% (n = 43, 12.4%).

Three study populations were defined:

1. The intent-to-treat (ITT) population, which consisted of all randomized subjects (n = 346), regardless of whether a subject received study treatment.

2. The per-protocol population, which consisted of subjects (n = 211) who satisfied the following criteria:
   - The 4-week abstinence status could be determined. Subjects who discontinued due to failure to quit smoking before week 7 were considered failures.
   - Absence of major protocol violations, including the study entry criteria.

3. The safety population, which consisted of all randomized subjects who received at least one study cigarette (n = 346).

17.1 Primary Efficacy Endpoint

The primary efficacy endpoint was 4 weeks of continuous abstinence from smoking. Abstinence rates were verified by self reports and exhaled CO less than 10 ppm. If at least one of these two conditions was not met, the subject failed to meet the primary endpoint (i.e., 4 weeks of continuous abstinence). At the determination of 4 week abstinence, the proportion of available subjects was 60.3% in Group 1, 49.1% in Group 2, and 61.4% in Group 3.

A treatment failure imputation was performed specifically for subjects who dropped out due to lack of efficacy and/or whose abstinence status could not be determined, and who were thus considered treatment failures. Using treatment failure imputation for the ITT population, the 4-week abstinence proportions were

32.8% for Group 1, 16.4% for Group 2, and 21.9% for Group 3. The treatment effects were statistically significant at the 0.05 significance level (p = 0.0449). A comparison of Group 2 to Group 3 did not reveal any statistical differences. Four-week abstinence proportions stratified by site revealed a significant site main effect (p = 0.0002). A logistic regression model was applied involving the collected baseline variables to explore the site effect. The model identified baseline saliva cotinine level, number of cigarettes smoked per day, years of smoking, and treatment group as factors that may have affected abstinence outcomes by site.

Four-week abstinence proportions using multiple imputation for the ITT population were 44.0% for Group 1, 29.3% for Group 2, and 28.6% for Group 3. The calculated 4-week abstinence proportion was significantly higher (p = 0.0297) in Group 1 compared with Group 3. A strong trend towards significance was noted when using the adjustment. No significant difference was observed when comparing Group 2 to Group 3. Stratification of 4-week abstinence proportions using multiple imputation for the ITT population by study site did not reveal a significant site main effect or treatment-by-site interactions.

Four-week abstinence proportions using treatment failure imputation for subjects who were discontinued due to lack of efficacy were 41.8% for Group 1, 22.9% for Group 2, and 26.0% for Group 3. Comparison among groups revealed a significant difference (p = 0.0169) between Groups 1 and 3 and no statistical difference between Groups 2 and 3 (p = 0.7455). Subsequent stratification by site revealed a significant site main effect.

Finally, 4-week abstinence proportions using the treatment failure imputation for the per-protocol population were 45.5% for Group 1, 20.0% for Group 2, and 27.8% for Group 3. Using the one-sided Fisher's exact test, the *p-value* was significant (p = 0.0213) both at the 0.05 significance level and upon using the

multiplicity adjustment when comparing Group 1 with Group 3. The *p-value* was 0.9017 when comparing Group 2 with Group 3. A significant site main effect was observed (p = 0.0054) involving 4-week abstinence proportions using the treatment failure imputation for the per-protocol population.

17.2 Secondary Efficacy Endpoints

17.2.1 Three-Month and Six-Month Abstinence Rates

Abstinence at 3 months (or 6 months) was defined as:

- No cigarettes smoked from Week 7 to Month 3 (or Month 6)
- Corrected exhaled CO < 10 ppm from Visit 6 to Visit 11 (or Visit 12)

At the determination of 3-month abstinence status (i.e., Visit 11), the proportion of available subjects was approximately 40% in Groups 1 and 3, and approximately 30% in Group 2. The proportion of available subjects further decreased with approximately 30% in Groups 1 and 3, and approximately 20% in Group 2 at the determination of 6-month abstinence status (i.e., Visit 12).

Using the treatment failure imputation for the ITT population, 3-month abstinence proportions were 17.2% in Group 1, 10.3% in Group 2, and 14.9% in Group 3; and 6-month abstinence proportions were 11.2% in Group 1, 7.8% in Group 2, and 9.6% in Group 3. No statistically significant differences were observed while comparing either Group 1 and Group 2 to Group 3 for 3-month and 6-month abstinence.

For the per-protocol population, 3-month abstinence proportions were 26.2% for Group 1, 12.7% for Group 2, and 21.1% for Group 3; and 6-month abstinence proportions were 16.7% for Group 1, 8.1% for Group 2, and 13.9% for Group 3. No significant differences were observed for 3-month and 6-month abstinence proportions when comparing either Group 1 or Group 2 to Group 3.

17.2.2 Corrected Exhaled CO Levels

Starting at Visit 7, or the determination of 4-week abstinence status, the mean corrected exhaled CO level was < 10 ppm for each treatment group. Three significant differences in mean corrected exhaled CO levels were noted: at Visit 3, corrected exhaled CO was higher in Groups 1 and 2 as compared to Group 3, and at Visit 4, corrected exhaled CO was higher in Group 1 as compared to Group 3. Interestingly, the mean number of total cigarettes smoked per day was also slightly higher in Groups 1 and 2 (respectively 29.7 and 29.9) compared to Group 3 (28.3). In addition, there was a trend observed when comparing Group 2 to Group 3 at Visit 4. These differences may be due to compensation behavior; subjects in Groups 1 and 2 may have experienced nicotine deprivation on Quest 1 (Visit 3), and subjects in Group 1 may have experienced nicotine deprivation on Quest 2 (Visit 4). These subjects may have tried to compensate for the lower nicotine content by smoking more study cigarettes.

17.2.3 Saliva Cotinine Levels

Results of saliva cotinine levels failed to show a trend for any treatment group. Saliva cotinine concentration levels were measured using Accutest NicAlert. Product labeling for Accutest NicAlert specified that saliva

levels in excess of 10 ng/mL indicated the use of tobacco products. However, saline cotinine levels did not match the exhaled, corrected CO levels (e.g., illustrated by the data obtained for Group 2 placebo patch usage at Visit 6 and beyond). In retrospect, this measurement should not have been included in the study since the method was not sufficiently sensitive to reach any valuable conclusions. The only information that may be learned from these data is as a measurement for subject compliance to the study treatment.

17.2.4 Subjective Questionnaires

Subjects completed the following subjective questionnaires throughout the course of the study: the Minnesota Nicotine Withdrawal Questionnaire, the Cigarette Evaluation Scale, the Sensory Questionnaire, and the Fagerström Test for Nicotine Dependence (FTND).

Nicotine Withdrawal Questionnaire

Subjects were asked to complete the Minnesota Nicotine Withdrawal Questionnaire at each study visit. The main statistical differences were noted at Visit 5, the quit date, when comparing Group 1 to Group 3. Specifically, at Visit 5:

- Subjects in Group 1 selected slight or mild depressed mood / sad more frequently than subjects in Group 3;
- Subjects in Group 1 indicated a higher rate of insomnia / sleep problems / awakening at night than subjects in Group 3;

- A significantly higher proportion of subjects in Group 1 reported being angry / irritable / frustrated than Group 3 subjects;
- Subjects in Group 1 indicated a higher rate of being anxious / nervous than subjects in Group 3;
- A significantly higher proportion of subjects in Group 1 reported difficulty concentrating than Group 3 subjects; and
- Subjects in Group 1 indicated a higher rate of increased appetite / hunger / weight problem than subjects in Group 3.

The increased rates observed in Group 1 as compared to Group 3 involving the various subcategories of the Minnesota Nicotine Withdrawal at Visit 5 may be related to a hyper-nicotine effect in Group 1. In Group 1, nicotine levels were steadily decreasing while using Quest 1 and Quest 2, and then the active 21mg nicotine patch and Quest 3 cigarettes were initiated at Visit 4. In contrast, Group 3 subjects were using sham-faded conventional cigarettes through Visit 5, and then added a placebo patch starting at Visit 4. Interestingly, at Visit 3, significantly more subjects in Groups 1 and 2 experienced desire or craving to smoke or a significantly more intense desire / craving to smoke than Group 3 subjects, following 2 weeks of using Quest 1 cigarettes. This phenomenon was not observed following 2 weeks of using Quest 2 at Visit 4, or using Quest 3 with or without the NRT patch at Visit 5.

Cigarette Evaluation Scale and Sensory Questionnaires

Subjects were asked to complete the Cigarette Evaluation Scale at Visits 1 through 5. Subjects reportedly derived less satisfaction and reported a significantly less favorable taste from Quest 2 and Quest 3 cigarettes than

their usual brand. Subjects on conventional cigarettes enjoyed the sensation in their throat considerably more than subjects smoking Quest 3. Significantly more subjects using conventional cigarettes reported feeling dizzy due to the last cigarette at Visit 3, and indicated a greater reduction in hunger for food than subjects smoking Quest 1 at Visit 5. Furthermore, Group 1 subjects on Quest 1 indicated considerable less effect on immediate reduction involving craving for cigarette than subjects using conventional cigarettes.

A Sensory Questionnaire was also administered at Visits 1 through 5 to rate the puffs from the last study cigarette smoked. Subjects smoking Quest 3 cigarettes liked the puffs of their last cigarette considerabley less, and indicated that the puffs were significantly less satisfying compared with subjects who smoked conventional cigarettes. Subjects smoking either Quest 1, Quest 2, or Quest 3 rated the nicotine level of the study cigarette significantly lower than subjects smoking conventional cigarettes. In addition, subjects rated the puffs of Quest 2 and Quest 3 considerably more dissimilar than their usual brand, compared to subjects using conventional cigarettes. Regarding the strength of puffs on the tongue, in the back of mouth and throat, windpipe, and chest, in general, only subjects in Group 1 reported a reduced strength while using Quest 1, 2, and 3 compared to Group 3, while no significant differences were observed when comparing Group 2 with Group 3.

Nicotine Dependence Questionnaire

The FTND was completed at Visits 1 through 5. Subjects were asked to answers 6 questions in order to calculate their level of nicotine dependence. No significant differences involving the individual questions

were observed when comparing Group 1 with Group 3 or Group 2 with Group 3.

17.3 Adverse Events

The proportion of treatment-emergent adverse events (AEs) was 61.2% in Group 1, 52.6% in Group 2, and 60.5% in Group 3. Serious AEs (SAEs) were reported for two subjects in Group 1 and for one subject in Group 3. None of the SAEs were considered treatment-related.

The proportion of subjects reporting at least one treatment-related AE was 44.0% in Group 1, 27.6% in Group 2, and 36.8% in Group 3. The most common treatment-related AE consisted of psychiatric disorders (most frequently specified as insomnia, abnormal dreams, irritability, and anxiety), nervous system disorders, and general disorders and administration site conditions. Headache was the most predominantly reported nervous system disorder. Application site disorders related to the NRT / placebo patch application included pruritus, dermatitis, discomfort, irritation, discoloration, and erythema.

18.0 CONCLUSIONS

The results of this study show that Quest with NRT is more effective than the standard of care NRT regimen in achieving abstinence at four weeks and suggest that Quest alone may be as effective as standard of care NRT. The magnitude of the difference observed (32.8% versus 21.9%) is a clinically relevant improvement. The benefit of Quest with NRT may be understated given that use of an Active Control cigarette in a sham-fading procedure in and of itself may have an effect on abstinence. The treatment effects were statistically significant at the 0.05 significance level and are close to significance. A series of sensitivity tests, employing a range of imputation methods for missing data, reinforce and support the primary analysis.

In the ITT population using treatment failure imputation, baseline saliva cotinine level, cigarettes per day, years of smoking, and treatment group assignment were all variables predictive of treatment success. Heavier smokers had less success quitting.

There were no SAEs attributable to the study product. There were three instances of patients developing dermatologic reactions while using the NRT patch. This was likely due to irritation, or possibly allergic dermatitis.

In general, as expected, subjects found the experience using Quest cigarettes less satisfying than traditional cigarettes. This was demonstrated by various responses to the Cigarette Evaluation Scale Questionnaire and the Sensory Questionnaire, where there were fewer subjects in Groups 1 and 2 with positive responses for questions relating to satisfaction, taste, and enjoyable sensation in the throat. There also appeared to be a small increase ("compensation") in the number of cigarettes consumed. Mean and median corrected exhaled CO levels for Groups 1 and 2 showed an increase between Visit 2 and Visit 3, and Visit 3 and Visit 4. Mean and median changes from baseline in corrected exhaled CO levels for Groups 1 and 2 were positive at Visit 3 and Visit 4; they were also slightly positive for Group 3 at Visit 4.

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