ATTACHMENT I: The unsigned DRAFT HED Chapter of the Nicotine and derivatives Reregistration Eligibility Decision Document presented to the HED Risk Assessment Review Committee (RARC2) on August 22, 2007 for consideration. 79 p. [Note: This document includes minor editorial modifications made after August 22, 2007.]

DRAFT

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

August xx, 2007

MEMORANDUM

SUBJECT: Nicotine and derivatives: HED Chapter of the Reregistration Eligibility Decision Document (RED). PC Code: 056702. Case #: 2460. DP Barcode: D341246.

Regulatory Action: Phase 1 Reregistration
Risk Assessment Type: Single Chemical Aggregate

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Attached is the Health Effects Division's Chapter to the Nicotine and derivatives Reregistration Eligibility Decision (RED) Document addressing the Human Health Risk Assessment for nicotine (PC 056702) or nicotine alkaloid or nicotine as a naturally occurring component of tobacco dust. Listed below are the companion disciplinary chapters to this risk assessment:

Occupational and Residential Exposure/Risk Assessment for the Nicotine and derivatives Reregistration Eligibility Decision (RED) Addressing the Fulex Nicotine Greenhouse Fumigator, B. Cropp-Kohlligian, D341249, 08/xx/2007.

Occupational and Residential Exposure/Risk Assessment for the Nicotine and derivatives Reregistration Eligibility Decision (RED) Addressing the Bonide Dog and Rabbit Repellant, B. Cropp-Kohlligian, D341897, 08/xx/2007.

Review of Nicotine Incident Reports, J. Blondell and M. Spann, D276938, 08/10/2001.

1.0 Executive Summary

A Human Health Risk Assessment is being conducted for Nicotine and derivatives (List B Reregistration Case #2460). Of the nicotine and derivatives listed as active ingredients in the Office of Pesticide Programs Information Network (OPPIN), which include nicotine (PC 056702), nicotine sulfate (PC 056703), and tobacco dust (PC 056704), only nicotine (PC 056702) is present as an active ingredient in currently registered products. Therefore, the active ingredients nicotine sulfate (PC 056703) and tobacco dust (PC 056704) will not be addressed further.

Nicotine or nicotine alkaloid or nicotine as a naturally occurring component of tobacco dust is present as an active ingredient (PC 056702) in three currently registered end-use products. Only two of these end-use products are being supported under reregistration. Fuller System, Inc. and Bonide Products, Inc. have recently informed the Agency (Use Closure for Nicotine RED, J. Bloom, 06/18/2007) that they intend to support the reregistrations of Fulex Nicotine Fumigator (EPA Reg. No. 1327-41) and Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465), respectively. [Note: According to OPPIN Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) was transferred from Faesy & Besthoff (EPA Reg. No. 779-29) on 08/16/2006.] Bonide Products, Inc. does not intend to support the reregistration of Bonide® Tobacco Dust (EPA Reg. No. 4-340) and has requested voluntary cancellation of this end-use product; hence this end-use product will not be discussed further.

In previous phases of the reregistration process for Nicotine and derivatives, Fuller Systems, Inc. and Bonide Products, Inc. held registrations but were not the registrants primarily responsible for generating data in support of this List B Reregistration Case (#2460). Phase 4 of the reregistration process was completed in 1990 and according to SRRD outstanding data requirements for nicotine (PC 056702), including toxicology and occupational and residential exposure (ORE) data, were identified in Data Call-Ins (DCIs), however, details concerning the communication of previously identified reregistration data requirements to Fuller Systems, Inc. and Bonide Products, Inc. are unknown to HED. Hence, HED defers to SRRD on the topic of regulatory history for nicotine.

Neither of the two end-use products which are being supported under reregistration are registered for use on food or feed; hence, any remaining tolerances listed under 40 CFR §180.167 are not being supported under reregistration and should be revoked. Also, HED notes that no Codex MRLs have been established for nicotine (PC 056702), nicotine sulfate (056703), or tobacco dust (PC 056704). This topic will not be discussed further.

HED notes that under 40 CFR §152.175, which concerns pesticides classified for restricted use, there is a regulation concerning all formulations of nicotine applied to cranberries; however, nicotine is not currently registered for use on cranberries and this regulation should be revoked. This topic will not be discussed further.

After considering the remaining uses of nicotine which are being supported under reregistration, the Health Effects Division (HED) and the Environmental Fate and Effects Division (EFED) have agreed that conducting a drinking water risk assessment is not appropriate. This topic will

not be discussed further.

Since neither of the two end-use products which are being supported under reregistration are registered for use on food or feed and given that HED and EFED have agreed that conducting a drinking water risk assessment is not appropriate, dietary (food + water) and aggregate (food + water + residential exposure) risk assessments were not conducted.

HED notes that since Nicotine and derivatives is a List B Reregistration Case, the Product Chemistry Chapter for the Nicotine and derivatives Reregistration Eligibility Decision (RED) is the responsibility of the Registration Division. This topic will not be discussed further.

Hazard Profile

There are no guideline toxicity studies available on nicotine and while there are numerous published studies in which nicotine was administered subcutaneously or intravenously, the utility of these studies to characterize the nicotine toxicity for this risk assessment is very limited. There are, however, a few studies in which nicotine or a salt of nicotine was administered by the oral or inhalation routes and these studies have been used to characterize the nicotine toxicity for this risk assessment. No dermal toxicity or dermal absorption studies using nicotine have been identified.

Nicotine is acutely toxic (Category I) by all routes of exposure (oral, dermal, and inhalation). The LD_{50} of nicotine is 50 mg/kg for rats and 3 mg/kg for mice. A dose of 40–60 mg can be a lethal dosage for adult human beings and doses as low as 1-4 mg can be associated with toxic effects in some individuals. Based on data collected with a 40% nicotine formulation, nicotine is only a slight dermal irritant (Category IV). It is reported that nicotine causes dermatitis (skin sensitization) in humans although data collected with a 40% nicotine formulation was not a skin sensitizer in the guinea pig.

Nicotine is an agonist at nicotinic receptors in the peripheral and central nervous system. It inhibits the function of acetylcholine receptors located at the neuromuscular junctions. In general terms, it causes stimulation of the ganglions in low doses but causes blockade at higher concentrations.

Nicotine in subchronic amounts administered to animals resulted in increased pancreatic biosynthesis and accumulation of digestive enzymes within the pancreas. It also enhances synthesis of cholesterol, triglycerides, phospholipids and free fatty acids in the liver and testes and lowers serum testosterone and estradiole levels suggesting gonadotoxic effects. Nicotine is hepatotoxic in some animal tests. It also adversely affects bone formation and decreases body storage of vitamin D.

Experimental data in male rats suggest that nicotine ingested chronically alters metabolic and endocrine factors that may be responsible, at least in part, for the development of gastrointestinal ulcers and pancreatitis. Chronic inhalation exposure to low level of nicotine (500 $\mu g/m^3$) for two years produced slight weight reduction and did not result in other adverse effects in rats.

Nicotine is an animal and human teratogen according to numerous studies. It has detrimental effect on general growth and development as well as on palatogenesis and ossification in mice fetuses prenatally exposed during gestation. It is also a developmental neurotoxicant. It produces biochemical changes in the fetal brain that result in abnormal behavior in the offspring of exposed animals. Experimental data in rats suggests that exposure to a high dose of nicotine in utero might cause a predisposition to diseases related to a dopaminergic dysfunction in the frontal cortex.

In some tests nicotine and its metabolites did not cause bacterial mutations nor did they increase the frequency of sister chromatid exchanges. In some other tests nicotine was found to induce chromosomal aberration.

According to the International Program on Chemical Safety (IPCS) review, literature reports indicate that nicotine is neither an initiator nor a promoter of tumors in mice. There is inconclusive evidence to suggest that cotinine, an oxidized metabolite of nicotine, may be carcinogenic in the rat (http://www.inchem.org/documents/pims/chemical/nicotine.htm).

Nicotine is extensively metabolized to a number of metabolites by the liver. Quantitatively the most important metabolite of nicotine in most mammalian species is the lactam derivative cotinine.

Tests in animals suggest that nicotine may adversely affect the immune system.

A subchronic oral rat toxicity study (Yuen *et al*. 1995) conducted with nicotine hydrogen tartrate was selected as a basis for estimating the incidental oral, dermal, and inhalation toxicity endpoints. Nicotine hydrogen tartrate was administered to pregnant and non-pregnant female rats in the drinking water for 10 days at 54 or 108 µmole/L (8.76, 17.52 mg/L; equivalent to 1.25 and 2.5 mg/kg/day assuming a rat consumes 50 mL of water per day) resulted in mild fatty change, mild focal necrosis and mild dark cell change (containing numerous prominent pore annuli in the nuclear membranes and the mitochondria appeared decreased in size with a decrease in mitochondrial granules and loss of aristae) in a dose proportional manner. Histopathological changes seen at the lower dose (1.25 mg/kg/day) were not statistically significant and this was considered to be a **NOAEL** and the **LOAEL** was 2.5 mg/kg/day. A margin of exposure of 1000 is applied to account for inter-species extrapolation (10X), intra-species variability (10X) and database uncertainty (10X).

Since the dermal and inhalation endpoints are based on the same toxicological effects, dermal and inhalation risks may be combined.

Fulex Nicotine Fumigator (EPA Reg. No. 1327-41)

Fulex Nicotine Fumigator (EPA Reg. No. 1327-41) is a ready-to-use formulation containing nicotine or nicotine alkaloid, as the sole active ingredient, and is used as an insecticide to control aphids and most thrips on ornamental plants grown in greenhouses. The product is formulated as a smoke generator in which nicotine is intended for release as a vapor. Based on the currently registered end-use product label (EPA Reg. No. 1327-41; dated 08/10/2005) and information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), non-dietary exposures for this use are expected to be short-term (1-30 days) and intermediate-term (1-6 months) in duration. Although there is the potential for nicotine to be applied year-round in greenhouses, daily long-term (>6 months) worker exposure is deemed unlikely.

Fulex Nicotine Fumigator (EPA Reg. No. 1327-41; label date 08/10/2005) is a restricted use pesticide and applications may be made by or under the direct supervision of a certified applicator (40 CFR §152.175). According to the currently registered label, applicators are required to wear: (1) coveralls over long-sleeve shirt and long pants, (2) **waterproof gloves**, (3) chemical-resistant footwear plus socks, (4) protective eyewear, (5) chemical resistant headgear for overhead exposure, and (6) a respirator with either an organic vapor-removing cartridge with a prefilter approved for pesticides (MSHA/NIOSH approval number prefix TC-23C) or a canister approved for pesticides (MSHA/NIOSH approval number prefix TC-14G). **Note: The currently registered label does not specify if the respirator is a half-face or full-face mask.**

Fulex Nicotine Fumigator (EPA Reg. No. 1327-41; label date 08/10/2005) contains 13.4% nicotine as a smoke fumigator available in 12 ounce and 6 ounce ready-to-use screw-capped canisters packaged with wire igniters (sparklers). Each 12 ounce canister contains 0.10 lbs a.i. and treats 20,000 ft^3. Each 6 ounce canister contains 0.05 lbs a.i. and treats 10,000 ft^3.

Fumigations are typically conducted overnight. Prior to fumigations, greenhouse vents are closed. The canisters are shaken and set in place with the screw caps removed. The wire igniters are lit and inserted into the open canisters for the purpose of burning the inert ingredients to produce smoke and vaporizing the nicotine. Sometime later, greenhouse vents are re-opened by handlers and canisters are re-collected. Canisters that did not ignite are re-capped and stored for later use. Post-fumigation re-entry is governed by Worker Protection Standards (WPS) ventilation requirements specified under 40 CFR 170.110(c)(3).

No nature of the residue data are available for this unique application method which alters nicotine's physical state and almost certainly form degradates. During fumigation, nicotine may be present as a vapor and possibly bound or adsorbed to particulate matter. Given the lack of sophistication of the ignition device (sparklers), without nature of the residue data, it is difficult to determine if nicotine is only vaporized and to some extent adsorbed to particulate matter or the extent to which it is decomposed as a result of the application method. Formation of significant compounds of interest such as N-nitrosonornicotine (NNN) and 4-(N-methyl-N-nitrosamino)-1-(3-pyridil)-1-butanone (NNK) cannot be excluded without supporting data. However, since exposure estimates in this risk assessment are based on maximum theoretical air concentration and surface residue data/calculations and default assumptions, all potential degradates of nicotine from the Fulex Nicotine [Greenhouse] Fumigator use have been included in the exposure

estimates of this risk assessment and the *de facto* hazard assumption is equivalent toxicity with nicotine.

The American Conference and Governmental Industrial Hygienists (ACGIH) have established a threshold limit value (TLV) as an 8-hour time-weighted average (TWA) of 0.5 mg/m^3 for nicotine. There is a skin notation; however, sufficient data were not available to recommend a Senisitizer (SEN) notation or carcinogenicity notation or a TLV as a short-term exposure limit (STEL). The Occupational Safety and Health Agency (OSHA) has established a permissible exposure limit (PEL) as an 8-hour time-weighted average (TWA) of 0.5 mg/m^3 for nicotine consistent with the ACGIH TLV and The National Institute for Occupational Safety and Health (NIOSH) concurs with the OSHA PEL and has established a value of 5 mg/m^3 for nicotine as a level that is immediately dangerous to life or health (IDLH).

Fulex Nicotine Fumigator (EPA Reg. No. 1327-41) Residential (Non-Occupational) Exposure/Risk Assessment

No residential (non-occupational) handler exposure scenarios have been identified for the Fulex Nicotine [Greenhouse] Fumigator use; however, residential (non-occupational) postapplication exposure scenarios *via* inhalation and dermal routes have been identified. These exposures are expected to be short-term in duration.

The Fulex Nicotine Fumigator (EPA Reg. No. 1327-41) is a restricted use product for use by and under direct supervision of a certified applicator (40 CFR §152.175); however, the current label does not prohibit application to privately owned greenhouses in residential settings by certified commercial applicators where it is possible for residents to be exposed following application and according to information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), the product is used in retail greenhouses where it is considered possible for non-occupational members of the general public to be exposed following application. These residential (non-occupational) postapplication exposures are addressed by the occupational postapplication risk assessment for this end-use product.

Fulex Nicotine Fumigator (EPA Reg. No. 1327-41) Occupational Handler Exposure/Risk Assessment

Occupational handler exposure scenarios *via* the inhalation route have been identified for the Fulex Nicotine [Greenhouse] Fumigator use and are expected to be short-term (1-30 days) and intermediate-term (1-6 months) in duration. Although there is the potential for nicotine to be applied year-round in greenhouses, daily long-term (>6 months) worker exposure is deemed unlikely.

Since short-term and intermediate-term risk estimates will be based on the same endpoint (1.25 mg/kg/day) and level of concern (1000), only short-term risks have been calculated.

Two major handler use patterns were identified: (1) opening/lighting of canisters, and (2) reentering after canisters are deployed but before the WPS ventilation requirements are met to open greenhouses vents and dispose of canisters (as specified under 40 CFR §170.3 *Handler*

(1)(vii). These two activities were considered as a single exposure scenario. The available data in the Pesticide Handlers Exposure Database (PHED) do not reflect these Fulex Nicotine [Greenhouse] Fumigator use patterns.

No mixing/loading methods are necessary for the Fulex Nicotine [Greenhouse] Fumigator use. Therefore, a mixing/loading exposure assessment was not performed.

Dermal exposures from a smoke formulation to handlers are assumed to be minimal relative to the exposures and risks from inhalation. Therefore, a dermal exposure assessment for handlers was not performed.

Since the duration of handler exposure is relative to the number of canisters needed for treatment based on the size of the individual greenhouse structure to be treated and the number of individual greenhouse structures to be treated per day at any given facility, exposure periods of 30 minutes, representing smaller greenhouse facilities and 60 minutes, representing larger greenhouse facilities, were used as estimations of exposure periods for handlers.

Inhalation MOEs greater than 1000 are not of concern to HED. Based on the currently registered end-use product label (EPA Reg. No. 1327-41; dated 08/10/2005), information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), theoretical air concentration data/calculations, and default assumptions, inhalation risks of concern (*i.e.*, MOEs less than 1000) were identified for individuals performing application activities both with and without respiratory protection (*e.g.*, half- and full-face respirators with chemical and particulate filter cartridges), except for those individuals using a self-contained breathing apparatus (SCBA). Moreover, inhalation risks of concern (*i.e.*, MOEs less than 1000) were identified for individuals using half- and full-face respirators with chemical and particulate filter cartridges for exposure periods of 1 and >3 minutes, respectively. These risk estimates may be considered conservative since they are based on theoretical air concentration data/calculations and default assumptions in the absence of acceptable chemical-specific data.

Fulex Nicotine Fumigator (EPA Reg. No. 1327-41) Occupational Postapplication Exposure/Risk Assessment

Postapplication exposure scenarios *via* inhalation and dermal routes have been identified for the Fulex Nicotine [Greenhouse] Fumigator use and are expected to be short-term (1-30 days) and intermediate-term (1-6 months) in duration. Although there is the potential for nicotine to be applied year-round in greenhouses, daily long-term (>6 months) worker exposure is deemed unlikely.

Since short-term and intermediate-term risk estimates will be based on the same endpoint (1.25 mg/kg/day) and level of concern (1000), only short-term risks have been calculated.

Inhalation and dermal MOEs greater than 1000 are not of concern to HED. All occupational postapplication exposure scenarios had dermal risks of concern (*i.e.*, MOEs less than 1000) at day-zero; however, inhalation risks were not of concern (*i.e.*, MOEs greater than 1000). For dermal risks, Restricted Entry Intervals of 40+ days would be required to achieve acceptable

MOEs. This risk estimate may be considered conservative since it is based on theoretical air concentration and surface residue data/calculations and default assumptions, including 100% dermal absorption, in the absence of acceptable chemical-specific data.

Note: The technical grade of the active ingredient nicotine is classified as a Category I toxicant based on acute oral toxicity data which would, under the Worker Protection Standard (WPS), require a minimum restricted entry interval (REI) of 48-hours (40 CFR §156.208(c)); however, this criteria for determining the REI does not apply to any product that is a fumigant (40 CFR §156.208(d)). Hence, the REI for the Fulex Nicotine [Greenhouse] Fumigator, if it is determined to be a fumigant, will be governed by the WPS ventilation criteria (40 CFR §170.110(c)(3)) and product-specific REI calculations. In the absence product-specific data collected in accordance with 40 CFR §158.390, these calculations have been based on theoretical data/calculations and default assumptions.

Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465)

Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) a ready-to-use formulation containing nicotine as a naturally occurring component of tobacco dust and is used as an animal repellant in outdoor settings. Based on the currently registered Bonide® Rabbit & Dog Chaser end-use product label (EPA Reg. No. 4-465; label date 12/28/2006), small packaging sizes (available in 1- and 3-pound packages), and information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), this product is intended for use by homeowners as a repellent *via* a barrier/perimeter treatment to prevent eastern cottontail rabbits (*Sylvilagus floridanus*) from eating and defecating on ornamental plants and domestic dogs (*Canis l. familiaris*) from defecating on ornamentals, including lawns. It may also be used around the perimeter of vegetable gardens. Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465; label date 12/28/2006) contains 0.35% nicotine (as a naturally occurring component of tobacco dust), as well as two other active ingredients, dried blood and naphthalene. **[NOTE: The nicotine content of tobacco dust in the end-use product is substantially less than the content normally found in dried tobacco leaves (up to 8% by weight according to the Merck Index) and in the tobacco found in cigarettes (on average, 1.5% by weight according to the 1988 report of the Surgeon General, entitled, "The Health Consequences of Smoking").]** Given that the nicotine in this product is contained in tobacco dust, the formulation is considered a dust for this risk assessment. Based on the currently registered Bonide® Rabbit & Dog Chaser end-use product label (EPA Reg. No. 4-465; label date 12/28/2006) and information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), non-dietary exposures to this product are expected to be short-term in duration.

HED notes that under 40 CFR §152.175, which concerns pesticides classified for restricted use, nicotine (alkaloid) as a liquid or dry formulations containing 1.5% and less is unclassified for all uses. Toxicity data provided for the Bonide® Rabbit & Dog Chaser has shown low acute toxicity by the oral, dermal, and inhalation routes (Category IV). It is a weak eye or dermal irritant (Category IV) and does not cause dermal sensitization in the guenea pig. *See Table A.2.1b.*

Maximum use rates for this product are not specified on the currently registered Bonide®

Rabbit & Dog Chaser end-use product label (EPA Reg. No. 4-465; label date 12/28/2006) and are difficult to determine. The product is available in 1 pound and 3 pound ready-to-use packages. According to the label, a 3-pound package of product will produce a band of product 1 inch wide and 85 feet long and a 1-pound package of product will produce a band of product 1 inch wide and 28 feet long. Both the 3-pound and 1-pound packages provide the same use directions. The product is sprinkled directly from the package in 2 or more inch-wide bands around areas to be treated. The product may be used throughout the year and repeat applications are as needed.

The label does not provide any distinction in use directions for professional (occupational) and non-professional (residential homeowner) applicators and no occupational postapplication scenarios have been identified. Therefore, only the residential handler exposures and risks have been assessed which should be protective of occupational handlers.

Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) Residential (Non-Occupational) Handler Exposure/Risk Assessment

It has been determined that there is a potential for dermal and inhalation exposure in residential settings during the application of Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465; label date 12/28/2006). Based on the currently registered Bonide® Rabbit & Dog Chaser end-use product label (EPA Reg. No. 4-465; label date 12/28/2006) and information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), non-dietary exposures to this product are expected to be short-term in duration.

Residential MOEs equal to or greater than 1000 are not of concern to HED. The short-term combined dermal and inhalation MOE for residential handlers who apply one three-pound package of the ready-to-use Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) was 1875 and therefore not of concern to HED. However, application of two or more three-pound packages would be of concern, since this is estimated to result in a combined dermal and inhalation MOE less than 1000. The concerns are primarily for dermal exposure/risk and HED notes that the inhalation/dermal risk estimates may be considered conservative since, in the absence of chemical-specific data, they are based on theoretical data/calculations and default assumptions, including 100% inhalation/dermal absorption, for this product containing nicotine as a naturally occurring component of tobacco.

Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) Residential (Non-Occupational) Postapplication Exposure/Risk Assessment

Because the use pattern results in applications to areas not frequented by children or in areas where maintenance would result in significant exposure, dermal and incidental oral assessments were not conducted. However, episodic ingestion of the material is considered reasonable due to packages which are not child resistant and which could be accessible to children prior to application or due to the potential for children to come in contact with the product postapplication.

Residential MOEs equal to or greater than 1000 are not of concern to HED. The short-term oral

MOE for episodic ingestion of the ready-to-use Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) containing nicotine (as a naturally occurring component of tobacco dust) was 1 and therefore is of concern to HED. Moreover, ingestion of more than 0.002 teaspoons of product by a toddler (15 kg) exceeds the level of concern (MOE less than 1000); however, this type of ingestion is considered an episodic event and not a routine behavior. Because HED does not believe that this would occur on a regular basis, our concern for human health is related to acute poisoning rather than short -term residue exposure.

2.0 Ingredient Profile

2.1 Summary of Registered/Proposed Uses

2.1.1 Fulex Nicotine Fumigator (EPA Reg. No. 1327-41)

See: Occupational and Residential Exposure/Risk Assessment for the Nicotine and derivatives Reregistration Eligibility Decision (RED) Addressing the Fulex Nicotine Greenhouse Fumigator, B. Cropp-Kohlligian, D341249, 08/xx/2007.

Fulex Nicotine Fumigator (EPA Reg. No. 1327-41; label date 08/10/2005) contains 13.4% nicotine as a smoke fumigator available in 12 ounce and 6 ounce ready-to-use screw-capped canisters packaged with wire igniters (sparklers). Each 12 ounce canister contains 0.10 lbs a.i. and treats 20,000 ft^3. Each 6 ounce canister contains 0.05 lbs a.i. and treats 10,000 ft^3. Since both the 12 ounce and 6 ounce canisters provide the same maximum application rate, hereafter, only the 12 ounce product will be use in exposure estimate calculations. Table 2.1.1 summarizes the use pattern and formulation specified in the end-use product used in greenhouses containing nicotine.

Table 2.1.1. Use Patterns and Formulations for the Fulex Nicotine [Greenhouse] Fumigator use of Nicotine.

Formulation	Method of Application	Use Sites	Application Rates	Timing of Application and Restrictions
Fulex Nicotine Fumigator Insecticide (13.4% ai) EPA Reg. No. 1327-41 Note: Available in ready-to-use 6- and 12-oz. screw-capped steel canisters with wire igniters which look and act like sparklers.	Smoke fumigator	Greenhouses on ornamental **plants** (to control aphids and thrips)	1 canister (12 oz)/ 20,000 ft^3 or 1 canister (6 oz)/ 10,000 ft^3 Note: Both canister sizes provide the same application rate.	At fumigation, greenhouse temperature between 70-90 F. Foliage and blossoms must be dry. Avoid rainy or windy days. Close all vents prior to use. Repeat treatment in one week if necessary. **Note: According to information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), in contradiction to the label restrictions, applications are typically made every 3-12 days. The actual minimum re-treatment interval (RTI) is therefore assumed to be 3 days.**

Given the lack of sophistication of the ignition device (sparklers), it is difficult to determine if nicotine is only vaporized (and to some extent decomposed) as a result of the application method producing nicotine vapor and possibly nicotine bound or adsorbed onto particulate matter or burned during the application method producing pyrolysis products of nicotine including N-

nitrosonornicotine (NNN) and 4-(N-methyl-N-nitrosamino)-1-(3-pyridil)-1-butanone (NNK). However, since exposure estimates in this risk assessment are based on maximum theoretical air concentration data/calculations and default assumptions, all potential degradates of nicotine from the Fulex Nicotine [Greenhouse] Fumigator use have been included in the exposure estimates of this risk assessment and the *de facto* hazard assumption is equivalent toxicity with nicotine.

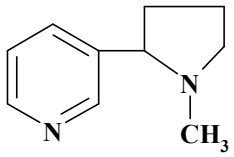
2.1.2 Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465)

See: Occupational and Residential Exposure/Risk Assessment for the Nicotine and derivatives Reregistration Eligibility Decision (RED) Addressing the Bonide Dog and Rabbit Repellant, B. Cropp-Kohlligian, D341897, 08/xx/2007.

Based on the currently registered Bonide® Rabbit & Dog Chaser end-use product label (EPA Reg. No. 4-465; label date 12/28/2006), small packaging sizes (available in 1- and 3-pound packages), and information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), this product is intended for use by homeowners as a repellent *via* a barrier/perimeter treatment to prevent eastern cottontail rabbits (*Sylvilagus floridanus*) from eating and defecating on ornamental plants and domestic dogs (*Canis l. familiaris*) from defecating on ornamentals, including lawns. It may also be used around the perimeter of vegetable gardens. Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465; label date 12/28/2006) contains 0.35% nicotine (as a naturally occurring component of tobacco dust), as well as two other active ingredients, dried blood and naphthalene. **[NOTE: The nicotine content of tobacco dust in the end-use product is substantially less than the content normally found in dried tobacco leaves (up to 8% by weight according to the Merck Index) and in the tobacco found in cigarettes (on average, 1.5% by weight according to the 1988 report of the Surgeon General, entitled, "The Health Consequences of Smoking").]** Given that the nicotine in this product is present as a naturally occurring component of tobacco dust, the formulation is considered a dust for this risk assessment. The product is available in 1 pound and 3 pound ready-to-use packages; applications are the same. A 3-pound package of product will produce a band of product 1 inch wide and 85 feet long. The product is sprinkled directly from the package in 2 or more inch-wide bands around areas to be treated. Maximum use rates in terms of lb ai/A/application are not specified on the product label; the reviewer estimates that a 3-pound package of product will provide a barrier/perimeter treatment around an area encompassing at most 144 ft^2. The product may be used throughout the year and repeat applications are as needed. The maximum number of treatments per season is not specified on the product label. Table 2.1.2 summarizes the use pattern and formulation specified in the end-use product containing nicotine (as a naturally occurring component of tobacco dust).

Table 2.1.2. Use Patterns and Formulations for the BonideRabbit and Dog Repellant.			
Formulation	Method of Application	Use Sites	Application Rates, Timing of Application, and Restrictions
Bonide® Rabbit & Dog Chaser EPA Reg. No. 4-465 (The product contains 0.35% nicotine and is present in this product as a naturally occurring component of tobacco dust; hence, the formulation is considered a dust for this risk assessment.) Note: Marketed in ready-to-use in 1 and 3-pound poly bags.	Rabbit and dog repellant applied by hand. The product is sprinkled onto treated area directly from the package.	Barrier/perimeter treatments for use by homeowners around residential lawns, flower gardens, vegetable gardens, ornamentals, trees and shrubs.	According to the label, a 3-pound package of product will produce a band of product 1 inch wide and 85 feet long and a 1-pound package of product will produce a band of product 1 inch wide and 28 feet long. The product is applied in 2 or more inch-wide bands around areas to be protected. **The reviewer estimates that a 3-pound package of product will provide a barrier/perimeter treatment around ≤144 ft^2 area as follows:** A 3-pound package of product will provide 42.5 linear feet of 2 inch-wide bands of barrier/perimeter treatment. If this product is applied in a closed circle it will encompass at most a 144 ft^2 of area (Area=πr^2) with a circumference (Circumference = $2\pi r$) of 42.5 ft. According to the label, before treatment, try to remove all traces of animal droppings and urine from the areas to be protected. Be careful not to apply product directly to foliage or stems. Repeat treatment as needed. More frequent applications will be needed if heavy rains, heavy snowfalls, hot weather, or high winds occur. Applications may be made throughout the year.

2.2 Structure and Nomenclature

Table 2.2. Test Compound Nomenclature.	
Compound	
Common name	Nicotine
IUPAC name	(*S*)-3-(1-methylpyrrolidin-2-yl)pyridine (according to allanwood.net)
CAS name	(*S*)-3-(1-Methyl-2-pyrrolidinyl)pyridine (according to the Merck Index)
CAS registry number	54-11-5
End-use products (EP)	Fulex Nicotine Fumigator (13.4% nicotine): EPA Reg. No. 1327-41) Bonide Rabbit & Dog Chaser (0.35% nicotine as a naturally occurring component of tobacco dust); EPA Reg. No. 4-465)

2.3 Physical and Chemical Properties

Nicotine is a colorless to pale yellow oily liquid, which slowly turns brown when exposed to air or light. It is hygroscopic and forms water-soluble salts including the hydrochloride, sulfate, and tartrate. Nicotine decomposes slightly at its boiling point.

Nicotine is a tertiary amine composed of a pyridine and a pyrrolidine ring. Nicotine may exist in two stereoisomers. Tobacco contains only (S)-nicotine, which is the more pharmacologically active form. Racemization is expected with combustion.

According to *Merck Index*, Nicotine comes from the dried leaves of *Nicotiana tabacum* and *N. rustica* where is occurs to the extent of 2 to 8%, combined with citric and malic acids. Commercial nicotine is entirely a byproduct of the tobacco industry.

According to a report of the Surgeon General (1988) entitled, "The Health Consequences of Smoking", on average, nicotine content of the tobacco used in American cigarettes is 1.5% (by weight).

Table 2.3. Physicochemical Properties of Nicotine.

Parameter	Value	Reference
Boiling point/range	247°C at 745 mmHg (partial decomposition)	Merck Index
pH		
Density	specific gravity 1.0097 at 20° referred to water at 4°	Merck Index
Water solubility		
Solvent solubility	Soluble in water, chloroform, alcohol, ether, kerosene, and oils	Merck Index
Vapor pressure	4.25×10^{-2} torr at 20°C	
Dissociation constant, pK_a		
Octanol/water partition coefficient, $Log(K_{OW})$		
UV/visible absorption spectrum		

3.0 Hazard Characterization/Assessment

Nicotine toxicity has been investigated extensively. This review is based on published literature studies to identify hazards from exposure to nicotine that will aid in assessing the risks from its limited pesticidal uses. None of the studies reviewed followed EPA guidelines.

Nicotine is a potent toxicant. It is an agonist at nicotinic receptors in the peripheral and central nervous system. It inhibits the function of acetylcholine receptors located at the neuromuscular junctions. In general terms, it causes stimulation of the ganglions in low doses but causes blockade at higher concentrations. The oral LD_{50} of nicotine is 50 mg/kg for rats and 3 mg/kg for mice. A dose of 40–60 mg can be a lethal dosage for adult human beings. Nicotine is readily absorbed by the skin making it equally toxic by the dermal route. Its high solubility both in polar and non-polar solvents and its low molecular weight make it an efficient skin penetrant. Workers handling and harvesting green tobacco develop "green-tobacco sickness" due to the dermal absorption of nicotine from the leaves. The average blood concentration in lethal cases is 29 μg/mL (0.18 mM).

It is reported that nicotine causes contact dermatitis (skin sensitization) in humans although a 40% nicotine formulation was not a skin sensitizer in the guinea pig.

Nicotine in subchronic amounts administered to animals resulted in increased pancreatic biosynthesis and accumulation of digestive enzymes within the pancreas. It also enhances synthesis of cholesterol, triglycerides, phospholipids and free fatty acids in the liver and testes and lowers serum testosterone and estradiole levels suggesting gonadotoxic effects. Nicotine is hepatotoxic in some animal tests. It also adversely affects bone formation and decreases body storage of vitamin D.

Experimental data in male rats suggest that nicotine ingested chronically alters metabolic and endocrine factors that may be responsible, at least in part, for the development of gastrointestinal ulcers and pancreatitis. Chronic inhalation exposure to low level of nicotine (500 $\mu g/m^3$) for two years produced slight weight reduction and did not result in other adverse effects in rats.

Nicotine is an animal and human teratogen according to numerous studies. It has detrimental effect on general growth and development as well as on palatogenesis and ossification in mice fetuses prenatally exposed during gestation. It is also a developmental neurotoxicant. It produces biochemical changes in the fetal brain that result in abnormal behavior in the offspring of exposed animals. Experimental data in rats suggests that exposure to a high dose of nicotine in utero might cause a predisposition to diseases related to a dopaminergic dysfunction in the frontal cortex.

In some tests nicotine and its metabolites did not cause bacterial mutations nor did they increase the frequency of sister chromatid exchanges. In some other tests nicotine was found to induce chromosomal aberration.

According to the International Program on Chemical Safety (IPCS) review, literature reports indicate that nicotine is neither an initiator nor a promoter of tumors in mice. There is

inconclusive evidence to suggest that cotinine, an oxidized metabolite of nicotine, may be carcinogenic in the rat (http://www.inchem.org/documents/pims/chemical/nicotine.htm).

Tests in animals suggest that nicotine may adversely affect the immune system.

3.1 Hazard and Dose-Response Characterization

3.1.1 Database Summary

There are no guideline toxicity studies available on nicotine. There are numerous published studies where nicotine was administered subcutaneously or intravenously. The utility of such studies in hazard characterization for the risk assessment of nicotine exposure is very limited. However, few studies were available where nicotine was administered orally or by inhalation. These (oral/inhalation) studies were used to characterize the nicotine toxicity for this current risk assessment. Only key studies used to derive or support endpoints of toxicity for the hazard characterization of nicotine are discussed below. All other studies are discussed in Appendix A.3

3.1.1.1 Studies available and considered (animal, human, general literature)

Acute Toxicity

The acute toxicity of nicotine is summarized in Appendix A Table 2.1. Nicotine is highly toxic. It is reported that as low as 60 mg ingested by an adult human being are fatal and that doses as low as 1-4 mg can be associated with toxic effects in some individuals (Saxena and Scheman, 1985). The oral LD_{50} of nicotine is 50 mg/kg for rats and 3 mg/kg for mice (Other published studies summarized in the tox profile in Appendix A.2. showed that mice tolerated higher doses without mortality). It is equally toxic by the oral and dermal routes. Workers handling and harvesting green tobacco absorb through their skins nicotine doses oozing from the wet leaves to produce frequently toxic symptoms of what is known as "green-tobacco sickness" (Gehlbach *et al*, 1974 and 1975; Ghosh *et al*, 1985; Jones and Goldy, 1998; Hipke, 1993; Boylan *et al* 1993; Quandt *et al*, 2000). A 40% formulation of nicotine known as Black Leaf 40 produced slight dermal irritation to the rabbit skin and was not a skin sensitizer in the guinea pig (MRID 41521101 & 41521102). However, nicotine is reported to cause contact dermatitis (skin sensitization) in humans (Bircher *et al*, 1991; Vincenzi *et al*, 1993).

Nicotine dermal patches applied topically (1 to 2 mg/kg/24 h) or orally (2.8 to 13.4 mg/kg over 25-57 h, with mean maximal plasma levels of 36 - 73 ng/mL) to dogs produced minimal clinical symptoms of excess salivation, emesis and vomiting. The acute oral LD_{50} for nicotine in dogs is at least 10-12 mg/kg (Matsushima *et el*, 1995).

Data derived from five fatal case reports indicated that the average blood concentration of nicotine was 29 ug/mL equivalent to a 0.18 mM mean lethal concentration (Baselt and Cravey, 1977 as cited in Jover *et al*, 1994).

Subacute/Subchronic Toxicity

In a study by Yuen *et al*, 1995, nicotine hydrogen tartrate was administered to pregnant (10±1 day pregnant, 16 rats/dose) and non-pregnant female (72±3 day old, 24 rats/dose) Sprague Dawley rats in the drinking water for 10 days at 0, 54 or 108 μmole/L (8.76, 17.52 mg/L; equivalent to 1.25 and 2.5 mg/kg/day assuming a rat consumes 50 mL of water per day). On the 10^{th} day before sacrifice, each group was subdivided into two subgroups. One subgroup was sacrificed by cervical dislocation without further treatment. The second subgroup of rats was administered subcutaneous dose of carbon tetrachloride at 6 g/kg, 4 hours before they were killed. Nicotine treatment resulted in mild fatty change, mild focal necrosis and mild dark cell change (containing numerous prominent pore annuli in the nuclear membranes and the mitochondria appeared decreased in size with a decrease in mitochondrial granules and loss of aristae) in a dose proportional manner. In the non-pregnant rats fed with 54 μmole/L of nicotine, there was a slight increase (not statistically significant) in the number of rats showing mild fatty change and mild necrosis compared with the control group. The livers of the 108 μmole/L non-pregnant rats showed significant pathological changes in all parameters assessed in comparison to the control rats with increases in degree of fatty change, dark cell change, and of focal and confluent necrosis. The latter three parameters were also significantly more severe when compared with the low dose group. Similar pathological changes were seen in the pregnant rats treated with nicotine, but were less severe than the effects seen in the non-pregnant rats. Thus in the low dose group, no increased hepatic changes were seen compared to controls. Pretreatment with nicotine aggravated the hepatotoxicity of carbon tetrachloride. Pregnant rats were more resistant to the hepatotoxicity of both materials. This study demonstrated the hepatoxicity of nicotine. A **LOAEL** of 108 μmole/L of nicotine (2.5 mg/kg/day) in drinking water is suggested by this study based on the histopathological changes produced and the **NOAEL** is 54 μmole/L (1.25 mg/kg/day).

In another study nicotine tartrate, administered orally to 10 New Zealand white male rabbits (2.4 mg/kg/day) in the drinking water for 25 weeks produced *in vivo* morphologic effect on endothelial cells in the aortic arch (Booyse *et al*, 1981). Fasting serum levels of glucose, triglycerides, total cholesterol, and LDL-cholesterol were significantly (<0.001) elevated in nicotine-treated rabbits. Endothelial cells from nicotine-treated arched areas (Evans-blue-stained) showed extensive changes such as increased cytoplasmic silver disposition, increased formation of microvilli, and numerous focal areas of "ruffled" endothelium (projections on cell surfaces).

Chronic Toxicity

Chronic exposure of Sprague Dawley female rats (initial weight 240 g) through inhalation to 0.0 (34 rats) or 501±151 μg/ m^3 of >99% pure nicotine/m^3 (68 rats), 20 h/day, 5 days/week for 2 years resulted in slightly depressed body weight (Waldum *et al*, 1996). Rats were weighed weekly and the body weight reduction in the nicotine treated rats was less than 6% compared to controls. Body weight gains in the treated rats were initially less than the controls (70% of the controls at 4 months) and became almost comparable at 24 months (91% of the controls). Rats were housed to a maximum of 8 rats in a cage. To avoid oral intake, food and water were available *ad libitum* only during non exposure periods. Rats from each cage were weighed in a

group once weekly. Nicotine air concentration in the inhalation chambers was determined once or twice weekly throughout the two year study period and was fairly constant. The administered dose of 501±151 μg/m^3 is equivalent to 0.34 ± 0.1 mg/kg/day (based on inhalation rate of 0.29 m^3/day and 350 g average rat weight). Nicotine concentration in the blood was determined 5 days after exposure, and at 6, 12, 18 and 24 months of exposure. Also at those intervals except for the 5 day interval, specified number of rats were examined grossly for tumors in the brain, lungs, gastrointestinal tract, live, kidney and ovaries and further examined histopathologically. Rats exhibiting unhealthy signs (bristling fur, emaciation, and shiny eyes) were withdrawn (7 controls (22%) and 10 nicotine exposed (16%) rats) and these were examined wherever possible. At the end of the study, the remaining rats-7 controls and 22 nicotine exposed- were sacrificed and examined for tumors and atherosclerosis. Nicotine plasma concentration after 5 days was 108.4 ± 55.1 ng/mL and remained fairly constant and after 24 months it was 129.8±43.0 ng/mL. All parameters measured were comparable to the controls except for elevated adenomas of pituitary gland (4/59) which was attributed to the neuroendocrine action of nicotine. There was no increase in mortality, in atherosclerosis or frequency of tumors in these rats compared with controls. Particularly, there were no microscopic or macroscopic lung tumors or any increase in pulmonary neuroendocrine cells. Throughout the study, however, the body weight of the nicotine exposed rats was reduced as compared with controls. Based on this study there was no indication of any harmful effect of nicotine when given in its pure form by inhalation and the **NOAEL** is 0.3 mg/kg/day. Other potential effects of nicotine inhalation in this study were published separately and no negative effects on bone mineral density, ultimate bending moment, ultimate energy absorption, stiffness, or deflection of the femurs examined were found except for depressed body weights (Syversen *et al*, 1999).

Developmental Toxicity

Nicotine (12 mg/kg/day) or nicotine plus caffeine (125 mg/kg/day) administered by intubation by water during gestation days 6-18 to female mice (7/group) had minimal ossification effects on the fetuses as measured by staging and measuring craniofacial bones, and counting ossification centra in sternbrae and in cervical and sacrococcygeal vertebrae (Leblebicioglu-Bekcioglu *et al*, 1995). Caffeine had a significantly greater effect on fetal growth and ossification than nicotine. In this study overnight bred ICR female mice weighing 25-26 g (20/group) were intubated three times a day (4 hours apart) with 125 mg/kg caffeine, 12 mg/kg nicotine or 125 mg/kg caffeine plus 12 mg/kg nicotine on gestation days 6-18. A control group of similar number was administered distilled water. These doses were selected on the basis of a range finding study where groups of female mice (7/group) were intubated for 12 consecutive days (three times a day to sustain stable daily blood levels) with 85 mg/kg caffeine plus 8 mg/kg nicotine, or 100 mg/kg caffeine plus 10 mg/kg nicotine or 125 mg/kg caffeine plus 12 mg/kg nicotine. Nicotine blood levels ranged from 6.1 -30.2 ng/mL (mean 20.1), 31.0 – 41.6 ng/mL (mean 35.2), 52.1 – 87.7 ng/mL (mean 70.4) at the 8, 10 and 12 mg/kg nicotine dose, respectively. Nicotine blood levels peaked within three days and remained steady during exposure indicating the rapid elimination. In the main study, all pregnant rats were sacrificed on day 18, and fetuses collected and subjected to detailed examination. No compound related mortality was reported. Nicotine intubated mice experienced hyperactivity for nearly 10 minutes after intubation, during the initial 2-3 days. This was especially noted after the third dose and this effect subsided after the third day. Three mice in the caffeine plus nicotine group and two mice from the nicotine group died during the first

three days. These deaths were attributed to the nicotine treatment. None of the caffeine treated mice died.

Mean fetal body fat was significantly increased in fetuses of rats administered nicotine (2.46 ± 0.18 mg/kg/day in drinking water) during pregnancy throughout gestation day 20 (Williams & Kanagasabai, 1984). Rate for maternal lypolysis were higher in the nicotine treated animals. Maternal body weights gains were significantly lower (77.2% of controls, $p < 0.001$).

Nicotine - delivering transdermal patches applied on the back of pregnant female rats resulted in 100% pregnancy failure in 2 animals treated with 3.5 mg/day during the entire pregnancy (GD 2-19) and 50% in 8 animals exposed to the same amount during the first trimester (GD 2-7) and 55% in 13 animals exposed to 1.75 mg/day during the entire pregnancy (Witschi *et al*, 1994). Litter size and pup weights were not affected by the nicotine treatment. Nicotine and cotinine plasma levels in the sacrificed animals were not detected in animals that had carried a patch during the first trimester of pregnancy. In animals exposed the entire pregnancy at 1.75 mg/day patches, 3 pregnant animals out of six had measurable nicotine levels (43 ± 22 ng/mL) and all had cotinine levels (100 ± 48 ng/mL). The non pregnant females of the 1.75 mg/day patches had 70 ± 57 ng/mL of plasma nicotine and 231 ± 84 ng/mL of plasma cotinine. The two non-pregnant animals exposed to 3.5 mg/day patches had nicotine plasma levels of 241 ± 51 ng/mL and cotinine levels of 302 ± 94 ng/mL.

Many studies have demonstrated that nicotine penetrates the fetal brain to cause various biochemical changes with behavioral consequences on the offspring. These studies are listed in the tox profile in Appendix A.2. In all of these studies, nicotine was administered subcutaneously or intraperitonealy. Performance deficits in both learned and innate behavioral measures throughout development and adulthood in offspring of animals exposed to nicotine during gestation have been reported. Doses as low as 0.25 mg/kg/day produced behavioral changes.

Genotoxicity

The genotoxic potential of nicotine and its metabolites has been evaluated in numerous published studies. In some tests nicotine induced chromosomal aberration (CA) and sister chromatid exchange (SCE) frequencies in Chinese hamster ovary (CHO) cells at concentrations as low as 375 µg/mL for CA and 150 µg/mL for SCE (Trivedi *et al*, 1990). Whereas, other tests indicated that nicotine and its major metabolites including cotinine were negative in the Salmonella mutagenicity assay and the CHO SCE assay at concentrations ranging from 0 to 1000 µg/mL, with and without S9 metabolic activation (Doolittle *et al*, 1995). However, gross chromosomal aberrations including fuzzy chromosomes (stickiness), aneuploidy and translocations were observed in mice receiving low tolerable doses (0.07-0.09 µg/total body weight injected to young mice (Bishun *et al*, 1972). Cotinine (a metabolite of nicotine and a biological monitoring marker of nicotine absorption in humans) was positive in the presence or absence of S9 metabolic activation in the bacterial luminescence genotoxicity test at 1.25 - 2.5 mg/mL (9- 30 h incubation) while, nicotine was not positive to 20 mg/mL concentrations for up to 40 hours of incubation (Yim & Hee 1995). Nicotine has been shown to cause concomitant genotoxic and antiapoptotic effect in human gingival fibroblasts (HGFs) in the cytokinesis-block micronucleus

(CBMN) test (Argentin and Cicchetti, 2004). Recent data indicate that nicotine exerts significant direct genotoxic effects in human lymphocytes *in vitro* (Kleinsasser *et al*, 2005). These tests are presented further in Appendix A.3. of this review.

3.1.1.2 Mode of action, metabolism, toxicokinetic data

"Nicotine is an agonist at nicotinic receptors in the peripheral and central nervous system. In man, as in animals, nicotine has been shown to produce both behavioral stimulation and depression. Pharmacodynamic studies indicate a complex dose response relationship, due both to complexity of intrinsic pharmacological actions and to rapid development of tolerance." (http://www.inchem.org/documents/pims/chemical/nicotine.html)

"Nicotine inhibits the function of acetylcholine receptors located at the neuromuscular junctions. In general terms, it causes stimulation of the ganglions in low doses but causes blockade at higher concentrations. The nicotinic acetylcholine receptors (named for their interaction with nicotine, and not to be confused with the muscarinic acetylcholine receptor) are 270kD proteins with 4 subunits located in the CNS. Under normal conditions, a change in calcium ion concentration releases acetylcholine from storage vesicles. Acetylcholine then crosses the synaptic cleft and binds to the alpha subunit of the nicotinic receptor causing conformational changes which opens an ion channel, allowing the passage of cations. This depolarizes the postsynaptic membrane initiating an action potential in the adjacent membrane, and thus a signal is transmitted. Nicotine stimulates, and then blocks the acetylcholine receptor, locking the ion channels in the open position and impairing signaling ability.

The metabolism and disposition kinetics of nicotine with focus on humans has been extensively reviewed by Hukkanen *et al*, 2005. In this review, evidence is presented to the rapid absorption of nicotine from the inhaled cigarette smoke and its rapid distribution via the blood stream to various tissues including the brain. However, nicotine is poorly absorbed from the stomach because it is protonated in the acidic gastric fluid, but is well absorbed in the small intestine, which has a more alkaline pH and a large surface area. Various formulations of nicotine replacement therapy (NRT) to aid in smoking cessation, such as nicotine gum, transdermal patch, nasal spray, inhaler, sublingual tablets, and lozenges, are buffered to alkaline pH to facilitate the absorption of nicotine through cell membranes. Absorption of nicotine from all NRTs is slower and the increase in nicotine blood levels more gradual than from smoking. Only nasal spray provides a rapid delivery of nicotine that is closer to the rate of nicotine delivery achieved with smoking. Following the administration of nicotine capsules or nicotine in solution, peak concentrations in the blood are reached in about 1 hour. Oral bioavailability is incomplete because of the hepatic first-pass metabolism.

Binding to plasma proteins is minimal (<5%). Nicotine is distributed extensively to body tissues. With the highest affinity for nicotine is in the liver, kidney, spleen, and lung and the lowest affinity in adipose tissue. In poisoning cases, nicotine was found to bind to brain tissues with high affinity, and the receptor binding capacity is increased in smokers compared with nonsmokers. The increase in the binding is caused by a higher number of nicotinic cholinergic receptors in the brain of the smokers. Nicotine accumulates markedly in gastric juice and saliva. Nicotine also accumulates in breast milk (milk/plasma ratio 2.9). Nicotine crosses the placental

barrier easily, and there is evidence for the accumulation of nicotine in fetal serum and amnionic fluid in slightly higher concentrations than in maternal serum. The plasma half-life of nicotine after intravenous infusion or cigarette smoking averages about 2 h. However, when half-life is determined using the time course of urinary excretion of nicotine, which is more sensitive in detecting lower levels of nicotine in the body, the terminal half-life averages 11 h (Hukkanen *et al*, 2005).

Nicotine is extensively metabolized to a number of metabolites by the liver. Six primary metabolites of nicotine have been identified. Quantitatively, the most important metabolite of nicotine in most mammalian species is the lactam derivative cotinine. In humans, about 70 to 80% of nicotine is converted to cotinine. Other pathways of nicotine metabolism are oxidation of the pyrrolidine ring, methylation of the pyridine nitrogen giving nicotine isomethonium ion (also called *N*-methylnicotinium ion) and glucuronidation. About 3 to 5% of nicotine is converted to nicotine glucuronide and excreted in urine in humans. Oxidative *N*-demethylation is a minor pathway in the metabolism of nicotine. Conversion of nicotine to nornicotine in humans has been demonstrated (Hukkanen *et al*, 2005).

A new cytochrome P450 mediated metabolic pathway for nicotine metabolism was recently reported by Hecht et al. (2000) and summarized by Hukkanen *et al*, 2005.

3.1.1.3 Sufficiency of studies/data

Although there are no guideline studies, there are few studies suitable for the characterization of risks from human exposure to nicotine.

3.2 Hazard Identification and Toxicity Endpoint Selection

3.2.1 Acute Reference Dose (aRfD) and Chronic Reference Dose (cRfD)

No acute or chronic dietary (food and drinking water) exposure to nicotine is expected based on the use patterns. Therefore no acute or chronic reference dose was selected for this assessment.

3.2.2 Incidental Oral (Short-Term)

The endpoint selected for this risk assessment is derived from subchronic oral rat toxicity study (Yuen *et al*. 1995) discussed below. In this study, histopathological changes seen at the lower dose (1.25 mg/kg/day expressed as nicotine (alkaloid) were not statistically significant and this was considered to be a **NOAEL**. The **LOAEL** was 2.5 mg/kg/day, expressed as nicotine (alkaloid). A margin of exposure of 1000 is applied for assessment of this type to account for inter-species extrapolation (10X), intra-species variability (10X), and database uncertainty (10X).

3.2.3 Dermal Absorption

There are no dermal absorption studies available with nicotine. Numerous studies indicate that nicotine is absorbed readily through the skin. Therefore, 100% dermal absorption factor is

assumed.

When free nicotine was applied to the backs of cats weighing 1.8-4.5 kg (treated area, 5-7 cm, in diameter was clipped with scissors and not shaven) at 2- 10 mL per cat, nicotine was fatal within a few minutes. On the other hand when three cats were exposed to 10 mL of nicotine sulfate they did not succumb suggesting that the sulfate is not readily absorbed by the cat. When one of the cats was treated with 10 mL of free nicotine, it immediately succumbed (Faulkner, 1933). In other tests with cats receiving dermal doses of 200 mg of nicotine or nicotine sulfate per cat, 81% of the cats receiving the nicotine base died within 21-195 minutes (Travell, 1960). Poisoning occurred very rapidly within 1-4 minutes including vomiting, salivation, swallowing difficulty, and increased rate of respiration. The remaining nicotine treated cats were moribund after 4 hours of exposure. Symptoms of the nicotine sulfate treated cats were milder and none of the animals died. This indicated the nicotine base is absorbed completely from the skin, while nicotine sulfate which is ionized is absorbed slightly. In experiments with dogs using nicotine dermal patches, nicotine was absorbed and produced clinical signs in 15% of the treated dogs with plasma concentrations reaching 43 ng/mL (Matsushima *et al,* 1995). Nicotine's high solubility in both polar and non polar solvents (log Kw = 1.17) and its low molecular weight (162.2 g/mole) make it theoretically efficient penetrant (ZORIN *et al*, 1999).

3.2.4 Dermal Exposure (Short-, Intermediate- and Long-Term)

No nicotine dermal toxicity studies were identified. The Yuen *et al*, 1995 oral toxicity study, discussed above was selected as a basis for estimating the dermal toxicity endpoint. In this study nicotine hydrogen tartrate was administered to pregnant and non-pregnant female rats in the drinking water for 10 days at 54 or 108 mmole/L (8.76, 17.52 mg/L; equivalent to 1.25 and 2.5 mg/kg/day expressed as nicotine (alkaloid) and assuming a rat consumes 50 mL of water per day) resulted in mild fatty change, mild focal necrosis and mild dark cell change (containing numerous prominent pore annuli in the nuclear membranes and the mitochondria appeared decreased in size with a decrease in mitochondrial granules and loss of aristae) in a dose proportional manner. Histopathological changes seen at the lower dose (1.25 mg/kg/day expressed as nicotine (alkaloid) were not statistically significant and this was considered to be a **NOAEL**. The **LOAEL** was 2.5 mg/kg/day, expressed as nicotine (alkaloid). A margin of exposure of 1000 is applied for assessment of this type to account for inter-species extrapolation (10X), intra-species variability (10X), and database uncertainty (10X).

The selected endpoint for this risk assessment is supported by another study where nicotine tartrate, administered orally to 10 New Zealand white male rabbits (2.4 mg/kg/day) in drinking water for 25 weeks produced *in vivo* morphologic effect on endothelial cells in the aortic arch (Booyse *et al*, 1981). Fasting serum levels of glucose, triglycerides, total cholesterol, and LDL-cholesterol were significantly (<0.001) elevated in nicotine-treated rabbits. Endothelial cells from nicotine-treated arched areas (Evans-blue-stained) showed extensive changes such as increased cytoplasmic silver disposition, increased formation of microvilli, and numerous focal areas of "ruffled" endothelium (projections on cell surfaces).

The selected endpoint is applicable to all exposure durations, since other studies have shown that nicotine is eliminated quickly from the body and its serum levels or its metabolites do not

accumulate with continuous dosing suggesting that its toxicity is more dependent on the dose than the exposure period.

3.2.5 Inhalation Exposure (Short-, Intermediate- and Long-Term)

The endpoint selected for this risk assessment is derived from the subchronic oral rat toxicity study (Yuen *et al*. 1995) discussed above. In this study, histopathological changes seen at the lower dose (1.25mg/kg/day expressed as nicotine (alkaloid) were not statistically significant and this was considered to be a **NOAEL**. The **LOAEL** was 2.5 mg/kg/day, expressed as nicotine (alkaloid). A margin of exposure of 1000 is applied for assessment of this type to account for inter-species extrapolation (10X), intra-species variability (10X), and database uncertainty (10X). This selected endpoint is applicable to all exposure durations, since other studies have shown that nicotine is eliminated quickly from the body and its serum levels or its metabolites do not accumulate with continuous dosing suggesting that its toxicity is more dependent on the dose than the exposure period.

The Waldum *et al* 1996 inhalation study discussed earlier suggested a NOAEL of 0.3 mg/kg/day based on lack of adverse effects. Although, this study was well conducted, it used one dose only which limits its use of selecting this NOAEL as a Point of Departure (POD) for toxicity. The effects seen were minimal reduced weight gain during the long exposure duration of the study.

3.2.6 Level of Concern for Margin of Exposure

Table 3.2.6 Summary of Levels of Concern for Risk Assessment of Nicotine.

Route	Short-Term (1 - 30 Days)	Intermediate-Term (1 - 6 Months)	Long-Term (> 6 Months)
Occupational (Worker) Exposure			
Dermal	1000	1000	NA
Inhalation	1000	1000	NA
Residential Exposure			
Dermal	1000	NA	NA
Inhalation	1000	NA	NA
Incidental Oral	1000	NA	NA

3.2.7 Classification of Carcinogenic Potential

According to the International Program on Chemical Safety (IPCS) review, literature reports indicate that nicotine is neither an initiator nor a promoter of tumors in mice. There is inconclusive evidence to suggest that cotinine, an oxidized metabolite of nicotine, may be carcinogenic in the rat (http://www.inchem.org/documents/pims/chemical/nicotine.htm). Nicotine has not been evaluated by the NCI National Toxicology Program, the International Agency for Research on Cancer and the EPA Integrated Risk Information System for its potential carcinogenicity.

3.2.8 Summary of Toxicological Doses and Endpoints for Use in Human Risk Assessments

Table 3.2.8. Summary of Toxicological Doses and Endpoints for Nicotine for Use in Occupational and Residential Human Health Risk Assessments

Exposure/ Scenario	Point of Departure	Uncertainty Factors	Level of Concern for Risk Assessment	Study and Toxicological Effects
Incidental Oral, short-term exposure (1-30 days)	NOAEL=1.25 mg nicotine alkaloid /kg/day	UF_A=10x UF_H=10x UF_{DB}=10x	Residential LOC for MOE = 1000	Yuen *et al*. 1995: oral study in drinking water LOAEL = 2.5 mg nicotine alkaloid/kg/day based on hepatotoxicity: mild fatty change, mild focal necrosis and mild dark cell change (containing numerous prominent pore annuli in the nuclear membranes and the mitochondria appeared decreased in size with a decrease in mitochondrial granules and loss of aristae) were seen in both pregnant and non-pregnant rats, but more severe in the non-pregnant rats.
Dermal, Short-Term (1-30 days) and Intermediate-Term (1-6 months)	NOAEL=1.25 mg nicotine alkaloid /kg/day	UF_A=10x UF_H=10x UF_{DB}=10x	Occupational and Residential LOC for MOE = 1000	Yuen *et al*. 1995: oral study in drinking water
Inhalation, Short-Term (1-30 days) and Intermediate-Term (1-6 months)	NOAEL=1.25 mg nicotine alkaloid /kg/day	UF_A=10x UF_H=10x UF_{DB}=10x	Occupational and Residential LOC for MOE = 1000	Yuen *et al*. 1995: oral study in drinking water
Cancer (oral, dermal, inhalation)	Classification: Nicotine has not been evaluated by the NCI National Toxicology Program, the International Agency for Research on Cancer and the EPA Integrated Risk Information System for its potential carcinogenicity. It has not been tested in rodents exempt for the Waldum *et al*, 1996 inhalation study which tested one dose only. According to the International Program on Chemical Safety (IPCS) review, literature reports indicate that nicotine is neither an initiator nor a promoter of tumors in mice. There is inconclusive evidence to suggest that cotinine, an oxidized metabolite of nicotine, may be carcinogenic in the rat.			

Point of Departure (POD) = A data point or an estimated point that is derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). UF_L = use of a LOAEL to extrapolate a NOAEL. UF_S = use of a short-term study for long-term risk assessment. UF_{DB} = to account for the absence of key date (i.e., lack of a critical study). MOE = margin of exposure. LOC = level of concern. N/A = not applicable.

3.3 Endocrine disruption

EPA is required under the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." Following recommendations of its Endocrine Disruptor and Testing Advisory Committee (EDSTAC), EPA determined that there was a scientific basis for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

When additional appropriate screening and/or testing protocols being considered under the Agency's EDSP have been developed, nicotine may be subjected to further screening and/or testing to better characterize effects related to endocrine disruption.

There is experimental evidence suggesting endocrine effects by nicotine. A few of these studies are discussed below. Other studies are listed in the toxicity profile.

Nicotine inhibited ovulation, estradiol production, and fertilization both *in vivo* and *in vitro* in rat models of ovulation when pregnant mare's serum gonadotropin (PMSG) - primed and human chorionic gonadotropin (hCG) - triggered rat ovaries were exposed to nicotine (ip injection of 6.25 ng/g animal weight) (Blackburn *et al*, 1994). A dose dependent reduction in oocytes within the fallopian tube was noted in nicotine treated rats ($p<0.001$). On the other hand cotinine, the primary nicotine metabolite, did not affect ovulation, estradiol production or fertilization in those tests.

Nicotine administration (1 mg/kg or 10 mg/kg, subcutaneously twice daily to rats for 1 or 2 weeks) produced alterations in catecholamines (CA) release, tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH), and the ability of isolated storage vesicles to incorporate 3H-epinephrine in the adrenal glands and these alterations persisted when nicotine administration was discontinued (Slotkin and Seidler, 1975).

Prenatal exposure to nicotine can interfere with the development of the male gonadal axis and with the organization of sexually dimorphic behavior (Lichtensteiger and Schlumpf 1985). Time-pregnant Sprague Dawley rats were implanted on gestational day (GD) 12 with an osmotic minipump containing either nicotine tartrate (delivered at a rate of 25 ug/100 g x hr), tartaric acid or saline. Others were sham-operated on GD 12 or left untreated. Male fetuses of all control groups displayed the characteristic rise in plasma testosterone at GD 18 (as compared to GD 17 and 19); this was abolished by nicotine. Adult offspring of untreated or tartaric acid-treated dams exhibited a marked sexual dimorphism in their preference for saccharin-containing drinking water at 0.06-0.25%. No such sex difference was seen in offspring of nicotine-treated rats. In controls, the sexes differed with respect to the proportion of rats with high saccharin

preference. In the group of males prenatally exposed to nicotine, the proportion of animals with high preference increased to the female level.

4.0 Public Health and Pesticide Epidemiology Data

4.1 Incident Reports

4.1.1 Fulex Nicotine Fumigator (EPA Reg. No. 1327-41)

See: Review of Nicotine Incident Reports, J. Blondell and M. Spann, D276938, 08/10/2001.

The following databases were searched by HED for poisoning incident data on the active ingredients nicotine (PC Code 056702) and nicotine sulfate (PC Code 056703): (1) OPP Incident Data System (IDS); (2) Poison Control Centers; (3) California Department of Pesticide Regulation; and (4) National Pesticide Telecommunications Network (NPTN).

Three nicotine related incident reports were identified in IDS. A pesticide incident occurred in 1994 (Incident #2796-52), when an individual, who was not wearing gloves, reported nausea and convulsions three hours after handling nicotine shreds. The individual was treated overnight in the hospital. A second pesticide incident occurred in 1998 (Incident #7981-1), when the owner of a home misused the smoke generator product to control roaches in the home. A woman and her children were exposed to the product and one of the children (a 10-year old male) reported coughing. A third pesticide incident occurred in 1999 (Incident #9175-1), when a pest control operator illegally used a nicotine-based bug bomb in a small apartment. A woman and her four children reported chest tightness, nausea, and coughing.

Results from the years 1993 through 1998 were acquired for 45 exposures to nicotine reported to Poison Control Centers. Cases involving exposures to multiple products were excluded. Only 12 cases were reported among children under six years of age and no cases among older children and adults exposed at their workplace. This was too few cases to warrant a detailed analysis. None of these cases reported a serious or even a moderate outcome. There were 31 non-occupational exposure cases among older children and adults. Of these cases, 16 had outcomes determined of which none were moderate or major cases.

Detailed descriptions of 20 cases submitted to the California Pesticide Illness Surveillance Program (1982-1999) were reviewed. In 12 of these cases, nicotine was used alone or was judged to be responsible for the health effects. Only cases with a definite, probable or possible relationship were reviewed. None of the individuals required hospitalization and two of the twelve individuals were reported to have taken 1 or 2 days off from work. Work activities included applicator, drift, field residue, and routine or unknown occupational activity. Drift was associated with more exposures than any other work activity. Illnesses included symptoms of difficulty breathing, throat tightness, headache, nausea, and eye irritation. Nicotine ranked 81st as a cause of systemic poisoning in California based on data for 1982 through 1994.

On the list of top 200 chemicals for which NPTN received calls from 1984-1991 inclusively, nicotine was ranked 178th with 14 incidents in humans reported and 1 incident in animals.

4.1.2 Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465)

The OPP Incident Data System (IDS) was searched by HED (email communication with N. Spurling, OPP/ITRMD/ISB, 07/25/2007) for poisoning incident data on the active end-use products EPA Reg. No. 4-465 (Bonide® Rabbit & Dog Chaser) and EPA Reg. No. 779-29 (according to OPPIN, transferred from Faesy & Bestoff to Bonide® Rabbit & Dog Chaser 08/16/2001). No incidents were reported in IDS for these end-use products.

5.0 Dietary Exposure/Risk Characterization

A dietary (food + water) risk assessment was not conducted. Neither of the two end-use products which are being supported under reregistration are registered for use on food or feed and HED and EFED have agreed that conducting a drinking water risk assessment is not appropriate.

6.0 Residential (Non-Occupational) Exposure/Risk Characterization

6.1 Fulex Nicotine Fumigator (EPA Reg. No. 1327-41)

See: Occupational and Residential Exposure/Risk Assessment for the Nicotine and derivatives Reregistration Eligibility Decision (RED) Addressing the Fulex Nicotine Greenhouse Fumigator, B. Cropp-Kohlligian, D341249, 08/xx/2007.

NOTE: 40 CFR 157.21(e) defines "residential use" as follows: "Residential use means use of a pesticide or device: (1) directly on humans or pets; (2) In, on, or around any structure, vehicle, article, surface, or area associated with the household, including but not limited to areas such as non-agricultural outbuildings, non-commercial greenhouses, pleasure boats and recreational vehicles; or (3) in or around any preschool or daycare facility."

No residential (non-occupational) handler exposure scenarios have been identified for the Fulex Nicotine [Greenhouse] Fumigator use; however, residential (non-occupational) postapplication exposure scenarios *via* inhalation and dermal routes have been identified. These exposures are expected to be short-term in duration.

The Fulex Nicotine Fumigator (EPA Reg. No. 1327-41) is a restricted use product for use by and under direct supervision of a certified applicator (40 CFR §152.175); however, the current label does not prohibit application to privately owned greenhouses in residential settings by certified commercial applicators where it is possible for residents to be exposed following application and according to information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), the product is used in retail greenhouses where it is considered possible for non-occupational members of the general public to be exposed following application. These non-commercial postapplication exposures are addressed by the occupational postapplication risk assessment for this end-use product. (*See Section 9.1.3*)

6.2 Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465)

See: Occupational and Residential Exposure/Risk Assessment for the Nicotine and derivatives Reregistration Eligibility Decision (RED) Addressing the Bonide Dog and Rabbit Repellant, B. Cropp-Kohlligian, D341897, 08/xx/2007.

Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) is a ready-to-use animal repellant for use in outdoor residential settings containing nicotine (as a naturally occurring component of tobacco dust). It is intended for use by homeowners as a repellent *via* a barrier/perimeter treatment to prevent eastern cottontail rabbits (*Sylvilagus floridanus*) from eating and defecating on ornamental plants and domestic dogs (*Canis l. familiaris*) from defecating on ornamentals, including lawns. It may also be used around the perimeter of vegetable gardens.
Based on use patterns, residential (homeowner) handlers may be exposed to nicotine (as a naturally occurring component of tobacco dust) while applying the product by hand. No postapplication scenarios have been identified for homeowners with the exception of potential concern for availability to homeowners in packages which are not child resistant and could be accessible to children prior to application or potential for children to come in contact with the product postapplication; hence, an episodic ingestion is also being assessed.

The registrant has not provided any chemical-specific data to assess the exposure for this risk assessment.

6.2.1 Residential (Homeowner) Handler Risks

It has been determined that there is a potential for dermal and inhalation exposure in residential settings during the application of Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465; label date 12/28/2006). Based on the currently registered Bonide® Rabbit & Dog Chaser end-use product label (EPA Reg. No. 4-465; label date 12/28/2006) and information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), non-dietary exposures to this product are expected to be short-term in duration.

Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465; label date 12/28/2006) contains 0.35% nicotine (as a naturally occurring component of tobacco dust). The product is sprinkled directly from the package in 2 or more inch-wide bands around areas to be treated. A 3-pound package of product will produce a band of product 1 inch wide and 85 feet long. The reviewer estimates that a 3-pound package of product will provide a barrier/perimeter around a 144 ft^2 area at most.

Residential MOEs equal to or greater than 1000 are not of concern to HED. The short-term combined dermal and inhalation MOE for residential handlers who apply one three-pound package of the ready-to-use Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) was 1875 and therefore not of concern to HED. However, application of two or more three-pound packages would be of concern, since this is estimated to result in a combined dermal and inhalation MOE less than 1000. The concerns are primarily for dermal exposure/risk and HED notes that the inhalation/dermal risk estimates may be considered conservative since, in the absence of chemical-specific data, they are based on theoretical data/calculations and default assumptions, including 100% dermal absorption, for this product containing nicotine as a naturally occurring component of tobacco. *See Table 6.2.1.*

6.2.1.1 Data and Assumptions for Handler Exposure Scenarios

The quantitative exposure/risk assessment developed for residential handlers (applicators only) is based on the following exposure scenario in the PHED Surrogate Exposure Guide, as a surrogate for application of this product:

· **Applicator**: Standard Operating Procedures (SOPs) for Residential Exposure Assessments (PHED Version 1.1) Wettable Powder, Open Mixing and Loading

Assumptions and Factors:

- Based on the currently registered Bonide® Rabbit & Dog Chaser end-use product label (EPA Reg. No. 4-465; label date 12/28/2006) and information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), no mixing/loading methods are necessary for this ready-to-use end-use product. Therefore, a mixing/loading exposure assessment was not performed.

- HED used the Standard Operating Procedures (SOPs) for Residential Exposure Assessments (PHED Version 1.1) Wettable Powder, Open Mixing and Loading to assess residential handler exposure. This scenario which considers a wettable powder formulation is deemed best suited to represent a residential handler applying a dust formulation by hand while wearing short pants, a short-sleeved shirt, and no gloves.

- The application rate is based on the currently registered Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465; label date 12/28/2006) end-use product label. **The label does not specify a maximum use rate in terms of lb ai/A. The reviewer has estimated that a 3-pound package of product, containing 0.35% nicotine (as the alkaloid), will provide a barrier/perimeter treatment around ≤144 ft^2 area.**

- Body Weight: An average adult body weight of 70 kg was used for short-term calculations, since the dermal and inhalation endpoints were based on effects that were not sex-specific.

Equations and Calculations:

- **Average Daily Dose (ADD):** Daily dose (inhalation or dermal) was calculated by normalizing the daily dermal or inhalation exposure value by body weight and accounting for dermal or inhalation absorption. Dermal and inhalation absorption factors of 100% were assumed. Daily dose was calculated using the following formula:

$$\text{ADD (mg/kg/day)} = \text{Daily Exposure (mg ai/day)} \times \left\{\frac{\text{Absorption Factor (\%/100)}}{\text{Body Weight (kg)}}\right\}$$

Where,

ADD	=	Average daily dose absorbed dose received from exposure to a pesticide in a given scenario (mg pesticide active ingredient/kg body weight/day)
Daily Exposure	=	Amount (mg ai/day) deposited on the surface of the skin that is available for dermal absorption or amount inhaled that is available for inhalation absorption;
Absorption Factor	=	A measure of the amount of chemical that crosses a biological boundary such as the skin or lungs
Body Weight	=	Body weight determined to represent the population of interest in a risk assessment.

- **Margin of Exposure (MOE):** the calculations of daily dermal dose and daily inhalation dose received by handlers were then compared to the appropriate endpoint (i.e., NOAEL) to assess the total risk to handlers for each exposure route within the scenarios. All MOE values were calculated separately for dermal and inhalation exposure levels using the following formula:

$$\text{MOE} = \frac{\text{NOAEL (mg/kg/day)}}{\text{ADD (mg/kg/day)}}$$

Where:

MOE = Margin of exposure value used by HED to represent risk or how close a chemical exposure is to being a concern (unitless)
ADD = Average daily dose is absorbed dose received from exposure to pesticide
NOAEL = Dose level in a toxicity study, where no observed adverse effects occurred in the study

- **Total MOE:** When the dermal and inhalation endpoints, effects and routes of exposure are the same the doses may be added together to determine a total dose and MOE using the following formula:

$$\text{TOTAL MOE} \quad \frac{\text{NOAEL (mg/kg/day)}}{\text{Dermal Dose (mg/kg/day)} + \text{Inhalation Dose (mg/kg/day)}}$$

Table 6.2.1. Residential Handler (Applicator Only) Short-term Exposure and Risk for Nicotine (as a naturally occurring component of tobacco dust).

Scenario	Use Site	Dermal Unit Exposure (mg/lb) [1]	Inhalation Unit Exposure (mg/lb) [1]	Application Rate [2] (lb ai/site/day)	Area Treated	Dermal Dose [3] (mg/kg/day)	Dermal MOE [5]	Inhalation Dose [4] (mg/kg/day)	Inhalation MOE [6]	Total MOE [7]
					Applicator					
Nicotine (0.35% a.i) **dust** Reg.# 4-465	In and around homes as a barrier/perimeter treatment to repel rabbits and dogs	4.4	0.0434	0.0105 (1 3-pound package)	42.5 linear feet; could provide a barrier/perimeter around a 144 ft^2 area (or 0.0033 acres) 42.5 ft/2π = 6.77 ft area = π(6.77 ft)^2 = 144 ft^2	6.6E-4	1894	6.5E-6	1.9E5	1875
				0.0210 (2 3-pound packages)	288 ft^2 area (or 0.0066 acres)	1.32E-3	947	1.3E-5	9.6E4	938

1 Standard Operating Procedures (SOPs) for Residential Exposure Assessments (PHED Version 1.1) Wettable Powder, Open Mixing and Loading; Dermal unit exposure (assumes short pants, short sleeved shirt and no gloves) = 4.4 mg/lb; Inhalation Unit Exposure = 0.0434 mg/lb.
2 Application Rate based on currently registered Bonide Rabbit & Dog Chaser end-use product label (EPA Reg. No. 4-465) and assuming 1, 5, or 10 3-pound packages of product are used per day. One 3-pound package of product contains 0.35% nicotine (as the alkaloid) or 0.0105 lb nicotine (as the alkaloid).
3 Short-term Dermal Dose (mg/kg/day) = [Rate (lb ai/A/day) x UE (mg /lb ai) / BW (70 kg)
4 Short-term Inhalation Dose (mg/kg/day) = [Rate (lb ai/A/day) x UE (mg /lb ai) / BW (70 kg)
5 Short-term Dermal MOE = [Dermal NOAEL (1.25 mg/kg/day)]/ Dermal Dose (mg/kg/day)
6 Short-term Inhalation MOE = [Inhalation NOAEL (1.25 mg/kg/day)] / Inhalation Dose (mg/kg/day)
7 Total MOE = NOAEL (1.25 mg/kg/day) / Dermal Dose (mg/kg/day) + Inhalation Dose (mg/kg/day)
Note: Dermal and Inhalation LOC is 1000.

6.2.2 Residential (Homeowner) Postapplication Exposure

Because the use pattern results in applications to areas not frequented by children or in areas where maintenance would result in significant exposure, dermal and incidental oral assessments were not conducted. However, episodic ingestion of the material is considered reasonable due to packages which are not child resistant and which could be accessible to children prior to application or due to the potential for children to come in contact with the product postapplication.

Residential MOEs equal to or greater than 1000 are not of concern to HED. The short-term oral MOE for episodic ingestion of the ready-to-use Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) containing nicotine (as a naturally occurring component of tobacco dust) was 1 and therefore is of concern to HED.

On average, the bulk density of the ready-to-use Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) is 35 lb/ft^3. This is equivalent to approximately 8.3 grams of product per tablespoon or 2.8 grams of product per teaspoon calculated as follows:

35 lb/ft^3 x 454 g/lb x 1ft^3/1915 tablespoons = 8.3 g/tablespoon or 2.8 g/teaspoon

Ingestion of more than 0.002 teaspoons of product by a toddler (15 kg) exceeds the LOC for the MOE (1000); however, this type of ingestion is considered an episodic event and not a routine behavior. Because HED does not believe that this would occur on a regular basis, our concern for human health is related to acute poisoning rather than short -term residue exposure. See Table 6.2.2.

6.2.2.1 Data and Assumptions for Episodic Oral Ingestion

This scenario was assessed using the residential SOP 2.3.1, Postapplication Potential Among Toddlers from Ingestion of Pesticide Granules from Treated Areas. This SOP provides a standard method for estimating postapplication doses among toddlers from incidental ingestion of pesticide granules.

Assumptions and Factors:

- Ingestion rate for dry pesticide formulation (granules) is 5 gram/day. This is based on poison specialist estimate used in the Rodenticide Cluster RED (1998) which assumed that a one year old child weighing 10 kg could consume approximately 5 grams of a granular formulation in one swallow. Note: This estimated ingestion rate (5 grams of product/day) is equivalent to approximately 1% (by weight) of a 1-pound package of Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) which may be applied as multiple one inch bands of product around the perimeter of lawns and gardens.

- Average weight of toddler is 15 kg

Equations and Calculations:

- **Potential Daily Dose (PDD):** Potential daily dose (oral) was calculated by normalizing the amount of chemical that could be ingested by body weight. Daily dose was calculated using the following formula:

  PDD = IgR x F x CF1 ÷ BW

  Where,

  PDD = Potential Daily Dose
  IgR = ingestion rate of dry pesticide formulation (grams/day)
  F = fraction of ai in dry formulation (%)
  CF1 = weight unit conversion factor to convert g units to mg for daily exposure (1,000 mg/g)
  BW = Body Weight (15kg)

- **Margin of Exposure (MOE):** The calculations of potential daily dose were then compared to the appropriate endpoint (i.e., NOAEL) to assess the risk using the following equation:

  Short-term Oral MOE =NOAEL (1.25 mg/kg/day) ÷ Potential Daily Dose (PDD)

Table 6.2.2. Postapplication Exposure and Risk for Episodic Ingestion

Scenario	IgR (g/day)	F	CF1 (mg/g)	Dose [1] (mg/kg/day)	MOE [2]
Episodic Ingestion	5	0.35%	1000	1.17	1
	0.005 (0.002 tsp)			0.00117	**1000**

1 Dose = IgR x F x CF1 ÷ BW

Where,

IgR = ingestion rate of dry pesticide formulation (grams/day)
F = fraction of ai in dry formulation (%)
CF1 = weight unit conversion factor to convert g units to mg for daily exposure (mg/g)
BW = Body Weight (15kg)

2 MOE = NOAEL (1.25 mg/kg/day)/Dose
Note: LOC is 1000.

6.3 Other (Spray Drift, etc.)

Spray drift is always a potential source of exposure to residents nearby to spraying operations. This is particularly the case with aerial application, but, to a lesser extent, could also be a potential source of exposure from the ground application method employed for nicotine. The Agency has been working with the Spray Drift Task Force, EPA Regional Offices and State Lead Agencies for pesticide regulation and other parties to develop the best spray drift management practices. On a chemical by chemical basis, the Agency is now requiring interim mitigation measures for aerial applications that must be placed on product labels/labeling. The Agency has completed its evaluation of the new database submitted by the Spray Drift Task Force, a membership of U.S. pesticide registrants, and is developing a policy on how to appropriately apply the data and the AgDRIFT computer model to its risk assessments for pesticides applied by air, orchard airblast and ground hydraulic methods. After the policy is in place, the Agency may impose further refinements in spray drift management practices to reduce off-target drift with specific products with significant risks associated with drift.

7.0 Aggregate Risk Assessments and Risk Characterization

An aggregate (food + water + residential exposure) risk assessment was not conducted. Neither of the two end-use products which are being supported under reregistration are registered for use on food or feed and HED and EFED have agreed that conducting a drinking water risk assessment is not appropriate.

8.0 Cumulative Risk Characterization/Assessment

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to nicotine and any other substances and nicotine does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that nicotine has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

9.0 Occupational Exposure/Risk Pathway

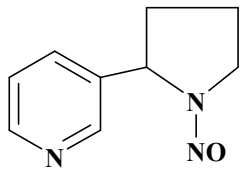
9.1 Fulex Nicotine Fumigator (EPA Reg. No. 1327-41)

See: Occupational and Residential Exposure/Risk Assessment for the Nicotine and derivatives Reregistration Eligibility Decision (RED) Addressing the Fulex Nicotine Greenhouse Fumigator, B. Cropp-Kohlligian, D341249, 08/xx/2007.

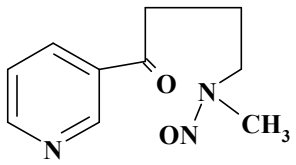
No nature of the residue data are available for this unique application method which alters nicotine's physical state and has the potential to form degradates. During fumigation, nicotine may be present as a vapor and possibly bound or adsorbed to particulate matter.

A nicotine pyrolysis study has not been submitted but according to the 1981 Report of the Surgeon General entitled, "The Health Consequences of Smoking; The Changing Cigarette", during tobacco curing, fermentation, and **burning**, nicotine gives rise to N-nitrosonornicotine (NNN) and 4-(N-methyl-N-nitrosamino)-1-(3-pyridil)-1-butanone (NNK). See structures below.

NNN



NNK



Given the lack of sophistication of the ignition device (sparklers), without nature of the residue data, it is difficult to determine if nicotine is only vaporized (and to some extent adsorbed to particulate matter) or decomposed as a result of the application method. If actually burned, formation of NNN and NNK cannot be excluded without supporting data.

Exposure estimates in this risk assessment are based on maximum theoretical air concentration data/calculations and default assumptions, all potential degradates of nicotine from the Fulex Nicotine [Greenhouse] Fumigator use have been included in the exposure estimates of this risk assessment and the *de facto* hazard assumption is equivalent toxicity with nicotine.

9.1.1 Regulatory Standards Fulex Nicotine Fumigator (EPA Reg. No. 1327-41)

The American Conference and Governmental Industrial Hygienists (ACGIH) have established a threshold limit value (TLV) as an 8-hour time-weighted average (TWA) of 0.5 mg/m^3 for nicotine.[1] This value is intended to minimize the potential for gastrointestinal disturbances, cardiovascular effects, and adverse central nervous system effects. A skin notation is based on the well-documented percutaneous absorption of nicotine and resultant systemic toxicity from handling uncured tobacco leaves[2] and in the manufacture[3] and application[4] of nicotine insecticides. Sufficient data were not available to recommend a Senisitizer (SEN) notation or carcinogenicity notation or a TLV as a short-term exposure limit (STEL).

The 8-hour intake of nicotine received by inhalation was calculated as 0.07 mg/kg/day; [5] based on the metabolism and controlled dosing of human volunteers[5,6] and on the NOAEL level of 1.14 mg/kg/day found in chronic studies in rodents.[7] In a chronic rat study (Wenzel and Richards), rats given 1.14 or 4.56 mg nicotine/kg/day for 34 weeks in their drinking water produced subtle biochemical changes (increased isocitric dehydrogenase and β-glucuronidase activities) in myocardiums only at the high dose.

The Occupational Safety and Health Agency (OSHA) has established a permissible exposure limit (PEL) as an 8-hour time-weighted average (TWA) of 0.5 mg/m^3 for nicotine[1] consistent with the ACGIH TLV and The National Institute for Occupational Safety and Health (NIOSH) concurs with the OSHA PEL.[1]

NIOSH has established a value of 5 mg/m^3 for nicotine as a level that is immediately dangerous to life or health (IDLH).[1] No inhalation toxicity data were identified on which to base and IDLH for nicotine. Therefore, the IDLH is based on acute oral toxicity data in humans[8] and animals.[8,9]

References

1. American Conference of Governmental Industrial Hygienists. (2004) 1995-1996 Threshold Limit Values (TLVs) for Chemical Substances and Physical Agents and Biological Exposure Indices (BEIs).
2. Gehlback, S.H.; Williams, W.A.; Perry, L.D.; et al.: Green-Tobacco Sickness; An Illness of Tobacco Harvesters. JAMA 229:1880-1883 (1974).
3. Lockhart, L.P.: Nicotine Poisoning. Br. Med. J. 1:246-274 (1933).
4. Haines, K.A.; Davis, L.G.: Protection of Workers Applying Nicotine alkaloid as a Concentrated Mist Spray. J. Econ. Entomol. 4:513 (1948).
5 Hayes, Jr., W.J.: Pesticides Studied in Man, pp. 86-91. Williams & Wilkins, Baltimore (1982).
6 Henningfield, J.E.; Miyasato, K.; Jesinski, D.R.: Abuse Liability and Pharmacodynamic Characteristics of Intravenous and Inhaled Nicotine. J. Pharmacol. Exp. Ther.234:1-12 (1985).
7 Wenzel, D.G.; Richards, M.H.: Effects of Chronic Nicotine, Acute, Hypoxia and Their Interactions on Myocardial Enzymes. Toxicol. Appl. Pharmacol. 16:656-667 (1970).
8 Lazutka, F.A.; Vasilyauskene, A.D.; Gefen, S.C.: Toxicological Evaluation of the Insecticide nicotine sulfate. Gig. Sanit. 34(5):3033 (1969).
9 Franke, F.E.; Thomas, J.E.: A note on the minimal fatal dose of nicotine for unanesthetized dogs. Proc. Soc. Exp. Biol. Med. 29:1177-1179 (1932).

9.1.2 Short-/Intermediate-/Long-Term (Non-Cancer) Handler Risk

Since short-term and intermediate-term risk estimates will be based on the same endpoint (1.25 mg/kg/day) and level of concern (1000), only short-term risks have been calculated. A summary of the short-term (non-cancer) inhalation risks for handlers is included in Table 9.1.2.

Inhalation MOEs greater than 1000 are not of concern. The short-term inhalation risk estimate resulted in a MOE less than 1000 for all individuals performing application activities except those using a self-contained breathing apparatus (SCBA) which is estimated to provide 99.99% protection (PF10,000). Moreover, inhalation risks of concern (*i.e.*, MOEs less than 1000) were identified for individuals using half- and full-face respirators with chemical and particulate filter cartridges for exposure periods of 1 and >3 minutes, respectively. This risk estimate may be considered conservative since it is based on theoretical air concentration data/calculations and default assumptions.

Fulex Nicotine Fumigator (EPA Registration No. 1327-41) contains 13.4% nicotine as a smoke fumigator for use as an insecticide in greenhouses for the prevention and/or treatment of aphids and most thrips. The application rate is one 12 ounce canister per 20,000 ft^3 or one 6 ounce canister per 10,000 ft^3. Each ready-to-use 12 ounce canister contains 0.10 lbs ai.

The application method is as follows:

- close all vents;
- shake each canister;
- distribute the canisters spaced equidistant in the center aisle of an ordinary greenhouse;
- remove top covers;
- starting with canister farthest from exit, light the sparkler at the bottom near the handle;
- insert lighted sparkler deeply into the contents of the canister;
- proceed to next canister, until all canisters are ignited.

Based on the number of applications indicated on the currently registered end-use product label (EPA Reg. No. 1327-41; dated 08/10/2005) and information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), handler exposures are expected to be short-term and intermediate-term in duration.

9.1.2.1 Data and Assumptions for Handler Exposure Scenarios

The quantitative exposure/risk assessment developed for commercial handlers is based on the following exposure scenario:

- **Applicator**: Smoke Generator Canister

The assumptions, parameters and factors used for the exposure calculations include:

- Based on the currently registered end-use product label (EPA Reg. No. 1327-41; dated 08/10/2005) and information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), no mixing/loading methods are necessary for these ready-to-use

end-use products. Therefore, a mixing/loading exposure assessment was not performed.

- The application rate for this product was based on the currently registered end-use product label (EPA Reg. No. 1327-41; dated 08/10/2005). The application rate was adjusted to pounds of active ingredient per 12 ounce canister. The application rate for a 12 ounce canister is 0.10 lb ai per 20,000 cubic feet, using the following equation:

  (12 ounces/canister)(1canister/20,000 ft^3)(1 lb/16 ounces)(0.134 ai) = 0.10 lb ai/20,000 ft^3

- The air concentration was calculated at the maximum application rate. The application rate was adjusted to an air concentration of 80.1 mg ai/m^3 (milligrams of active ingredient per cubic meters). The air concentration was calculated using the following equation:

  (0.10 lb ai/20,000 ft^3)(35.3 ft^3/m^3)(454,000 mg/lb) = 80.1 mg ai/m^3

- The same nicotine air concentration is assumed to be encountered by handlers when they apply/light smoke canisters and when they enter the treated greenhouse to open vents and dispose of canisters. These two activities are considered as a single exposure scenario.

- A single handler could treat multiple greenhouses per day. The exposure period for handlers would depend on the number of canisters used to treat an individual greenhouse and the number of individual greenhouses treated per day. Therefore, handler exposure periods per day are estimated at 30 minutes or less for smaller greenhouse facilities and up to an hour for larger facilities.

- For handlers (*i.e.*, those who ignite the canisters as well as those who enter before the WPS ventilation criteria are met to open vents and collect spent canisters), dermal exposures are assumed to be negligible relative to the exposures and risks from inhalation. This assumption is based on the use pattern where potential dermal exposure is limited to possible contact with nicotine : (1) while opening the canisters and inserting the sparkler, (2) an accidental spill during lighting of a canister or retrieval of an unlit canister, and (3) possible contact with residue on the outside of a spent canister. These dermal exposures are expected to be relatively infrequent and of relatively short duration in comparison with the estimated inhalation exposure times. Therefore, a dermal exposure assessment for handlers was not performed.

- Handler exposure assessments for inhalation routes of exposure were completed by HED using no respirator (baseline), 10PF respirator, 50 PF respirator, and 10,000PF respirator. While the currently registered end-use product label specifies that handlers must use a respirator with either an organic vapor-removing cartridge with a prefilter approved for pesticides (MSHA/NIOSH approval number prefix TC-23C) or a canister approved for pesticides (MSHA/NIOSH approval number prefix TC-14G), the label does not specify if the respirator is a half-face or full-face mask.

- Body Weight: An average adult body weight of 70 kg was used since the dermal and inhalation endpoints were based on effects that were not sex-specific.

- The maximum air concentration levels potentially encountered by handlers assumes that during fumigation all of the active ingredient in the smoke canister enters the greenhouse air at the maximum label application rate.

- Air Concentration Equations:

Inhalation Dose (mg/kg/day) = (C_a x BR x ET)/BW
Where,
C_a = air concentration at time of application (time = 0)
BR = adult breathing rate (1 m^3/hr)
ET = exposure time; assumed to comprise the time to ignite canisters and re-enter after the fumigation but before the WPS ventilation criteria are met to open vents and dispose of canisters
(smaller greenhouse 30 min/day = 0.50 hr/day)
(larger greenhouse 60 min/day = 1.0 hr/day)
BW = body weight (70 kg)

- Margin of Exposure (MOE) Equation:

MOE = Inhalation NOAEL (mg/kg/day)/ Inhalation Dose (mg/kg/day)
Where,
Short-term and Intermediate-term Inhalation NOAEL = 1.25 mg/kg/day
Note: Inhalation LOC is 1000.

Table 9.1.2. Short-Term Occupational (Non-cancer) Handler Inhalation Exposure and Risk for Nicotine Used in Greenhouses. [1]

Mitigation Level	C_a (mg ai/m^3)	BR (m^3/hr)	ET (hr/day)	BW (kg)	Inhalation Dose [1] (mg/kg/day)	Inhalation MOE [2]
Baseline No Respirator	80.1	1	0.5	70	0.57	2
			1.0		1.14	1
PF10 Respirator Half-face organic-vapor-removing respirator providing 90% protection	8.01	1	0.5	70	0.057	22
			1.0		0.114	11
	8.01	1	0.011 (<1 minutes)	70	0.00125	1000
PF50 Full-face organic-vapor-removing respirator providing 98% protection	1.6	1	0.5	70	0.01	125
			1.0		0.02	63
	1.6	1	0.055 (~3 minutes)	70	0.00125	1000
PF10,000 Self-contained breathing apparatus (SCBA) providing 99.99% protection	0.008	1	1.0	70	0.0001	12500

Note: Shaded areas provide estimates of exposure time with PPE at which the estimated Inhalation MOE is at or above the LOC (1000).

1 Inhalation Dose (mg/kg/day) = [C_a (mg ai/m^3) x BR (m^3/hr) x ET (hr/day)] / BW (70 kg)
Where,
C_a = air concentration at time of application (time = 0)
BR = adult breathing rate (1 m^3/hr)
ET = exposure time; assumed to comprise the time to ignite canisters and re-enter after the fumigation but before the WPS ventilation criteria are met to open vents and dispose of canisters.
(smaller greenhouse 30 min/day = 0.50 hr/day)
(larger greenhouse 60 min/day = 1.0 hr/day)
BW = body weight (70 kg)

2 MOE = Inhalation NOAEL (mg/kg/day) / Inhalation Dose (mg/kg/day)
Where,
Short-and Intermediate-term Inhalation NOAEL = 1.25 mg/kg/day
Note: Inhalation LOC is 1000.

9.1.3 Short-/Intermediate-/Long-Term (Non-Cancer) Postapplication Risk

A summary of the dermal exposure and risk during postapplication activities is included in Table 9.1.3.1. Based on the currently registered end-use product label (EPA Reg. No. 1327-41; dated 08/10/2005) and information provided by the registrant (Use Closure for Nicotine RED, J. Bloom, 06/18/2007), postapplication exposures are expected to be short-term and intermediate-term in duration. Short-term dermal postapplication MOEs were below 1000 (the level of concern) on the day of treatment. This risk estimate may be considered conservative since it is based on theoretical surface residue levels/calculations and default assumptions, including 100% dermal absorption, in the absence of acceptable chemical-specific data. For dermal risks, a Restricted Entry Interval of 40+ days is required to achieve acceptable MOEs.

A summary of the inhalation exposure and risk during postapplication activities is included in Table 9.1.3.2. The MOE after 10 air changes (a ventilation option from the Worker Protection Standard (WPS)) is 3049 and does not exceed the level of concern (MOE is greater than 1000). This risk estimate may be considered conservative since it is based on theoretical air concentration data/calculations and default assumptions in the absence of acceptable chemical-specific data. While the other WPS ventilation options are not quantifiable without assumptions for greenhouse size and ventilation rates, it is assumed, because the WPS ventilation options are considered equally protective, that the resulting exposure/risk is equal for all six ventilation options.

9.1.3.1 Data and Assumptions for Dermal Postapplication Exposure Scenarios

Exposures during postapplication activities were estimated using dermal transfer coefficients from the Science Advisory Council For Exposure: Agricultural Reentry Task Force (ARTF) Ornamental Plants Transfer Coefficients, April 2002, summarized in Table 9.1.3.1.1, and the following assumptions:

- Application Rate = 2.178 lb ai/A

- The application rate for this product was based on the currently registered end-use product label (EPA Reg. No. 1327-41; dated 08/10/2005). The application rate was adjusted to pounds of active ingredient per acre. The application rate for an assumed 10 foot greenhouse ceiling is 2.178 lb ai/acre, using the following equation:

  (0.10 lb ai/canister)(1 canister/20,000 ft^3)(43,560 ft^2/acre)(10 ft ceiling) = 2.178 lb ai/acre

- Exposure Duration = 8 hours per day

- Body Weight = 70 kg

- Fraction of a.i. retained on foliage is assumed to be 20% (0.2) on day zero (= % dislodgeable foliar residue, DFR, after initial treatment). This fraction is assumed to further dissipate at the rate of 10% (0.1) per day on following days. These are standards values established by HED's Science Advisory Council (SAC) for Exposure.

Table 9.1.3.1.1. Anticipated Postapplication Activities and Dermal Transfer Coefficients.			
Crops	**Transfer Coefficients (cm^2/hr)**	**Activities**	**Reference**
Ornamentals	175	greenhouse hand pinching ornamentals; nurseries activities	MRID 453445-01; ARTF Study No. ART039
	400	harvest (workers moved plants to trucks and reorganized the gallon pots or containers)	MRID 454695-02; ARTF Study No. ART044
	5100	hand harvesting cut flowers	ExpoSAC Meeting minutes 01/26/2006-

The information in the table is based on proprietary and non-proprietary data.

Equations/Calculations:

The following equations were used to calculate dermal risks for workers performing postapplication activities:

- **Disoldgeable Foliar Residue (DFR)**
  DFR_t (ug/cm^2) = Application Rate (lb ai/acre) x F x $(1-D)^t$ x 4.54E8 µg/lb x 2.47E-8 acre/cm^2
  Where:
  DFR_t = dislodgeable foliage residue on day "t" (ug/cm^2)
  F = fraction of ai retained on foliage (0.2 unitless)
  D = fraction of residue that dissipates daily (0.1 unitless)

- **Dermal Dose $_t$ = $\frac{DFR_t \text{ (µg/cm}^2\text{) x 1E-3 mg/µg x Tc (cm}^2\text{/hr) x ET (hrs)}}{\text{BW (kg)}}$**

  Where,
  t = number of days after application day (days)
  DFR_t = dislodgeable foliage residue on day "t" (ug/cm^2)
  Tc = transfer coefficient (cm^2/hr)
  ET = exposure time (hr/day)
  BW = body weight (kg)

- **MOE = Dermal NOAEL (mg/kg/day)/ Dermal Dose (mg/kg/day)**
  Where,
  Short-and Intermediate-term Dermal NOAEL = 1.25 mg/kg/day
  Note: Dermal LOC is 1000.

9.1.3.2 Data and Assumptions for Inhalation Postapplication Exposure Scenarios

According to the Worker Protection Standard (40 CFR Parts 170.110) greenhouse re-entry following application of a pesticide applied as a smoke is governed by one of six ventilation options when no inhalation exposure level is specified on the label:

- 10 air changes; or
- 24 hours with no ventilation; or
- 2 hours mechanical ventilation; or
- 4 hours passive ventilation; or
- 11 hours no ventilation with 1 hour mechanical ventilation; or
- 11 hours no ventilation with 2 hours passive ventilation.

Postapplication inhalation exposure and risk were calculated for re-entry based on the assumption that the area air concentration will follow pseudo-first order kinetics upon ventilation (i.e., air changes or ventilation rate) [ExpoSAC Meeting Minutes, 01/26/2006]. The following equation was used for this calculation:

$C_{AC} = C_i \times 0.5^{(\#\ ACH/0.693)}$
Where,
C_{AC} = air concentration after number of air changes of interest (mg ai/m^3)
C_i = initial air concentration at time of application (80.1 mg ai/m^3)
ACH = number of air changes (10)

Inhalation Dose (mg/kg/day) = (C_{AC} x BR x ET)/BW
Where,
C_{AC}= air concentration after number of air changes of interest
BR = adult breathing rate (1 m^3/hr)
ET = exposure time (8 hr/day)
BW= body weight (70 kg)

MOE = Inhalation NOAEL (mg/kg/day)/ Inhalation Dose (mg/kg/day)
Where,
Short- and Intermediate-term Inhalation NOAEL = 1.25 mg/kg/day
Note: Inhalation LOC is 1000.

Table 9.1.3.1. Non-cancer Dermal Postapplication Exposure and Risk for Nicotine Used in Greenhouses.						
Crops	DAT[1]	DFR[2] (ug/cm^2)	Dermal Dose[3] (mg/kg/day)		Dermal MOE[4]	
			Low (Tc=175)	High (Tc= 5100)	Low	High
Ornamentals, Baseline	0	4.88	0.0976	2.844	13	<1
	3 (RTI[5])	3.56	0.0712	2.075	18	<1
	40	0.0721	0.00144	0.042	868	30

1 DAT = Days after treatment
2 DFR = Dislodgeable Foliar Residue
3 Dermal Dose = [DFR (ug/cm^2) x Tc (cm^2/hr) x 0.001 mg/ug x 8 hrs/day] ÷ Body Weight (70 kg)
4 Dermal MOE = NOAEL (1.25 mg/kg/day)/Dermal Dose
Note: Dermal LOC is 1000.
5 RTI = Minimum Retreatment Interval

Table 9.1.3.2. Non-cancer Inhalation Postapplication Exposure and Risk for Nicotine Used in Greenhouses.								
Exposure Scenario (Scenario #)	C_i (mg/ai/m^3)	#ACH	C_{AC} [1] (mg ai/m^3)	BR (m^3/hr)	ET (hr/day)	BW (kg)	Inhalation Dose [2] (mg/kg/day)	Inhalation MOE [3]
Greenhouse Re-entry	80.1	10	0.0036	1	8	70	0.00041	3049

1 $C_{AC} = C_i \times 0.5^{(\# ACH/0.693)}$

Where,

C_{AC} = air concentration after number of air changes of interest (mg ai/m^3)

C_i = initial air concentration at time of application (80.1 mg ai/m^3)

ACH = number of air changes (10)

2 Inhalation Dose (mg/kg/day) = [C_{AC} (mg ai/m^3) x BR (m^3/hr) x ET (hr/day)] / BW

Where,

C_a= air concentration at time of application (time = 0)

BR= adult breathing rate (1 m^3/hr)

ET= exposure time (8 hr/day)

BW = body weight (70 kg)

3 Inhalation MOE = Inhalation NOAEL (1.25 mg/kg/day) / Inhalation Dose (mg/kg/day)

Note: Inhalation LOC is 1000.

9.2 Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465)

See: Occupational and Residential Exposure/Risk Assessment for the Nicotine and derivatives Reregistration Eligibility Decision (RED) Addressing the Bonide® Dog & Rabbit Chaser, B. Cropp-Kohlligian, D341897, 08/xx/2007.

The Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) end-use product label does not provide any distinction in use directions for professional (occupational) and non-professional (residential homeowner) applicators and no occupational postapplication scenarios have been identified. The residential handler exposures and risks have been assessed (*see Section 6.2.*1) which should be protective of occupational handlers.

10.0 Data Needs and Label Recommendations

10.1 Toxicology

870.1100	Acute Oral Toxicity
870.1200	Acute Dermal Toxicity
870.1300	Acute Inhalation Toxicity
870.2400	Primary Eye Irritation
870.3200	21-day dermal toxicity study in rats
870.3465	90-day inhalation study-duration reduced to 21 days
870.3700a	Prenatal developmental - rodent
870.3800	Reproduction and fertility effects

10.2 Occupational and Residential Exposure

Fulex Nicotine Fumigator (EPA Reg. No. 1327-41)

1. In accordance with 40 CFR 156.212(e), the Personal Protective Equipment (PPE) should be based on acute toxicity of the end-use product. HED notes that the label recommends use of waterproof gloves for applicators. HED recommends that this be revised to chemical-resistant gloves.

2. Label(s) must specify respirator type (half-face, full-face, other).

NOTE: The technical grade of the active ingredient nicotine is classified as a Category I toxicant based on acute oral toxicity data which would, under the Worker Protection Standard (WPS), require a minimum restricted entry interval (REI) of 48-hours (40 CFR §156.208(c)); however, this criteria for determining the REI does not apply to any product that is a fumigant (40 CFR §156.208(d)). Hence, the REI for the Fulex Nicotine [Greenhouse] Fumigator, if it is determined to be a fumigant, will be governed by the WPS ventilation criteria (40 CFR §170.110(c)(3)) and product-specific REI calculations. In the absence product-specific data collected in accordance with 40 CFR §158.390, these calculations have been based on theoretical data/calculations and default assumptions.

NOTE: Although not a data requirement, the registrant should be aware that refinements to the exposure estimates would be possible with acceptable nature of the residue data to determine the residues of concern and area air monitoring and foliar dislodgeable residue data to provide better estimates of the level(s) of exposure to the residue(s) of concern. It is expected that none of these types of studies require the participation of human subjects; however, protocols for these studies must be evaluated by the HSRB before studies are initiated.

Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465)

1. The product contains 0.35% nicotine (as a naturally occurring component of tobacco dust). **The nicotine content of tobacco dust in the end-use product is substantially less than the content normally found in dried tobacco leaves (up to 8% by weight according to the Merck Index) and in the tobacco found in cigarettes (on average, 1.5% by weight according**

to the 1988 report of the Surgeon General, entitled, "The Health Consequences of Smoking"). The nicotine content of Bonide® Rabbit & Dog Chaser (EPA Reg. No. 4-465) must be substantiated by preliminary analysis (830.1700) and certified limits (830.1750) product chemistry data for the active ingredient, nicotine (alkaloid).

2. Label should be amended to specify a maximum use rate in terms of lb ai/A/application and seasonal limitations.

References:

AGENCY MEMORANDA CITED IN THIS DOCUMENT

DP Barcode: D276938
Subject: Review of Nicotine Incident Reports. Chemical #056702 and #056703.
From: J. Blondell and M. Spann
To: B. Cropp-Kohlligian
Dated: 08/10/2001
MRIDs: None

DP Barcode: None
Subject: Use Closure for Nicotine RED
From: J. Bloom
To: Nicotine RED team
Dated: 06/18/2007
MRIDs: None

DP Barcode: D341249
Subject: Nicotine: Occupational and Residential Exposure/Risk Assessment for the Nicotine and derivatives Reregistration Eligibility Decision (RED) Addressing the Fulex Nicotine Greenhouse Fumigator Insecticide.
From: B. Cropp-Kohlligian
To: J. Bloom
Dated: 08/xx/2007
MRIDs: 46165801

DP Barcode: D341897
Subject: Nicotine (as a naturally occuring component of tobacco dust): Occupational and Residential Exposure/Risk Assessment for the Nicotine and derivatives Reregistration Eligibility Decision (RED) Addressing the Bonide Rabbit and Dog Repellent.
From: B. Cropp-Kohlligian
To: J. Bloom
Dated: 08/xx/2007
MRIDs: None

MRID REFERENCES

46165801 Krake, A. (1997) Health Hazard Evaluation Report 96-0032-2649: Fulex Nicotine Fumigator. Project Number: 96/0032/2649. Unpublished study prepared by Cornell University. 20 p.

Appendix A: Toxicology Assessment

A.1 Toxicology Data Requirements

The requirements (40 CFR 158.340) for non food for NICOTINE are in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Test	Technical	
	Required	Satisfied
870.1100 Acute Oral Toxicity	yes	no
870.1200 Acute Dermal Toxicity	yes	no
870.1300 Acute Inhalation Toxicity	yes	no
870.2400 Primary Eye Irritation	yes	no
870.2500 Primary Dermal Irritation	yes	yes
870.2600 Dermal Sensitization	yes	yes
870.3100 Oral Subchronic (rodent)	NR	-
870.3150 Oral Subchronic (nonrodent)	NR	-
870.3200 21-Day Dermal	Yes	no
870.3250 90-Day Dermal	NR	-
870.3465 90-Day Inhalation (21 – day)	Yes	no
870.3700a Developmental Toxicity (rodent)	Yes	no
870.3700b Developmental Toxicity (nonrodent)	NR	-
870.3800 Reproduction	Yes	no
870.4100a Chronic Toxicity (rodent)	NR	-
870.4100b Chronic Toxicity (nonrodent)	NR	-
870.4200a Oncogenicity (rat)	NR	-
870.4200b Oncogenicity (mouse)	NR	-
870.4300 Chronic/Oncogenicity	NR	-
870.5100 Mutagenicity—Gene Mutation - bacterial	Yes	Yes[$]
870.5300 Mutagenicity—Gene Mutation - mammalian	Yes	Yes[$]
870.5xxx Mutagenicity—Structural Chromosomal Aberrations	Yes	Yes[$]
870.5xxx Mutagenicity—Other Genotoxic Effects	Yes	Yes[$]
870.6100a Acute Delayed Neurotox. (hen)	NR	-
870.6100b 90-Day Neurotoxicity (hen)	NR	-
870.6200a Acute Neurotox. Screening Battery (rat)	NR	-
870.6200b 90-Day Neuro. Screening Battery (rat)	NR	-
870.6300 Develop. Neuro	NR	-
870.7485 General Metabolism	NR	-
870.7600 Dermal Penetration	NR	-
Special Studies for Ocular Effects		
Acute Oral (rat)	NR	-
Subchronic Oral (rat)	NR	-
Six-month Oral (dog)	NR	-

[$] Published studies satisfy this requirement.

A.2 Toxicity Profiles

TableA.2.1a. Acute Toxicity Profile – Nicotine				
Guideline No.	Study Type	MRID No./Reference	Results	Toxicity Category
Non-Guideline	Acute oral – rat Acute oral - muse	IPCS INCHEM, 1991*	LD_{50} = 50 mg/kg LD_{50} = 3 mg/kg	I I
Non-Guideline	Acute oral – dog	Matsushima *et el*, 1995	LD_{50} = 10-12 mg/kg	I
Non-guideline	Acute i.p. - mice	Priestly and Plaa 1976	LD_{50} = 13.5 mg/kg	I
870.1200	Acute dermal - rabbit	NA		
870.1300	Acute inhalation - rat	NA		
870.2400	Eye irritation - rabbit	NA		
870.2500	Dermal irritation – rabbit (40% nicotine formula)	41521101	Slightly irritant	IV
870.2600	Skin sensitization (40% nicotine formula)	41521102	Not a skin sensitizer	

* http://www.inchem.org/documents/pims/chemical/nicotine.htm

Table A.2.1b Acute Toxicity Profile - F&B Rabbit and Dog Chaser (mixture of tobacco, naphthalene and sterilized cattle blood)				
Guideline No.	Study Type	MRID(s)	Results	Toxicity Category
870.1100	Acute oral: rat	43523301	LD_{50} = > 5000 mg/kg	IV
870.1200	Acute dermal: rabbit	42631101	LD_{50} = greater than 2020 mg/kg	IV
870.1300	Acute inhalation: rat	42631102	LC_{50} = >5.39 mg/L	IV
870.2400	Acute eye irritation: rabbit	42631103	minimally irritating	IV
870.2500	Acute dermal irritation: rabbit	42631104	Mildly irritating	IV
870.2600	Skin sensitization: guinea pig	42640001	Not sensitizer	NA

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Non-guideline	Subacute Pregnant and non-pregnant female rats	Yuen *et al*. 1995 Nicotine tartrate in drinking water for 10 days at 54 or 108 μmole/l (8.76, 17.52 mg/l = 1.25 or 2.5 mg/kg/day)	Resulted in mild fatty change, mild focal necrosis and mild dark cell change (containing numerous prominent pore annuli in the nuclear membranes and the mitochondria appeared decreased in size with a decrease in mitochondrial granules and loss of aristae) in a dose proportional manner statistically significant at 2.5 mg/kg/day. The **NOAEL** is 2.5 mg/kg/day and the **NOAEL** is 1.25 mg/kg/day.
Non-guideline	Subchronic Sprague Dawley Rats	Lau *et al*. 1990 adult male rats (185-225 g initial weight) were implanted with nicotine pellets containing 0, 5, 15 or 50 mg nicotine (delivering 0, 9.9, 29.8, and 99.2 ug/hr, respectively) for up to 12 weeks (equivalent to 0.0, 1.2, 3.6, 12.0 mg/kg/day)	Increased pancreatic enzyme biosynthesis and accumulation of digestive enzymes within the pancreas were reported. Plasma nicotine at the 50 mg dose reached a steady state level of 76±19 ng/mL and cotinine exceeded 300 ng/mL. Electron microscopy of pancreas from rats treated with the 50 mg nicotine dose revealed intracytoplasmic vacuoles appearing after 3 weeks of treatment, and persisting throughout the remaining experimental period (evidence of morphological damage). Nicotine treatment and durations did not have an effect on body weight, pancreatic weight, DNA, RNA or protein concentrations. A **LOAEL** of 12 mg/kg/day based on pancreatic morphologic change is suggested by the study findings and a **NOAEL** is 3.6 mg/kg/day..
Non-guideline	Subchronic Sprague Dawley Rats	(Dubick *et al*, 1988) adult male rats (190-200 g) receiving nicotine via a time–release pellet at a rate of 1.65 ug/min)equivalent to 12 mg/kg/day) for 3 weeks	Showed that increases in digestive pancreatic enzymes (amylase, trypsin and chymotrypsin; 51%, 28%, 35% higher) were noted compared to controls. Final body weight or pancreatic weight was not affected.
Non-guideline	Subchronic CF-1 Swiss derived mice	(Priestly and Plaa 1976) Male mice, 20-25 g initial weight) consuming nicotine HCl in their drinking water (25 mg/L; equivalent to 7 mg/kg/day) daily for 2-3 months or injected i.p. with 5 mkg once or for 3 weeks	no evidence of hepatotoxicity as measured by serum glutamic-pyruvic transaminase or serum alkaline phosphatase activities. The LD_{50} of nicotine HCl administered i.p. in mice was 13.5 mg/kg. Nicotine did not modify the hepatotoxic response of CCl4 or the less potent hepatotoxins chloroform or 1,1,1-trichloroethane nor the cholestatic effect of α-naphthylisothiocyanate was modified.
Non-guideline	Subchronic Sprague Dawley Male Rats	Kavitharaj and Vijayammal, 1999 Nicotine, subcutaneously at 0.0, 0.6 mg/kg/day for 21 days, or 0.6 mgk nicotine for 21 days + mecamylamine at 0.8 mgk on Days 21, 23, and 25 (6 rats/group, 1.5 months old, 120-150 g weight) sacrificed on Day 35.	Administration of nicotine produced enhanced synthesis of cholesterol, triglycerides, phospholipids and free fatty acids in the liver and testes and lower serum testosterone and estradiol levels suggesting gonadotoxic effects. Body weight gain at sacrifice time in the nicotine treated rats was less than the control rats (22.14 ± 0.86 g vs 34.28 ± 1.61 g in the controls). The activity of the lipogenic enzymes (NADPH generating enzymes) was higher in the liver, but not altered in the testes. Mecamylamine (a known inhibitor of nicotine) counteracted the effects seen in rats treated with nicotine only.

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Non-guideline	Subchronic Adult Female Rats	Fung *et al*, 1999 nicotine 3.0 or 4.5 mg/kg/day (9-10 rats/group) by subcutaneous osmotic minipumps	had significantly lower levels of 25-hydroxyvitamin D than controls. The high dose nicotine group had smaller vertebral areas and a lower bone mineral content and significantly lower tibial endocortical mineral apposition rate than the controls. Nicotine serum levels at sacrifice time were 60 ± 6 and 85 ± 5 ng/mL at the low and high dose, respectively.
Non-guideline	Subchronic Male New Zealand white rabbits	Booyse *et al*, 1981 Nicotine tartrate, administered orally (2.4 mg/kg/day) in drinking water for 25 weeks to 10 rabbits	produced *in vivo* morphologic effect on endothelial cells in the aortic arch. Fasting serum levels of glucose, triglycerides, total cholesterol, and LDL-cholesterol were significantly (<0.001) elevated in nicotine-treated rabbits. Endothelial cells from nicotine-treated arched areas (Evans-blue-stained) showed extensive changes such as increased cytoplasmic silver disposition, increased formation of microvilli, and numerous focal areas of "ruffled" endothelium (projections on cell surfaces).
Non-guideline	Subchronic Sprague Dawley Male Rats	Chowdhury *et al*, 1990 nicotine ingested for 16 weeks at 50 and 200 mg/l in drinking water equivalent to 7 and 28 mg/kg/day	alters metabolic, endocrine, and pathologic factors that may be responsible, at least in part, for the development of gastrointestinal ulcers and pancreatitis. Endocrinological studies showed that the plasma levels of CCK were significantly increased with nicotine but the amylase secretory response of pancreatic acinar cells was inhibited in response to CCK-8 and carbachol. Prominent loss of gastric mucosal surface was found in nicotine-treated animals with gross microscopic evidence of bleeding ulcers. All of the metabolic parameters except body weight gain were reversed upon nicotine withdrawal. Histopathologic data showed a partial but not a complete recovery of the pancreatic acinar cell morphology and gastric mucosal surface following 4 weeks of nicotine withdrawal.
870.3200	21/28-Day dermal toxicity (species)	NR	
870.3250	90-Day dermal toxicity (species)	NR	

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Non-Guideline	Chronic, Inhalation Female rats	Waldum *et al*, 1996 inhalation to 501±151 µg/m^3 , 20 h/day, 5 days/week for 2 years (equivalent to 0.3 ± 0.09 mg/kg/day)	resulted in slightly depressed weight (Waldum *et al*, 1996). Nicotine plasma concentration after 5 days was 108.4 ± 55.1 ng/mL and remained fairly constant and after 24 months it was 129.8±43.0 ng/mL. All parameters measured were comparable to the controls except for elevated adenomas of pituitary gland (4/59) which was attributed to the neuroendocrine action of nicotine. There was no increase in mortality, in atherosclerosis or frequency of tumors in these rats compared with controls. Particularly, there were no microscopic or macroscopic lung tumors or any increase in pulmonary neuroendocrine cells. Throughout the study, however, the body weight of the nicotine exposed rats was reduced as compared with controls.
Non-Guideline	Developmental Mouse	Saad *et al*, 1990, 1991 Pregnant CD-1 mice, exposed to ip injection of 0.1% nicotine sulfate at a dose of 1.67 mg/kg body weight/day on gestational days 6-15	Depressed maternal weight gain and fetal weight. Fetal crown-rump length fetal head dimensions were significantly reduced. Histological examination revealed that 9.6% of fetuses of nicotine injected mothers presented clefts of the palate (none in the control). Nicotine treatment also had teratogenic effects on first molar odontogenesis in the mouse. The mesiodistal diameter of molar tooth germs of nicotine treated fetuses without cleft palate was significantly less than those of controls.
Non-Guideline	Developmental Rat	Chowdhury & Bromage 2000 experimental details of nicotine treatment were not presented	dental asymmetries (calculated as a size difference between a tooth and its antimere) were significantly increased while occlusal areas were significantly decreased in nicotine-exposed rats compared to control rats. Females tending to exhibit the deleterious effects of nicotine more so than males
Non-Guideline	Developmental Mouse	Nasrat *et al*, 1986 Nicotine administered subcutaneously to mice during gestation at ≥ 450 µg/kg/day dose	Nicotine increased the perinatal mortality. The male to female ratio was in favor of females in the nicotine treated mice during the 2nd and 3rd trimester. When large doses of the drug were given especially in the second and third trimesters of gestation, there was a significant shortening of the gestation period.
Non-Guideline	Developmental Mouse	Leblebicioglu-Bekcioglu *et al*, 1995 Nicotine (12 mkg or nicotine plus caffeine (125 mkg) by intubation GD6-18. (7/group)	Nicotine had minimal ossification effects on the fetuses as measured by staging and measuring craniofacial bones, and counting ossification centra in sternbrae and in cervical and sacrococcygeal vertebrae. Caffeine had greater effect.
Non-Guideline	Developmental Rat	Williams & Kanagasabai, 1984. 2.46 ± 0.18 mg/kg/day in drinking water. GD 0-20	Mean fetal body fat was significantly increased in fetuses of rats administered nicotine during pregnancy. Rate for maternal lypolysis were higher in the nicotine treated animals. Maternal body weights gains were significantly lower (77.2% of controls, $p < 0.001$).

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Non-Guideline	Developmental Rat	(Witschi *et al*, 1994) Nicotine - delivering transdermal patches applied on the back of pregnant female rats 3.5mg/day (2 rats GD 2-19; 8 rats GD 2-7; 3 rats GD 2-5) 1.75 mg/day patch (13 rats GD 2-19; 4 rats GD 2-7)	Nicotine –caused 100% pregnancy failure in animals treated GD2-19 with 3.5 mg/day and 50% in animals treated GD 2-7, and 55% in animals exposed GD2-19 to 1.75 mg/day. Litter size and pup weights were not affected. Nicotine and cotinine not detected in animals exposed the first trimester of pregnancy. In animals exposed the entire pregnancy at 1.75 mg/day patches, 3 pregnant animals out of six had measurable nicotine levels (43 ± 22 ng/mL) and all had cotinine levels (100 ± 48 ng/mL). The non pregnant females of the 1.75 mg/day patches had 70 ± 57 ng/mL of plasma nicotine and 231 ± 84 ng/mL of plasma cotinine. In the two animals exposed to 3.5 mg/day patches and became nonpregnant, nicotine plasma levels were 241 ± 51 ng/mL and cotinine levels of 302 ± 94 ng/mL.
Non-Guideline	Developmental Rat (Sprague Dawley	Slotkin *et al*, 1986 Subcutaneous injection of nicotine (3 mg/kg/day from gestation day 4 -20) to timed pregnant Sprague Dawley rats (41 control and 57 nicotine treated)	significant reduced maternal weight gain and increased maternal mortality (14%) and fetal resorptions (13%). Litter size was not affected. Biochemical changes in the fetal brain (elevation of fetal ornithine decarboxylase activity, suppression of DNA synthesis (most profound in cerebellum), desynchronization of the ontogenctic patterns of DNA, RNA and proteins in every brain region)
Non-Guideline	Developmental Rat (Sprague Dawley)	Muneoka *et al*, 1999 Subcutaneous injections of nicotine (3 or 6 mg/kg/day, divided into two doses) on GD 7-20 to pregnant rats (10/group)	Abnormal behavior such as ataxic gate, decrease in spontaneous activity, creeping, or salivation in pregnant rats (3 or 6 mg/kg/day) and convulsion and ptosis (6 mg/kg), decreased maternal body weight at GD 14 (both groups) and reduced offspring body weight. Significant decreases in dihydroxyphenylacetic acid (DOPAC) content in the neocortex and in both the neocortex and in the midbrain plus pons medulla, respectively.
Non-Guideline	Developmental Rat	Hussein *et al*, 2007 nicotine dose of 6 mg/kg/day administered to Sprague Dawley time-mated rats from gestation day 3 through 21 by osmotic mini pump	resulted in nicotine plasma concentrations of 115-174 ng/mL. Maternal hematocrit was not affected by nicotine administration, or the number of pups per litter, pup weight, placental weight or the weight of various pups' organs. Maternal weight changes were comparable in the nicotine treated and control groups. Maternal plasma coticosterone concentrations were not affected by the nicotine infusion versus the control rats.

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Non-Guideline	Developmental Rat	Zahalka *et al* 1993 Nicotine's effects on ontogeny of postsynaptic muscarinic M1-receptors in rat striatum and hippocampus were investigated after continuous maternal infusions (15-21 rats/treatment) of 2 mg/kg/day or 6 mg/kg/day from GD 4 through 20.	Brain region weights were unaffected. Postnatal development of striatal M1-receptor binding, as identified with [^{3}H]pirenzepine, was significantly impaired with either of the fetal nicotine regimens. Treatment with 2 mkg also produced alterations in striatal receptor affinity state, characterized by enhanced ability of an agonist (oxotremorine-M) to displace [^{3}H]pirenzepine; raising the dose to 6 mkg masked the affinity shift by affecting G-protein regulatory mechanisms, such that addition of the GTP analog, GppNHp, produced a larger decrease in agonist affinity. In the hippocampus, no such effects on receptor binding, affinity state, or G-protein regulation were seen with either regimen.
870.3800	Reproduction and fertility effects (species)	NA	
870.4100a	Chronic toxicity (species)	NA	
870.4100b	Chronic toxicity (dog)	NA	
870.4200	Carcinogenicity (rat)	NA	
870.4300	Carcinogenicity (mouse)	NA	
Gene Mutation Non-Guideline		Tests by the R. J. Reynolds Tobacco Co. (Doolittle *et al*, 1995)	Nicotine and its major metabolites: cotinine, nicotine-N'-oxide, cotinine-N-oxide, and trans-3'-hydroxycotinine in the Salmonella mutagenicity assay (strains TA98, TA100, TA1535, TA1537, and TA1538) at 0 to 1000 µg/plate and in the Chinese hamster ovary sister-chromatid exchange (SCE) assay at 0 to 1000 µg /mL, with and without S9 metabolic activation did not increase the frequency of mutations or the frequency of SCEs.
Cytogenetics Non-Guideline	Bacterial luminescence genotoxicity test	Yim & Hee 1995	Cotinine was positive in the presence or absence of S9 at 1.25 - 2.5 mg/mL (9- 30 h incubation). Nicotine was not positive to 20 mg/mL concentrations for up to 40 hours of incubation. Nicotine/cotinine mixtures were still positive at physiological concentrations, with potentiation relative to cotinine alone with and without S9

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Other Effects Non-Guideline	Chromsomal aberration (CA) and sister chromatid exchange (SCE)	Trivedi *et al*, 1990	Nicotine induced chromsomal aberration (CA) and sister chromatid exchange (SCE) frequency in a dose and duration dependent manner and at concentrations comparable to the saliva levels of nicotine achieved during tobacco chewing. Statistically significant elevations in CA frequency were observed with nicotine concentrations > 375 μg/mL; SCE frequencies were increased significantly > 150 μg/mL. Nicotine (150 μg/mL) and in combination with arecoline (an areca nut alkaloid) incubated in Chinese hamster ovary (CHO) cell line (-S9) increased the SCE frequency/cell. Nicotine alone at 300 μg/mL did not increase the frequency of CHE/cell, but in combination with arecoline it did at ≥90 μg/mL.
Genotoxicity Non-Guideline	Chromosomal aberrations	Bishun *et al*, 1972	Nicotine was cytotoxic at 1.5 - 2.0 μg/mL to human leucocytes in culture *in vitro* without producing any chromosome damage. However, gross chromosomal aberrations including fuzzy chromosomes (stickiness), aneuploidy and translocations were observed in the mice receiving low tolerable doses of the drug (0.07 - 0.09 μg/total body weight injected to 5 weeks-4 month old mice in saline , two weekly injections for 3 weeks before sacrifice and preparing the bone marrow from the femora.
Genotoxicity Non-Guideline	DNA damage	Kleinsasser *et al*, 2005 The genotoxicity of nicotine was tested with the alkaline single-cell microgel electrophoresis (Comet) assay using human lymphocytes and target cells from lymphatic tissue of the palatine tonsils from healthy patients.	Nicotine exerted significant direct genotoxic effects in human target cells *in vitro*. One hour exposure to nicotine at 0.125, 0.25, 0.5, 1, 2, and 4 mM induced a statistically significant dose-dependent increase of DNA migration up to 3.8-fold and 3.2-fold in tonsillar cells and lymphocytes, respectively. The minimum concentration eliciting significant DNA damage was 0.5 mM nicotine. The genotoxic effect was confirmed in a second series of experiments using nicotine of high purity from two different suppliers. Finally, DNA damage by nicotine was compared in cells incubated in medium strictly adjusted to neutral pH, with nonadjusted medium becoming alkaline with increasing nicotine concentrations. Again no differences in DNA migration were observed. However, no differences in DNA damage were observed in cells from smokers and nonsmokers incubated without nicotine. The lack of higher DNA damage in smokers compared to nonsmokers could be a question of nicotine dose, rapid DNA repair, or interactions with other smoke constituents.
Genotoxicity Non-Guideline	Human nasal epithelia.	Sassen *et al* 2005	Nicotine produced genotoxic effects in human nasal epithelia.

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Genotoxicity Non-Guideline	Human gingival fibroblasts (HGFs)	Argentin and Cicchetti, 2004	Nicotine caused concomitant genotoxic and antiapoptotic effect in human gingival fibroblasts (HGFs) in the cytokinesis-block micronucleus (CBMN) test. Treatment of HGFs with nicotine, at a concentration of 1 uM, caused a statistically significant increase of micronucleus (MN) frequency at the tested time intervals, while no change was detected in cell growth under the same conditions. Preincubation of HGFs with 1 uM nicotine strongly attenuated staurosporine (STP)-induced apoptosis. Cultures exposed to nicotine showed an increase of reactive oxygen species, as determined by increased levels of 2,7-dichlorofluorescein (DCF). When cells were prelabeled with N-acetyl-cysteine (NAC), a substrate for glutathione synthesis, and catalase (CAT), the oxygen free radical scavenger, a significant reduction in cytogenetic damage was observed.
870.6200a	Acute neurotoxicity screening battery	NA	
870.6200b	Subchronic neurotoxicity screening battery	NA	
870.6300 Non-Guideline	Developmental neurotoxicity	Temocin *et al*, 1993 subcutaneous injection of nicotine (0.125 - 0.375 mg/kg)	Decreased the endurance time in swimming exercise significantly (10 minute after the injection). At 0.125 mg/kg nicotine, the endurance time remained unchanged, while at the doses of 0.25 and 0.375 mg/kg, it decreased significantly ($p < 0.05$ and $p < 0.01$, respectively). This effect was antagonized by pretreatment with hexamethonium 5 mg/kg s.c.
Non-Guideline	Developmental neurotoxicity	Johns *et al* (1992) 0, 0.5, 1.5, or 2.5 mg/kg of nicotine hydrogen tartrate, injected 2X daily throughout gestation period (15/dose).	Offspring exhibited performance deficits in both learned and innate behavioral measures throughout development and adulthood. Offspring birth weight was not affected by treatment nor the 32 day weight gain. Initial weight, weight gain, gestation length, number of live or dead offspring, or mean food consumption during pregnancy did not differ significantly in the treated groups versus the controls. Dams receiving the nicotine injections reacted with aversion.

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Non-Guideline	Developmental neurotoxicity	Navarro *et al*, 1989 2 mg/kg of nicotine infused per day from gestational days 4 through 20	Infusing pregnant rats with a nicotine dose that did not interfere with maternal weight gain or resorption rate resulted in sufficient nicotine penetrating the fetal brain to cause persistent alterations in [^{3}H] nicotine binding sites, abnormalities of cellular development [assessed by measurements of ornithine decarboxylase (ODC) activity and deoxyribonucleic acid (DNA)], and impairment of development of peripheral noradrenergic projections (assessed by kidney norepinephrine levels).
Non-Guideline	Developmental neurotoxicity	Levin *et al*, 1993 2 mg/kg of nicotine subcutaneous injection per day from gestational days 4 through 21	Prenatal nicotine exposure caused subtle alterations in cognitive performance of the offspring which were magnified by challenges of nicotinic and adrenergic systems.
Non-Guideline	Developmental neurotoxicity	Fung, 1988 1.5 mg/kg/day, nicotine tartrate subcutaneous implanting of pregnant rats) during gestation	14 – day old male and female offspring of rats exposed to nicotinedemonstrated an increase in spontaneous locomotor activity compared with controls. The total number of pups born to the treated group was significantly less than the controls.
Non-Guideline	Developmental neurotoxicity	Peters *et al*, 1979 60 - 80 days old offspring of rats treated with nicotine in drinking water (6 mg/kg/day intake) four weeks before mating and during pregnancy and throughout nursing and 6 weeks after weaning	Increased spontaneous motor activity in the light which was not prevented by cross-fostering to control dams at birth. The paternal rats had a marked reduction in body weight gain (55% of controls for males and 63% of controls for females after 4 month of nicotine treatment). The dams were more active during the day and exhibited a reduced plasma corticosterone response to stress. Male but not female offspring of nicotine treated rats were significantly lighter at birth than control males.
Non-Guideline	Developmental neurotoxicity	Peters & Ngan, 1982 Nicotine at 0, 1.5, 3 or 6.0 mg/kg per day; subcutaneously one week prior to mating and throughout gestation	subtle neurological changes which are manifested as behavioral alterations in the newborn (the righting reflex, temperature regulation, adherence to the inclined screen, and in organ/body weight ratios for brain, heart, lung, liver, and kidney) and adult offspring (prolonged time required and an increase in number of mistakes made, during food maze testing and an increased brain protein content).
Non-Guideline	Developmental neurotoxicity	Roy & Sabherwal, 1998 pregnant rats were injected ip with nicotine (2.5 mg/kg/ day, given in two divided doses) from GD 6 to term and pups were delivered at term normally	Reduction in cortical thickness and decreased cell size, decreased dendritic branching and increased dendritic spine density, irregular arrangement of cisternae of rough endoplasmic reticulum, paucity of free ribosomes, and frequent cytoplasmic vacuoles of the somatosensory cortex were observed in the pup up to postnatal day 40. Morphological changes in the hippocampus (significant reduction in the neuronal area of nicotine-exposed brains in the dentate gyrus, CA3, and CA1 regions) also resulted from the prental nicotine treatment, which may contribute to the behavioral abnormalities.

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Non-Guideline	Developmental neurotoxicity	Ajarem & Ahmad, 1998 daily subcutaneous injections of nicotine (0.5mg/kg) into the nape of the neck during pregnancy	significant reduced postnatal body weight gain, as well as significant delay in eye opening, in the appearance of body hairs, and in sensory motor reflexes. Motor activity was significantly stimulated in early adulthood of mouse pups prenatally exposed to nicotine, and had long-lasting hyperactive effects on mice.
Non-Guideline	Developmental neurotoxicity	Marks *et al*, 1985 four inbred strains of mice (BALB, C57BL, DBA, C3H), nicotine administered ip at a single dose of 0, 0.5, 1.0 or 2.0 mg/kg	Generally nicotine administration increased the respiration rate, decreased the body temperature and the heart rate, and a decline in the Y-maze crosses and rears at different rates in the various strains. The startle response was increased in the C3H strain only.
Non-Guideline	Developmental neurotoxicity	McFarland *et al*, 1991 Nicotine given at an acute subcutaneous injection to neonatal rats as nicotine dibitartrate (3 mg/kg of nicotine base) at 1, 3, 8, 10 or 15 days of age	Nicotine inhibited DNA synthesis in neonatal rat brain regions as assessed by the incorporation of [3H]thymidine into DNA of the brain tissues. The inhibition potency correlated to the concentration of nicotinic receptors: midbrain + brainstem ≥cerebral cortex > cerebellum. The inhibitory effect of nicotine was also seen in fetal brain on gestational day 20 after injection of nicotine (3 mg/kg) to pregnant rats.
Non-Guideline	neurobehavioral teratology	Seidler *et al*, 1996 developing rats (1, 7, 14 and 21 days old) challenged acutely with nicotine (0.3 mg/kg, i.p.) and the release of catecholamines was evaluated *in vivo* in three brain regions that differ in nicotinic receptor concentrations.	Nicotine did not stimulate catecholamine release at birth, but developed the capacity to do so in parallel with the ontogeny of nicotinic cholinergic receptors in the midbrain+brainstem and in the forebrain. In the cerebellum, no response was obtained at any age. Changes in sensitivity to nicotine were also seen that corresponded to ontogenetic changes in endogenous cholinergic tone, suggesting that receptor desensitization occurs normally during developmental stages in which neuronal activity is high. The absence of a catecholamine response to nicotine at birth in the rat indicates that neurobehavioral teratology associated with fetal nicotine exposure does not reflect secondary actions mediated through catecholamines. However, because brain development in the neonatal rat corresponds to fetal stages in man, the onset of these mechanisms may be relevant to human fetal exposure.
Non-Guideline	Endocrine effects *in vivo* and *in vitro* rat models	Blackburn *et al*, 1994 pregnant mare's serum gonadotropin (PMSG) - primed and human chorionic gonadotropin (hCG) - triggered rat ovaries exposed to nicotine (ip 6.25 ng/g animal weight)	Nicotine inhibited ovulation, estradiol production, and fertilization both *in vivo* and *in vitro* in rat models. A dose dependent reduction in oocytes within the fallopian tube was noted in nicotine treated rats ($p<0.001$). On the other hand cotinine did not affect ovulation, estradiol production or fertilization in those tests.

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Non-Guideline	Endocrine effects rats	Slotkin and Seidler, 1975 Nicotine at1 mg/kg or 10 mg/kg, subcutaneously twice daily for 1 or 2 weeks)	Nicotine produced alterations in catecholamines (CA) release, tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH), and the ability of isolated storage vesicles to incorporate 3H-epinephrine in the adrenal glands and these alterations persisted when nicotine administration was discontinued.
Non-Guideline	Endocrine effects Time-pregnant Sprague Dawley rats	Lichtensteiger and Schlumpf 1985 Nicotine tartrate delivered in an osmotic minipump at a rate of 25 ug/100 g/ hr	Male fetuses of all control groups displayed the characteristic rise in plasma testosterone at GD 18 (as compared to GD 17 and 19); this was abolished by nicotine. Adult offspring of untreated or tartaric acid-treated dams exhibited a marked sexual dimorphism in their preference for saccharin-containing drinking water at 0.06-0.25%. No such sex difference was seen in offspring of nicotine-treated rats.
Non-Guideline	Endocrine effects	Ehlers *et al*, 1997 Nicotine hydrogen-tartrate (Sigma) dissolved in distilled water and added to the milk diet (0 mg, 1 or 4mg/kg/day) administered to neonatal rat pups from PN4 through PN12 with an artificial rearing paradigm; Nicotine. All rats were weaned at PN21 and housed in pairs. Thirty one suckle controls (17 males, 14 females), 23 artificially reared rats (14 males, 9 females), 22 1 mg/kg/day rats (12 males, 10 females), and 23 4 mg/kg/day animals (16 males, 7 females) were used in this study.	Nicotine exposure altered responses of the P3 component of the event-related potential (ERP), recorded in dorsal hippocampus, to changes in stimulus parameters. A significant reduction in the response to the noise tone as compared with the level of the infrequently presented tone also was seen in the P3B component. No effects of drug exposure were found on the N1 component in any lead, although artificial rearing produced specific effects on the latency of the N1 component in cortex. No significant differences among treatment groups were found on any of the EEG-dependent variables. Female rats overall were found to have significantly higher electroencephalography (EEG) amplitudes than the males. However, no overall effects of gender were found on any ERP component. These studies suggest that neonatal nicotine exposure specifically reduces the electrophysiological response of the hippocampus to changes in auditory stimuli
Non-Guideline	Endocrine effects Sprague Dawley rats	Slawecki *et al*, 2000 Male rats were exposed to 6.0 mg/kg/day nicotine via gastric infusion using an artificial rearing, “pup-in-the-cup,” technique for 6 consecutive days PND 4–9. At adulthood, EEG and auditory ERPs were recorded from the cortex and hippocampus	Examination of the hippocampal EEG revealed significantly decreased power in the 1–2-Hz frequency band of nicotine-treated rats. In addition, there was a significantly attenuated P300 ERP response to a noise tone in the nicotine-treated rats compared to controls. These data indicate that neonatal nicotine exposure alters functional activity in the hippocampus of adult rats. These effects are likely to be the result of synaptic disorganization in the hippocampus, and indicate that neonatal nicotine exposure exerts teratogenic effects on the developing central nervous system, particularly the hippocampus, which persist into adulthood.

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Non-Guideline	Immune system	Maritz & Woolward, 1992 nicotine at1 mg/kg bw/d from GD 7 until weaning three weeks after birth)	resulted in a very low elastic tissue content in the lungs of 1- and 7-day old rat pups compared with those of the controls. This may interfere with normal lung development since elastic tissue is part of the lung connective tissue structure and is involved in the formation of alveoli. Impaired elastic synthesis may thus make the rat pups more susceptible to lung diseases such as emphysema
Non-Guideline	Immune system	Geng *et al*, 1995 Nicotine at 1 mg/kg to young adult rats by intradermal implantation for 3-4 weeks	Nicotine inhibited both the T-dependent and T-independent AFC responses and proliferation to anti-CD3. Significantly fewer NT T cells entered the S and G2/M phases than CON T cells, indicating an arrest in the GO/G1 phase. B and T cells were unable to elevate the intracellular calcium levels normally in response to ligation of antigen receptors, although Ca^{2+} responses of salivary gland cells to acetylcholine were normal. Serum cotinine levels of 219 ± 40 ng/mL were comparable to average human smokers.
Non-Guideline	Immune system	Basta *et al*, 2000 pregnant rats treated with nicotine (6 mg/kg/day) from GD 4-20 subcutaneously	Nicotine suppressed splenocyte responsiveness to Concanavalin A or lipopolysaccharide in offspring and remained subresponsive to stimulation well into adulthood. Nicotine treatment also resulted in reduced maternal weight gain during early gestation, increased resorptions, increased offspring body weight and significantly elevated offspring spleen weights. In combination with ethanol, the observed effects were more severe.
Non-Guideline	Immune system *In vitro* T-cells from female mice	Zhang & Petro, 1996 T-cells (splenic mononuclear cells) exposed to nicotine at 1-100 µg/100 mL)	Exposure of T-cells (splenic mononuclear cells from female mice) to physiological concentration of nicotine (1-100 µg/100 mL) can alter T cell expression of CD28 and CTLA-4 and the CD4 T cell cytokine expression pattern

Table A.2.2	Subchronic, Chronic and Other Toxicity Profile of Nicotine		
Guideline No.	**Study Type**	**MRID No. (year)/ Classification /Doses**	**Results**
Non-Guideline	Metabolism and pharmacokineti	Hukkanen *et al*, 2005	Review article. Rapid absorption of nicotine from the inhaled cigarette smoke and rapid distribution via the blood stream to various tissues including the brain within 10 to 20 seconds, faster than iv. Poorly absorbed from stomach - protonated in the acidic gastric fluid, but well absorbed in the small intestine - more alkaline pH and a large surface area. Oral ingestion of nicotine capsules or nicotine in solution, peak concentrations in the blood are reached in about 1 hour. Nicotine crosses the placental barrier easily, and there is evidence for the accumulation of nicotine in fetal serum and amnionic fluid in slightly higher concentrations than in maternal serum. The plasma half-life of nicotine after iv infusion or cigarette smoking averages about 2 h. However, when half-life is determined using the time course of urinary excretion of nicotine, which is more sensitive in detecting lower levels of nicotine in the body, the terminal half-life averages 11 h. Nicotine is extensively metabolized – cotinine.
Non-Guideline	Dermal penetration (species)	Zorin *et al*, 1999	*In vitro* tests of nicotine's permeability through human skin using Franz' diffusion cells demonstrated the dermal penetration of nicotine. Flux depended on the nicotine concentration in a non-linear fashion, with the highest flux at 50% w/w nicotine-water solution. The lowest flux was in the acidic nicotine solution and ethanol solution. Penetration continued after rinsing the nicotine from the donor compartment, indicating that the skin acts as reservoir for continued absorption.
Non-Guideline	Special studies: Daily intake of nicotine from cigarette smoke	Benowitz znd Jacob III, 1984	Daily intake of nicotine in a group of 22 smokers averaged 37.6 ± 17.7 mg (10.5 – 78.6 mg). Nicotine intake per cigarette averaged 1.04± 0.36 mg. Among several markers measured (carboxyhemoglobin level, blood cotinine concentration), afternoon (4:00 pm) blood nicotine level was the best marker for measuring nicotine intake in this group of smokers.

A.3 Supplementary Toxicity Studies

Subchronic Toxicity

Experimental data in male rats suggest that nicotine (free base) ingested for 16 weeks at 50 or 200 mg/L in drinking water (3 and 14 mg/kg/day calculated on water intake) alters metabolic and endocrine factors that may be responsible, at least in part, for the development of gastrointestinal ulcers and pancreatitis (Chowdhury *et al*, 1990). The plasma levels of CCK (cholecystokinin) were significantly increased with nicotine intake but the amylase secretory response of pancreatic acinar cells was inhibited in response to CCK-8 (cholecystokinin–octapeptide) and carbachol. Prominent loss of gastric mucosal surface was found in nicotine-treated animals with gross microscopic evidence of bleeding ulcers. Body weight gain in the nicotine treated groups was significantly lower than in the controls at every measurement point. At the end of treatment period, body weight gain in the low and high dose groups was approximately 74% and 53% of the controls, respectively. Nicotine treatment was accompanied by significantly lower food and water intake. All of the metabolic parameters except body weight gain were reversed upon nicotine withdrawal. Histopathologic data showed a partial but not a complete recovery of the pancreatic acinar cell morphology and gastric mucosal surface following 4 weeks of nicotine withdrawal.

When adult male rats (185-225 g weight) were implanted subcutaneously with nicotine pellets containing 0, 5, 15 or 50 mg nicotine (delivering 0, 9.9, 29.8, and 99.2 ug/hr, respectively; equivalent to 0.0, 1.2, 3.6, 12.0 mg/kg/day) for up to 12 weeks, increased pancreatic enzyme biosynthesis and accumulation of digestive enzymes within the pancreas were reported (Lau *et al*. 1990). Plasma nicotine concentrations at the 50 mg dose reached a steady state level of 76±19 ng/mL and cotinine exceeded 300 ng/mL. No effect on enzyme secretion was observed in rats treated with 15 mg nicotine pellets, in rats treated with 15 mg pellets, CCK-8-mediated amylase, trypsinogen, and chymotrypsinogen secretion was maximally stimulated at 1.5 weeks and then decreased to near control levels. In rats treated with 50 mg nicotine pellets, enzyme release in response to CCK-8 was also higher at 1.5 weeks, peaked at 3 weeks and then decreased, such that at 12 weeks, enzyme release was at or below control levels. Electron microscopy of pancreas from rats treated with the 50 mg nicotine pellets revealed intracytoplasmic vacuoles appearing after 3 weeks of treatment, and persisting throughout the remaining experimental period (evidence of morphological damage). Nicotine treatment and durations did not have an effect on body weight, pancreatic weight, DNA, RNA or protein concentrations. From this study, a **LOAEL** based on pancreatic morphogic damage is the 50 mg nicotine pellet corresponding to a daily dose of 12 mg/kg and a **NOAEL** is 3.6 mg/kg/day. Earlier work from the same laboratory (Dubick *et al*, 1988) showed that adult male rats receiving nicotine via a time–release pellet at a rate of 1.65 ug/min for 3 weeks, increases in digestive pancreatic enzymes (amylase, trypsin and chymotrypsin) were noted compared to controls. Final body weight or pancreatic weight was not affected.

Male mice (CF-1 Swiss derived, 20-25 g initial weight) consuming nicotine

hydrochloride in their drinking water (25 mg/l; equivalent to 7 mg/kg/day) daily for 2-3 months or injected i.p. with 5 mg/kg/day acutely or for 3 weeks showed no evidence of hepatotoxicity as measured by serum glutamic-pyruvic transaminase or serum alkaline phosphatase activities (Priestly and Plaa 1976). The LD_{50} of nicotine HCl administered i.p. in mice was 13.5 mg/kg. Acute or subchhronic administration of nicotine did not modify the hepatotoxic response of carbon tetrachloride (potent hepatotoxin) or the less potent hepatotoxins chloroform or 1,1,1-trichloroethane nor the cholestatic effect of α-naphthylisothiocyanate was modified.

Nicotine administration may adversely affect bone formation and decrease body storage of vitamin D (Fung *et al*, 1999). Female rats (7-month old; 9-10/group) administered nicotine (nicotine hydrogen tartrate) at 0, 3.0 or 4.5 mg/kg/day dissolved in saline solution by subcutaneous osmotic minipumps with a delivery rate of 0.25 ul/hr for three months had significantly lower levels of 25-hydroxyvitamin D in the two nicotine groups than controls. The high dose nicotine group had smaller vertebral areas and a lower bone mineral content than the controls. Tibial endocortical mineral apposition rate was also significantly lower in the high dose nicotine group than in the control group. Nicotine serum levels at sacrifice time were 60 ± 6 ng/mL, and 85 ± 5 ng/mL at the low and high dose, respectively.

Nicotine tartrate, administered orally to 10 New Zealand white male rabbits (2.4 mg/kg/day) in their drinking water for 25 weeks produced *in vivo* morphologic effect on endothelial cells in the aortic arch (Booyse *et al*, 1981). It was estimated that each rabbit consumed about as much as nicotine per day (2.4 mg/kg/day) as a person smoking 1- 2 packs of cigarettes per day. Fasting serum levels of glucose, triglycerides, total cholesterol, and LDL-cholesterol were significantly (<0.001) elevated in nicotine-treated rabbits. Endothelial cells from nicotine-treated arched areas (Evans-blue-stained) showed extensive changes such as increased cytoplasmic silver disposition, increased formation of microvilli, and numerous focal areas of "ruffled" endothelium (projections on cell surfaces). Serum blood levels of nicotine were not measured in this study.

Chronic Toxicity

Dietary feeding of nicotine sulfate (0.0%, 0.00625%, 0.01255%, 0.025%, 0.05%, 0.1%, or 0.2% as nicotine base of the diet), nicotine tannate (0.0%, 0.1%, 0.2%, or 0.4% of the diet) and nicotine bentonite (0.0%, 0.0625%, 0.1255%, 0.25%, 0.5%, 1.0%, or 2.0% of the diet) to rats (initial weight 50-60 g) for 300 days resulted in retarded growth due to nicotine toxicity and lower food consumption (Wilson & DeEds, 1936) with a NOAEL of 0.006% nicotine base (4 mg/kg/day). Retarded growth in rats was reversed upon discontinuing the nicotine diets and rats resumed normal growth. Rats receiving the nicotine sulfate as 0.1% base and above died within few days. It was also demonstrated that "the toxicity in terms of nicotine base was about the same for two nicotine salts and the bentonite clay formulation" tested. Nicotine bentonite for this study was prepared by mixing one part of nicotine base with nine parts of finely powdered Wyoming bentonite and mixing thoroughly. The bentonite nicotine complex was not merely an adsorption complex, but rather some chemical bonding. The nicotine was not removed by extraction

with alkali and only small amounts were removed by water, but was readily extracted with dilute acid.

Developmental Toxicity

Pregnant CD-1 mice (N=19), exposed to ip injection of 0.1% nicotine sulfate at a dose of 1.67 mg/kg body weight/day on gestational days 6-15 had significantly depressed maternal weight gain (37% decrease on GD18) and fetal weight (64% decrease) compared to the controls. Fetal crown-rump length fetal head dimensions (width, height and circumference) were significantly reduced. Histological examination revealed that 9.6% of fetuses of nicotine injected mothers presented clefts of the palate, whereas none of the control fetuses had that anomaly. Nicotine treatment also had teratogenic effects on first molar odontogenesis in the mouse. The mesiodistal diameter of molar tooth germs of nicotine treated fetuses without cleft palate was significantly less than those of controls (Saad *et al*, 1990 & 1991). It was suggested that nicotine, or its metabolic byproducts, interfere with normal interaction between the epithelial and mesenchymal components of the developing tooth.

Subcutaneous injection of nicotine to timed pregnant Sprague Dawley rats (41 control and 57 nicotine treated) (3 mg/kg/day from gestation day 4 -20) resulted in significant reduced maternal weight gain and increased maternal mortality (14%) and fetal resorptions (13%). Litter size was not affected. Nicotine exposure produced biochemical changes in the fetal brain (elevation of fetal ornithine decarboxylase activity, suppression of DNA synthesis (most profound in cerebellum), desynchronization of the ontogenctic patterns of DNA, RNA and proteins in every brain region) (Slotkin *et al*, 1986).

Subcutaneous injections of nicotine (3 or 6 mg/kg/day, divided into two doses) on GD 7-20 to pregnant rats (10/group) resulted in abnormal behavior such as ataxic gate, decrease in spontaneous activity, creeping, or salivation in pregnant rats (3 or 6 mg/kg/day) and convulsion and ptosis (6 mg/kg), decreased maternal body weight at GD 14 (both groups) and reduced offspring body weight. These nicotine exposures also resulted in significant decreases in dihydroxyphenylacetic acid (DOPAC) content in the neocortex and in both the neocortex and in the midbrain plus pons medulla, respectively, without any effects on the other brain regions such as the hypothalamus or striatum. Norepinephrine, serotonin, or 5-hydroxy-3-indolacetic acid levels were not affected. These data demonstrated that prenatal nicotine exposure induced disturbances in the dopaminergic system in the young adult period. Furthermore, the region-specific reductions in the DOPAC content suggests that the exposure to a high dose of nicotine in utero might cause a predisposition to diseases related to a dopaminergic dysfunction in the frontal cortex (Muneoka *et al*, 1999).

A recent study questioned developmental effects of nicotine reported in the scientific literature (Hussein *et al*, 2007). They found that a nicotine dose of 6 mg/kg/day administered to Sprague Dawley time-mated rats from gestation day 3 through 21 by osmotic mini pump resulted in nicotine plasma concentrations of 115-174 ng/mL. This is 3-10X higher than published values observed in heavy smokers (10-50 ng/mL) and

pregnant mothers (2-10 ng/mL). The total nicotine dose of 6 mg/kg/day in the rat was considered to be equivalent to smoking 480 and 560 cigarettes per day (1 mg NIC per cigarette) by pregnant women weighing 60 and 70 kg, respectively, which is unrealistic. Maternal hematocrit was not affected by nicotine administration, or the number of pups per litter, pup weight, placental weight or the weight of various pups' organs. Maternal weight changes were comparable in the nicotine treated and control groups. Maternal plasma coticosterone concentrations were not affected by the nicotine infusion versus the control rats. Measurements of plasma nicotine concentration in this study demonstrated that total nicotine clearance increases towards the second trimester but remains unchanged thereafter, whereas the plasma concentrations decrease. Thus plasma nicotine concentrations are maintained for at least two thirds of the gestation period and only a marginal decrease is observed during the third trimester. In earlier studies in CD-1 mice by Paulson *et al* (1989), fetal retardation was observed at plasma nicotine concentrations of 218-1284 ng/mL corresponding to 60 mg/kg/day, but not at lower doses of 12 or 36 mg/kg/day corresponding to 100 and 400 ng/mL.

Genotoxicity

Nicotine was found to induce chromsomal aberration (CA) and sister chromatid exchange (SCE) frequency in Chinese hamster ovary cells (CHO) in a dose and duration dependent manner and at concentrations comparable to the saliva levels of nicotine achieved during tobacco chewing. In one experiment CHO cells were incubated with free base nicotine (analytical grade from Sigma Chem. Co.) at 625 µg/mL or 1000 µg/mL for 2 or 4 hours. In a second experiment, CHO cells were incubated with free base nicotine (analytical grade from Sigma Chem. Co.) at 150 µg/mL, 250 µg/mL, 375 µg/mL, 500 µg/mL or 625 µg/mL for 24 and 48 hours for CA and SCE analysis, respectively. Statistically significant elevations in CA frequency were observed with nicotine concentrations ≥375 µg/mL, whereas, SCE frequencies were increased significantly ≥150 µg/mL concentrations (Trivedi *et al*, 1990). Nicotine (150 µg/mL) and in combination with arecoline (an areca nut alkaloid) incubated in Chinese hamster ovary (CHO) cell line (without S9 metabolic activation) increased the SCE frequency/cell. Nicotine alone at 300 µg/mL did not increase the frequency of chromosomal aberrations/cell, but in combination with arecoline it did at ≥90 µg/mL (Trivedi *et al*, 1993).

Nicotine was cytotoxic at 1.5 - 2.0 µg/mL to human leucocytes in culture *in vitro* without producing any chromosome damage. However, gross chromosomal aberrations including fuzzy chromosomes (stickiness), aneuploidy and translocations were observed in mice receiving low tolerable doses of the drug (0.07 -0.09 µg/total body weight injected to 5 weeks-4 month old mice in saline, two weekly injections for 3 weeks before sacrifice and preparing the bone marrow from the femora (Bishun *et al*, 1972).

Nicotine has been shown to cause concomitant genotoxic and antiapoptotic effect in human gingival fibroblasts (HGFs) in the cytokinesis-block micronucleus (CBMN) test (Argentin and Cicchetti, 2004). Treatment of HGFs with nicotine, at a concentration of 1 uM, caused a statistically significant increase of micronucleus (MN) frequency at the tested time intervals, while no change was detected in cell growth under the same

conditions. Preincubation of HGFs with 1 uM nicotine strongly attenuated staurosporine (STP)-induced apoptosis. Cultures exposed to nicotine showed an increase of reactive oxygen species, as determined by increased levels of 2,7-dichlorofluorescein (DCF). When cells were prelabeled with N-acetyl-cysteine (NAC), a substrate for glutathione synthesis, and catalase (CAT), the oxygen free radical scavenger, a significant reduction in cytogenetic damage was observed.

Cotinine (a metabolite of nicotine and a biological monitoring marker of nicotine absorption in humans) was positive in the presence or absence of S9 in the bacterial luminescence genotoxicity test at 1.25 - 2.5 mg/mL (9- 30 h incubation). In contrast, nicotine was not positive to 20 mg/mL concentrations for up to 40 hours of incubation. Nicotine/cotinine mixtures were still positive at physiological concentrations, with potentiation relative to cotinine alone with and without S9 (Yim & Hee 1995).

Tests by the R. J. Reynolds Tobacco Co. (Doolittle *et al*, 1995) on nicotine and four of its major metabolites: cotinine, nicotine-N'-oxide, cotinine-N-oxide, and trans-3'-hydroxycotinine in the Salmonella mutagenicity assay (strains TA98, TA100, TA1535, TA1537, and TA1538) at concentrations ranging from 0 to 1000 micrograms/plate and in the Chinese hamster ovary sister-chromatid exchange (SCE) assay at concentrations ranging from 0 to 1000 micrograms/mL, with and without S9 metabolic activation showed that none of the five compounds increased the frequency of mutations or the frequency of SCEs.

Recent data indicate that nicotine exerts significant direct genotoxic effects in human target cells *in vitro* (Kleinsasser *et al*, 2005). The genotoxicity of nicotine was tested with the alkaline single-cell microgel electrophoresis (Comet) assay using human lymphocytes and target cells from lymphatic tissue of the palatine tonsils from healthy patients. One hour exposure to nicotine at 0.125, 0.25, 0.5, 1, 2, and 4 mM induced a statistically significant dose-dependent increase of DNA migration up to 3.8-fold and 3.2-fold in tonsillar cells and lymphocytes, respectively. The minimum concentration eliciting significant DNA damage was 0.5 mM nicotine. The genotoxic effect was confirmed in a second series of experiments using nicotine of high purity from two different suppliers. Finally, DNA damage by nicotine was compared in cells incubated in medium strictly adjusted to neutral pH, with nonadjusted medium becoming alkaline with increasing nicotine concentrations. Again no differences in DNA migration were observed. However, no differences in DNA damage were observed in cells from smokers and nonsmokers incubated without nicotine. The lack of higher DNA damage in smokers compared to nonsmokers could be a question of nicotine dose, rapid DNA repair, or interactions with other smoke constituents. Further work by Sassen *et al* (2005) confirmed the genotoxic effects of nicotine on human nasal epithelia.

Many studies have demonstrated that nicotine penetrates the fetal brain to cause various biochemical changes with behavioral consequences on the offspring. These studies are listed in the tox profile in Appendix A.2. In all of these studies, nicotine was administered subcutaneously or intraperitonealy. Performance deficits in both learned and innate behavioral measures throughout development and adulthood in offspring of

animals exposed to nicotine during gestation have been reported. Doses as low as 0.25 mg/kg/day produced behavioral changes.

Immune System Toxicity

There is considerable evidence suggesting immune toxicity by nicotine. Few of these studies are discussed below. Other studies are listed in the tox profile in Appendix A.2.

Maternal nicotine exposure (1 mg/kg body mass/d from GD 7 until weaning three weeks after birth) resulted in a very low elastic tissue content in the lungs of 1- and 7-day old rat pups compared with those of the controls. This may interfere with normal lung development since elastic tissue is part of the lung connective tissue structure and is involved in the formation of alveoli. Impaired elastic synthesis may thus make the rat pups more susceptible to lung diseases such as emphysema (Maritz & Woolward, 1992).

Nicotine at 1 mg/kg (administered to young adult rats by intradermal implantation for 3-4 weeks), inhibited both the T-dependent and T-independent AFC responses and proliferation to anti-CD3. Significantly fewer NT T cells entered the S and G2/M phases than CON T cells, indicating an arrest in the GO/G1 phase. B and T cells were unable to elevate the intracellular calcium levels normally in response to ligation of antigen receptors, although Ca^{2+} responses of salivary gland cells to acetylcholine were normal. Serum cotinine levels of 219 ± 40 ng/mL were comparable to average human smokers (Geng *et al*, 1995).

Prenatal nicotine exposure can cause long-term suppression of the proliferative response of offspring immune cells (Basta *et al*, 2000). Thus in offspring of pregnant rats treated with nicotine (6 mg/kg/day) from GD 4-20 subcutaneously, nicotine suppressed splenocyte responsiveness to Concanavalin A or lipopolysaccharide and remained subresponsive to stimulation well into adulthood. Nicotine treatment also resulted in reduced maternal weight gain during early gestation, increased resorptions, increased offspring body weight and significantly elevated offspring spleen weights. In combination with ethanol, the observed effects were more severe.

Exposure of T-cells (splenic mononuclear cells) from female mice to physiological concentration of nicotine (1-100 µg/100 mL) can alter T cell expression of CD28 and CTLA-4 and the CD4 T cell cytokine expression pattern (Zhang & Petro, 1996).

A.4 References

Registrant References:

MRID 41521101. Naas, D. (1990) Primary Dermal Irritation Study in Albino Rabbits with Nicotine, Black Leaf 40: Lab Project Number: WIL-149014. Unpublished study

prepared by WIL Research Laboratories, Inc. April 2, 1990. 19 p.

MRID 41521102. Naas, D. (1990) Skin Sensitization Study in Albino Guinea Pigs with Nicotine, Black Leaf 40: Lab Project Number: WIL-149015, unpublished study prepared by WIL Research Laboratories, Inc., April 2, 1990.. 38 p. 30 pages.

MRID 42631101. Kuhn, J. (1992) Acute Dermal Toxicity Study in Rabbits: F&B Rabbit & Dog Chaser: Lab Project Number: 9643-92. Stillmeadow, Inc. Unpublished. 11 p.

MRID 42631102. Holbert, M. (1992) Acute Inhalation Toxicity Study in Rats: F&B Rabbit & Dog Chaser: Lab Project Number: 9644-92. Stillmeadow, Inc. Unpublished. 17 pages.

MRID 42631103. Kuhn, J. (1992) Primary Eye Irritation Study in Rabbits: F&B Rabbit & Dog Chaser: Lab Project Number: 9645-92. Stillmeadow, Inc. Unpublished. 17 p.

MRID 42631104. Kuhn, J. (1992) Primary Dermal Irritation Study in Rabbits: F&B Rabbit & Dog Chaser: Lab Project Number: 9646-92. Stillmeadow, Inc. Report no. 9646-92. Unpublished. 12 pages.

MRID 42640001. Kuhn, J. (1993) Dermal Sensitization Study in Guinea Pigs: F&B Rabbit & Dog Chaser: Lab Project Number: 9647-92. Stillmeadow, Inc. Unpublished. 17p.

MRID 43375601. Kuhn, J. (1994) Dermal Sensitization study in Guinea Pigs: F&B Rabbit & Dog Chaser: Amendment: Lab Project Number: 9647-92. Stillmeadow, Inc. Unpublished. 10 p.

MRID 43523301. Kuhn, J. (1994) Acute Oral Toxicity Study in Rats: (F & B Rabbit and Dog Chaser): Final Report: Lab Project Number:1607-94. Stillmeadow, Inc. **MRID 43523301.** Unpublished. 11 pages.

MRID 43874001. Bennick, J. (1995) F & B Rabbit and Dog Chaser: Acute Inhalation Toxicity Study in Rats: Final Report: Lab Project Number: 2376-95: S9-FF81-3. Stillmeadow, Inc. Unpublished. 10 P.

Literature References:

Ajarema, J S. and Ahmad M. 1998. Prenatal Nicotine Exposure Modifies Behavior of Mice Through Early Development. Pharmacology Biochemistry and Behavior, 59 (2) : 313-318.

Argentin G; ; Cicchetti R. 2004. Genotoxic and Antiapoptotic Effect of Nicotine on Human Gingival Fibroblasts. TOXICOLOGICAL SCIENCES **79,** 75–81.

Armitage, AK; DM Turner. 1970. Absorption of Nicotine in Cigarette and Cigar Smoke through the Oral Mucosa. Nature, 226:1232-1232.

Ballard T; Ehlers J; Freund E; Auslander M; Brandt V; Halperin W. 1995. Green Tobacco Sickness: Ocuupational Nicotine Poisoning in Tobacco Workers. Archives of Environmental Health, 50(5): 384-389.

Basta PV; Basham KB ; Ross WP; Brust ME; Navarro HA. 2000. Gestational Nicotine Exposure alone or in Combination with Ethanol Down-modulates Offspring Immune Function. Int J Immunopharmacol, 22(2):159-69.

BENOWITZ NL; JACOB III P. 1984. Daily intake of nicotine during cigarette smoking. Clinical Pharmacology and Therapeutics 35(4):499-504.

Benowitz NL; Lake T; Keller KH; Lee BL. 1987. Prolonged absorption with development of tolerance to toxic effects after cutaneous exposure to nicotine. Clin. Pharmacol. Ther., 42:119-120.

Bircher AJ Howald H Rufli T. 1991. Adverse skin reactions to nicotine in a transdermal therapeutic system. Department of Dermatology. Contact Dermatitis, 25(4):230-6.

.

Bishun NP Lloyd N Raven RW Williams DC. 1972. The in vitro and in vivo cytogenetic effects of nicotine. Acta Biol Acad Sci Hung, 23(2):175-80.

Blackburn CW; Peterson CA; Hales HA; Carrell DT; Jones KP; Urry RL; Peterson CM. 1994. Nicotine, but not cotinine, has a direct toxic effect on ovarian function in the immature gonadotropin-stimulated rat. Reprod Toxicol, 8(4):325-31.

Booyse FM; Osikowicz G; Quarfoot AJ. 1981. Effects of chronic oral consumption of nicotine on the rabbit aortic endothelium. Am J Pathol, 102(2):229-38.

Boylan B.; Brandt V.; Muehlbauer J.; Auslander M.; Spurlock C.; Finger R. 1993. Green tobacco sickness in Tobacco harvesters - Kentucky. Journal of the American Medical Association, 269(21):2722-2724.

Chowdhury IG; Bromage TG. 2000. Effects of fetal exposure to nicotine on dental development of the laboratory rat. Anat Rec, 258 (4):397-405.

Chowdhury P; Hosotani R ; Chang L; Rayford PL. 1990. Metabolic and pathologic effects of nicotine on gastrointestinal tract and pancreas of rats. Pancreas; 5(2): P222-9.

Doolittle DJ; Winegar R; Lee CK; Caldwell WS; Hayes AW; de Bethizy JD . 1995. The genotoxic potential of nicotine and its major metabolites. Mutat Res. 344(3-4):95-102.

Dubick MA, Palmer R, Lau PP, Morrill PR, Geokas MC. 1988. Altered exocrine pancreatic function in rats treated with nicotine. Toxicol. Appl. Pharmacol. 96: 132-139.

Ehlers CL; Somes C;Thomas J; Riley EP. 1997. Effects of neonatal exposure to nicotine on electrophysiological parameters in adult rats. Pharmacol Biochem Behav: 58(3):713-20.

FAULKNER, J.M. 1933. Nicotine poisoning by absorption through the skin. Journal of the American Medical Association 100(21):1664-1665.

Fèarm G. 1993. Contact allergy to nicotine from a nicotine patch. Contact Dermatitis; 29(4):14-5.

Fung YK. 1988. Postnatal behavioural effects of maternal nicotine exposure in rats. J Pharm Pharmacol. 40(12):870-2.

Fung Y.K.; Iwaniec U.; Cullen D.M.; Akhter M.P.; Haven M.C.; Timmins P. 1999. Long-term effects of nicotine on bone and calciotropic hormones in adult female rats Pharmacology and Toxicology, 85(4):181-187.

Garcia-Estrada H, Fischman CM. 1977. Unusual Case of Nicotine Poisoning. Clin. Toxicol., 10(4) :391-393.

Gelbacch, GH; Williams; W-A; Peny; LD.; et al. 1974. Grean-Tobacco Sickness: An Illness of Tobacco Harvesters, JAMA 229:1880-1863.

Gehlbach SH; Williams WA; Perry LD; Freeman JI; Langone JJ; Peta LV; Van Vunakis H.. 1975. Nicotine absorption by workers harvesting green tobacco. Lancet 1(7905):478-480.

Geng Y; Savage SM; Johnson LJ; Seagrave J; Sopori ML. 1995. Effects of nicotine on the immune response. I. Chronic exposure to nicotine impairs antigen receptor-mediated signal transduction in lymphocytes. Toxicol Appl Pharmacol. 135(2):268-78.

Ghosh SK; Parikh JR; Gokani VN; Rao NM; Doctor PB. 1985. Occupational Health Problems Among Tobacco Processing Workers: A Preliminary Study. Archives of Environmental Health, 40(6):318-321.

Hipke M.E. 1993. Green tobacco sickness. Southern Medical Journal 86(9): 989-992.

Hussein H; Farkas S; MacKinnon Y; Ariano RE; Sitar DS; Hasan SU. 2007. Nicotine dose–concentration relationship and pregnancy outcomes in rat: Biologic Plausibility and implications for future research. Toxicology and Applied

Pharmacology 218: 1–10.

Johns JM; Walters PA; Zimmerman LI . 1993. The effects of chronic prenatal exposure to nicotine on the behavior of guinea pigs (Cavia porcellus). J Gen Psychol; 120(1):49-63.

Jones MS; Goldy DA. 1998. Green tobacco sickness. Nurse Pract. 23:15-16.

Jover R.; Ponsoda X.; Castell J.V.; Gomez-Lechon M.J. 1994. Acute cytotoxicity of ten chemicals in human and rat cultured hepatocytes and in cell lines: correlation between in vitro data and human lethal concentrations. Toxicology in Vitro, 8(1):47-54.

Kavitharaj N.K.; Vijayammal P.L. 1999. Nicotine administration induced changes in the gonadal functions in male rats, Pharmacology 5(1):2-7.

Kleinsasser NH, Sassen AW, Semmler MP, Harre´us UA, Licht AK, Richter E. 2005. The Tobacco Alkaloid Nicotine Demonstrates Genotoxicity in Human Tonsillar Tissue and Lymphocytes. TOXICOLOGICAL SCIENCES 86(2), 309–317.

Lau PP; Dubick MA; Yu GS; Morrill PR; Geokas MC. 1990. Dynamic changes of pancreatic structure and function in rats treated chronically with nicotine. Toxicol Appl Pharmacol. 104(3):457-65.

Leblebicioglu-Bekcioglu B; Paulson R.B; Paulson JO; Sucheston ME; Shanfeld J; Bradway S.D. 1995. Effects of caffeine and nicotine administration on growth and ossification of the icr mouse fetus. Journal of Craniofacial Genetics and Developmental Biology, 15(3):146-156.

Levin ED; Briggs SJ; Christopher NC; Rose JE. 1993. Prenatal nicotine exposure and cognitive performance in rats. Neurotoxicol Teratol. 15(4):251-60.

Lichtensteiger W; Schlumpf M. 1985. Prenatal nicotine affects fetal testosterone and sexual dimorphism of saccharin preference. Pharmacol Biochem Behav; 23(3):439-44.

McFarland BJ; Seidler FJ; Slotkin TA. 1991. Inhibition of DNA Synthesis in Neonatal Rat Brain Regions Caused by Acute Nicotine Administration. Developing Brain Research, 58:223-229.

Manoguerra AS, Freeman D. 1983. Acute Poisoning from the Ingestion of *Nicotiana glauca*. J Toxicol Clin Toxicol; 19(8):861-4.

Marks, MJ; Romm E; Bfaler S; Collins AC. 1985. A test battery for measuring nicotine effects in mice. Pharmacology *Biochemistry and* Behavior 23(2):325-330.

Maritz GS; Woolward K. 1992. Effect of maternal nicotine exposure on neonatal lung elastic tissue and possible consequences. S Afr Med J, 81(10):517-9.

Matsushima D; Prevo ME; Gorsline J 1995. Absorption and adverse effects following topical and oral administration of three transdermal nicotine products to dogs. Journal of Pharmaceutical Sciences, 84(3):365-369.

Muneoka K; Nakatsu T; Fuji J; Ogawa T; Takigawa M. 1999. Prenatal administration of nicotine results in dopaminergic alterations in the neocortex. Neurotoxicol Teratol; 21(5):603-9.

Nasrat HA; A1-Haachim GM; Mahmood FI.1986. Perinatal Effects of Nicotine. Biol. Neonate 49: 8-14

Paulson, R., Shanfeld, J., Sachs, L., Price, T., Paulson, J., 1989. Effect of smokeless tobacco on the development of the CD-1 mouse fetus. Teratology 40, 483–494.

Peters MA; Ngan LL. 1982. The effects of totigestational exposure to nicotine on pre- and postnatal development in the rat. Arch Int Pharmacodyn Ther; 257(1):155-67.

Peters DA; Taub H; Tang S. 1979. Postnatal effects of maternal nicotine exposure. Neurobehav Texaco; 1(3):221-5.

Priestly BG; Plaa GL. 1976. Hepatic function after acute of subchronic nicotine administration in untreated mice and mice treated with hepatotoxic chemicals. Arch Int Pharmacodyn Ther; 223(1):132-41.

Quandt SA; Arcury TA; Preisser JS; Norton D; Austin C. 2000. Migrant farm workers and green tobacco sickness: New issues for an understudied disease, American Journal of Industrial Medicine, 37(3):307-315.

Roy TS; Andrews JE; Seidler FJ; Slotkin TA. 1998. Nicotine evokes cell death in embryonic rat brain during neurulation. J Pharmacol Exp Ther. 287(3):1136-44.

Roy TS; Sabherwal U. 1994. Effects of prenatal nicotine exposure on the morphogenesis of somatosensory cortex. Neurotoxicol Teratol; 16(4):P411-21.

Saad AY; Gartner LP; Hiatt JL. 1990. Teratogenic effects of nicotine on palate formation in mice. Biol Struct Morphog; 3(1):31-5.

Saad AY; Gartner LP; Hiatt JL. 1991. Teratogenic effects of nicotine on first molar odontogenesis in the mouse. Acta Morphol Hung, 39(2):87-96.

Sassen AW; Richter R; Semmler MP; Harre´us UA; Gamarra F; Kleinsasser NH. 2005. Genotoxicity of Nicotine in Mini-Organ Cultures of Human Upper Aerodigestive Tract Epithelia. Toxicological Sciences 88(1), 134–141.

Seidler FJ; Albright ES; Lappi SE; Slotkin TAI. 1994. In search of a mechanism for

receptor-mediated neurobehavioral teratogenesis by nicotine: catecholamine release by nicotine in immature rat brain regions. Dev Brain Res; 82(1-2):1-8.

SLAWECKI, C. J., J. D. THOMAS, E. P. RILEY AND C. L. EHLERS. 2000. Neonatal Nicotine Exposure Alters Hippocampal EEG and Event-Related Potentials (ERPs) in Rats
Pharmacology Biochemistry and Behavior, 65(4): 711–718.

Slotkin TA; Greer N; Faust, J; et al. 1986. Effects of Maternal Nicotine Injections on Brain Development in the Rat. Brain Res. Bull. 17: 41-50.

Slotkin TA; Seidler FJ. 1975. Acute and Chronic Effects of Nicotine on Synthesis and Storage of Catecholamines in the Rat Adrenal Medulla. Life Sciences, 16:1613-1622.

Sudan BJL; Levin ED. 1995. Nicotine skin patch treatment and adverse reactions: skin irritation, skin sensitization, and nicotine as a hapten. Journal of Clinical Psychopharmacology, 15(2):145-146.

Syversen U; Nordsletten L; Falch JA; Madsen JE; Nilsen OG; Waldum HL. 1999. Effect of lifelong nicotine inhalation on bone mass and mechanical properties in female rat femurs. Calcif Tissue Int; 65(3):246-9.

Temocin S; Erenmemisoglu A; Suer C; Beydagi H. 1993. Effect of nicotine on swimming exercise in rats. Jpn J Physiol; 43(4):567-70.

Travell J. 1960. Absorption of nicotine from various sites. Annals of the New York Academy of Sciences 90(l):13-30.

Trivedi AH; Dave BJ; Adhvaryu SG. 1990. Assessment of genotoxicity of nicotine employing in vitro mammalian test system. Cancer Lett; 54(1-2):89-94.

Trivedi AH; Dave BJ; Adhvaryu SC. 1993. Genotoxic effects of nicotine in combination with arecoline on CHO cells. Cancer Letters 74(1-2):105-110.

Vincenzi C; Tosti A; Cirone M; Guarrera M; Cusano F. 1993. Allergic contact dermatitis from transdermal nicotine systems. Contact Dermatitis; 29(2):104-5.

Waldum HL; Nilsen OG; Nilsen T; Rorvik H; Syversen V; Sanvik AK; Haugen OA; Torp SH; Brenna E. 1996. Long-term effects of inhaled nicotine. Life Sci; 58(16):1339-46.

Williams CM; Kanagasabai T. 1984. Maternal adipose tissue response to nicotine administration in the pregnant rat: effects on fetal body fat and cellularity. Br J Nutr; 51(1):7-13.

Wilson RH; DoEds E. 1936. Chronic Nicotine, Toxicity. Feeding of Nicotine Sulfate,

Tannate, and Bentonite. J. Ind.Hyg. Toxicol. 18:553-564.

Witschi H; Lundgaard SM; Rajini P; Hendrickx AG; Last JA. 1994. Effects of exposure to nicotine and to sidestream smoke on pregnancy outcome in rats. Toxicol Lett 71(3):279-86.

Yim SH; Hee SQ. 1995. Genotoxicity of nicotine and cotinine in the bacterial luminescence test. Mutation Research - Environmental Mutagenesis and Related Subjects, 335(3):275-283.

Yuen ST; Gogo AR Jr; Luk IS; Cho CH; Ho JC; Loh TT. 1995. The effect of nicotine and its interaction with carbon tetrachloride in the rat liver. Pharmacol Toxicol; 77(3):225-30.

Zhang S; Petro TM. 1996. The effect of nicotine on murine CD4 T cell responses. Int J Immunopharmacol; 18(8-9):467-78.

Zorin S; Kuylenstierna F; Thulin H. 1999. In vitro test of nicotine's permeability through human skin. Risk evaluation and safety aspects. Annals of Occup. Hygiene; 43(6):405-413.