Final Term Project (Expanded Written Report):
NICOTINE
Toxicology- Winter 2007

INTRODUCTION

Nicotine is a neurotoxicant that is most popularly known as the (most) addictive substance in cigarettes and other tobacco products. However, smokers are not the only population that should consider the danger of nicotine toxicity. It is a naturally occurring substance in several plant species, but by no means do its natural origins make it benign. In fact, it is one of the most potent drugs in the alkaloid family—more so than cocaine—and can inflict serious damage on gastrointestinal and respiratory systems, as well as causing serious developmental defects in fetuses receiving nicotine *in utero*.

COMMON AND SCIENTIFIC NAMES OF TOXICANT

Nicotine is an alkaloid (meaning it is a nitrogenous base) derived primarily from the tobacco plant, *Nicotiana tabacum*. Although it is colorless and odorless as a solid, the Center for Disease Control describes it as "pale yellow to dark brown" "with a fishlike odor when warm" in its liquid form (CDC website). Nicotine typically constitutes about five percent of the tobacco plant, however, it is also present in smaller amounts in other members of the nightshade family, *Solanaceae*, such as tomatoes, eggplant, potatoes, coca, and some pepper species. Its chemical name is 3-(1-Methyl-2-pyrrolidyl)pyridine.

CHEMICAL STRUCTURE

Nicotine is a pyrrolidine compound, meaning it has five-part ring structure consisting of four carbon atoms and one nitrogen atom. The specific chemical formula of nicotine is $C_{10}H_{14}N_2$.

ROUTES OF EXPOSURE

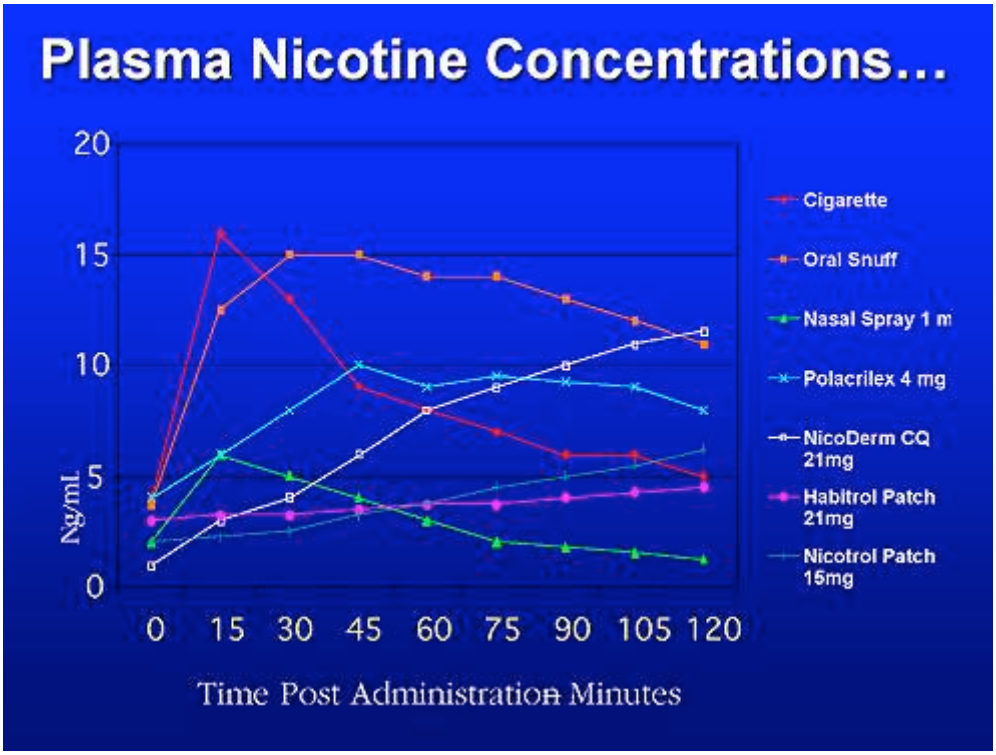
The most common route of exposure is inhalation through cigarette smoking, but ingestion and skin absorption are also possible. It is not uncommon for infants and children to incur nicotine intoxication by eating as little as one cigarette or three cigarette butts, and while these instances tend to result in intoxication, smoking does not produce acute toxicity because the smoker does not actually inhale all the nicotine in each cigarette. Cigarettes generally contain 8 to 20 milligrams of nicotine, but because the substance burns at a low temperature, a large portion is lost to combustion and only a small remainder (about one milligram) actually enters the body. (While the smoker may not feel the toxic effects of nicotine use immediately, maternal cigarette smoking has been shown to cause developmental defects to fetuses *in utero*.)

The likelihood of nicotine exposure via passive smoking (second-hand smoke) is very low, since most of the nicotine is burned off and even for the smoker, no acute inhalation toxicity is available because there are almost no conceivable circumstances in which it would occur (CDC).

Intoxication can also occur after eating foods that have been contaminated with nicotine, which happened on a large scale in January 2003 when about 100 people in Michigan became ill after a grocery store employee contaminated 200 pounds of beef with the insecticide Black Leaf 40, which contains nicotine as an additive (Boulton et al., 2003). Nicotine is easily absorbed through the alveoli in the lungs, as well as mucus

membranes in the nose and gums, which is how the substance is absorbed via chewing tobacco, nicotine inhalers and nicotine gum (Texas Medical Association). Skin absorption from smoke does not pose a serious threat, but nicotine patches effectively use this route of exposure.

(Below is a graph of concentrations and time distribution of plasma nicotine in various nicotine substances)



(from The Texas Medical Association- http://www.texmed.org/Template.aspx?id=1751)

No particular demographic seems to be in danger of acute nicotine intoxication; it is not a particularly high risk for any type of labor or geographic area. The most at-risk populations are probably children, who are the most likely to consume cigarettes or cigarette butts, and possibly those using nicotine-containing insecticides. Cigarette smokers and other tobacco users are at risk for chronic toxicity.

ADVERSE HEALTH EFFECTS

ACUTE EFFECTS

It can be difficult to isolate the effects of nicotine on humans in observational studies because it is often inextricably linked to cigarette smoking, which involves thousands of other harmful or potentially harmful chemicals. Nicotine overdoses often occur when children and babies eat cigarettes or cigarette butts. The effects of ingestion include nausea, vomiting, diarrhea, convulsions, depressed respiration and more, including death in some cases.

Nicotine triggers the release of adrenaline into the blood as well as glucose, an example of the 'fight or flight' response. In addition, it inhibits the release of insulin, the hormone that is supposed to remove the excess glucose from the blood. This excess glucose attributes to the slight hyperglycemia that may accompany nicotine use (in addition to the release of norepinephrine, hyperglycemia may explain why nicotine may curb the appetite because the brain interprets high blood sugar levels as a sign that the person is not hungry) (Texas Medical Association). The release of adrenaline into the blood may also marginally raise metabolic rates, but only for a short time since nicotine does not stay in the body for long.

The stimulation of cholinergic neurons triggers the release of dopamine in the brain's reward pathways, which are strongly associated with addiction. The brain learns that each time the body is exposed to nicotine, the reward pathways will be activated and so it craves nicotine. The drug also has the effect of increasing brain activity, alertness, concentration, and improving mood and memory (Texas Medical Association).

CHRONIC EFFECTS

Some long-term effects of nicotine use include elevated levels of low density lipoprotein (LDL), which is popularly known as the 'bad' type of cholesterol, leading to clogged arteries and ultimately cardiovascular disease, such heart attacks, or strokes. Chronic nicotine use has also been linked to decreased serotonin uptake in the hippocampus and prefrontal cortex regions of the brain in rats (Awtry et al., 2003)

PHYSICAL AND CHEMICAL PROPERTIES CONTRIBUTING TO TOXICITY

Nicotine has the ability to pass through the blood brain barrier very easily, and takes only about seven seconds to enter the brain after passing through the alveoli in the lungs when inhaled, the most common route of exposure. Nicotine acts on the cell by binding to, opening and passing through ion channels in the plasma membrane of the central nervous system, which normally bind to acetylcholine, and producing the same response as the neurotransmitter by mimicking it. In this case, the channels are called nicotinic acetylcholine receptors (nAChRs).

Acetylcholine is responsible for such important functions as controlling breathing and heart rate and relaying communication between the brain and muscles, as well as relating to learning and memory; nicotine's effects on the latter play a crucial role in teratology. Generally speaking, one reason nicotine is so toxic is that even though both acetylcholine and nicotine cause receptor activity to spike, the brain regulates levels of acetylcholine, while it has no mechanism to do so for nicotine (CRIS).

Nicotine is highly toxic, more so than many drugs that have more dangerous reputations, like its alkaloid cousin, cocaine. According to the Center for Disease Control, 50 to 60 milligrams of nicotine, which is roughly the amount found in three to four

cigarettes, can be enough to kill a healthy adult (CDC). In these cases, death is due to paralysis of the respiratory system.

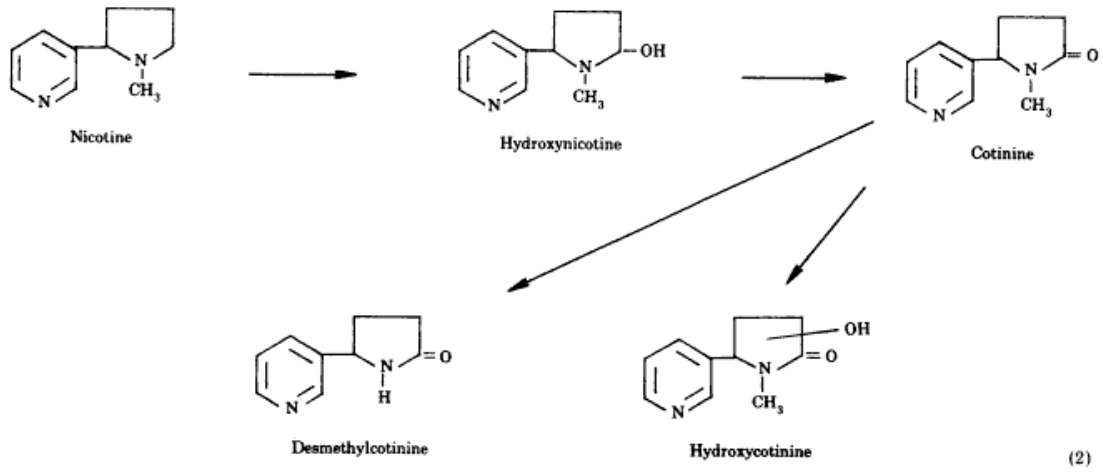
Because the chemical structure of nicotine is fairly simple and provokes a strong neurological response, scientists have designed and tested a number of similarly structured substances, but theses analogs have not proved comparable to nicotine in terms of the activity they induce (Soloway).

METABOLISM

The enzymes CYP2A6 and to a lesser extent CYP2B6, which are cytochrome P450 enzymes, metabolize about 80 percent of the nicotine that enters the body. The resulting metabolites are cotinine and nicotine oxide, which are excreted in urine. Any remaining nicotine passes through the kidneys and is removed from the blood to be excreted through urination as well. Nicotine has a short half-life; in about an hour, half of the nicotine that has entered the human body is degraded or gone. Cotinine has a 24-hour half-life.

This is not to say that everyone metabolizes nicotine at the same rate. Scientists have connected different metabolism rates to the cytochrome P450 enzyme CYP2A6. In 2005, researchers at the Scripps Research Institute finished mapping the protein's structure, in the hopes of creating a drug that could inhibit its activity and therefore decrease nicotine cravings in smokers. The idea is that with substantially fewer active enzymes in the body to degrade nicotine, the metabolic process will be slowed and less nicotine will be required to achieve equal or longer periods of satisfaction (Bardi, 2005).

(Below is a diagram of nicotine metabolism.)



(from Soloway, 1976)

Exposure via the retina or tongue also result in quick absorption while it is slower when ingested, as it is a fairly common additive in insecticides (Black Leaf 40 incident in Michigan 2003) or absorbed through the skin or tissue, as with chewing tobacco (CDC).

COMMON USES

Nicotine sulfate is a common additive to agricultural pesticides, although its use ha decreased significantly in the United States in recent decades. In August 2002, the Environmental Protection Agency revoked tolerances for "residues of nicotine-containing compounds used as insecticides" on 66 types of fruits, vegetables and other agricultural products (GPO CFR). According to EPA, the tolerances were revoked because there not been any active food use registrations under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) for those 66 foods since 1994 and the agency's view is that continuing to post unnecessary tolerances could encourage possible abuse of pesticides. The only remaining tolerances for nicotine are for tomatoes, cucumbers and lettuce grown in the US, for which nicotine residues are allowable up to levels of two parts per million (GPO CFR). Commercially, two main varieties of nicotine-containing botanical

insecticides exist: those that contain carbon, hydrogen and oxygen and those with a nitrogen presence (Soloway, 1976). 40 percent nicotine sulfate solution is widely available; popular brands include the botanical pesticides Black Leaf 40 and Tender Leaf Plant Insect Spray. Nicotine is also listed as an additive in one type of dog repellent (PAN).

CASE STUDIES: NICOTINE ADMINISTERED *IN UTERO*

NEUROLOGICAL IMPACTS

Nicotine has been shown to have a profoundly negative effect on fetuses *in utero* (Bardy et al., 1993). Theodore A. Slotkin of the Duke University Medical Center introduces his study 'Developmental Neurotoxicants: Nicotine and Chlorpyrifos' by challenging the traditional teratological concept that the most susceptible time for a fetus to experience the negative effects of drugs falls within the first trimester. In fact, he contends, the impacts of drug exposure on brain development can be observed as early as the embryonic stage and into adolescence (Slotkin, 1999). Two of the most common causes of chemical damage to the fetus are nicotine and insecticides, which may also potentially contain nicotine.

MECHANISM FOR INDUCING PRENATAL TOXICITY

When maternal cigarette smoking conveys nicotine to the fetus, it acts as a cholinergic agonist, meaning that it binds to the nicotinic cholinergic receptor and mimics acetylcholine to trigger neurotransmitter activity. While a certain amount of cholinergic stimulation is necessary to fetal brain development, overstimulation, especially at the

wrong time in development, can lead to "developmental anomalies" like halting neuronal mitosis too early, which amounts to low cell counts and reduced synaptic activity in the brain of the fetus (Slotkin).

Maternal cigarette smoking has been associated with spontaneous abortion, Sudden Infant Death Syndrome (SIDS), and later in life, learning disabilities, and behavioral problems including Attention Deficit Disorder (ADD), although these cannot be attributed solely or definitively to nicotine because cigarettes contain thousands of dangerous chemicals, plus a number of other confounding variables like the link between maternal cigarette smoking and other risk-taking behavior as well as the likelihood of being on the lower end of the socioeconomic spectrum .

Slotkin tested the effects of nicotine in pregnant rats for his experiment and found that in rats that had received 2 to 6 mg/kg/day prenatally (the level that replicates the system of a heavy smoker) and found evidence of high orthinine decarboxylase levels in the forebrain and cerebellum, which span from early to late in the developmental process. That enzyme is a biomarker for cell damage. In another alarming finding, nicotine was observed to trigger genes in the fetus that are linked to apoptosis (programmed cell death). This is a direct contradiction to the "neuroprotective effect" nicotine exposure exhibits in adults, which seems to protect against "injury-induced" apoptosis (Slotkin).

The effects of nicotine administered prenatally again proved severe in the DNA synthesis of the rats that were exposed to the toxicant. Chronic use was not necessary to produce enduring negative effects, Even one dose produced a steep and hours-long slump in DNA synthesis, especially cells in the nictotinic cholinergic receptor-rich parts of the brain (Slotkin).

(diagram of cholinergic activity, neurtotransmitter impact on gene transcription)

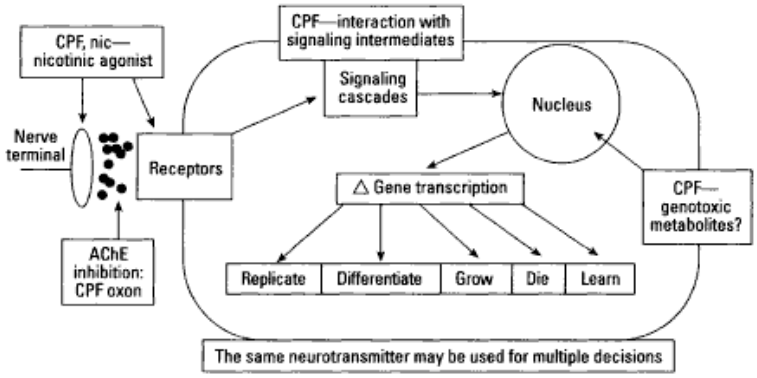


Figure 1. Cholinotoxicant targeting of cell development. Abbreviations: AChE, acetylcholinesterase; CPF, chlorpyrifos; Nic, nicotine. During development, neurotransmitters, through their receptors and associated signaling cascades, control the genes that influence differentiation. Depending on the context in which stimulation occurs, the same neurotransmitter can promote cell replication, can elicit a switch from replication to differentiation, can promote or arrest cell growth, can evoke apoptosis, or can program the genes that determine the future responsiveness of the cell to external stimulation. Nicotine targets nicotinic cholinergic receptors located on target cells, directly evoking changes in gene expression. Presynaptic nicotinic receptors that modulate release of other neurotransmitters produce secondary alterations of target cell development through the actions of these other transmitters on their respective receptors, signaling cascades and gene expression (*39*). Chlorpyrifos through its active oxon metabolite inhibits acetylcholinesterase, preventing the breakdown of acetylcholine and thus enhancing cholinergic activity. In addition, chlorpyrifos can exhibit agonistlike properties, opening and then desensitizing nicotinic cholinergic receptor/ion channels (*81*), can interact with signaling intermediates such as G-proteins and adenylyl cyclase (*80,82,83*), or can produce oxidative damage to DNA (*84,85*).

(from Slotkin, 1999)

In cases where the fetus is exposed to insecticides, the toxicant increases cholinergic activity by acting as a cholinesterase inhibitor, thereby preventing the enzyme from breaking down acetylcholine into choline and acetic acid by way of hydrolysis (Slotkin).

MUSCULOSKELETAL IMPACTS

A Danish study published in 2006 linked prenatal “nicotine substitute”-use (by non-tobacco smoking mothers) to an increase in congenital malformations (versus non-prenatal nicotine substitute-using women. The majority of the problems were related to musculoskeletal development, including several hip displacements (Morales-Suárez-Varela et al., 2006). This was an unusual study because most studies addressing fetal results of nicotine use have cigarette smoke as the route of exposure. Although additional

chemicals in cigarettes are cited as possibly confounding the results, there is still little research on the effects of solely nicotine in human fetuses, primarily because such a study would be unethical.

Even in this study, which did a good job of attempting to isolate nicotine as the sole variable through the use of stringent methods, the authors acknowledged that "During the pregnancy, the smoking members of the cohort were younger, had a lower body weight, a higher alcohol intake, and were less educated than nonsmokers" (Morales-Suárez-Varela et al.). However, smoking during pregnancy is apparently common in Denmark and there is little social stigma associated with it, unlike in the United States, so the authors thought self-reporting of smoking habits was likely to be accurate, another beneficial factor to this particular study (Morales-Suárez-Varela et al.).

More definitively, nicotine has been shown to inhibit palate fusion of fetuses in human, animal, and in vitro studies (causing cleft palates). The authors mentioned that they observed lower instances of urinary, eye, ear, face, and neck malformations but did allow that this may have been because the presence of these malformations triggered spontaneous abortion, the incidence of which increases with nicotine exposure (Morales-Suárez-Varela et al.).

METHODS TO REDUCE RISK

Maternal cigarette smoking occurs in about one fourth of pregnancies in the United States, according to two reports published in the 1990's (Bardy et al.). Since the congenital malformations associated with prenatal nicotine exposure are some of its most hazardous and entirely preventable effects, abstinence from smoking during pregnancy is

key. Additionally, nicotine use may cause abortion of the fetus in the embryonic phase, before the mother even knows she is pregnant, but these instances are difficult to measure for obvious reasons (Morales-Suárez-Varela et al.). Keeping cigarettes and other forms of nicotine out of reach of children is another important step in preventing intoxication. 90 percent of cases involving nicotine toxicity occur in children under five (Lavoie et al. 1991). The acute oral toxicity threshold in humans is 5 mg/m^3, which requires very little nicotine to produce toxicity in children and babies (CDC).

People using nicotine-based pesticides should exercise extra caution, making sure to avoid dermal exposure or contact with the eyes or inhalation. Some kind of mask to cover the nose and mouth may be a good investment. If nicotine pesticides are used on fruits or vegetables, people should be sure to wash them thoroughly to remove all residues. Additionally, any store-bought produce should be washed thoroughly. In cases like the 2003 Michigan beef contamination event, there were no precautions the victims could have taken to prevent becoming ill, since the nicotine was absorbed into the meat and could not be washed or cooked out (Boulton et al.).

CONCLUSION

Nicotine is a widely available, highly potent neurotoxicant that should not be viewed lightly. By imitating acetylcholine, it stimulates neurotransmitter activity and produces a brief dopamine high, using reward pathways to ingratiate itself to the brain, which with repeated exposure, learns to crave the substance. However, nicotine toxicity produces a range of detrimental effects from nausea and vomiting, to muscle spasms and seizures, to respiratory paralysis and in fetuses, it has been linked to apoptosis,

spontaneous abortion, severe birth defects, and even infant death. Studies have shown that fetal nicotine exposure causes more damage than fetal cocaine exposure (Slotkin, 1998). Almost all nicotine toxicity is avoidable by maintaining abstinence from smoking, keeping nicotine products out of children's reach and exercising caution when using products that contain nicotine or nicotine residues.

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