TSNAs in Burley and Dark Tobacco

Anne Jack, Lowell Bush, and Andy Bailey

What Are TSNAs?

Nitrosamines are nitrogenous compounds, some of which are carcinogenic. They are found in a wide range of food and cosmetic products, as well as in tobacco. Tobacco-specific nitrosamines (TSNAs) are so called because they are formed only from tobacco alkaloids, and found only in tobacco leaves and in the particulate phase of tobacco smoke. With the current emphasis on the health risks of tobacco, TSNA reduction has become a major issue for the tobacco industry.

Several TSNAs have been identified, but interest has focused on the four most important: NNK, NNN, NAT, and NAB. Of these, NNN is the most important in burley and dark tobacco.

How Are TSNAs Formed?

Negligible amounts of TSNAs are present in freshly harvested tobacco. They are mainly formed during curing, specifically during the late yellowing to early browning stage. Typically this occurs over a two-week period between the third and fifth week after harvest, but can be earlier or later depending on curing conditions.

TSNAs are formed by the nitrosation of tobacco alkaloids (addition of a nitrogen and an oxygen atom to the alkaloid molecule). NNN is formed by the nitrosation of the alkaloid nornicotine. The nitrosating agent in air-cured tobacco is usually nitrite, derived from the reduction of leaf nitrate by the action of microbes during curing. In fire-cured tobacco, the nitrosating agents are both nitrite and any of several nitrogen oxides (NOx) formed during the fire-curing process. Both the alkaloid and the nitrosating agent are necessary for the formation of TSNAs. Any practices or conditions that increase the accumulation of either of these groups of compounds would be expected to increase TSNAs.

Factors Affecting TSNA Accumulation

Three main factors affect the amount of TSNA accumulation:

- The amount of specific alkaloid precursor. In the case of burley and dark tobaccos, this is nornicotine, and it is mainly determined by the amount of conversion of nicotine to nornicotine in the seedlot used. Screened or "LC" seed has been selected for low conversion, and we have shown that this results in significantly lower TSNAs.
- The amount of nitrosating agent. Nitrite is the main nitrosating agent for air-cured tobacco and is determined by the microbial populations reducing the leaf nitrate to nitrite. The microbial populations are affected by curing conditions, particularly during the first 35 days of curing. The amount of leaf nitrate, determined by available soil nitrogen, has little direct effect on the amount of leaf nitrite; any effect is probably indirect, through the effect of nitrate on the thickness and drying rate of the leaf. With the levels of nitrate found in the normal production range, the main effect of applied nitrogen fertilizer on TSNAs is through the effect on alkaloid level. During fire-curing, nitrogen oxides (NOx) are the nitrosating agent and are the result of combustion of wood during firing.
- The amount of total alkaloids/nicotine. The relative amount of nornicotine depends on conversion, and the absolute amount depends on the amount of nicotine originally present. The higher the nicotine, the higher the absolute amount of nornicotine (because there is more nicotine available to be converted to nornicotine), and consequently the higher the potential for TSNA accumulation. The amount of total alkaloid is determined partly by environmental conditions, such as rainfall, and partly by agronomic practices, such as fertilization, topping, maturity at harvest, etc.

If any of these factors (conversion, nitrosating agent, total alkaloids) are reduced, TSNAs are reduced (Figure 1).

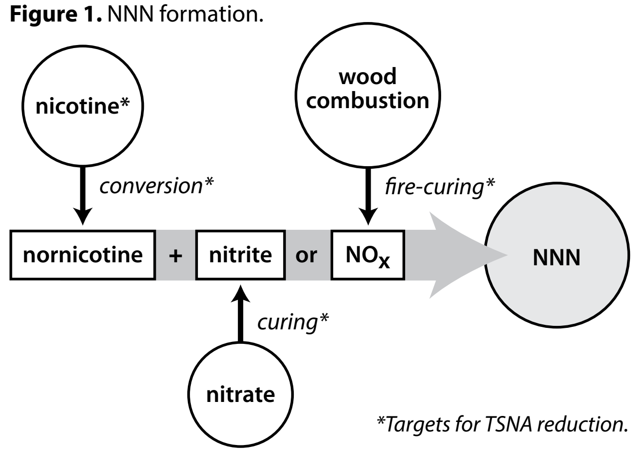
Seed Screening

Reducing the amount of nornicotine precursor for NNN is the single most effective step in reducing TSNA accumulation. Figure 2 illustrates the difference in NNN between two varieties, a non-commercial high converter and a screened low converter.

There are very low inherent levels of nornicotine in the green plant; it is mainly formed by the conversion of nicotine to nornicotine during curing. The ability of plants to convert nicotine is under genetic control and most modern varieties have been selected for minimum conversion.

In the US, all the public varieties have been screened; i.e., the foundation seed was selected for low conversion, which is indicated by "LC" (low converter) in the variety name (for example, TN 90LC, KT 204LC). Many other varieties also have this designation. Some varieties do not have the LC designation, but "screened seed" is indicated on the seed pack. All seed of commercially viable varieties has now been screened, and there should be no unscreened seed sold in the domestic seed market.

Figure 1. NNN formation.



Prior to universal seed screening, many seedlots had relatively high conversion, and consequently the potential for high TSNA formation. There has been a considerable reduction in TSNAs in recent years as a direct result of seed screening.

What the Grower Can Do

The most important step in TSNA reduction, the use of LC or screened seed, has been taken for U.S. tobacco growers. All seed on the domestic market is now screened, and all contracts with major tobacco companies now require the grower to use LC or screened seed.

Variety

To some extent, there seem to be inherent differences between some burley varieties in their potential to accumulate TSNAs, differences that are not explained by conversion levels. These differences are small, but they do appear to be real. For example, it appears that KT 204LC often has lower TSNA levels than some other varieties (Figure 3). We do not yet understand the mechanism for these varietal differences. Like all factors affecting TSNAs, these differences are not always apparent. They are dependent to a large extent on the environmental growing and curing conditions; differences are more likely to be apparent under conditions conducive to higher TSNA accumulation.

To date, no varietal differences in TSNA accumulation have been observed in dark tobacco varieties.

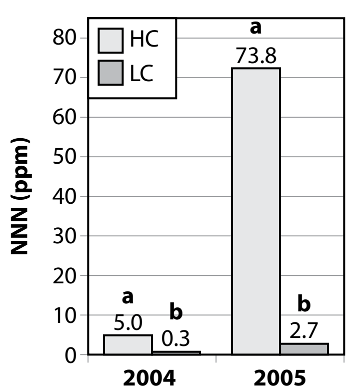


Figure 2. NNN (ppm) for high converter (HC) and low converter (LC) burley varieties over two years. Within each year, bars with different letters are significantly different at the 5% level.

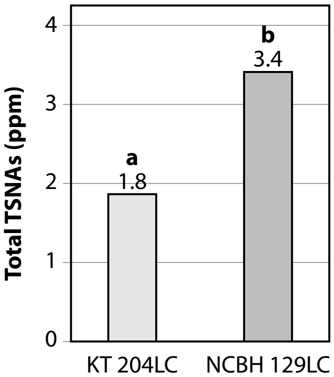


Figure 3. Total TSNAs (ppm) for KT 204LC and NCBH 129LC; mean over two years. Bars with different letters are significantly different at the 5% level.

What the Grower Can Do

Variety choice is a minor consideration in relation to TSNA accumulation.

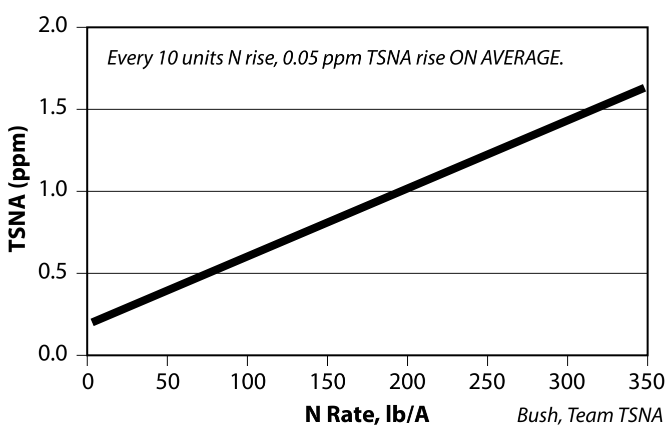
Choose the variety most suited to local conditions, paying particular attention to the disease spectrum. If KT 204LC meets the requirements, the choice of this variety may contribute to lowering TSNAs.

Fertilization

Nitrogen fertilization has a considerable impact on TSNA accumulation in the leaf, but the effect is probably indirect; nitrate is probably not directly involved in TSNA synthesis in the leaf. Nitrate affects TSNA levels mainly through its effect on alkaloid levels, and also through the effect on the body and drying rate of the leaf. However, high nitrate in the leaf is undesirable because additional TSNAs may be accumulated during storage and cigarette smoking.

Many studies have found large differences in TSNAs between very high and very low nitrogen rates. However, within the normal production range, the effect was observed to be much smaller and often inconsistent. Growing and curing conditions can play a large role in determining how nitrogen rates affect TSNAs, even when the rates are extreme. Only when many studies were pooled were researchers able to show a clear relationship between the amount of applied nitrogen and TSNA accumulation. Figure 4 shows the strong linear trend for TSNAs to increase with increasing nitrogen. On average, TSNAs will increase 0.05 ppm for every 10-pound per acre increase in applied nitrogen.

Figure 4. TSNAs vs. nitrogen rates for three years, five locations.



The total amount of applied nitrogen is the critical factor, regardless of whether it is all applied as a pretransplant application or is split between pretransplant and sidedressing. Sidedressing does not appear to cause a significant increase in TSNAs, as long as it is applied at the recommended time. Applying sidedress nitrogen later than six weeks after transplanting could increase TSNA levels under some conditions.

There is no clear link between nitrogen source and TSNAs.

Fat stems can increase TSNAs by retaining moisture in the leaf stem. Fat stems can be caused by late uptake of nitrogen (late sidedressing or a dry period followed by rain shortly before harvest) and by the use of muriate of potash fertilizers.

While the amount of applied nitrogen is important, the most important factor is the amount of available nitrogen, which depends partly on the soil type. We have measured large differences in TSNA accumulation between growing locations, where higher TSNAs were associated with heavier, more fertile soil. It is necessary to exercise particular care when fertilizing these heavier soils.

What the Grower Can Do

Judicious fertilizer application is one of the more feasible steps a grower can take to reduce TSNAs.

- Apply no more nitrogen than is necessary for the crop. Apart from minimizing TSNAs, there are many other good reasons to avoid excessive nitrogen—not the least of which is cost. Excess nitrogen can also cause disease problems and contribute to groundwater pollution, and it does not increase yield.
- If sidedressing, apply nitrogen within four to five weeks after transplanting.
- Avoid spring applications of muriate fertilizers. If muriate fertilizers are used, they should be applied in the fall. Chloride also has an adverse effect on quality and causes the cured leaf to be more hygroscopic (moisture absorbing).
- Exercise particular care when fertilizing heavier soils.

Topping

Any effect of topping on TSNAs is indirect through the effect on alkaloid levels. Topping early and/or low increases alkaloids and would be expected to increase TSNAs. We do not have much data on this topic, but indications are that differences are small and unlikely to have much impact, especially with low converters.

What the Grower Can Do

The effect of topping on TSNA accumulation is relatively minor. Top as recommended for best yield and quality (see Topping and Sucker Control on page 44).

Maturity at Harvest

Several studies have shown that TSNAs increase with increased maturity at harvest. Earlier studies used unscreened seed, and we know that conversion increases with increased maturity. Current results show a similar, but smaller, response with the low converter varieties now in use. The increase in TSNAs with increased maturity is due mainly to the higher alkaloids in later harvested tobacco; alkaloids increase steadily after topping.

What the Grower Can Do

Weather and availability of labor to cut the crop often limit the grower's choice of harvest date, but to the extent possible, harvest at the maturity for best yield and quality.

- **Burley.** Typically the best compromise between yield and quality is approximately three and a half to four weeks after topping.
- **Dark air-cured.** Five to six weeks after topping–some early maturing varieties may require earlier harvest.
- **Dark fire-cured.** Six to seven weeks after topping–some early maturing varieties may require earlier harvest.

Harvesting Practices

Field-wilting longer than necessary can, under some conditions, increase TSNAs. Figure 5 shows the TSNA accumulation in burley tobacco field-wilted for three and six days. These increases are small and are not always apparent, but it is advisable not to field-wilt burley longer than three days, as this can have a detrimental effect not only on TSNA accumulation, but also on leaf quality.

What the Grower Can Do

Weather and availability of labor often dictate when the tobacco can be housed, but house burley tobacco as soon as possible, ideally within a few days of cutting.

Air-Curing

Growing season and curing environment play a very large role in TSNA accumulation. Figure 2 shows the effect of season on a high and a low converter variety. At this site, 2005 was a year very conducive to TSNA accumulation; 2004 was not. The more than tenfold difference between years was due solely to environmental differences, as the same seed and growing practices were used in both years. Note that the low converter variety in 2005 (when conditions were highly conducive) still had lower TSNAs than the high converter in 2004 (when conditions were very unfavorable for TSNA formation).

The main factors affecting air-curing in relation to TSNAs are temperature, relative humidity, and air movement.

- Higher temperatures increase TSNAs because biological and chemical reactions are faster at higher temperatures.
- Higher humidity increases TSNAs because it is favorable for the nitrite-producing microbes and the leaf remains alive and active longer during curing, allowing more conversion of nicotine to nornicotine. Thus, with the increased nitrite and nornicotine available, more TSNA is formed.
- Increased air movement decreases TSNAs mainly by increasing the drying rate of the leaf.

High humidity and high temperatures result in high TSNAs and often in houseburn. Low temperatures or low humidity result in low TSNAs but green or piebald tobacco. The conditions best for optimal quality (moderate temperatures and 72 to 75% relative humidity, i.e., a long, slow cure) are also favorable for TSNA accumulation. Under these conditions, TSNAs levels will

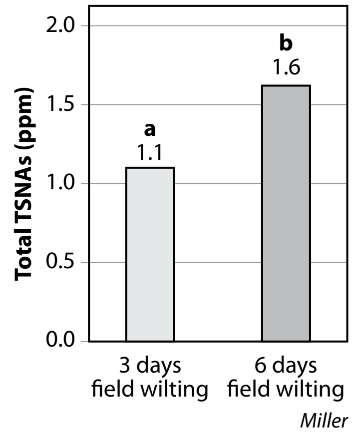


Figure 5. Total TSNAs (ppm), means across varieties, for burley tobacco field-wilted for 3 and 6 days after cutting. Bars with different letters are significantly different at the 5% level

be unacceptable if there is any appreciable amount of conversion. However, TSNAs will usually be acceptable if conversion is low and curing is well managed. Low converters can have significant amounts of TSNAs in conducive conditions if the curing is not properly managed (see Figure 2, low converter in 2005). The challenge is to produce quality tobacco with acceptable levels of TSNAs.

Tests have shown that TSNAs in outdoor burley curing structures are very similar to those in a conventional barn (Figure 6) if they are in the same vicinity and experience similar environmental conditions.

The location and orientation of a barn can have a considerable effect on TSNAs by affecting the amount of ventilation. There can be big differences in TSNAs between tobaccos cured in different barns. TSNAs will tend to be lower in exposed barns on ridges and higher in barns in protected hollows with limited air movement.

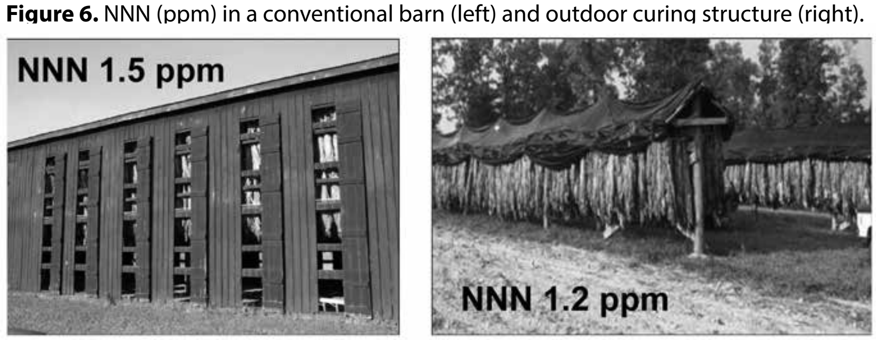
Various barn modifications have been tested, but none have yet resulted in a practical and economical system to consistently reduce TSNAs while producing quality tobacco.

What the Grower Can Do

Attention has focused on ventilation, because there is little that a grower can do to control ambient humidity and temperature during air-curing. However, ventilation can be manipulated to a limited extent if quality is to be maintained. Managing curing specifically for very low TSNAs will often result in poor quality tobacco, so the best curing management is a balance between enough humidity for good quality and enough ventilation to minimize TSNA formation. Take the following steps:

- Space plants evenly on sticks, and space sticks evenly on the rails.

Figure 6. NNN (ppm) in a conventional barn (left) and outdoor curing structure (right).



- Avoid packing sticks too closely (actual stick spacing will vary with barn design and size of tobacco).
- Manage vents to ensure adequate but not excessive ventilation.

Fire-Curing

Fire-curing of dark tobacco involves the burning of hardwood slabs and sawdust on the floor of the barn during curing. Although fire-curing barns have bottom and top ventilators, they are typically much tighter than air-cured barns and most have metal siding. Many fire-cured barns are also equipped with fans in the top of the barn that can be used to increase ventilation early in the cure. Although differences in barn design and the fire-curing process itself allow more control over curing conditions and less influence of outside weather conditions, the growing season and curing environment still play a major role in TSNA accumulation in fire-cured tobacco.

Fire-curing allows more potential for TSNA accumulation than air-curing. Higher temperatures are involved, which increases the speed of biological and chemical reactions, and nitrogen oxide (NO_x) gases are produced by the burning of wood, which increases nitrosation of tobacco alkaloids. However, some basic management practices for fire-curing can reduce the potential for high TSNA formation.

Avoid packing sticks too closely in the barn, as this can lead to poor cured leaf quality, losses in cured leaf weight, poor or uneven smoke finish on leaves, and higher TSNAs.

Ideally, start firing within seven days after housing. Avoid firing the tobacco more or longer than necessary to produce cured leaf with acceptable quality and marketability. Growers should strive to keep barn temperatures below 130°F, even during the drying stage of the cure. Ideally, tobacco should not be kept at 130°F longer than four to five days; by seven days at this temperature, TSNAs would be expected to increase.

Artificial casing with overhead misting systems or steamers is often required for takedown in dark fire-cured tobacco due to the extremely dry condition of the tobacco after curing is complete. This is particularly true with first cures in double-crop curing, where takedown needs to occur quickly following curing. Research has shown that use of overhead misting systems at takedown may result in lower TSNAs than steam.

What the Grower Can Do

The most effective steps a grower can take are to minimize the effects of high temperatures (which increase the speed of TSNA-forming reactions) and wood combustion (which increases the amount of nitrosating agent). Do the following:

- Fire dark tobacco no more than necessary.
- Ideally, start firing within seven days after housing.
- Strive to keep barn temperatures in fire-cured barns below 130°F.
- Ideally, do not keep temperatures at 130°F for longer than four to five days.
- Space plants evenly on sticks, and place sticks evenly on the rails.
- Avoid packing sticks too closely.
- Use minimal artificial casing.
- Consider using overhead misting systems instead of steam when artificial casing is needed in fire-cured tobacco.

Control of Microbes

The nitrite-producing microbes are ubiquitous and cannot be avoided. They are endophytic (inside the leaf), which makes application of any treatment very difficult.

Many chemicals and biological agents have been tested, but none of them has yet resulted in a practical control method. Correct curing will help to control microbes.

What the Grower Can Do

At this point, there is no treatment to directly control the nitrifying microbes. Manage curing for production of high quality, full flavor and aroma tobacco, and avoid houseburn conditions that are conducive to microbial activity.

Moisture and Storage

Studies have shown that housing wet tobacco can increase TSNAs, as can storing high-moisture tobacco. It is difficult to control the moisture content of tobacco when using artificial methods of casing, such as steam or water sprays, and over-application of water to cured leaf can result in unsafe moisture levels during storage. For this reason, it is better to use natural casing if possible.

TSNAs generally increase with time in storage, although this is less evident in low converter tobacco. Tobacco should therefore not be left in storage longer than necessary.

What the Grower Can Do

The following steps will help to minimize the effects of moisture on the nitrite-producing microbes:

- To the extent possible, do not house tobacco with free moisture on the leaves.
- Allow air-cured tobacco to come into case naturally if possible. If using artificial casing, avoid over-applying moisture.
- Use minimal artificial casing for fire-cured tobacco, and consider using overhead misting systems instead of steam.
- Strip, bale, and deliver tobacco as soon as possible to avoid any extra time in storage.
- Keep moisture in the cured tobacco as low as possible, ensuring that it is below the level specified in the contract.

Best Management Practices for Minimizing TSNAs

TSNA formation is a very complex process, and one cannot consider any of the factors contributing to it in isolation. All of these factors interact, and that is why different treatments sometimes result in TSNA differences and sometimes do not. These practices will contribute to lowering TSNAs:

- Use LC or screened seed.
- Choose the most suitable variety with the appropriate disease resistance package—if KT 204LC meets other requirements, the choice of this variety may help to lower TSNAs.
- Use no more nitrogen than necessary to optimize yield.
- Avoid spring applications of muriate fertilizers.
- If sidedressing, apply nitrogen within four to five weeks after transplanting.
- Top correctly.
- Harvest at correct maturity, ideally about three and a half to four weeks after topping for burley, about five to six weeks

for dark air-cured, and about six to seven weeks for dark fire-cured.

- House burley tobacco as soon as possible, ideally within a few days of cutting.
- To the extent possible, do not cut or house tobacco with free moisture on the leaves.
- Manage air-curing carefully, ensuring adequate but not excessive ventilation, and avoid houseburn.
- Avoid overpacking the barn, and space sticks and plants on the sticks evenly.
- Fire dark tobacco no more than necessary.
- Ideally, start firing dark fire-cured tobacco within seven days after housing.
- Strive to keep barn temperatures in fire-cured barns below 130°F.
- Ideally, do not keep temperatures in fire-cured barns at 130°F for longer than four to five days.
- Allow burley tobacco to come into case naturally and use minimal artificial casing for dark tobacco, ideally misting systems instead of steam.
- Do not leave tobacco in storage longer than necessary; strip, bale, and deliver tobacco as soon as possible.
- Keep moisture in the leaf as low as possible; do not put high-moisture tobacco into storage, and do not deliver tobacco with moisture higher than specified in the contract.