Desktop study of emission factors for urease inhibitors for nitrogen fertiliser

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Desktop study of emission factors for urease inhibitors for nitrogen fertiliser

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1. Executive Summary

We conducted a literature review to examine the contribution of urease inhibitors for N fertiliser to emissions reductions in New Zealand's national greenhouse gas inventory and develop methods to describe how ammonia (NH_3) emissions from pastoral agriculture soils can be reduced using urease inhibitors. Unlike nitrous oxide (N_2O), NH_3 itself is not a greenhouse gas. However, it acts as a secondary source of N_2O and thus contributes to global warming and ozone depletion in the atmosphere.

A number of chemicals have been tested as potential inhibitors of soil urease activity, for use with urea fertilisers over the last 30 years. However, only two groups, namely Hydroxamic acids and Phosphorodiamidates, have gained importance as potent urease inhibitors (UIs). Among the compounds studied and evaluated, N-(n-butyl) thiophosphoric triamide (nBTPT) sold under the trade name Agrotain® is currently the most promising and effective UI when mixed with urea or urine.

Based on the available literature an application rate of 0.025% w/w nBTPT is optimum for reducing NH_3 emissions from temperate grasslands. New Zealand studies where nBTPT (0.025% w/w) was applied reduced NH_3 emissions on average by 43% from urea and by 48% from urine and resulted in an overall increase of 6.5% in pasture production when compared to urea alone.

Currently New Zealand N_2O inventory uses the IPCC default values of 0.1 and 0.2 for $Frac_{GASF}$ (fraction of total fertiliser emitted as NO_x and NH_3) and $Frac_{GASM}$ (fraction of total nitrogen excretion emitted as NO_x and NH_3). In a recent review of New Zealand specific $Frac_{GASM}$ and $Frac_{GASF}$ emission factors, Sherlock et al. (2008) have recommended a New Zealand specific value of 0.1 $Frac_{GASM}$ and $Frac_{GASF}$ be considered for adoption. The application of nBTPT with N-fertilisers and urine further reduces the amount of NH_3 emission and reduces the values $Frac_{GASF}$ and $Frac_{GASM}$. To our knowledge, no country has revised its emission factors to account for the effect of nBTPT application with urea or onto soils.

Based on our above estimates of reductions in NH_3 emission from NBPT treated urea application, a New Zealand specific value of 0.06 for $Frac_{GASF}$ is recommended for adoption where fertilisers containing urease inhibitor, NBPT, are applied. We recommend that where NBPT is applied as recommended, $Frac_{GASF}$ should be calculated as follows:

$$\mathbf{FracGASF = [(FN_{UI}) \times 0.06] + [(FN - FN_{UI}) \times 0.10]} \qquad \textbf{(1)}$$

where $\mathbf{Frac_{GASF}}$ is the fraction of total fertiliser N emitted as NH_3, **FN** is the total amount of applied fertiliser N, $\mathbf{FN_{UI}}$ is the amount of applied fertiliser N treated with urease inhibitor, nBTPT.

Changing the $Frac_{GASF}$ from 0.1 to 0.06 for the current use of 18.4 Gg N of SustaiN (urea containing with nBTPT) reduces indirect N_2O emissions by 0.012 Gg, which equates to 3.6 Gg $CO_{2\text{-equiv.}}$ However, assuming all the urea is applied with NBPT in New Zealand, changing the $Frac_{GASF}$ from 0.1 to 0.06 will reduce the indirect N_2O emissions by 0.14 Gg, which equates to 43.4 Gg $CO_{2\text{-equiv.}}$

Currently, there are no New Zealand data on direct application of nBTPT or the effect of nBTPT containing urea fertilisers applied concurrently or as close as possible to the deposition of urine-N on NH_3 emissions. Therefore, it is not possible to estimate the effect of urease inhibitors on emission reductions from excretal N inputs during grazing. This report also outlines the aspects of urease inhibitors that need to be further evaluated including their effectiveness in different soil types, at a range of soil temperatures and soil moistures, and the mode of application of nBTPT to urine patches and the frequency of its application to provide quantitative estimates on emission reductions from excretal N inputs during grazing.

2. Introduction

2.1. Background and rationale

Urea is the form of N fertiliser used most widely in New Zealand pastures and it is the most susceptible to loss via ammonia volatilisation. When it is applied to soil, urea undergoes hydrolysis, catalysed by the enzyme urease to form ammonium carbonate [$(NH_4)_2CO_3$] (Eq. 1), which in turn, being unstable, dissociates into ammonium (NH_4^{+}) and carbonate (CO_3^{2-}) ions (Eq. 2). The CO_3^{2-} ions release hydroxyl (OH^-) ions (Eq. 3), thereby resulting in a high pH close to the site of hydrolysis.

$$(NH_2)_2CO + 2H_2O \rightarrow (NH_4)_2CO_3 \quad \text{(Eq. 1)}$$

$$(NH_4)_2CO_3 \rightarrow 2NH_4^{+} + CO_3^{2-} \quad \text{(Eq. 2)}$$

$$CO_3^{2-} + H_2O \rightarrow HCO_3^{-} + OH^{-} \quad \text{(Eq. 3)}$$

Ammonia volatilisation or emission is simply a chemical reaction that occurs only under alkaline conditions, i.e. when the soil pH is high (>7.5) (Bolan et al. 2004). The effect of pH on the amount of NH_4^+ and ammonia (NH_3) formed is crucial in determining the fate of fertiliser and animal excreta N. Ammonium ions dissociate into gaseous NH_3, which is subjected to volatilisation losses (Eq. 4).

$$NH_4^{+} + OH^{-} \rightarrow NH_3 \uparrow + H_2O \text{ (pKa 9.24)} \quad \text{(Eq. 4)}$$

Temperature, soil water content, and other factors have some controlling influence on the potential loss of urea-N by this process. A necessary prerequisite for NH_3 volatilisation is a supply of free NH_3 near the soil surface. The conversion of NH_4^+ ions to NH_3 (Eq. 4) is thus the major process regulating the potential loss of NH_3 from soils.

Unlike nitrous oxide (N_2O), NH_3 itself is not a greenhouse gas. However, it acts as a secondary source of N_2O, by deposition and then conversion to NO_3 and N_2 through nitrification and denitrification, and thus contributes to global warming and ozone (O_3) depletion in the atmosphere. NH_3 emissions also represent agronomic losses and may cause eutrophication and soil acidification after deposition on water and soil. Various approaches have been used to mitigate the economic and environmental impacts of NH_3 losses. One such approach is the use of urease inhibitors (UIs). Recently in New Zealand there has been increasing interest in the use of commercially formulated UIs (e.g., SustaiN) to reduce the loss of N through leaching and gaseous emissions,[1] and thus enhance plant productivity.

It has been suggested (Bolan et al. 2004; Saggar et al. 2005, 2008, 209; Singh et al. 2008) that the ideal inhibitor for use in agricultural soils should:

- specifically block an enzymatic reaction (e.g., UIs should block urease enzyme during urea hydrolysis)
- remain in close contact with N compounds (e.g., UIs must move with urea molecules that are not readily absorbed by soil)
- not adversely affect other beneficial soil organisms and higher plants
- remain effective in the soil for several weeks after urea-N input through fertiliser addition and urine deposition
- not be toxic to animals and humans at the levels used to inhibit nitrification effectively
- be cost-effective to use.

This project will examine the contribution of UIs amended to N fertiliser to reduction of emissions in New Zealand's national greenhouse gas inventory. It will also determine the activity data (quantities of inhibitors, soil types, regions, grazing regimes) needed and the means of incorporation into the national agricultural inventory based on our current understanding of the effect of urease inhibitor on nitrogen transformation processes and gaseous emissions of N. To refine New Zealand's agricultural emissions inventory, this project will provide estimates of the effect of urease inhibitors applied to pastoral soils on emission reductions and suggest a methodological approach to quantify these emission reductions.

2.2. Objectives

The aims of the proposed project are to:

1. review the national and international published research and unpublished data on the effect of urease inhibitors for nitrogen fertiliser on gaseous losses of nitrogen (N);

[1] Zaman M, Nguyen ML, Blennerhassett JD, Quin BF (2007) Reducing NH_3, N_2O and NO_3 –N losses from a pasture soil with urease or nitrification inhibitors and elemental S-amended nitrogenous fertilizers. *Biol. Fertil. Soils* DOI 10.1007/s00374-007-0252-4

2. assess the impact of potential emissions reductions on the national inventory;

3. determine the requisite activity data and means of incorporation into the national agricultural inventory.

2.3. Outcomes

The outcomes of the study are to:

- provide a framework for estimating the effect of urease inhibitors on relevant emission factors within the national greenhouse gas inventory and their uncertainty based on available relevant overseas and New Zealand published and unpublished data.

- recognise key gaps in activity data for national emissions reduction estimates.

- provide recommendations for targeted research, if required, to fill the key gaps in emission factor and activity data knowledge.

3. Literature Review

Urea is the most common source of fertiliser-N used world-wide and urea-based fertilisers make up almost half the world's nitrogen (N) market. Continued growth is expected owing to urea's high-analysis and its ability to be applied as a dry granule or a urea containing solution. However, the most important negative impact from an increased use of urea-based fertilisers (solid or liquid) would be to increase ammonia (NH_3) emissions to the atmosphere. Urea applied to soils in the form of fertilisers or in urine applied hydrolyses, i.e. it reacts with soil moisture and breaks down. The enzyme urease, which is produced by soil microbes, facilitates the reaction. High temperatures and high biological activity at the soil surface promote rapid hydrolysis of urea to NH_3 and carbonate species, leading to large NH_3 losses (up to 30% of applied N). If this conversion takes place below the soil surface, the NH_3 is almost instantaneously converted to NH_4^+-N, which is bound to soil particles. If the conversion takes place on the soil surface or on the surface residues, there is potential for the NH_3 gas to escape into the atmosphere in a process called ammonia volatilisation or emission.

Ammonia emissions depend on the environmental conditions at the time of application. Soil temperature, soil moisture, amount of surface plant residue, soil pH, and length of time between application and the first rain event or irrigation are all factors that determine the total amount of N that can potentially be lost by volatalisation. Ammonia emission losses can be substantially reduced if a urease inhibitor is used with the fertiliser (Bundy & Bremner 1973). Urease inhibitors slow the conversion of urea to the NH_4^+ by inhibiting the urease enzyme, which reduces NH_4^+ concentration in the soil solution and hence lowers the potential for NH_3 emission. Slowing the hydrolysis of urea allows more time for it to diffuse away from the application site. It also increases the probability that rain or irrigation will dilute the urea and NH_4^+ concentration at the soil surface and increase its dispersion in the soil subsequently retaining NH_3 in the soil.

3.1. Urease Activity

The urea in fertiliser and animal urine is usually hydrolysed within a few days by an enzyme termed urease, which is present in many plants and plant litter (Freney & Black 1988) and in most species of bacteria, yeast and fungi. The enzyme catalyses the hydrolysis of urea to NH_4^+ and carbamate ions which then decompose to CO_2 and NH_3.

The active site of urease enzyme contains two nickel (II) atoms linked by a carbamate bridge. Two imidazole N atoms are bound to each Ni atom; a carboxylate group and a water molecule fill the remaining coordination site of the metal ion. The ability of soil bacteria and fungi to hydrolyse urea varies: soil bacteria hydrolyse between 17% and 70 % of urea, while soil fungi hydrolyse between 78% and 98% (Lloyd & Sheaffe 1973). Although soil urease enzyme is considered to be of microbial origin there is evidence that some soil urease activity may be derived from plants (Frankenberger & Tabatabai 1982). However, there is no direct evidence for the production of urease by plant roots.

The urease activity of soils is associated with organic matter (O'Toole et al. 1982; Reynolds et al. 1985; Kissel & Cabrera 1988): as the organic matter content of soil decreases with depth, so too does urease activity (Bremner & Mulvaney 1978; Mulvaney & Bremner 1981). Urease activity is greater in grassland than in cultivated soils (O'Toole et al. 1985; Reynolds et al. 1985; Whitehead & Raistrick 1993), which probably relates to differences in organic matter and microbial activity.

Hydrolysis of urea is temperature dependent and increases with soil temperature over the range of 0–40^{o}C (Vlek & Carter 1983), though slight hydrolysis has been detected at sub-zero temperatures (Bremner & Mulvaney 1978). It is also affected by urea concentration, soil water and soil pH. The optimum pH for urea hydrolysis is between 6.0 and 7.0 (Kissel & Cabrera 1988).

3.2. Mechanism of Inhibition of Urease

Thousands of chemicals have been tested as potential inhibitors of soil urease activity, for use with urea fertilizers. These can be classified according to their structures or according to their binding modes with urease, and mostly fall into three groups: (i) reactive organic or inorganic compounds (e.g., alk(en)yl thiosulfinate, aydroquinone, p-Benzoquinone) that react with sulfhydryl (mercapto) groups in the urease enzyme; (ii) metal chelating compounds (e.g., caprylohydroxamic acid, acetohydroxamic acid) that cause inhibition due to complex formation with one of the Ni atoms at the active site of urease; and (iii) competitive inhibitors (e.g., hydroxyurea, phosphoroamides, phenyl phosphorodiamidate PPDA, N-(n-butyl)phosphorothioic triamide NBPT) that resemble urea molecule (structural analogue), and bind to the active site of urease enzyme. Amtul et al. (2002) divided UIs into (i) substrate-analogue inhibitors, and (ii) non-substrate-like or mechanism-based inhibitors, depending on their binding modes.

Substrate-analogue inhibitors have structural similarities to urea and inhibit urease by competing for the same active site on the enzyme. Thiourea, methylurea, hydroxyl urea, and numerous hydroxamic acids are the main examples of the substrate-analogue UIs.

Non-substrate analogue inhibitors do not have any close structural similarity with urea, but they interfere with the enzyme's catalysis mechanism, leading to enzyme inactivation. These compounds are also called "mechanism-based" inhibitors, e.g., imidazoles and sulphydryl reagents like p-chloromercuribenzoate, polyhydric phenols, aminocresols and quinones (e.g., p-benzoquinone, 2-5 demethylbenzoquinone).

A number of UIs have been studied and tested over the last 30 years, but the following two groups have gained importance during the last few years as potent UIs:

Hydroxamic Acids:

Hydroxamic acid [R-CONH-OH, R-C(OH)=NOH] (HXA) derivatives characterized by a terminal O=C-NHOH functionality were discovered by (Kobashi et al. 1962). Since then a range of hydroxamic acids have been designed and examined (Gale & Atkins 1969; Nervig & Kadis 1976; Kobashi et al. 1980). The best studied hydroxamate and the prototype of this class of inhibitors is acetohydroxamic acid (AHA), which inhibits ureases from *Clostridium sordelli, E. coli, Morganella morganii, Proteus mirabilis, Proteus vulgaris, Providencia rettgeri, Staphylococcus aureus* (Rosenstein et al. 1981) and many other micro-organisms, as well as ureases from soil (Pugh & Waid 1969). AHA is a stable synthetic lead (Pb) molecule, weakly acidic and highly soluble in water, which structurally resembles urea. Hydroxamic acids are effective metal chelates and their mechanism of inhibition involves binding to the metal ions of the active site of enzyme.

Phosphorodiamidates:

The synthetic phosphorodiamidates are more potent than HXA and can be successfully used to inhibit the urease activity of ureolytic bacteria in soil (Byrnes et al. 1983; Martens & Bremner 1984; Kobashi et al. 1985; Liao & Raines 1985; Bremner et al. 1986; Rao & Ghai 1986). The strong interaction between urease and phosphoroamide compounds may result from the electrostatic stabilization and structural similarity of phosphoroamide (tetrahedral geometry) that may mimic an intermediate state in enzymatic catalysis. Many compounds have been studied and evaluated (Mulvaney & Bremner 1981; Martens & Bremner 1984; Broadbent et al. 1985; O' Connor & Hendrickson 1987), though most have shown limited potential as fertilizer amendment due to problems of low effectiveness, lack of sustained action, or lack of stability in fertilizer. N-(n-butyl) thiophosphoric triamide (nBTPT) is currently the most promising and effective at low concentrations when mixed with urea (Bremner & Chai 1986; Joo et al. 1987). nBTPT is not an active UI and must be converted in the soil to its oxygen analogue N-(n-butyl) phosphoric triamide (nBPTO), which is the actual UI (Christianson et al. 1990). The conversion of nBTPT to its oxygen analogue nBPTO is rapid, occurring within minutes/hours in aerobic soils (Byrnes & Freney 1995), but it can take several days in the floodwater of tropical soils. nBPTO forms a tridentate ligand with the urease enzyme, blocking the active site (Manunza et al. 1999).

4. Effect of N-(N-Butyl) Thiophosphoric Triamide (nBTPT)

4.1. Ammonia loss

Studies conducted in New Zealand and overseas and reviewed here have shown that urea containing fertilisers can lose up to 30% or more of their N if not rapidly incorporated into the soils. According to Black et al. (1985) the rate of NH_3 losses from applied urea increases with increasing rate of urea-N application. Emissions range from ~11% at rates of urea-N typically applied by New Zealand pastoral farmers (i.e. ≤ 45 kg N/ha) but reach ~30% at rates of 300kg N/ha (MAF report by Sherlock et al. 2008). Ammonia losses are expected to be low from urine deposition as compared with those from surface applied urea as most of the urea in urine deposited from grazing animals in pasture soils is incorporated into soil. Compilation of the data using aspirated chambers from studies conducted in New Zealand by Sherlock et al. (2008) suggest the direct average NH_3-N emissions from urine applied to pasture soils are 15.9%.

Urea in fertilisers or in urine would be more effective if an economical and efficient method was devised to reduce NH_3 loss. One method of reducing losses is to use a urease inhibitor that retards the hydrolysis of urea by soil urease and allows the urea to diffuse deeper into the soil. Much of the NH_3 then released would be retained by the soil. The most readily available urease inhibitor nBTPT, is sold as Agrotain®, which is formulated as a green clear liquid containing 25% of the active ingredient nBTPT. The nBTPT is in a mixed solvent consisting of 10% by weight of N methyl-pyrrolidone with the balance consisting of non-hazardous solvent and inert ingredients (IMC-Agrico 1997). This can be used to impregnate urea granules. Agrotain is marketed by Incitec Pivot Ltd in the following formulations: (*i*) Green Urea 14, which contains 45.8% N as urea and Agrotain @ 5.0 L/t to reduce the loss of NH_3 for up to 14 days; and (*ii*) Green Urea 7, which contains 45.9% N as urea and Agrotain @ 3.0 L/t to reduce NH_3 volatilisation for up to 7 days (Incitec Pivot Ltd 2006). In New Zealand the only commercial product available is SustaiN (Green and Rapid S) where granulated urea is coated with Agrotain @1L/t urea, and then mixed with ammonium sulphate in the case of Rapid S (http://www.summitquinphos.co.nz).

Here we review the overseas and New Zealand studies on the use of most commonly urease inhibitor NBPT.

Overseas studies

Studies of nBTPT as urease inhibitor appear to have started with Bremner and Chai (1986) who reported that nBTPT is considerably more effective than phenylphosphorodiamidate (PPD) as a soil urease inhibitor and merits consideration as a fertiliser amendment for retarding hydrolysis of urea fertilizer in soil. Following this publication numerous experiments were conducted to measure the effectiveness of nBTPT as a urease inhibitor when applied with fertiliser in controlled laboratory conditions, upland soil systems (crops), lowland soils (flooded rice), though only a few were conducted under pastoral systems. In the US alone, urea plus nBTPT field trials were conducted in 33 states including 61 research institutions. To date 1340 trials have been conducted worldwide involving different crops such as corn (660 trials), wheat (260 trials), rice, sugarcane, cotton, etc. (Gordon Welch, Agrotain International, New Zealand Pers. Comm.; Appendix 1). In a review of five years studies of nBTPT across USA, Hendrickson (1992) concluded that nBTPT, commercially known as 'Agrotain', has a good potential to improve the effectiveness of urea-based fertilisers. It was most effective in reducing NH_3 emissions from surface applications of both urea and urea ammonium nitrate (UAN), when applied to high pH soils in Manitoba (Grant et al. 1996) and residue covered tropical wet soils (Schlegel et al., 1986; Clay et al 1990).

As indicated above there have been many overseas studies of nBTPT with different fertilisers. The enormous amount of available literature on nBTPT mainly deals with agricultural production systems in tropical climates, which are of little relevance to New Zealand's temperate climate and grazed pastoral systems. Additionally, a high proportion of the literature involves forms of fertilisers that are not widely used in New Zealand, we have generally limited this review to include only those studies where urea was incorporated or surface applied with nBTPT to pasture soil (Table 1). Unlike the large number of studies on fertiliser-N amended with nBTPT, we could not find any international work on the effectiveness of nBTPT in reducing direct NH_3 losses from urine.

Of most relevance to New Zealand conditions are various laboratory and field trials carried out by Watson et al. (1990, 1994 a & b, 1998, 2008) in Ireland with nBTPT treated urea on temperate grassland soils. All these studies conclude that NBPT is not only highly effective in reducing total NH_3 emissions and the maximum daily emission rate, but also in delaying the time at which maximum NH_3 flux occurs. In experiments conducted by Watson et al. at different times, it was found that the percentage reduction of NH_3 emission from nBTPT when applied to urea varied with the rate of nBTPT application. The percentage reduction in emissions increased with an increase in the rate of nBTPT addition from 0.01 to 0.5% (on a urea weight basis, w/w). At 0.01% (w/w) application rate emission reduction was 28.4% (average of 16 different soil types) under laboratory conditions (1994a), 50.4% under field conditions (1994b); and at 0.05% (w/w) rate it was 68% under laboratory conditions (average of 16 soil types) (1994a) and 83% under field conditions (1994b). At the highest rate of 0.5% (w/w) the reduction was 97% (1994a) and 82.8% (1998). Their studies also show there is no significant effect of previous nBTPT applications on the subsequent efficacy of nBTPT in reducing NH_3 emission from urea (Watson et al. 1998). Watson et al. recently conducted another laboratory incubation study to assess the effect of nBTPT on NH_3 losses from urea with 0, 0.01, 0.025, 0.05, 0.075 and 0.1% (w/w) of nBTPT in four contrasting soils at 3 different soil temperatures (Watson et al. 2008). The average % reduction in NH_3 emissions from all the soils and temperatures was 61.2%, 69.9%. 74.2%, 79.2% and 79.8% for 0.01, 0.025, 0.05, 0.075 and 0.1% nBTPT rates, respectively, which led to the conclusion that commercially there is little additional benefit in using concentrations above 0.025% nBTPT (w/w) on a range of grassland soils.

Results of another series of field experiments in the UK show that the addition of Agrotain applied at 0.025, 0.05 or 0.1% w/w nBTPT to solid urea, in the grassland and tillage soils, on average reduced NH_3 emissions by 70% (range 41–100%) (Chadwick et al. 2005).

These studies did not show significant differences (P>0.05) in reduction of NH_3 emissions between different addition rates of Agrotain (i.e. 0.05 vs. 0.1% or 0.025 vs. 0.05% nBTPT). They also show no differences (P>0.05) in NH_3 emissions reduction when NBPT was either coated on to the urea granules or incorporated within the granules (i.e. in the melt).

In contrast to these studies, there was no significant effect of nBTPT on reduction of NH_3 emissions from two Spanish grassland soils receiving 70 kg N ha^{-1} as urea or cattle slurry under low (summer) water filled pore space (WFPS; 20–60%) and high (winter) WFPS (50–90%) (Menendez et al. 2009). However, the NH_3 emissions under low WFPS conditions were 32% lower in the urea (reduced from 3.32 to 2.33 kg NH_3-N ha^{-1}) and 38% lower in the slurry (reduced from 12.61 to 7.84 kg NH_3-N ha^{-1}) treatments with nBTPT. It appears that the high magnitude of NH_3 loss from slurry as compared with urea and large differences in replicates among the treatments may have masked the level of significance in average emissions reductions with nBTPT. Furthermore, under high WFPS little NH_3 (up to 0.70 kg NH_3-N ha^{-1}) was emitted from soil because of the heavy rainfall immediately after fertiliser application that may have leached the urea to lower depth. These results suggest nBTPT may be less effective in soils where fertiliser application is immediately followed by heavy rainfall and/or irrigation.

New Zealand studies

The first unpublished experiments in New Zealand to assess the effect of urease inhibitors were carried out in Canterbury by Scott Black and his colleagues at Lincoln University in the mid-1980s (MAF report; Sherlock et al. 2008). These studies proved nBTPT to be a more effective urease inhibitor than PPD, resulting in NH_3 emission reduction from 55.4–85% when applied at 0.01 to 1.0% (w/w) with urea (100 kgN/ha).

There was little New Zealand research on urease inhibitors between the mid-1980s and 2000. Between 1990 and 2005 there was a 6-fold increase in fertiliser N usage in New Zealand from 51 787 to 308 406 tonnes N, mainly due to intensification of New Zealand pastures. The increasing fertiliser N input to grazed pastures rekindled the debate on its impact on atmospheric, terrestrial and aquatic environments (Parliamentary Commissioner for the Environment Report 2004), and the interest to assess the impact of N transformation inhibitors on N losses. Currently, the majority of the nitrogen fertiliser used is granulated urea, which makes up ~82.9% of the sales on a unit of N basis; and Agrotain is the only urease inhibitor commercially available in New Zealand.

Most of the work on the effect of nBTPT in reducing NH_3 losses from New Zealand pasture soils with urea and urine application, both under glasshouse and field conditions, has been recently published in four papers (Singh et al. 2008; Zaman et al. 2008, 2009; Meneer et al. 2008). In a glass house study, Singh et al. (2009) used nBTPT- containing commercial urea fertilisers (Sustain Green: urea coated with Agrotain @1L agrotain/tonne urea and Sustain Yellow: Sustain Green plus 4% S^{o}) and Agrotain to study the effect of nBTPT in reducing NH_3 losses from urea and urine, respectively. They found a 27% and a 42% reduction in NH_3 losses with the application of Sustain Green at 600 and 100 kg N/ha compared with urea applied at the same rates.

A 22% reduction in NH_3 emissions was observed from urine-amended pasture soil cores (476 kg N/ha) treated with Agrotain (@1L/t of urea equivalent).

In a field trial on pasture soil, Zaman et al. (2008) observed a 45% lower NH_3 emission from soil that had received Sustain Green at 150kg N/ha compared with soil receiving the same rate of urea. When Sustain Green was applied together with a nitrification inhibitor (DCD), the combination resulted in a 29% reduction in NH_3 loss compared with straight urea, indicating DCD slightly reduced the effectiveness of nBTPT. In another field study by Zaman et al. (2009) in 2005, the application of Agrotain (@1L/t urea equivalent) with urine (600 kg N/ha) to pasture soil resulted in reduction of NH_3 losses by 29%, 93% and 31%, over autumn, spring and summer seasons respectively. However, the reduction in NH_3 emissions declined to 14%, 77% and 8% over autumn, spring and summer when Agrotain was applied with DCD (@1L/t urea equivalent).

In another field lysimeter experiment with a free-draining pumice soil, Meneer et al. (2008) showed 64% lower ($P<0.05$) NH_3 loss from urine+Agrotain than the urine alone (775 kg N/ha). Overall NH_3-N loss from urine-N over the 20-day period was reduced from 109 to 39 kg N/ha. In two trials conducted by Ballance AgriNutrients in 2008, nBTPT applied @ 0.025%, 0.05% and 0.1% (w/w) to urea (100 kg N/ha) reduced average NH_3 emissions by 41% from a volcanic ash soil (Sprosen & Ledgard 2008) and by 59–62% from Manawatu silt loam soil (Theobald et al. 2008).

Table 1 Reduction in ammonia volatilisation with application of nBTPT in temperate climate both overseas and New Zealand

nBTPT Concentration (w/w) urea weight basis	N source (kg N /ha)	Emission Reduction (%) NH_3	Country	*T_{max} delay (days)	References
0.5%	Urea – 100	76.5	Ireland - temperate: Field	5	Watson et al. 1990
0.01% 0.05% 0.10% 0.25% 0.50%	Urea – 100	52% 83% 88% 96% 97%	Ireland - temperate Field study	0.58 1.13 1.34 1.67 2.14	Watson et al. 1994b
0.01% 0.058% 0.28%	Urea - 100	28.4% 67.9% 87%	Ireland - temperate Laboratory study	n.a.	Watson et al. 1994a
0.1%	Urea – 100	83%	Ireland Laboratory study	3.3	Watson et al. 1998
0.025%	Urea – 100, 600 Urine – 450	42%, 27% 22%	New Zealand – Glasshouse study	6,1 1	Singh et al. 2009
0.025% 0.025%+DCD	Urea – 150	45% 29%	New Zealand – Field	0	Zaman et al. 2008
17 kg Agrotain/ha	Urine – 775	64%	New Zealand – Field	2	Meneer et al. 2008
0.01% 0.025% 0.05% 0.075% 0.10%	Urea – 100	61.2% 69.9% 74.2% 79.2% 79.8%	Ireland – Laboratory study	2.8 3.5 3.7 4.6 5.2	Watson et al. 2008
0.2%	Urea – 70	29% (N.S.)	Spain – temperate Field study	n.a.	Menendez et al. 2009
0.025%	Urea – 100	41%	New Zealand – Field	1	Spronson & Ledgard 2008
0.025%	Urea – 100	59%	New Zealand – Field	7	Theobald et al. 2008
3.2 kg (or 3 L) Agrotain/ha	Urine – 600 (3 seasons)	29%, 93%, 31%	New Zealand – Field	0-1	Zaman et al. 2009

*T_{max} is the delay the time (days) at which maximum NH_3 flux occurs

4.2. Effect on plant production

Overseas studies

In a number of field studies with nBTPT-amended urea applied to maize (Hendrickson 1992), rice (Buresh et al. 1988), orchardgrass (*Dactylis glomerata L*) (Bundy & Oberle 1988) and Kentucky bluegrass (Joo et al. 1991), led to increases in yield and N uptake compared to unamended urea. In 21 field experiments, mostly in the Midwest US, nBTPT increased average yields of maize by 750 kg/ha (9%) at an average fertilisation rate of 100 kg N/ha (Hendrickson 1990, 1992). To achieve equivalent yields without nBTPT amendment required an additional 80 kg N/ha fertiliser. The average increase for 78 trials over 5 years at all rates of N was 270 kg of grain per hectare (4%). In southern Illinois, maize yields were increased by an average of 9% for 13 experiments with broadcast urea and 14% for 9 experiments in which urea was band placed on the soil surface (Varsa et al. 1993). A complete list of number of experiments conducted with nBTPT worldwide is given at the end of the report (Appendix. 1). However, very few international studies on the effect of nBTPT on pasture production in a temperate climate have been conducted and these are summarized in the following section.

Temperate climate

Field studies with nBTPT conducted in Northern Ireland temperate grassland (Watson et al. 1990) show nBTPT (0.5% w/w) significantly increased the pasture dry matter yield (DM yield) of the amended urea treatment by 8.8%. Further field trials (Watson et al. 1994b) evaluated a range of nBTPT concentrations (0.01, 0.05, 0.1, 0.25 and 0.5% nBTPT w/w) to determine the optimum application rate for temperate grassland under a range of environmental conditions in a given year. They showed that 0.05% concentration of nBTPT overall increased DM yield by 9 c ompared with urea alone (100 kg N/ha) with a range of 3–9% response for all the concentrations (Table 2). In a long-term experiment at the Agricultural Research Institute, Hillsborough, urea and urea amended with 0.1 and 0.5% (w/w) nBTPT were applied three times a year over 3 years at a rate of 300 kg N /ha/yr. The increase in DM yield during 1994, 1995 and 1996 was 8.0%, 2.7% and 10.0% for 0.1 % nBTPT and 15.1%, 9.8% and 9.2% for 0.5% nBTPT (w/w), respectively (Watson et al. 1998). According to this study, there was no indication of any long-term adverse effect of repeated nBTPT applications on pasture production. In contrast, both a UK study (Chadwick et al. 2005) and a Spanish study (Menendez et al.

2009) did not find significant increase in grass yield from application of nBTPT-amended urea compared with unamended urea (Table 2).

New Zealand studies

In New Zealand, Quin et al. (2005) showed a 10% increase in pasture yield from Sustain (0.025% nBTPT w/w) compared with standard urea at 30 and 60 kg N/ha application rates. In subsequent studies conducted at five different sites, Sustain was applied at 50 kg N/ha after every two pasture cuts, or in split applications of 25 kg N/ha after every pasture cut, over periods of 6–16 months. The increase in DM yield from application of nBTPT-amended urea compared with standard urea ranged from 3% to 9% at Morrinsville, and 6% to 13% at Canterbury (Ramakrishnan et al. 2008).

Martin et al. (2008) applied Agrotain-amended urea and standard urea at rates of 25, 50 75 and 100 kg N/ha to dairy pasture during wet summer conditions, and observed no pasture growth response to the Agrotain. In this study, 42 mm rain fell immediately after fertiliser application, and resulted in the urea and its breakdown products being washed into the soil. By contrast, in the dry conditions of the spring and autumn trials, the yield advantage of Agrotain-amended urea over standard urea ranged from 0% to 9%, with an average of 5%, similar to data presented by Stafford et al. (2008) and data given above. Martin et al. (2008) observed that the yield advantage in both spring and autumn trials from Agrotain-treated urea increased with increasing rate of fertiliser application, i.e. 2% at 25 kg N/ha to 10% at 100 kg N/ha. In another field trial conducted on a dairy farm in Hamilton, total pasture production increased by 17% and 15% for urea+Agrotain and urea+Agrotain+DCD (150 kgN/ha) compared with urea alone (Zaman et al. 2008) (Table 2). The application of Agrotain (3 L/ha) to urine (600 kg N/ha) in a field plot study conducted over three seasons (autumn, spring and summer) by Zaman et al. (2009) resulted in an increase in pasture production as compared to urine alone by a maximum of 9% in spring. In this experiment, the combined effect of DCD and Agrotain was slightly higher than the effect of application of individual inhibitors on pasture production with maximum of 11% increase in pasture production in spring.

Table 2 Effect of NBPT application on pasture yield when applied with either urea or urine in temperate conditions

nBTPT (% w/w) to Urea	N source (kg N/ha/y)	Type of crop	% increase in DM yield	Country	References
0.5%	Urea – 100	Perennial ryegrass	8.8%	Ireland – temperate	Watson et al. 1990
0.01% 0.05% 0.10% 0.25% 0.50%	Urea – 100	Perennial ryegrass	6.56% 8.90% 3.94% N.S. 5.90% 4.38% N.S.	Ireland – temperate	Watson et al. 1994b
0.1% 0.1% 0.5%	Urea – 60 Urea – 300	Perennial ryegrass	-0.9% - 5% (2 % mean) 7.16% 11.4%	Ireland – temperate	Watson et al. 1998
0.025%	Urea – 25, 50, 100	Kikuyu grass	0.6 - 17% (10 % mean)	Northland – New Zealand temperate	Hunt & O'Connor 2003
0.25%,0.5%, 1.0%	Urea – 100	Grass	No effect	UK – temperate	Chadwick et al. 2005
0.025%, 0.0375%, 0.0625%, 0.075%	Urea – 100, 200	Pasture	No effect	New Zealand – temperate	Hawke & Hunt 2005
0.025%	Urea – 120, 240	Ryegrass-white clover	7.05–16.3% (10.3% mean)	New Zealand – temperate	Blennerhassett et al. 2006
0.025%		Ryegrass-white clover	3–9% (6.2% mean) 6–13 % (9.7% mean)	Moronsville, Canterbury New Zealand	Ramakrishnan et al. 2008
0.025%	Urea – 150	Ryegrass-white clover	17%	New Zealand – temperate	Zaman et al. 2008
0.025%	Urea – 25, 50, 75, 100	Pasture	–4.14–9% (4% mean)	New Zealand – temperate	Martin et al. 2008
0.2%	Urea – 70	Cut grassland	No effect	Spain – temperate	Menendez et al. 2009
0.025% & 0.075%	Urea 25–120	Pasture	–5–36% (8% mean)	New Zealand – temperate	Smith et al. 2009
0.025%	Urine – 600	Pasture	6–9% (7% mean)	Manwatu –New Zealand- temperate	Zaman et al. 2009
Overall			**6.5%**		

A yield response to urease inhibitor application could be expected only when the pasture is able to utilize the surplus N conserved by the inhibitor when environmental conditions are otherwise conducive to substantial NH_3 losses from surface applied urea. Ballance Agri-Nutrients conducted a number of experiments (unpublished data) over 2003–06 where along with other products, Sustain was compared with urea on a site in Northland with Kikuyu dominant pastures (Hunt & O'Connor 2003), two sites in Northland and Rotorua (Hawke & Hunt 2005) and nine sites through out New Zealand (Smith et al. 2007). Hawke and Hunt (2005), measuring pasture yield responses to urea applied at 100 and 200 kg N/ha with and without different doses of Agrotain (as SustaiN), found no significant yield responses to different rates of Agrotain at either rate of urea application, at any cut (point in time), or in total production at both the sites. Smith et al. (2007) reported that only two sites out of nine, one in Waikato (9% increase) and the other in Canterbury (7% increase), showed a significant difference in total spring pasture production in response to application of Sustain compared with urea. Overall, Sustain gave 8% larger response than urea (range 5–36%) during spring production across all sites an overall average increase of 6.5% (Table 2).

In summary, the benefit of using Sustain to reduce NH_3 emission is most likely realised in dry conditions when fertiliser N is broadcast rather than incorporated into the soil. The reviewed data for varying rates of nBTPT with surface applied urea showed that the reduction in NH_3 emissions increased with increasing rate of nBTPT, but followed the law of diminishing returns (Table 1). Overall, the weighted mean % reduction in NH_3 emissions with nBTPT application above or equal to 0.2% w/w concentration to surface applied urea is ~ 63% (Table 1). Watson et al. (2008) suggested that commercially there is no additional benefit in using >0.025 % of nBTPT w/w. In New Zealand, the commercial product SustaiN contains nBTPT at the recommended concentration of 0.025% w/w. When considering the New Zealand data, the reduction in NH_3 emissions averaged 43% from nBTPT application rate of 0.025% w/w with urea and ~48% from nBTPT with urine.

5. Factors Affecting nBTPT Efficacy

Many years of research has established that nBTPT can be effective in reducing the loss of NH_3 from urea based fertilisers. The rate of reduction in this loss from urea with the use of nBTPT is regulated by a range of environmental conditions and soil characteristics. The following section describes how the soil and climatic conditions influence the effectiveness of nBTPT.

Soil properties

Among the factors that affect NH_3 emissions from soils are urease activity, soil pH, temperature, soil moisture content, soil characteristics, wind speed and rate and method of urea application. In a study on 13 diverse surface soils with a wide range in properties incubated at 20ºC for 7 days with urea and nBTPT (0.01% w/w), the ability of nBTPT to retard urea hydrolysis was significantly correlated with organic C content (r = – 0.70**), total N content (r = –0.76**), cation-exchange capacity (r = –0.67*), sand content (r = 0.61*), clay content (r = –0.63*) and surface area (r = –0.66*) (Bremner & Chai (1986) but soil urease activity (Bremner & Chai 1986) and soil pH (Bronson et al. 1989) had little influence.

As organic C content and total N content are indices of organic matter content and cation-exchange capacity is closely related to organic matter content, it was concluded in the above studies that organic-matter content accounts for the most of the observed variation in effectiveness of nBTPT. Other workers have also suggested that effectiveness of nBTPT is reduced with high percentages of organic C in soil or amendment of soil with plant residues (Carmona et al. 1990; Wang et al. 1991; Watson et al. 1994a).

In an extensive laboratory study with 16 different soil types, Watson et al. (1994a) showed that the five soil properties, i.e. titratable acidity, moisture content, pH, urease activity, and CEC, contributed significantly to the variation of effectiveness of nBTPT in soils. As many of these soil properties are highly correlated to one another, it was concluded that the response of increasing inhibitor concentration in reducing NH_3 emission was the highest in soil with low organic matter content, high pH and low buffering capacity.

These soil conditions also lead to high NH_3 emissions from urea. nBTPT clearly has considerable potential to improve the efficiency of urea from these grassland soils. The majority of New Zealand pastoral soils are characterised by high organic matter contents, low pH and high buffering capacity. It might be expected, therefore, that NH_3 emissions from urea and urine on pastoral soils would be low, and the potential for reducing NH_3 emissions with nBTPT would also be low. However, the data on NH_3 emissions reduction resulting from application of nBTPT (29 to 93%, Table 2) show a large potential to improve the effectiveness of urea in New Zealand pastures.

No direct relationship was obtained between inhibition of urea hydrolysis and reduction in NH_3 loss, this led Carmona et al. (1990) to suggest that nBTPT delayed urea hydrolysis thereby increasing diffusion of urea into the soil, and reducing NH_3 concentrations on the soil surface. Thus the effect of nBTPT on NH_3 loss is also dependent on the diffusion characteristics of urea in the soil and urease activity. Christianson et al. (1993) found nBTPT more effective in sandy soils with low pH and low buffering capacity than a clay soil with high pH. Interestingly, in an experiment with 3 different soil types incubated with different dose rates of nBTPT, Antisari et al. (1996) found that soil with higher sand content (81%) and organic matter content (2.30% organic C) needed high concentrations of nBTPT to reduce NH_3 losses compared with soil with high clay content (35%) and lower organic matter content (1.76% organic C). The findings of the studies reported above suggest that the efficiency of nBTPT as an inhibitor depends on the combination and interaction of soil physical and chemical properties rather than one single factor.

Amount of nBTPT applied

In laboratory studies an increasing rate of nBTPT application with urea increased inhibition of urease activity (Bremner & Chai 1986; Carmona et al. 1990; Antisari et al. 1996) and reduced in NH_3 emissions but followed the law of diminishing returns (Watson et al. 1994a). The optimum concentration of nBTPT for temperate grassland production was reported to be 0.1% (w/w). Watson et al. (2008) reported there was little commercial benefit in using nBTPT concentrations above 0.025%.

Field trials investigating nBTPT performance at varying rates under temperate conditions are limited (Watson et al. 1994b, 2000), but the results tend to agree with laboratory studies. A 2-year field-study in Canada showed NH_3 emissions over 12 days and 21 days decreased in the order 0% > 0.05% > 0.15% ≥ 0.10% NBPT w/w when applied with urea (Rawluk et al. 2001). Results of numerous field trials conducted in the USA with nBTPT amended urea over 20 years with corn, wheat, barley, rice, cotton, grain sorghum, ryegrass, and sugarcane generally show the 0.14% nBTPT w/w increased the yield and N uptake compared to unamended urea (Trenkel 1997; Watson 2000).

Soil temperature

The time delay of maximum urea hydrolysis rate and the reduction in NH_3 emissions by nBTPT depend on soil temperature. Generally, nBTPT effectiveness decreases with an increase in soil temperature (Watson et al. 1994; Carmona et al. 1990). An incubation study of the effect of nBTPT (0.01% w/w) on urea hydrolysis in six different soils incubated at 10º, 20º, 30º and 40º C for 3, 7 and 14 days showed that the inhibitory effect of nBTPT on urea hydrolysis decreased markedly (on an average from 74% to 24%) with an increase in temperature from 10º to 40º C. The effect of temperature on inhibition of urea hydrolysis by nBTPT was considerably greater after 14 days than after 3 or 7 days (Bremner & Chai 1986). Carmona et al. (1990) also observed the relative effectiveness at nBTPT concentrations 0.01%, 0.05% and 0.10% declined as the temperature increased from 18º to 32ºC. For example, 0.01% nBTPT w/w reduced NH_3 loss by 81% at 18ºC but by only 42% at 32ºC, and a similar pattern was observed for urea hydrolysis. Research findings of other workers also show a decrease in nBTPT effectiveness at higher temperatures (Clay et al. 1990; Bremner et al. 1991; Rawluk et al. 2001), which can be explained by increased urease activity with temperature. Under these conditions a greater concentration of nBTPT is required to achieve a level of inhibition equivalent to when loss potential is low. At higher temperatures, the hydrolysis rate may surpass the rate of nBTPT conversion to nBPTO, or the rate of inhibitor degradation may be more rapid.

A more recent laboratory study with four different soil types at three different temperatures (5ºC, 15ºC and 25ºC), Watson et al. (2008) reported that the percentage reduction in NH_3 loss was lower at 15ºC (61%) than at 5ºC (83%) or at 25ºC (74%). There was no evidence that the percentage inhibition at 15ºC could be increased by higher concentrations of nBTPT as observed in other studies reported above. These authors have not provided any explanation why the percentage inhibition at 15ºC was lower than at both a lower and a higher temperature. Our understanding of how the biotic and abiotic factors influence the transformation of nBTPT to nBPTO and its subsequent stability in soil is limited because little research has been done on these aspects. It is an important area and should be considered worthy of further investigation.

6. Other Implication

Effect on nitrification/denitrification

Wang et al. (1991) conducted incubation studies to determine the inhibitory effects of nBTPT on nitrification and denitrification in soil by adding ammonium and nitrite ions respectively. They found no significant influence on both processes when nBTPT was applied at the rate of 0.40% w/w. In contrast, Watson et al. (1994a) found that nBTPT applied at 0.28% (w/w) significantly lowered NO_2-N (Bremner & Chai 1989) and NO_3-N concentration in the soil suggesting reduced rates of nitrification.

However, the above results could not be repeated when Watson et al. (2008) applied nBTPT at increasing rates from 0.01% to 0.10% (w/w) with no significant effect on NO_3^--N concentration in the soil which is in agreement with other workers (Bundy & Bremner 1974; Bremner et al. 1986). Watson et al. (2008) suggested the inhibitor may have a small effect on either immobilization or other N cycle processes that need further investigation. In a lysimeter study conducted by Meneer et al. (2008), Agrotain applied at17 kg/ha with urine resulted in increased accumulation of NO_3-N (39 kg N/ha) in the 150–450 mm soil depth over 19 kg N/ha in the urine control. NO_3-N can be taken up by plants or alternatively may be leached if drainage occurs during a rainfall event.

Effect on soil microbes

Earlier studies have shown that nBTPT not only slows down urea hydrolysis in soil but also rapidly alters the osmotic strength of the soil solution (Bundy & Bremener 1974; Kieft et al. 1987). The decreased osmotic shock along with delayed urea hydrolysis might reduce the flush of mineral N and thus decrease the organic N mineralization associated with the microbial biomass. However, Benerjee et al. (1999) did not find any significant impact of nBTPT on soil biomass in clay loam and sandy loam soils during a 2-year micro-plot study. Their findings are supported by recent field-plot studies of Zaman et al. (2009), who obtained no significant differences in soil microbial biomass C and N over autumn and spring seasons with and without Agrotain applied with urine. Because Agrotain only affects the specific activity of urease, the enzyme that hydrolyses urea, and is only effective for a short period of time of 7–14 days. It does not appear to affect soil microbial biomass (Benerjee et al. 1999; Zaman et al. 2009).

Phytotoxicity

Solution culture studies done by workers like Bollard et al. (1968), Harper (1984) and Matsumoto et al. (1966) demonstrated that urea can be taken up as an intact molecule. Normally urea is rapidly hydrolysed to NH_4^+-N in soil so plants would rarely take up urea as the intact molecule. The studies conducted by Watson et al. (1990) reported tip-scorch on the leaves of plants receiving urea (100 kg N/ha)+0.5% nBTPT that increased with increasing urea-N application rate and concentration of nBTPT from 0.01% to 0.5% (Watson & Miller 1996). ^{15}N recovery, along with other evidence in this study with ryegrass, suggested that when urea hydrolysis is delayed it is more likely that the intact urea molecule is taken up by plants.

An accumulation of urea within plant tissue has been reported to cause leaf-tip necrosis. In these cases, urea accumulation resulted from a reduction in soybean leaflet urease activity induced by nickel deficiency (an essential component of urease) (Eskew et al. 1983), by spraying a urea solution onto the foliage of winter wheat (Warden & Kettlewell 1993) or by inhibition of soil urease activity by inhibitors (Krogmeier et al. 1989, 1991). In the study conducted by Watson and Miller (1996), it was not clear that the phytotoxicity was due to urea or a result of pH fluctuations within the plant tissue following its hydrolysis by shoot urease. However, the phytotoxicity observed was transient and unlikely to have any long-term adverse effect on growth as new developing leaves were unaffected. No such phytotoxicity symptoms were reported in any of the other studies reviewed.

Effect of combined application of urease and nitrification inhibitors

Few studies have attempted to examine the effect of combined application of Agrotain and DCD. The general theory behind use of DCD as a nitrification inhibitor is that it reduces N_2O emissions and nitrate leaching from urea-based fertilisers and urine spots by keeping N in NH_4^+ form for longer. However, the use of DCD can sometimes enhance the NH_3 volatilisation losses. Therefore, it is assumed that the combined application of two inhibitors can enhance the efficiency of applied urea or urine.

Two New Zealand field studies testing the above hypothesis show that application of combined inhibitors to surface applied urea increased the NH_3 emissions by 29%, while reducing NH_3 emissions from urine application by 14%, 78% and 8.6% in autumn, spring and summer seasons, respectively (Zaman et al. 2008, 2009).

However, this reduction from urine with combined inhibitors was lower for each season than that from Agrotain applied separately.

In summary, the available overseas and New Zealand research on the use of urease inhibitor nBTPT in peer-reviewed literature and unpublished reports reported here suggests that in temperate climates different levels of nBTPT application with urea reduce average NH_3 emissions from surface applied urea by 63% and an effective rate of nBTPT application is 0.025% w/w with urea or urine. The efficiency of nBTPT varied with the the N source, soil type (texture, pH, organic matter content and soil N status) and other environmental conditions (temperature, rainfall, wind velocity). New Zealand studies involving optimum nBTPT application (0.025% w/w) with urea and urine resulted in an average 43% and 48% reduction in NH_3 emissions, respectively, and the average increase in pasture production was 6.5% and 7% (based on only one study), respectively.

7. Method Development for Estimating Urease Inhibitor's Effect on Nitrous Oxide Emissions

Ammonia is produced and emitted from fertilisers that contain or produce ammonium – N (NH_4^+-N) and from animal excreted-N (urine and dung). Ammonia itself is not a greenhouse gas but when re-deposited on land acts as an indirect source of N_2O. New Zealand's N_2O inventory currently uses the IPCC default values of 0.1 and 0.2 for $Frac_{GASF}$ (fraction of total fertiliser emitted as NO_x and NH_3) and $Frac_{GASM}$ (fraction of total nitrogen excretion emitted as NO_x and NH_3). In a recent review of New Zealand specific $Frac_{GASM}$ and $Frac_{GASF}$ emission factors, Sherlock et al. (2008) have provided sufficient justification to recommend a New Zealand specific value of 0.1 for $Frac_{GASM}$ and $Frac_{GASF}$ to be considered for adoption. The recommended changes in $Frac_{GASM}$ from 0.2 to 0.1 is expected to reduce New Zealand's N_2O inventory by 2.455 Gg N_2O.

Application of the urease inhibitor nBTPT with N-fertilisers and urine further reduces the amount of NH_3 emission and thus could provide justification to further reduce the values of $Frac_{GASF}$ and $Frac_{GASM}$. To our knowledge, no other country has revised its emission factors to account for the effect of nBTPT application with urea or onto soils.

nBTPT is sold as Agrotain®, which is formulated as a green clear liquid containing 25% of the active ingredient nBTPT. In New Zealand commercial urea fertilisers containing Agrotain (SustaiN Green and Rapid S) are applied to grazed pasture soils (http://www.summitquinphos.co.nz). Although SustaiN has been applied to dairy, sheep, beef and cropping soils, its effect on the reducing NH_3 emissions (via $Frac_{GASF}$) is currently not accounted for in the New Zealand N_2O inventory.

Application rate for urease inhibitor

Based on the peer reviewed literature we conclude that an nBTPT application rate of 0.025% w/w with urea would most effectively reduce NH_3 emissions from temperate grasslands.

Influence of urease inhibitor NBPT

When nBTPT (0.025% w/w) was applied with urea to four New Zealand pastoral soils studies, NH_3 emission was reduced by 27–59 % (n = 5, mean 43 %, Std. Dev. 11.4) (Spronson & Ledgard 2008; Singh et al. 2009; Zaman et al. 2008, 2009). These statistics define the uncertainty of urease inhibitor application to New Zealand soils with respect to reductions in NH_3 emissions. The mean value of 43% supports our recommendation of changing $Frac_{GASF}$ from 0.10 to 0.06 for the fertiliser-N applied with urease inhibitor nBTPT.

Method of estimation of $Frac_{GASF}$

The effects of urease inhibitors are calculated using revisions of $Frac_{GASF}$ (fraction of total fertiliser emitted as NO_x and NH_3) from 0.1 to 0.06 for the amount of fertiliser N applied with urease inhibitor nBTPT. Consequently, when nBTPT is applied as recommended, we revise $Frac_{GASF}$ for the national inventory as follows:

$$\mathbf{FracGASF = [(FN_{UI}) \times 0.06] + [(FN - FN_{UI}) \times 0.10]} \qquad \textbf{(1)}$$

where **$Frac_{GASF}$** is the fraction of total fertiliser N emitted as NH_3, **FN** is the total amount of applied fertiliser N, **FN_{UI}** is the amount of applied urea fertiliser N treated with urease inhibitor, nBTPT.

[Note: These calculations take account of NH_3 emissions ($Frac_{GASF}$ = 0.06) from nBTPT treated urea-N i.e., FN_{UI} and NH_3 emissions ($Frac_{GASF}$ = 0.10) from the total amount of fertiliser N (FN) used minus nBTPT treated urea. The agstats on FN_{UI} will be provided by the fertiliser industry].

8. Effect of Urease Inhibitors on Inventory Estimates

There are no available data on NH_3 emissions following application of nBTPT to urine patches in a grazed pasture and the frequency of its application. Application of nBTPT containing urea fertilisers concurrently or as close as possible to the deposition of urine-N may reduce NH_3 emissions, but little research has been carried out on this. Therefore, it is not possible to estimate the effect of urease inhibitors on emission reductions from excretal N inputs during grazing. Here we consider just the potential reductions in NH_3 emission from surface applied nBTPT-amended containing urea fertilisers which contribute towards a reduction in $Frac_{GASF}$.

Based on the peer-reviewed literature and our above reported estimates of reductions in NH_3 emission, a New Zealand specific value of 0.06 for $Frac_{GASF}$ is recommended for adoption where fertilisers containing urease inhibitor, nBTPT are apllied

In New Zealand, there has been a six-fold increase in fertiliser N use, from 51.787 Gg ($Gg = 10^9$ g) of N in 1990 to 308.406 Gg of N in 2005 (MfE 2007). Best estimate of current urea usage in New Zealand is around 480–500 Gg (220–230 Gg of N) including around 40 Gg (18.40 Gg N) of SustaiN. Currently, the majority of this fertiliser (~80%) is applied to dairy pastures. Urea and SustaiN are both applied @ 25–40 kg N/ha per application or a total of 150–250 kg N/ha/yr. The market price of SustiaN is generally $100/tonne extra than standard urea. Our figures on Urea and SustaiN come from expert judgement (J. Blennerhasset, pers. comm. 2009). These figures suggest that in 2009 only around 8% of urea fertiliser used in New Zealand contain the urease inhibitor nBTPT.

Changing the $Frac_{GASF}$ from 0.1 to 0.06 for the current 18.4 Gg N of SustaiN reduces the annual NH_3-N emission by 0.74 Gg from 1.84 Gg to 1.10 Gg. The IPCC default emission factor for indirect N_2O emissions from volatilising N is 0.01 kg N_2O-N/kg NH_x-N (IPCC 2000, Table 4.18). To convert this to N_2O we multiply by 1.57. Thus the reduction in indirect N_2O emissions due to current application of SustaiN fertiliser is 0.012 Gg per year. This difference of 0.012 Gg of N_2O equates to 3.6 Gg $CO_{2\text{-equiv.}}$

Should all the urea be applied with NBPT in New Zealand, changing the $Frac_{GASF}$ from 0.1 to 0.06 will reduce the annual NH_3-N emission by 9 Gg from 22.5 Gg to 13.5 Gg, resulting in indirect N_2O emissions reduction of 0.14 Gg. This difference of 0.14 Gg of N_2O equates to 43.4 Gg $CO_{2\text{-equiv.}}$

9. Discussions and Conclusions

Studies conducted in New Zealand and overseas and discussed in the earlier sections of this report have shown that under certain conditions urea-containing fertilisers can lose up to 30% or more of their N by NH3 volatilisation if not incorporated into the soils. Ammonia itself is not a greenhouse gas but when re-deposited on land it acts as an indirect source of N_2O. Currently New Zealand N_2O inventory uses the IPCC default value of 0.1 for $Frac_{GASF}$ (fraction of total fertiliser emitted as NO_x and NH_3).

A large body of research has established that NH_3 emission losses can be substantially reduced if a urease inhibitor is used with the fertiliser or cattle urine. Urease inhibitors slow the conversion of urea to NH_4^+ by inhibiting the urease enzyme, which reduces NH_4^+ concentration in the soil solution and hence lowers the potential for NH_3 emission. This also allows more time for urea to diffuse away from the application site or for rain or irrigation to dilute urea and NH_4^+ concentrations at the soil surface and increase its dispersion in the soil subsequently retaining NH_3 in the soil.

Urease inhibitor, N-(n-butyl) thiophosphoric triamide (nBTPT) sold under the trade name Agrotain® is currently the most promising and effective in reducing the amount of NH_3 emission and thus reducing the values $Frac_{GASF}$ and $Frac_{GASM}$. The rate of reduction in this loss from urea with the use of nBTPT is regulated by a range of environmental conditions and soil characteristics.

Based on research on the use of urease inhibitor nBTPT in peer-reviewed literature and unpublished reports and reported here it is evident that a nBTPT application rate of 0.025% w/w with urea or urine most effectively reduces NH_3 emissions from temperate grasslands.

New Zealand studies involving optimum nBTPT application (0.025% w/w) with urea and urine show an overall reduction in NH_3 emissions of 43% and 48% from urea and urine, respectively. The average increase in pasture production involving nBTPT application with urea and urine was 6.5% and 7% (only based on one study), respectively.

In the above reported experiments, Agrotain was mixed with urine before application, thus giving a better chance to the active ingredient to interact with urine.

The single application of nBTPT with urine resulted in an overall 48% reduction in NH_3 emission. The effectiveness of Agrotain in soil varies with the soil organic matter content, texture, pH, soil N status and microbial activities of the soils. nBTPT does not kill microbes, but inhibits the activity of urease enzyme for a period of 1–2 weeks. As the effect of nBTPT diminishes, the amount of urease enzymes is built up quickly. Thus the effect of nBTPT directly applied to pasture soils is likely to last up to 2 weeks. Little New Zealand and overseas research has been conducted to evaluate the mode of application of nBTPT to urine patches and the frequency of its application required to determine the potential for direct use of nBTPT in pastures. Therefore, we are only able to assess the impact of nBTPT applied with urea in reducing NH_3 emission and its indirect effect on N_2O.

Based on the peer-reviewed literature and our above estimates of reductions in NH_3 emission, a New Zealand specific value of 0.06 for $Frac_{GASF}$ is recommended for adoption where fertilisers containing urease inhibitor, NBPT, are applied. Changing the $Frac_{GASF}$ from 0.1 to 0.06 for the current use of 18.4 Gg N of SustaiN reduces indirect N_2O emissions by 0.012 Gg, which equates to 3.6 Gg $CO_{2\text{-equiv.}}$

However, assuming that in New Zealand all the urea is applied with nBTPT, changing the $Frac_{GASF}$ from 0.1 to 0.06 will reduce the indirect N_2O emissions by 0.14 Gg, which equates to 43.4 Gg $CO_{2\text{-equiv.}}$

If a dollar value were to be placed on $CO_{2\text{-equiv}}$ of N_2O emissions reduction from the current and potential use (all urea), based on the current $CO_{2\text{-equiv}}$ value of ~€15.23 + 0.13 (http://www.pointcarbon.com/trading/25 May 2009) or ~NZ$35.00 per tonne, reduced N_2O emissions from current urea fertilisers containing urease inhibitor nBTPT in New Zealand would offset 18 Gg $CO_{2\text{-equiv}}$ during the commitment period (2008–2012), equivalent to a saving of about NZ $0.63 million.

Our recommendations on changing the $Frac_{GASF}$ from 0.1 to 0.06 are based only on an average emissions reduction value from three New Zealand studies on two allophanic soils with high organic C levels and one non-allophanic soil with low organic C level and urea application rates of 100 and 150 kg N/ha. It is not possible to assess the relative contribution of the key soil and environmental factors (e.g., soil organic C, temperature and moisture) towards the response rate of nBTPT in reducing NH_3 emission and to quantitatively account these in a national inventory from this existing New Zealand information. As more information on the effectiveness of nBTPT across a combination of soil temperature and moisture on soils contrasting in organic C becomes available more accurate parameter estimates could be developed for modelling the effectiveness of nBTPT at regional and national scales.

Apart from the small reductions in indirect N_2O emissions inventory, there are additional environmental benefits (not considered here) and productivity gains. A cost benefit, provided by the fertiliser industry (J. Blennerhasset, pers. comm. 2009) and reported below, highlights the increased pasture growth benefit from nBTPT-treated N fertilisers that may make urease inhibitors an even more viable option. For example, SustaiN currently costs $100/t more than urea. Economic analysis shows that for farmers to recoup the premium of the product, N responses (kg DM/kg N) from SustaiN need to increase by at least 6% in dairy situations and 8% in sheep operations (assuming a dairy payout of $4.55/kg MS and a lamb schedule of $5.20). The average improvement in N response across all known New Zealand SustaiN trials is in excess of 25% (J. Blennerhasset, pers. comm. 2009), showing farmers will, on average, more than recover the premium of the product in extra production. Reductions in GHG emissions from using nBTPT treated urea can therefore be regarded as being achieved with no extra cost.

10. Recommendations

Urease inhibitor, nBTPT application rate of 0.025% w/w with urea most effectively reduces NH_3 emissions from temperate grasslands. Application of urea containing nBTPT to New Zealand pastoral soils resulted in average 43% reduction in NH_3 emission. Based on the peer-reviewed literature and our above estimates of reductions in NH_3 emission, a New Zealand specific value of 0.06 for $Frac_{GASF}$ is recommended for adoption where fertilisers containing urease inhibitor, nBTPT are applied and $Frac_{GASF}$ should be calculated as follows:

$$\mathbf{Frac_{GASF} = [(FN_{UI}) \times 0.06] + [(FN - FN_{UI}) \times 0.10]} \qquad \textbf{(1)}$$

where $\mathbf{Frac_{GASF}}$ is the fraction of total fertiliser N emitted as NH_3, **FN** is the total amount of applied fertiliser N, $\mathbf{FN_{UI}}$ is the amount of applied fertiliser N treated with urease inhibitor, nBTPT.

In the absence of New Zealand data on direct application of nBTPT or the effect of nBTPT containing urea fertilisers concurrently or as close as possible to the deposition of urine-N on NH_3 emissions, it is not possible to estimate the effect of urease inhibitors on reductions in $Frac_{GASM}$ related to NH_3 emissions from excretal N inputs during grazing. Further research is needed to evaluate the mode of application of nBTPT to urine patches and the frequency of its application to provide quantitative estimates on emission reductions from excretal N inputs during grazing.

The requirements for the use of urease inhibitor nBTPT (Agrotain) are similar to those for nitrification inhibitor DCD, i.e. a requirement for accurate and verifiable records of the sale from the fertiliser industry and treated pasture/land/soils area from the farmers. Long-term record storage and availability for independent review are also required. A GPS system that could be future proof and suitable for accreditation of farm-scale carbon credits and IPCC audit, and might have wider application to monitoring N use and losses from farms, associated with the application proposed for DCD by Kelliher et al. (2007), seems ideal.

11. Future Research Needs

In New Zealand, urease inhibitors have been proposed as management alternatives to reduce NH_3 emissions and to provide greater N availability to the pasture plant. The use of commercially formulated urease inhibitors (UIs; [N(n-butyl) thiophosphoric triamide], nBTPT) (e.g., SustaiN) is encouraged to reduce the loss of N through gaseous emissions. nBTPT reduces the rate of urea hydrolysis to NH_4^+ but urea hydrolysis can not be inhibited indefinitely by nBTPT. The value of nBTPT for mitigating NH_3 emission losses in grazed pastures will depend on their rate of biodegradation and persistence in soils. Research by Summit-Quinphos suggests that generally nBTPT is likely to last in soils up to 2 weeks (J. Blennerhassett, pers. comm.), the period during which NH_3 is emitted from urea-N.

Soil temperature and moisture and soil organic C levels are obviously key factors that affect the rate of NH_3 emission and its % reduction with the inhibitor. Results from only three New Zealand field studies and a glasshouse study clearly show that nNBPT is less effective in high C allophonic soils in reducing NH_3 emissions from urea (41–45%) compared with the reductions (59%) from a low C

Manawatu silt loam soil. However, it is difficult to assess the relative contribution of soil temperature and moisture from this existing New Zealand information as the key soil and environmental factors influencing nBTPT efficiency.

More information on the effectiveness of nBTPT across a combination of soil temperature and moisture on soils contrasting in organic C is needed to quantitatively estimate reductions in NH_3 emission.

The only three New Zealand field studies involving nBTPT used a single application rate of 100 or 150 kg N/ha. No research has been conducted involving the lower application rates of 25 and 50 kg N/ha to reach (?) any conclusion on how the effectiveness of nBTPT will differ with these rates of Urea-N. This aspect needs to be considered in future studies.

Finally, to evaluate the mode of application of nBTPT to urine patches and the frequency of its application to determine the potential for direct use of nBTPT in New Zealand pastures field research is required.

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13. Appendices

Appendix 1: Details of research projects involving the application of urease inhibitor, Agrotain (nBTPT) conducted in US

State	Research Facility	Researcher	Type of Study	Research Topic
Alabama	Auburn University	J. T. Touchton	Field Trials - Yield	Improving Nitrogen Efficiency for Ryegrass with AGROTAIN Urease Inhibitor
	Auburn University	J. T. Touchton	Field Trials - Yield S. Hawkins	Improving Nitrogen Efficiency for Winter Wheat with AGROTAIN Urease Inhibitor
	Auburn University	Elizabeth Guertal	Volatility - Lab	Comparative Study Evaluating the Volatilization of Different Products Under Ambient Conditions
	Auburn University	Elizabeth Guertal	Volatility - Lab	Comparative Study Evaluating the Volatilization of Different Products Under Cool Temperature Conditions
	Auburn University	J. T. Touchton	Field Trials - Yield	Use of the Urease Inhibitor N-(n-butyl) Thiophosphoric Triamide for use of Corn Production
	Auburn University	K. F. Bronson	Field Trials - Yield	Use of Urease Inhibitor N-(n-buty) Thiophosphoric Triamide in Corn Production on a Loamy Sand
	Auburn Universtiy	C. W. Wood	Field Trials - Yield	Utilization of NBPT Treated Urea in a Corn Cropping System In Alabama
	Auburn University	J. T. Touchton	Field Trials - Yield	Nitrogen Efficiency for Corn
	Auburn University	J. T. Touchton	Field Trials - Yield	Use of a Urease Inhibitor N-(n-buty) Thiophosphoric Triamide for Corn Production
	Auburn University	K. F. Bronson	Field Trials - Yield	Control of Ammonia Volatilization with N-(n-buty) Thiophosphoric Triamide in Loamy Sands
	Auburn University	J. T. Touchton	Field Trials - Yield	Nitrogen Efficiency for Wheat

State	Research Facility	Researcher	Type of Study	Research Topic
	Auburn University	Charles Mitchell	Field Trials - Yield	Alternative N Sources for Cotton
	Auburn University	Charles Burmester	Field Trials - Yield	Alternative N Sources for Corn
Arizona	Arizona State University	M. J. Ottman	Field Trials - Yield	Use of Agrotain to Prevent Urea Volatilization in Irrigated Wheat Production
	University of Arizona	Jeffrey Silvertooth	Field Trials - Yield	Cantaloupe Response to Agrotain
Arkansas	University of Arkansas	B. R. Well R. J. Norman	Field Trials - Yield	Fertilizer Nitrogen Uptake by Rice from Granular Urea or Urea, Ammonium or Nitrate in a UAN
	University of Arkansas	R. J. Norman	Field Trials - Yield	Influence of Nitrogen Fertilizer Source, Application Rate and Timing on Grain Yields of Delayed Flood Rice
	University of Arkansas	J. S. McConnell	Field Trials - Yield	Response of Cotton to Urea Nitrogen Fertilization Under Furrow Irrigated and Dry Land Conditions
	University of Arkansas	D. Oostehuis	Field Trials - Yield	Field Evaluation of Foliar-Applied Fertilizers on the Growth and Yield of Cotton
	University of Arkansas	R. Norman	Field Trials - Yield	A comparison of Super U and Urea Applied at Different Application Times and Rates to Drill-Seeded, Delayed-Flood Rice
	University of Arkansas	J. Robbins	Turf Field Trials	Turf Study with UFLEXX, UMAXX, SuperU and Agrotain Treatments
	University of Arkansas	J. S. McConnell	Field Trials - Yield	Response of Cotton to SuperU and Urea Nitrogen Fertilization Under Furrow Irrigated and Dry Land Conditions
California	University of California, Davis	Jeff Mitchell	Field Trials - Yield	Investigate the Potential Benefits of Agrotain and AgrotainPlus for Field Corn Production in California's Sacramento Valley -Multiple Year Study

State	Research Facility	Researcher	Type of Study	Research Topic
	University of California, Davis	Don May	Field Trials - Yield	Onion Nitrogen Rate and Sources - Multiple Year Study
Colorado	Colorado State University	D. G. Westfall	Field Trials - Yield	Evaluation of AGROTAIN Urease Inhibitor Under Corn Production
	Colorado State University	Jerry Johnson	Field Trials - Yield	Agronomic Management for Consistent Wheat Quality in Eastern Colorado
	Colorado State University	D. G. Westfall	Field Trials - Yield	Evaluation of Urease Inhibitors Under Corn
	Agricultural Research Service	Ardell Halvorson	Field Trials - Leaching and Denitrification	Impacts of Stabilized Nitrogen on Reducing Air and Water Quality Impacts
	Irrigation Research Foundation	Charles Corley	Field Trials - Yield	Research on the Impacts of Stabilized Nitrogen on Irrigated Corn
Florida	University of Florida	Fred Rhoads	Field Trials - Yield	Agrotain Cotton Experiment
	University of Florida	Jerry B. Sartain	Denitrification Study	Evaluation of the Effect of Dicyandiamide, NBPT, on N Leaching Losses from Urea and UAN Based Fertilizer
	Unviersity of Florida	Jerry B. Sartain	Leaching Study	Effect of N Source on Leaching Characteristics of Turfgrass
Georgia	University of Georgia	G. Harris	Field Trials - Yield	Nitrogen Source Study
	University of Georgia	G. Harris	Field Trials - Yield	Evaluation of Agrotain Nitrogen Stabilizer for Cotton Production in the Southeast
	University of Georgia East Georgia Ext. Center	George Boyhan	Field Trials - Yield	Vidalia Onion Fertility Experiment
	University of Georgia	C. L. Neely	Field Trials - Yield	Evaluation of the Urease Inhibitor AGROTAIN Under Wheat Production

State	Research Facility	Researcher	Type of Study	Research Topic
	University of Georgia	Malcolm E. Sumner	Field Trials - Yield	Evaluation of the Urease Inhibitor NBPT Under wheat and Corn Production
	University of Georgia	Lewis L. Goodroad	Field Trials - Yield	Evaluation of Urease Inhibitor on Winter Small Grain
	Growmark	S. Parks	Field Trials - Yield	Field Evaluation and Demonstration of NBPT
	Micro Macro International	H. Mills	Leaching and Phytotoxic Study	Evaluation of Leaching and Phytotoxic Characteristics of Fertilizers in Turfgrass
Idaho	Idaho State University	Bob Mahler	Field Trials - Yield	Evaluation of Agrotain on Winter Wheat Production
	Cytozyme Laboratory	K. Whiting	Field Trials - Yield	Management Strategies to Enhance N Retention of N-Fertilizer Applications with Corn
	Idaho State University	B. Brown	Field Trials - Yield	Agrotain Evaluation for Irrigated Soft White Winter Wheat
Illinois	University of Illinois	B. Hoeft	Field Trials - Yields	Evaluation of Agrotain Urease Inhibitor
	University of Illionis	F. Below	Field Trials - Yield	Use of Agrotain to Improve the Efficiency of Surface Applied Urea in No-Till Corn
	Southern Illinois University	E. Varsa	Field Trials - Yield	Effect of Agrotain on No-Till Corn
	Parkland College	K. Wittler	Field Trials - Yield	Agrotain Urease Inhibitor Nitrogen Study
	Growmark	S. Parks	Field Trials - Yield	Field Evaluation and Demonstration of Agrotain on No-Till Corn
	Southern Illinois University	E. Varsa	Field Trials - Yield	An Evaluation of Urease Inhibitor Technology as a Nitrogen Management Tool in No-Till Corn Production
	University of Illinois	K. Barber	Field Trials - Yield	Response of Winter Wheat to Agrotain

State	Research Facility	Researcher	Type of Study	Research Topic
	Brownstone			Impregnated Urea
	University of Illinois	S. Ebelhar	Field Trials - Yield	Comparative Study of Products and Combination of Products
	University of Illinois	S. Ebelhar	Field Trials - Yield	Effects of a New Inhibitor on No-Tillage Corn After Soybeans in Southern Illinois
	University of Illinois	S. Ebelhar	Field Trials - Yield	Evaluation of New Nitrogen Fertilizer Technologies for Corn
	University of Illionis	S. Ebelhar	Field Trials - Yield	Nitrogen Source and Rate Effects on Corn in Southern Illinois
	Universtiy of Illinois	F. Below	Field Trials - Yield	Sources, Amendments and Application Systems for Nitrogen Fertilization of No-Till Corn
	Universtiy of Illinois	F. Below	Field Trials - Yield	Nitrogen Sources and Amendments for No-Till Corn
	University of Illionis	S. Ebelhar	Field Trials - Yield	Nitrogen Mangement with NBPT on Tillage Systems
	University of Illinois	David J. Wehner	Field Trials - Yield	Urease Inhibitor Study
	University of Illinois	R. Hoeft	Field Trials - Yield	Effect of N Source and Rate, and Urease Inhibitor on the Yield of Corn
	Universtiy of Illinois	K. Barber	Field Trials - Yield	Evaluation of the Effect of Time of NBPT - Treated Urea on Grain Yield of Winter Wheat
	University of Illinois	S. Ebelhar	Field Trials - Yield	Nitrogen Management for Corn after CRP
	Logan's Agri Service	E. Logan	Field Trials - Yield	Nitrogen Source Study with Agrotain on Corn
	Parkland College	M. A. Anderson	Field Trials - Yield	Evaluation of Various Nitrogen Fertilizers and Various Application Times/Methods on Corn Growth

State	Research Facility	Researcher	Type of Study	Research Topic
	Southern Illinois University	E. Varsa	Field Trials - Yield	The Effect of N Fertilizers and N-(n-buty) Thiosphosphoric Triamide - Amended N Sources on No-Till Corn - 3 Year Study
	Southern Illinois University	E. Varsa	Field Trials - Yield	Effect of NBPT and DCD Amended Urea and Ammonium Nitrate on No-Till Corn
	Southern Illinois University		Turf Field Trials	Improved Zoysia Response with UMAXX
	Southern Illinois University	E. Varsa	Field Trials - Yield	No-Till Corn Yield as Affected by N Fertilizers, Agrotain and Rotation
	Southern Illinois University	E. Varsa	Field Trials - Yield	Effect of NBPT and DCD Amended Urea, Alone and in Combinations on No-Till Corn
	Southern Illinois University	S. Ebelhar	Field Trials - Yield	Evaluation of Urease Inhibitor Technology as a Nitrogen Management Tool in No-Till Corn and Wheat Production
	Southern Illinois University	K. L. Diesburg	Turf Field Trials	Effect of Nutrient Sources, Biostimulants, and Soil Modifiers on Zoysiagrass Turf Quality
	Southern Illinois University	K. L. Diesburg	Turf Greenhouse Trials	Leaf Burn in Greenhouse Conditions of Perennial Ryegrass, Kentucky Bluegrass, and Bentgrass from Urea, AgricoTurf and AgricoTurf with Agrotain
	Southern Illinois University	K. L. Diesburg	Turf Field Trials	Effect of Nutrient Source upon Zoysiagrass
	Southern Illinois University	E. Varsa	Field Trials - Yield	Effect of NBPT Amended Urea and UAN on No-Till Corn in Southern Illionis
	Southern Illinois University	E. Varsa	Field Trials - Yield	Effect of Urease Inhibitors and Nitrogen Rates on No-Till Corn Yield at Two Locations
	Southern Illinois University	E. Varsa	Field Trials - Yield	Comparison of Super Urea, SuperN and NBPT Treated N Sources with Non-Amended N Fertilizers on No-Till Corn as Influenced by Placement and N Rates

State	Research Facility	Researcher	Type of Study	Research Topic
	Southern Illinois University	E. Varsa	Field Trials - Yield	An Evaluation of Urease Inhibitor Technology as a Nitrogen Tool in No-Till Corn Production
	University of Illinois	S. Ebelhar	Field Trials - Yield	Evaluation of New Nitrogen Fertilizer Technologies
	University of Illinois	S. Ebelhar	Field Trials - Yield	A Comparative Study of N Application Rates at Five Locations Across Illinois
	University of Illinois	S. Ebelhar	Field Trials - Yield	Comparative Study of N Inhibitors and Combination of Inhibitors at a Single N Rate
	University of Illinois	S. Ebelhar	Field Trials - Yield	Comparative Study with Various N Application Rates
	University of Illinois	S. Ebelhar	Field Trials - Yield	Effects of a New Inhibitor on No-Tillage Corn after Soybeans in Southern Illinois
	IMC Agribusiness	B. Urbanowicz	Field Trials - Yield	Soybean Sidedress Study
Indiana	Purdue University	S. Hawkins	Field Trials - Yield	No-Till Surface Applied Agrotain Impregnated Urea Evaluation
	Purdue University	D. Mengel	Field Trials - Yield	Evaluation of Agrotain Nitrogen Stabilizer
	Purdue University	D. Mengel	Field Trials - Yield	Management of Urea Nitrogen in No-Till Corn Production
	Purdue Unversity	D. Mengel	Field Trials - Yield	Evaluation of Agrotain Impregnated Urea in a No-Till Corn Production System
	Purdue University	D. Mengel	Field Trials - Yield	The Use of Urease Inhibitors as a Tool to Enhance Nitrogen Use Efficiency
	Purdue University	D. Mengel	Field Trials - Yield	Urease Inhibitor Trials
	Purdue University	S. Hawkins	Field Trials - Yields	Evaluation of Surface Applied Dry Material on

State	Research Facility	Researcher	Type of Study	Research Topic
				No-Till
	Purdue University	D. Mengel	Field Trials - Yields	New Ideas in Nitrogen Management
	Purdue University	D. Mengel	Field Trials - Yields	SEPAC Urease Inhibitor
	Purdue University	D. Mengel	Field Trials - Yield	Agrotain in No-Till Corn
	Purdue University	D. Mengel	Field Trials - Yields	Evaluation of SuperN and SuperU in a No-Till Corn Production System
	Purdue University	S. Hawkins	Field Trials - Yield	Evaluation of Possible Interaction of NBPT Urea with Accent (nicosulfuron) Herbicide or Accent and Counter (Terbufos) Insecticide
	Vincennes	C. Mansfield	Field Trials - Yield	Evaluation of Several Different Sources of Nitrogen for Soft, Red Winter Wheat Grain Production
	Purdue University		Turf Trials - Response	Response of Kentucky Bluegrass to Various Nitrogen Sources
Iowa	Iowa State University	R. Killorn	Field Trials - Yield	Comparison of Nitrogen Fertilizer Sources for Corn Production in Iowa
	Agricultural Custom Research Service	B. Schou	Field Trials - Yield	Effect of Agrotain Impregnated Urea and Mixed with UAN on No-Till Corn
	Agricultural Custom Research Service	B. Schou	Application Rate	NBPT Rate Definition with 46-0-0 and 32-0-0 Urea on Corn at Waterloo, Iowa
	Agricultural Custom Research Service	B. Schou	Compatibility Test	Agrotain Herbicide Compatibility Test
	Agricultural Custom Research Service	B. Schou	Field Trials - Yield	Corn Study with Stabilized Nitrogen
	Growmark	S. Parks	Field Trials - Yield	Field Evaluation and Demonstration of Agrotain on No-Till Corn

State	Research Facility	Researcher	Type of Study	Research Topic
	Terra Research Station	S. Barnhart	Field Trials - Yield	Agrotian Urease Inhibitor No-Till Corn Nitrogen Trial
	Iowa State University	Alfred M. Blackmer	Field Trials - Yield	Grain Yield as effected by N Rate and NBPT at Six Locations in Iowa
	Iowa State University	R. Killorn	Field Trials - Yield	Comparison of Nitrogen Fertilizer Sources for Reduced-Tillage Corn Production
	Iowa State University	N. Christian	Turf Field Trials	NBPT Field Study
	Iowa State University	R. Killorn	Field Trials - Yield	Corn Yield Response to Non-Incorporated Urea Fertilizer with NBPT - 2 Year Study
	Iowa State Universtiy	R. Killorn	Field Trials - Yield	Comparison of Nitrogen Fertilizer Sources for Corn Production in Iowa - 2 Year Study
	Iowa State University	N. Christian	Turf N-15 Study	Recovery of a Urea-Derived N-15 by Kentucky Bluegrass Turf Treated with N-(n-buty) Thiophosphoric Tiamide
	Iowa State University	Diane M. Panetta	Animal Waste	Management Strategy Impacts on Ammonia Volatilization from Swine Waste
	Terra Research Station	S. Barnhart	Field Trials - Yield	NBPT Urease Inhibitor No-Till Nitrogen Trial
	Terra Research Station	S. Barnhart	Field Trials - Yield	Nitrogen Source Study with Agrotain
	Terra Research Station	S. Barnhart	Field Trials - Yield	Evaluation of Agrotain in UAN Solutions on No-Till Corn
Kansas	Kansas State University	R. Lamond	Field Trials - Yield and Protein	Nitrogen Management for No-Till Corn Production - Four Year Study
	Kansas State University	R. Lamond	Field Trials - Yield and Protein	N Management for No-Till Sorghum Production - Four Year Summary

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	Kansas State University	R. Lamond	Field Trials - Yield and Protein	Four Year Responses of Agrotain on Bromegrass
	Kansas State University	Alan Schlegel	Field Trials - Yield	Effect of Urea Fertilizer and Urease Inhibitor NBPT on Corn Emergence and Early Growth
	Kansas State University	Alan Schlegel	Field Trials - Yield	Nitrogen Management Study
	Kansas State University	Alan Schlegel	Field Trials - Yield	Effect of Nitrogen Source, Nitrogen Rate, NBPT and Time of Nitrogen Application on Grain Yield of Ridge Till Corn
	Kansas State University	Alan Schlegel	Field Trials - Yield	Effect of Nitrogen Placement, Nitrogen Rate and NBPT on Corn Emergence and Early Growth
	Kansas State University	R. Lamond	Field Trials - Yield	Effects of Nitrogen Management and Tillage on Grain Sorghum
	Kansas State University	John Pair	Turf Field Trials	Influence of Nitrogen Carriers on Tall Fescue and Ryegrass
	Kansas State University	R. Lamond	Field Trials - Yield	Nitrogen Rates and Sources for Bromegrass
	Kansas State University	Alan Schlegel	Field Trials - Phytotoxicity	Reduction in Phytotoxicity from Urea Fertilizer by the Urease Inhibitor N-(n-buty) Thiosphosphoric Triamid - 2 year Study
	Kansas State University	Barney Gordon	Field Trials - Yield	Nitrogen Management for No-Till Corn and Grain Sorghum Production
	Kansas State University	Alan Schlegel	Field Trials - Phytotoxicity	Reduction in Phytotoxicity from Urea Fertilizer by The Urease Inhibitor N-(n-buty) Thiosphosphoric Triamid
	Kansas State University	R. Lamond	Field Trials - Yield	Nitrogen Rates and Source for No-Till Grain Sorghum, No-Till Corn and Bromegrass
	Kansas State University	J. Pair	Turf Field Trials	Comparisons of Various Fertilizers on Turf Type Fall Fescue

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	Kansas State University	K. Kelly	Field Trials - Yield	Wheat Response to Agrotain Treated Urea
	Kansas State University	R. Lamond	Field Trials - Yield	Nitrogen Management for No-Till Corn and Grain Sorghum Production
	Kansas State University	R. Lamond	Field Trials - Yield	Nitrogen - Tillage Sorghum Study
Kentucky	University of Kentucky	W. Frye	Field Trials - Yield	Improved Efficiency of Urea Fertilizer with Agrotain on No-Till Corn - Four Year Study
	Miles Farm Supply	W. Brown	Field Trials - Yield	Effect of Agrotain with Urea on No-Till Corn - Five Year Summary
	University of Kentucky	Richard Gates	Animal Waste	Effect of Enzyme Inhibitors on Ammonia Emissions From Broiler Houses
	University of Kentucky	W. Frye	Field Trials - Yield	Improved Efficiency of Urea Fertilizer with Agrotain on Fescue - Five Year Study
	Wheat Tech	C. Bowley	Field Trials - Yield	Nitrogen Management Study
	University of Kentucky	L. Murdock	Field Trials - Yield	Yield Response of No-Till Corn to Agrotain Nitrogen Stabilizers
	University of Kentucky	K. Wells	Field Trials - Yield	Field Evaluation of SuperU for Production of No-Till Corn - 2 Year Study
	University of Kentucky	G. Schwab	Field Trials - Yield	Nitrogen Volatilization Study - Multiple Year Study
	Miles Farm Supply	John Hagan	Field Trials - Yield	Nitrogen Management Trial on Corn
	Miles Farm Supply		Field Trials - Yield	Nitrogen Management on Conventional Tillage Wheat
	Miles Farm Supply	Travis Drake	Field Trials - Yield	Nitrogen Management Trial on Corn

State	Research Facility	Researcher	Type of Study	Research Topic
	Miles Farm Supply	John Hagan	Field Trials - Yield	Nitrogen Management Trial on Corn using Various N Rates
	University of Kentucky	L. Murdock	Field Trials - Yield Protein	Comparative Study of N Inhibitors and Combination of Inhibitors at a Single N Rate
	University of Kentucky	Bob Pearce	Field Trials - Yield	Determine the Substitution of SuperU for Ammonium Nitrate
	University of Kentucky	L. Murdock	Field Trials - Yield	Nitrogen Rates, Timing and Sources on No-Till Wheat
	University of Kentucky	W. Brown	Field Trials - Yield	Nitrogen Additive Trial
	University of Kentucky	W. Brown	Field Trials - Yield	Use of Agrotain with UAN on No-Till Wheat
	Miles Farm Supply	P. Logsdon	Field Trials - Yield	Effect of Agrotain with Urea on No-Till Corn
	IMC Agrico	Allen Sutton	Field Trials - Yield	Nitrogen Source Study Using Agrotain at Various Application Dates
	IMC Agrico	Allen Sutton	Field Trials - Yield	No-Till Sidedress Study
	IMC Agrico	Allen Sutton	Field Trials - Yield	Soybean Sidedress Study
	IMC Agrico	Allen Sutton	Field Trials - Yield	Liquid NBPT Comparisons
	IMC Agrico	Allen Sutton	Field Trials - Yield	Corn pH Study
	IMC Agrico	Allen Sutton	Field Trials - Yield	Rate Definition Study
	IMC Agrico	Allen Sutton	Field Trials - Yield	Liquid Snyergy Evaluation; Dry Synergy Evaluation in Bean Residue; Ammonium Chloride Evaluation; Dry Synergy in Corn Residue; Liquid Synergy Comparison Corn Residue; Liquid Synergy Evaluation on Corn Residue; SuperN Comparison

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	IMC Agrico	Allen Sutton	Field Trials - Yield	NBPT response in Different Soil pH's with Winter Wheat
	IMC Agrico	Allen Sutton	Field Trials - Yield	Wheat Urease Inhibitor
	IMC Agrico	Allen Sutton	Field Trials - Yield	Wheat Dry NBPT Study
	IMC Agrico	Allen Sutton	Field Trials - Yield	Nitrogen Source Study
Louisiana	Louisiana State University	W. B. Hallmark	Field Trials - Yield	Effect of Agrotain, Urea Nitrogen Rates and Placement on Plant Cane Yields
	Louisana State University	W.B. Hallmark	Field Trials - Yield	Effect of Agrotain Urease Inhibitor and Urea Nitrogen Rates and Placement on First Stubble Cane Yields - 2 Year Study
	Louisiana State University	W. B. Hallmark	Field Trials - Yield	Effect of Agrotain Urease Inhibitor and Urea Nitrogen Rates and Placement on Second Stubble Cane Yields
	Louisiana State University	P. Bollich	Field Trials - Yield	Evaluation of Agrotain as a Urease Inhibitor in Drill Seeded Lemont Rice
	Louisiana State University	W. B. Hallmark	Field Trials - Yield	Effect of a Nitrogen Stabilization Package and Urea Nitrogen Rates and Placement on Sugar Cane Yields
	Louisiana State University		Turf Trials - Response	Tifgreen Bermuda Grass Response to Various Nitrogen sources
Maryland	University of Maryland	V. A. Bandel	Field Trials - Yield	Nitrogen Source and Urease Inhibitor Research on No-Tillage Corn
	University of Maryland	F. R. Mulford	Field Trials - Yield	Effect of Agrotain Addition to Urea and UAN on No-Till Corn - 6 Year Study
	University of Maryland	V. A. Bandel	Field Trials - Yield	No-Till Corn Fertilization - 2 Year Study
	University of Maryland	F. R. Mulford	Field Trials - Yield	Evaluation of Urea and UAN With and Without

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				Agrotain Urease Inhibitors on No-Till Corn Compared to Ammonium Nitrate
	Wye Research & Education Center		Field Trials - Yield	No-Tillage Corn N Source Study
	University of Maryland	F. R. Mulford	Field Trials - Yield	Use of ConserveN Soil to Treat Poultry Manure (Broiler and Pelletized) in Corn Production
	University of Maryland	F. R. Mulford	Field Trials - Yield	Fall and Spring Applications of Poultry Manure on Wheat with and without AgrotainPlus
	University of Maryland	F. R. Mulford	Field Trials - Yield	Nitrogen Source Study with Agrotain on No-Till Corn
	University of Maryland	F. R. Mulford	Field Trials - Yield	Effect of Agrotain Addition and Application Timing on No-Till Corn
	University of Maryland	F. R. Mulford	Field Trials - Yield	Agrotain Study on No-Till Corn
	University of Maryland	F. R. Mulford	Field Trials - Yield	Nitrogen Source Study with No-Till Corn - 2 Year Study
	University of Maryland	F. R. Mulford	Field Trials - Yield	Influence of Agrotain Urease Inhibitor on Corn Production
	University of Maryland	F. R. Mulford	Field Trials - Yield	Effect of AgrotainPlus to Stabilize Poultry Manue Applied to Wheat
	University of Maryland	F. R. Mulford	Field Trials - Yield	Efficiency of Nitrogen Sources and Agrotain Urease Inhibitor on Wheat - Six Year Study
	University of Maryland	F. R. Mulford	Field Trials - Yield	Efficiency of Nitrogen Sources and Agrotain Urease Inhibitor on Barley
	University of Maryland	F. R. Mulford	Field Trials - Yield	Effect of SuperN to Stabilize Poultry Manure Applied to Wheat - Multiple Year Study

State	Research Facility	Researcher	Type of Study	Research Topic
	Universtiy of Maryland	F. R. Mulford	Field Trials - Yield	N Placement Study With and Without Agrotain in Wheat Production
	University of Maryland	F. R. Mulford	Field Trials - Yield	Comparative Trial Utilizing Various Rates of N Fertilizer in a No-Till System
	University of Maryland	F. R. Mulford	Field Trials - Yield	Efficiency of Nitrogen Sources and Agrotain Nitrogen Stabilizer on No-Till Corn
Michigan	Michigan State University	M. Vitosh	Field Trials - Yield	Urease Inhibitor Study on Corn
Minnesota	Universtiy of Minnesota	M. Schmitt	Field Trials - Yield	Urea Application Strategies on Corn Grain Yields and Stalk Nitrate-N Concentrations
	University of Minnesota	G. Malzer	Field Trials - Yield	The Impact of Agrotain on Corn Production
	University of Minnesota	G. Malzer	Field Trials - Yield	Preliminary Study on the Impact of NBPT on Corn Production
	University of Minnesota	M. Schmitt	Field Trials - Yield	Urea - NBPT Study
	University of Minnesota	J. Oolman	Field Trials - Yield	Agrotain and Urea for Enhanced Soybean Yield
Missouri	University of Missouri	G. J. Smith	Field Trials - Yield	Evaluation of Urease Inhibitor Amended Urea for No-Till Corn
	University of Missouri	R. Joost	Field Trials - Yield	Efficiency of Urea Materials for Nitrogen Fertilization of Established Caucasian Bluestem - Three Year Study
	Wheat Tech	C. Bowley	Field Trials - Yield	Nitrogen Management 2 Year Study
	University of Missouri	D. D. Buchholz	Field Trials - Yield	Urease and Nitrification Inhibitors in Continous No-Till Corn
	University of Missouri	D. D. Buchholz	Field Trials - Yield	Evaluation of Urease Inhibitor Amended Urea for No-Till Corn

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	Wheat Tech	C. Bowley	Field Trials - Yield	Nitrogen Management Study with Agrotain
	Wheat Tech	S. Jones	Field Trials - Yield	Effect of Agrotain Urease Inhibitor on Wheat
	University of Missouri	Bruce Burdick	Field Trials - Yield	Comparative Study of Various Products, Application Rates and Different Tillage Systems
	University of Missouri	Peter Scharf	Field Trials - Yield	Innovative Technologies for Nutrient Management - 2005
	University of Missouri	Peter Scharf	Field Trials - Yield	Innovative Technologies for Nutrient Management - 2006
Nebraska	University of Nebraska	R. Ferguson	Field Trials - Yield	Demonstration of Agrotain Impregnated Urea on Furrow Irrigated Ridge-Tilled Corn
	University of Nebraska	D. H. Sanders	Field Trials - Yield	Effect of Agrotain on No-Till Corn - 3 Year Study
	University of Nebraska	R. Ferguson	Field Trials - Yield	Ridge Till Corn and Urea Hydrolysis Response to Agrotain
	University of Nebraska	R. Ferguson	Field Trials - Yield	The Effects of a Urease Inhibitor on Volatile Ammonia Loss and Urea Hydrolysis on Irrigated Ridge Till Corn
	University of Nebraska	R. Ferguson	Field Trials - Yield	N Fertilization Study Comparing Urea to Agrotain Treated Urea
	University of Nebraska	R. Ferguson	Field Trials - Yield	Effect of Agrotain on Ridge-Till Corn
	University of Nebraska	R. Ferguson	Field Trials - Yield	Nitrogen Source Study with Agrotain
	University of Nebraska	R. Ferguson	Field Trials - Yield	Ridge-Till Corn and Urea Hydrolysis Response to NBPT
	University of Nebraska	R. Ferguson	Field Trials - Yield	Comparison of Nitrogen Rate, Sources, Application Methods and the Urease Inhibitor NBPT for

State	Research Facility	Researcher	Type of Study	Research Topic
				Irrigated, Ridge-Till Corn
	Agriculture Research Service	Vincent Varel	Animal Waste	Conservation of Nitrogen in Cattle Feedlot Waste with Urease Inhibitors
	Agriculture Research Service	Vincent Varel	Animal Waste	Cpmbination of a Urease Inhibitor and a Plant Essential Oil for Control of Fecal Coliforms and Emissions of Odor and Ammonia From Cattle Waste
	Agriculture Research Service	Vincent Varel	Animal Waste	Influence of Thynol and a Urease Inhibitor on Coliform Bacteria, Odor, Urea and Methane from a Swine Production Manure Pit
	University of Nebraska	Timothy L. Murphy	Field Trials - Yield	Ridge-Till Corn and Urea Hydrolysis to Agrotain
New Jersey	Rutgers Universtiy	James Walworth	Field Trials - Yield	Urease Inhibited Urea as a Nitrogen Source for Corn
	Rutgers University	Richard White	Turf Field Trials	Urease Inhibited Urea as a Nitrogen Source for Kentucky Bluegrass
North Carolina	North Carolina State University	M. Nguegium	Field Trials - Yield	Effect of Agrotain Urease Inhibitor on Corn Yield
	North Carolina State University	Phillip Westerman	Nitrogen Loss	Effect of NBPT on Nitrogen Loss from Livestock/ Poultry Manure
	North Carolina State University	Jack V. Baird	Field Trials - Yield	Effect of a Selected Urease Inhibitor (NBPT) on Corn Yield - 2 Year Study
Ohio	Ohio State University	J. Johnson	Field Trials - Yield	Response of a Urease Inhibitor on Corn Yield - 2 Year Study
	Spectrum Analytic	M. G. Molly	Field Trials - Yield	Nitrogen Source Study with Agrotain Urease Inhibitor on No-Till Corn

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	Ohio State University	Donald J. Eckert	Field Trials - Yield	Evaluation of NBPT on Corn
	Producer	Rick Fruth	Field Trials - Yield	Nitrogen Study on Impacts of Topdressing Wheat Using AgrotainPlus Treated UAN
	Ohio State University		Turf Field Trials	Soluble Nitrogen Fertility
	Ohio State University	John R. Street	Turf Field Trials	Effect of Various Bluegrass Growth and Quality Study
	Ohio State University	Walter Schmidt	Field Trials - Yield	Nitrogen Source Study
	Spectrum Analytic	M. G. Molly	Field Trials - Yield	Nitrogen Source Field Plots
	IMC Agribusiness	B. Urbanawich	Field Trials - Yield	Nitrogen Source Study with Agrotain on Corn
	IMC Agribusiness	B. Urbanawich	Field Trials - Yield	Soybean Sidedress Study
	IMC Agribusiness	B. Urbanawich	Field Trials - Yield	Agrotain Research
	Ohio State University		Field Trials - Yield	Determine if Nitrogen Additives (primarily urease inhibitors) could be used to Improve Corn Grain Yield and Nitrogen Use Efficiency
Oklahoma	Oklahoma State University	Jeff Edwards	Field Trials - Yield	Comparative Study on Spring Nitrogen Application Top Dressed on Wheat
	Oklahoma State University	Bill Raun	Field Trials - Yield	Evaluate the Effectiveness of UAN Treated with AgrotainPlus on Corn Grain Yield and Nitrogen Use Efficiency
	Oklahoma State University	Chris Rice	Field Trials - Yield and Protein	Forage Research using Agrotain on Bermuda Hay
Pennsylvania	Pennsylvania State Universtiy	R. H. Fox	Field Trials - Yield	Management and Urease Inhibitor Effects on Nitrogen Use Efficiency in No-Till Corn

State	Research Facility	Researcher	Type of Study	Research Topic
	Pennsylvania State University	R. H. Fox	Field Trials - Yield	Increasing Nitrogen Use Efficiency of Surface Applications of Urea-Ammonium Nitrate Solutions (UAN) to No-Till Corn
	Pennsylvania State University	R. H. Fox	Field Trials - Yield	Optimizing Nitrogen Fertilizer Efficiency of UAN Applied to No-Till Corn
	Pennsylvania State University	D. V. Waddington	Turf Field Trials	N-88 Nitrogen Source Test - 2 Year Study
	Pennsylvania State University	R. H. Fox	Volatility Study	Ammonia Volatilization Loss from Surface-Applied Urea Containing Fertilizers Measured by a Simplied Micro-Meteorological Method
	Pennsylvania State University	R. H. Fox	Field Trials - Yield	Management and Urease Inhibitor Effects on Nitrogen Use Efficiency in No-Till Corn
South Carolina	Clemson University	Jay Chapin	Field Trials - Yield	Fertility Effects on Wheat Yield
	Westvaco Forest Research	N. M. Berenyi	Field Trials	Nitrification and Urease Inhibitor Trials
South Dakota	South Dakota State	R. Gelderman	Field Trials - Yield	Agrotain Response on No-Till Wheat
Tennessee	University of Tennessee	M. M. Mullen	Field Trials - Yield	No-Till Corn Production with Agrotain Urease Inhibitor and Surface Applied Urea-N Fertilizers
	University of Tennessee	J. Bradley	Field Trials - Yield	Evaluation of Spoked Wheels vs. Cast Iron Press Wheels and Agrotain Coated Urea vs. Ammonium Nitrate Demonstration on No-Till Cotton
	University of Tennessee	M. M. Mullen	Field Trials - Yield	NBPT Research
	University of Tennessee	J. Bradley	Field Trials - Yield	Evaluation of Agrotain in Urea in No-Till Cotton
	University of Tennessee	D. Howard	Field Trials - Yield	Grey Leaf Spot Evaluation as Affected by P-K Fertilization and Varieties in a No-Tillage System

State	Research Facility	Researcher	Type of Study	Research Topic
	University of Tennessee	J. Bradley	Field Trials - Yield	Nitrogen Source Study with Agrotain
	University of Tennessee	J Bradley	Field Trials - Yield	Side Dress Application of Agrotain Urea vs. Ammonium Nitrate on Cotton
	University of Tennessee at Martin	Richard Joost	Field Trials Nitrate Toxicity Protein	Comparison Study of Agrotain, SuperU and Ammonium Nitrate Relative to Protein Levels and Nitrate Accumulation in the Plant
Texas	Texas A & M	Fred Turner	Field Trials - Yield	Minimum tillage Rice to Agrotain and Agrotain Plus DCD
	Texas A & M	Vincent Haby	Field Trials - Yield	Comparison Study Evaluating N Application to Hybrid Bermudagrass Pasture
	Texas A & M	Vincent Haby	Field Trials - Yield	Comparison Study Evaluating Various N Application Rates to Hybrid Bermudagrass Pasture
	West Texas A & M	D. B. Parker	Animal Waste	Rate and Frequency of Urease Inhibitor Application for Minimizing Ammonia Emissions from Beef Cattle Feedyards
	Texas A & M	Jeffrey L. Ullman	Animal Waste	Remedial Activities to Reduce Atmospheric Pollutants from Animal Feeding Operations
Utah	Cytozyme Laboratory	K. Whiting	Field Trials - Yield	Management Strategies to Enhance N Retention of N-Fertilizer Applications with Corn
	Cytozyme Laboratory	K. Whiting	Field Trials - Yield	Effect of Various N-Fertilizer Enhancing Products on Grain Yield Research Plus
Virginia	Virginia Tech	M. Alley	Field Trials - Yield	Evaluation of Agrotain Urease Inhibitor on Wheat
Washington	Washington State University	Rich Koenig	Field Trials - Yield Volatility	The Influence of Agrotain on Ammonia Volatilization from Urea in Grass Seed Production Systems

State	Research Facility	Researcher	Type of Study	Research Topic
West Virginia	Putnam County Extension	W. Bennett	Field Trials - Yield	Pelleted Lime Urea and Agrotain Tobacco Research
Wisconsin	University of Wisconsin	L. Bundy	Field Trials - Yield	Evaluation of a Urease Inhibitor Used with Surface Applied Urea in Corn Production
	Growmark	S. Parks	Field Trials - Yield	Field Evaluation and Demonstration of Agrotain on No-Till Corn
	University of Wisconsin	F. Rossi	Turf Field Trials	Burn Potential for AgricoTurf with Agrotain on Selected Turfgrass Varieties
	University of Wisconsin		Color and Growth Response	Turfgrass Color and Growth Responses to the Initial Applications of Seven New Fertilizers
	University of Wisconsin	Carrie Laboski	Field Trials - Yield	Comparative Study to Evaluate N Fertilizer Products for the Effectiveness of Yield
	University of Wisconsin		Turf Field Trials	Brentgrass Trials Comparing Different N Sources
	University of Wisconsin	Carrie Laboski	Cost Comparison	Does it Pay to Use Nitrification and Urease Inhibitors?

Appendix II: Details of research projects involving the application of urease inhibitor, Agrotain (nBTPT) conducted in US

Country	Research Facility	Researcher	Type of Study	Research Topic
Argentina	INTA Balcarce Experimental Station	N. Darwich	Field Trials - Yield	Corn Fertilization Experiments with Agrotain - 2 Year Study
	INTA Balcarce Experimental Station	N. Darwich	Field Trials - Yield	Urease Inhibitor Experiments
	INTA Balcarce Experimental Station	N. Darwich	Field Trials - Yield	Urease Inhibitors Use in Corn Fertilization Experiments
	INTA	R. Melgar	Field Trials - Yield Volatility	Evaluation of the Effect of the Inhibitor Urease NBPT (N-(n-buty) tiophosphoric acid triamide) on the Efficiency of the Urea as Fertilizer
	INTA	R. Melgar	Field Trials - Yield Volatility	Evaluation of the Effect of the Inhibitor Urease NBPT (N-(n-buty) tiophosphoric acid triamide) on the Efficiency of the Urea as Fertilizer in Wheat
	INTA	R. Melgar	Field Trials - Yield	Evaluation of the Effect of Urease Inhibitor on the Efficiency of Urea as a Fertilizer in Corn
Australia	Elders Merchandise		Field Trials - Yield	Evaluation of Seed Placement of Agrotain on Wheat
	Elders Merchandise		Field Trials - Yield	Evaluation of Agrotain on Wheat
	Western Australia No-Till Farmers Association	Bill Crabtree	Field Trials - Yield Toxicity	Urea Additives for Reduced Drilled Urea Toxicity for Canola and Wheat
	Griffith University	P. Saffigna	Field Trials - Volatility	Field Evaluation of NH3 Loss with Agrotain Treated Urea when Surface Applied to Sugar Cane Residue
	Pivot	C. Reynolds	Field Trials - Yield	Triticale Variety and Agrotain Trail on Rice Stubble in Dryland

Country	Research Facility	Researcher	Type of Study	Research Topic
Brasil	Instituto Agronomico de Campinas	Hector Cantarella	Field Trials - Yield Volatility	Efficiency and Recovery of N by Sugarcane Fertilized with Urea or Agrotain Treated Urea Multiple Year Study
	International Plant Nutrition Institute	T. Yamada	Field Trials - Yield	Effects of Three Systems of Weed Control and Applications of Nitrogen Fertilizer as Urea and Agrotain Treated Urea
	International Plant Nutrition Institute	T. Yamada	Field Trials - Yield	Effects of Different Sources of N Placed on Plant Emergence and Yield in Wheat in Campo Maurao, PR, Brasil
	Fundacao de Apoio Tecnologico a Cafeicultura PROCAFE	Antonio Wander Rafael Garcia	Laboratory Trials	Compare the Efficiency of Fertilization of Urea and Agrotain Urea in Coffee under Controlled Conditions
	Instituto Agronomico de Campinas	Hector Cantarella	Field Trials - Yield Volatility	The Effect of Agrotain Incorporated to Urea on Losses of NH3 from Volatilization and Crop Production
	Instituto Agronomico de Campinas	Hector Cantarella	Field Trials - Yield Volatility - Lab	Evaluation of the Urease Inhibitor Agrotain on the Efficiency of Urea Fertilizer under Brazilian Soil Conditions - 3 Year Study
		L. A. Henkes	Field Trials - Yield	Sarandi, Rio Grande Do Sul - Brasil, Agrotain in No-Till Corn
		A. Simionato	Field Trial - Yield	Campo Mourao, Parana-Brasil, Agrotain in No-Till Wheat
	EMBRAPA	Walkyria Bueno Scivittaro	Field Trials Volatility	Volatilization Losses of Ammonia in Flooded Rice Production in Brasil
	Instituto Agronomico de Campinas	Hector Cantarella	Laboratory Trials Volatility	Study the Efficiency of Urease and Nitrification Inhibitors Added to UAN in a Brasilian Soil at 25º C
	FUNDACAO ABC	Volnei Pauletti	Field Trials	Ammonium Volatilization in No-Till System, Campos Gerais-Parana; Roles of Nitrogen and

Country	Research Facility	Researcher	Type of Study	Research Topic
				Timing of Application
		T. M. L. Contin	Field Trials - Yield	Urea Treated with Urease Inhibitor NBPT on Sugarcane Harvested without Burning
	IAC, Center of Soils and Agroenvironmental Resources	Gabriel Barth	Field Trials Volatility	N-NH3 Volatilization Related to the Sources and Rates of Nitrogen Applied on Sugarcane Straw
	Instituto Agronomico de Campinas	Hector Cantarella	Field Trials - Yield Volatility	Ammonia Volatilization from Urease Inhibitor-Treated Urea Applied To Sugarcane Trash Blankets
Canada	Agriculture and Agri-Food Canada	C. A. Grant	Field Trials - Field	Urease Inhibitor, Fertilizer Placement and Tillage Effects in Crop Production
	WESTCO	John Harapiak	Field Trials - Yield Toxicity	Toxicity of Seed Placed Agrotain Relative to Urea on Barley - 2 Year Study
	Saskatchewan Soil Conservation Association	Ken Sapsford	Field Trials - Yield	Evaluation of Seed Placed Nitrogen on Canola Seed Emergence
	University of Guelph	X. Zhang	Field Trials - Yield	Initial Report on the Effect of USG with Stabilized Nitrogen on the Early Growth of Corn on Sandy Soil
	University of Guelph	X. Zhang	Field Trials - Yield	The Effect of Agrotain on Reduction the Adverse Effects of Urea Super Granule with the Early Growth of Corn Plant
	Nutrite Research Centre	P. Fournier	Field Trials - Yield	Two Year Response of Agrotain on Corn
	Agriculture and Agri-Food Canada	C. A. Grant	Field Trials Volatility	Volatile Losses of NH3 from Surface Applied Urea and Urea Ammonium Nitrate with and without Agrotain Urease Inhibitor
	Agriculture and Agri-Food Canada	C. A. Grant	Field Trials - Yield	Effect of Seed Placed Urea and Agrotain on Emergence and Growth of Barley - 3 Year Study

Country	Research Facility	Researcher	Type of Study	Research Topic
	Agriculture and Agri-Food Canada	C. A. Grant	Field Trials - Yield	Optimizing Rate and Source of N Fertilizer in No-Till Canola
	University of Manitoba	Eryn Williamson	Field Trials Nitrification	Use of NBPT-DCD Formulated Urea to Reduce N2O Emissions and N Losses from Fall Banded Fertilizer March 2007
	Agriculture and Agri-Food Canada	C. A. Grant	Field Trials - Yield	Use of Agrotain with Surface Dribble Banded and Broadcast Urea and UAN in Production of Hard Red Spring Wheat Under Zero-Till
	Agriculture and Agri-Food Canada	C. A. Grant	Field Trials - Yield Protein	Use of Agrotain with UAN or Urea In-Crop Applications for Protein Enhancement in Wheat - 2 Year Study
	University of Guelph	Todd Rideout	Animal Waste	Efficacy of Various Microbial Urease Inhibitors on Controlling Ammonia Emissions from Swine Manure Slurry
	Agriculture and Agri-Food Canada	C. A. Grant	Field Trials - Yield	Uptake of Foliar or Soil Application of N15 Labeled Urea Solution at Anthesis and its Effect on Wheat Grain Production
	Agriculture and Agri-Food Canada	C. A. Grant	Field Trials - Yield	Effects of Placement of Urea with a Urease Inhibitor on Seedling Emergence, N Uptake and Dry Matter Yield of Wheat
	Agriculture and Agri-Food Canada	C. A. Grant	Field Trials - Yield	Optimizing Rate and Source of N Fertilizer in No-Till Wheat
	Agriculture and Agri-Food Canada	C. A. Grant	Field Trials Volatility	The Effect of Moisture, Temperature and Soil Characteristics on Volatilization, Soil Movement and Plant Uptake of N Applied as Urea and Urea Ammonium Nitrate, with and without the Addition of Agrotain - 2 Year Study
	Guelph Turfgrass Institute		Turf Field Trials	Color Response of Kentucky Turf Fertilized with

Country	Research Facility	Researcher	Type of Study	Research Topic
			Color	Agrotain International Slow Release Fertilizers
	Manitoba Ag	J. Heard	Field Trials - Yield	Winter Wheat Fertility Study
	Prairie Turfgrass Research Centre, Olds College	M. A. Anderson	Turf Field Trials	The Evaluation of Various Controlled Release Fertilizers for Use on Turf
	University of Manitoba	Don Flatten	Field Trials Nitrification	Study Looking at the Use of Inhibitors to Reduce N2O Gases in Agricultural Nitrogen Applications
	Pembina Coop	Justin Daymond	Field Trials - Yield	Effects of Surface Application on Winter Wheat and Seed Place Safety with Canola
	Domain Coop	Ken McMullen	Field Trials - Yield	Side by Side SurfaceTrials using Agrotain Treated 28-0-0 UAN
	Nova Scotia Agricultural College	David Percival	Field Trials Volatility	Assessing Environmental Losses of N Due to Volatilization
	AGRICORE UNITED	Tom Jensen	Field Trials	Develop a System to Apply Nitrification Inhibitor In Anhydrous Ammonia Zone
	WESTCO	Rigas Karamanos	Field Trials - Yield	Effects of Stabilized Nitrogen on Fall Banding and Spring Pre-Plant Applications
	Philom Bio		Field Trials - Yield	Side by Side Grower Trials Demonstrating Surface Application and Seed Place Safety
	University of Manitoba	Stefan Cenkowski	Laboratory Trials	The Effects of Drying Agents, DTE and Sipernat, for Seed Place Safety
	Kelbrn Research Farm	James Richardson	Field Trials - Yield	Winter Wheat Surface Trials and Seed Place Safety Trials
	Exxon Chemicals Canada	Les Carstens	Field Trials - Yield	Agrotain Treated Fertilizer for Seed Application
	Exxon Chemicals Canada	Les Carstens	Field Trials Volatility	Soil Urease Inhibition by Agrotain
	Agriculture and Agri-Food Canada	C.A. Grant	Field Trials	Use of Agrotain Urease Inhibitor and ATS with

Country	Research Facility	Researcher	Type of Study	Research Topic
				Surface Dribble Banded and Broadcast Urea and UAN in Production of Hard Red Spring Wheat Under Zero Till
	AAFRD	Calvin Yoder	Field Trials - Yield	Comparison of Urea and Urea + Agrotain Nitrogen Fertilizer Applications on Creeping Red Fescue See Yields - September 2006
China	Chinese Academy of Agricultural Sciences	Wang Xiaobin	Field Trials - Yield	Effects of Placement of Urea with a Urease Inhibitor on Seedling Emergence, N Uptake and Dry Matter Yield of Wheat
England	Oxford Agricultural Trials	J. Ward	Field Trials	Urea + Urease Inhibitor for Wheat
France	IFA	M.E. Trenkel	Publication	Improving Fertilizer Use Efficiency, Controlled-Released and Stabilized Fertilizers in Agriculture
Germany	BASF AG	Gregor Pasda	Field Trials - Yield	Results with Agrotain in Europe
	Hydro Agri Centre for Plant Nutirtion	M. Basten	Field Trials - Yield	Response of Maize to NBPT Amended Urea in Italy
	Hydro Agri Centre for Plant Nutrition	M. Basten	Field Trials - Yield	Agronomic Performance of NBPT in Field Trials from Germany, France and Italy on Winter Wheat
	Hydro Agri Centre for Plant Nutrition	M. Basten	Laboratory Trials Volatility	Evaluation of the Impact of Different NBPT Rates on the Reduction of Volatilization Losses Based on Laboratory Trials
	Hydro Agri Centre for Plant Nutrition	M. Basten	Field Trials - Yield	Efficacy of Urea + NBPT Results of Winter Wheat Trials in Germany
	Yara Research Center	M. Basten	Field Trials - Yield	Efficacy of NBPT Amended Urea Results from Winter Wheat Field Trials Conducted in Germany, 2004
Ireland	Department of Ag for Northern Ireland - Belfast	C. J. Watson	Field Trials - Yield Volatility	Effectiveness of the Urease Inhibitor Agrotain for Improving the Efficiency of Urea for Ryegrass Production

Country	Research Facility	Researcher	Type of Study	Research Topic
	Department of Ag for Northern Ireland - Belfast	C. J. Watson	Field Trials - Yield Volatility N15 Study	Agronomic Assessment and N15 Recovery of Urea Amended with The Urease Inhibitor Agrotain for Temperate Grassland
	Department of Ag for Northern Ireland - Belfast	C. J. Watson	Laboratory Trials Volatility	Soil Properties Affecting the Ability of the Urease Inhibitor Agrotain to Reduce Ammonia Volatilization From Surface-Applied Urea
	Department of Ag for Northern Ireland - Belfast	C. J. Watson	Field Trials - Yield	Short Term Effects of Urea Amended with the Urease Inhibitor Agrotain on Perennial Ryegrass
Italy	University of Torino	P. Balsari	Laboratory Trials Volatility	Measurement of Ammonia Emission from Yara Chemical Fertilizer
	Hydro Agri Center for Plant Nutrition	M. Basten	Field Trials - Yield	Efficacy of Urea + NBPT Results from Winter Wheat Trial Conducted in Bologny, Italy
Malaysia	United Plantations Berhad	Aziz Bidin	Field Trials - Yield	Effect of the Urease Inhibitor, Agrotain, on Flood Water Characteristics and Rice Yield
Mexico		V. Zapata	Field Trials - Yield	Response of Wheat to Fertilization with Common Urea and Urea with the Urease Inhibitor Agrotain
		R. N. Excobar	Field Trials - Yield	Response of Sorghum and Rice to Nitrogen Fertilizers, Sulfur and to the Urease Inhibitor Agrotain
Netherlands	Nutrient Management Institute	D. W. Bussink	Field Trials - Yield	Toetsing Voorjaarsmeststoffen op Grassland (Report in Dutch)
New Zealand	Summit-Quinphos	Long Nguyen	Field Trials	Evaluation of Nitrogen Fertilizers with and without Nitrogen Inhibitors and Elemental Sulfur under Field Conditions
	Massey University Palmerston North	Jagrati Singh	Field Trials - Yield	The Role of Nitrification Inhibitors in Mitigating Nitrogen and Cation Losses in Grazed Pasture

Country	Research Facility	Researcher	Type of Study	Research Topic
	Summit-Quinphos	M. Zaman	Field Trials	Increasing the Utilisation of Urea Fertiliser by Pasture
	Centre for Soil and Environmental Quality	H. J. Di	Field Trials	Effects of Temperature and Application Rate of a Nitrification Inhibitor, Dicyandiamide (DCD), on Nitrification rate and Microbial Biomass in a Grazed Pasture Soil
	AgResearch Woodlands Research Station	L. C. Smith	Field Trials	The Effectiveness of Different Nitrification Inhibitor Formulations in Limiting Nitrate Accumulation in a Southland Pastoral Soil
	Summit-Quinphos	Bert Quin	Field Trials	Reducing Nitrogen Losses from Dairy Pastures
Phillipines		R. J. Buresh	Field Trials - Yield	Effect of Two Urease Inhibitors on Floodwater Ammonia Following Urea Application to Lowland Rice
		R. J. Buresh	Field Trials - Yield	Field Evaluation of Two Urease Inhibitors with Transplanted Lowland Rice
Thailand		S. Phongpan	Field Trials - Yield	Use of Phenylphosphorodiamidate and Agrotain to Reduce Ammonia Loss and Increase Grain Yield Following application of Urea to Flooded Rice
	Suphanburi Rice Experiment Station	J. R. Freney	Field Trials - Yield	Effect of Urease, Nitrification and Algal Inhibitors on Ammonia Loss and Grain Yield in flooded Rice in Thailand
Turkey	Selcuk University	F. Bayrakli	Field Trials - Yield	Control of Ammonia Volatilization from Surface Applied Urea in the Field on Sugar Beets
	Selcuk University	F. Bayrakli	Field Trials - Yield	Control of Ammonia Volatilization from Surface Applied Urea in the Field on Wheat
United Arab Emirates	UAE University	A. A. Soaud	Laboratory Trials Volatility	Evaluation of Chemical Amendments to Reduce Ammonia Volatilization from Solid Urea Fertilizer Applied to Sandy Soils with Different $CaCO_3$ Content

Appendix III: Summary of the research that has been conducted on the Agrotain Family of Products

Domestic Studies: 33 States, 61 Institutions, 125 Researchers

International Studies: 18 Countries, 45 Institutions, 56 Researchers

Crops Studied:1,340 Trials,
Corn (660 Trials), Wheat (260 Trials), Rice, Sugarcane, Cotton, Sorghum, Cantaloupe, Onions, Bromegrass, Tobacco, Coffee, Canola, Barley, Sugarbeets, Fescue, Ryegrass, Kentucky Bluegrass, other Turf and pasture.

Studies Include: Volatility, Leaching, Nitrification, Yield, Protein, N15, Phytotoxicity