

# LABORATORY EVALUATION OF UREASE INHIBITORS 2-NPT AND nBTPT IN REDUCING AMMONIA EMISSIONS FROM CATTLE URINE APPLIED IN DAIRY-GRAZED PASTURE SOILS

**Kamal P. Adhikari<sup>1</sup>, Surinder Saggar<sup>1,2</sup>, James A. Hanly<sup>1</sup> & Danilo F. Guinto<sup>3</sup>**

<sup>1</sup> *Environmental Sciences Group, School of Agriculture & Environment, Massey University, Palmerston North 4442, New Zealand*

<sup>2</sup> *Ecosystems & Global Change, Manaaki Whenua Landcare Research, Palmerston North 4442, New Zealand*

<sup>3</sup> *Formerly with Ballance Agri-Nutrients Limited, Hewletts Road, Mount Maunganui, Tauranga, New Zealand  
(Email: [adhikari.kamal5@gmail.com](mailto:adhikari.kamal5@gmail.com))*

## Abstract

This laboratory study compares the effectiveness and longevity of the urease inhibitor N-(2-Nitrophenyl) phosphoric triamide (2-NPT) with the commonly used N-(n-butyl) thiophosphoric triamide (nBTPT), such as Agrotain<sup>®</sup>, in reducing ammonia (NH<sub>3</sub>) emissions from pasture soils. The study was conducted using two dairy-grazed pasture soils (Recent, Rangitikei soil and Allophanic, Egmont soil) with contrasting organic carbon (C) levels. Four replicates of each of the following treatments were applied to the soils at the start of the experiment; i) No inhibitor (control), ii) nBTPT (0.025% of total urine N), iii) 2-NPT (0.025% of total urine N), iv) 2-NPT (0.050% of total urine N), and v) 2-NPT (0.075% of total urine N). The amounts of inhibitors applied were calculated based on total N content in urine collected for the first urine application. Ammonia emissions were measured at 4 stages after applying urine to all of treatments; a) immediately before inhibitor application (first urine application), b) 29 days after inhibitor application, c) 56 days after inhibitor application, and d) (b) + urine reapplication at 60 days after inhibitor application. The results showed that the inhibitors significantly reduced total NH<sub>3</sub> emissions from applied urine at stage (a) from both soils, but the difference between inhibitors was not significant. Both of the inhibitors also significantly reduced total NH<sub>3</sub> emissions when urine was applied at stage (b) on the Rangitikei soil with there being a significantly greater reduction from using 2-NPT compared to nBTPT. Treatment effects were not significant for the Egmont soil. The 2-NPT continued to reduce NH<sub>3</sub> emissions in the measurement period following the urine application at stage (c) on the Rangitikei soil but not with nBTPT. However, no reduction in emissions was observed during stage (d) by either of the inhibitors. These results show that 2-NPT has the ability to extend the longevity of urease inhibition compared to the more commonly used inhibitor nBTPT.

## Introduction

Intensification of dairy farming in New Zealand (NZ) over the last 3 decades has resulted in a substantial increase in the national dairy herd size from 2.3 million cows in 1985–86 to 5.0 million in 2015–16 (an increase of 115%) (Livestock Improvement Corporation Limited and DairyNZ Limited 2016). This has resulted in a significant rise in the volumes of urine deposited by grazing cattle onto pasture soils, from 17.8–21.4 million m<sup>3</sup> yr<sup>-1</sup> to 38.3–46.0

million  $\text{m}^3 \text{yr}^{-1}$  (assuming average urination volume – 2.1 L and frequency of urination – 10 to 12 events  $\text{day}^{-1}$  (Selbie et al. 2015)) and as a consequence, higher ammonia ( $\text{NH}_3$ ) emissions. Urine patches cover approximately 20–30% of the grazed pasture area annually and contain the equivalent rate of 200–2000  $\text{kg N ha}^{-1}$  (Selbie et al. 2015). Cattle urine-N contains about 80% urea-N (Zaman et al. 2007) and an average of 15.9% of urine-N deposited can be lost as  $\text{NH}_3$  emissions (Sherlock et al. 2008) and, in some cases, up to 25.7% (Laubach et al. 2012) depending on soil and climatic conditions. These emissions not only represent economic losses but also have negative impacts on health and the wider environment.

Urease inhibitor, N-(n-butyl) thiophosphoric triamide (nBTPT), is one of the more promising and effective approaches for reducing  $\text{NH}_3$  emissions by inhibiting soil urease activity (UA) when applied with cattle urine (Saggar et al. 2013). Following application, nBTPT is quickly transformed in soil to its oxygen analogue N-(n-butyl) phosphoric triamide (NBPTO, actual urease inhibitor) (Creason et al. 1990), which then forms a tridentate ligand with the active site (2 nickel (Ni) atoms) of the urease enzyme (Manunza et al. 1999) and causes a slowing of urea hydrolysis. N-(n-butyl) phosphoric triamide is not a sufficiently stable compound (Hendrickson and Douglass 1993), and its inhibitory effect on soil UA and  $\text{NH}_3$  emissions is effective only for a relatively short period of time of 7–14 days (Saggar et al. 2009), which will potentially inhibit  $\text{NH}_3$  emissions from cattle urine deposited during only 1 grazing event. Thus, in the context of dairy-grazed pasture, to reduce the  $\text{NH}_3$  emitted from the deposition of urine following each grazing event, regular applications of nBTPT is required, which is neither practically feasible nor very economical. It is therefore essential to explore alternative urease inhibitory compounds that have longer stability and effectiveness in dairy pasture soils.

Some previous studies reported that micronutrients such as copper (Cu) and zinc (Zn) are effective in inhibiting soil UA over a longer duration (8-12 weeks) compared to nBTPT (Hemida et al. 1997; Wyzkowska et al. 2006). However, our recent study showed that Cu and Zn are ineffective at inhibiting soil UA in NZ dairy-grazed pasture soils rich in organic carbon (C) (Adhikari et al. 2017). Recent researches have reported N-(2-Nitrophenyl) phosphoric triamide (2-NPT) performs better than nBTPT to inhibit soil UA and  $\text{NH}_3$  emissions. Domínguez et al. (2008) reported that nBTPT showed very low urease inhibition of less than 40% while 2-NPT had inhibition of about 65% after 10 days of incubation of acidic soil. There was no reduction in soil UA by nBTPT after 30 days of incubation of soil with pH 4.5, but there was a 12% reduction with 2-NPT. Similarly, the reductions in soil UA by nBTPT and 2-NPT after 30 days of incubation of soil with pH 8.4 were 43% and 83%, respectively. The inhibitors were applied at the rate of 0.5% of urea-N (w/w). The reduction in urea hydrolysis at 25 days after the application of 2-NPT in combination with urea, at the rate of 0.05% of urea-N (w/w), was 70% compared to urea alone (Hucke et al. 2010). They indicated that 2-NPT turned out to be a highly promising urease inhibitor and suggested that it could be used at concentrations ranging from 0.001 to 10% (w/w) with urea-based fertiliser or added to feed urea or to the animal effluent in animal enclosures. N-(2-Nitrophenyl)-phosphoric triamide is very stable during storage and has been shown to decrease  $\text{NH}_3$  emissions from granulated urea by 26 – 83% relative to urea alone when applied at the concentration of 0.075% of urea-N (w/w) to winter wheat (Ni et al. 2014). A similar result has been reported by Schraml et al. (2009), who stated that the addition of 2-NPT decreased  $\text{NH}_3$  emissions by 49 – 75% relative to urea alone in a grassland soil. The application of 2-NPT with fertiliser urea reduced  $\text{NH}_3$  emissions by 74 – 84% for a urease inhibitor concentration of 0.075 % of total N, by 69 – 88% for a concentration of 0.1% of total N, and by 70 – 100% for a concentration of 0.15 % of total N compared to urea alone, indicating that 0.075 % of total N could be considered an efficient level of application (Schraml et al. 2016). Other than this,

there is minimal international or NZ information on the effectiveness and longevity of 2-NPT in reducing NH<sub>3</sub> emissions from both fertiliser urea and cattle urine urea. Therefore, the objective of this study was to determine and compare the effectiveness and longevity of 2-NPT with nBTPT in reducing NH<sub>3</sub> emissions from cattle urine applied to dairy-grazed pasture soils.

## Materials and Methods

### *Experimental details*

Two dairy-grazed pasture soils (0-10 cm soil depth; a Manawatu soil (Recent, Rangitikei soil) and a Taranaki soil (Allophanic, Egmont soil) with contrasting soil organic C were used to determine and compare the effectiveness and longevity of 2-NPT with nBTPT in reducing NH<sub>3</sub> emissions from cattle urine applied to pasture soils. The experiments were conducted in an incubator room at 20°C. Physical and chemical properties of soils used are presented in Table 1.

**Table 1** Selected physical and chemical properties of dairy-grazed pasture soils studied

Soil properties	Rangitikei soil	Egmont soil
pH water	5.5	5.4
Maximum water holding capacity (%)	17.5	53.3
Total C (%)	1.8	9.7
Total N (%)	0.2	1.0
Sand (%)	60	40
Silt (%)	30	40
Clay (%)	10	20
Soil urease activity (mg kg <sup>-1</sup> soil h <sup>-1</sup> )	16.8	61.5
Exchangeable bases:		
Exchangeable K (meq 100 g <sup>-1</sup> )	0.3	0.6
Exchangeable Ca (meq 100 g <sup>-1</sup> )	5.8	12.3
Exchangeable Mg (meq 100 g <sup>-1</sup> )	0.8	1.9
Exchangeable Na (meq 100 g <sup>-1</sup> )	0.1	0.3
CEC (meq 100 g <sup>-1</sup> )	15.1	42.9

A 108 ml air-dried soil (7.8 cm soil depth) sample was weighed in a plastic container (diameter 4.2 cm) and then was placed inside a 1 L glass Agee jar. The 5 treatments applied in quadruplicate were: i) No inhibitor (control), ii) nBTPT (0.025% of total urine N), iii) 2-NPT (0.025% of total urine N), iv) 2-NPT (0.050% of total urine N), and v) 2-NPT (0.075% of total urine N). The amounts of inhibitors were calculated based on total N content in urine collected for the first urine application. Ammonia emissions were measured at 4 stages after applying urine: a) immediately before inhibitor application (first urine application), b) 29 days after inhibitor application, c) 56 days after inhibitor application, and d) (b) + urine reapplication at 60 days. The application depth of urine was 10 mm for the first 3 stages and 7.2 mm for stage (d). Initial moisture content after urine application was recorded by weighing the plastic container when the soil was at 60% of water holding capacity. This moisture content was maintained by adding deionised water for the first 3 stages. However, initial moisture content after urine application at stage (d) was at water holding capacity for the Rangitikei soil and 82% of water holding capacity for Egmont soil, and these moisture levels were maintained throughout the experiment. A previously evaluated passive sampling technique was used for measuring NH<sub>3</sub> emissions. This included closing the Agee jar with an

air tight lid, which had attached to it an air dried glass microfiber borosilicate filter paper (4.2 cm diameter, 0.6  $\mu\text{m}$  pore size and 0.21 mm thickness) that was soaked in 0.5 M sulphuric acid to trap emitted  $\text{NH}_3$ . Samples were taken daily for the first 7 days (by replacing the filter paper) and then periodically up to day 31. Filter papers were placed in plastic containers containing 10 mL of deionised water at the time of sampling. The plastic containers were then shaken for 20 min at 220 rpm with a horizontal shaker to elute the ammonium ( $\text{NH}_4^+\text{-N}$ ) from filter paper. Extracts were stored at 4°C until they were analysed for  $\text{NH}_4^+\text{-N}$  concentration. A parallel experiment was set up to measure the changes in surface soil pH, after applying urine immediately before inhibitor application, using 70 mL soils held in a plastic container (5 cm soil depth). The plastic container was then placed inside a 1 L glass Agee jar to create a similar conditions as in the  $\text{NH}_3$  measurement experiment. Soil pH was measured using a HI99121 Direct Soil Measurement pH Portable Meter (Hanna Instruments). The application rate and chemical properties of urine used in the soil pH experiment was the same as stage (b) of  $\text{NH}_3$  experiment. The application rates of urine, equivalent to total N in  $\text{kg ha}^{-1}$ , and chemical properties of urine used in all of the experiments are presented in Table 2.

**Table 2** Application rate and chemical properties of cow urine used in the study

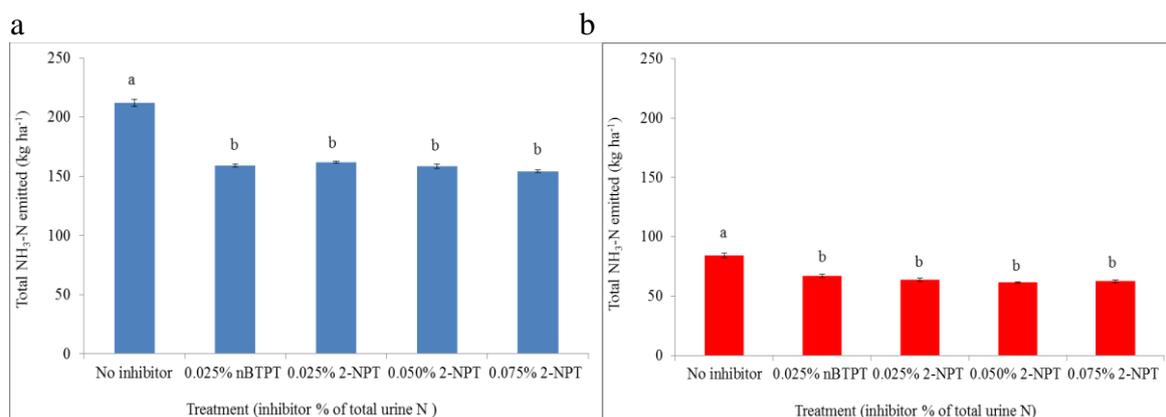
Stage and urine application time	N applied ( $\text{kg ha}^{-1}$ )	pH	Total N ( $\text{g L}^{-1}$ )	Urea-N ( $\text{g L}^{-1}$ )
a) Immediately before inhibitor application	593	8.2	5.9	4.7
b) 29 days after inhibitor application	580	8.4	5.8	4.4
c) 56 days after inhibitor application	1120	8.3	11.2	9.8
d) 29 days (b) and then reapplied at 60 days after inhibitor application	580 + 808	8.3	11.2	9.8

### *Statistical analysis*

The data for  $\text{NH}_3$  emissions were analysed using Analysis of Variance (ANOVA) to detect any significant difference and treatment means were compared using Tukey's Studentized Range (HSD) Test. All of the analyses were conducted using Statistical Analysis System software (SAS 9.4,  $p < 0.05$ ).

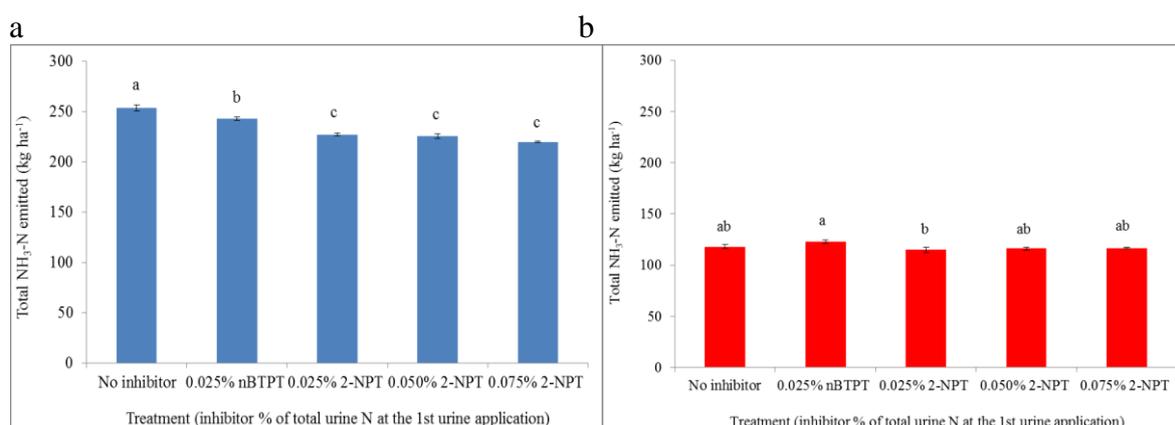
### **Results**

The total  $\text{NH}_3$  emitted from the Rangitikei soil was significantly higher compared to Egmont soil for the no inhibitor control treatment at all stages. Both of the inhibitors significantly reduced total  $\text{NH}_3$  emissions from urine applied immediately before inhibitor application, for both soils. The emissions reduction was 23.7 - 27.3 % for the Rangitikei soil (Fig. 1a) and 20.6 - 27.2% for the Egmont soil (Fig. 1b), compared to the no inhibitor control treatment. However, the difference between inhibitors was not significant.



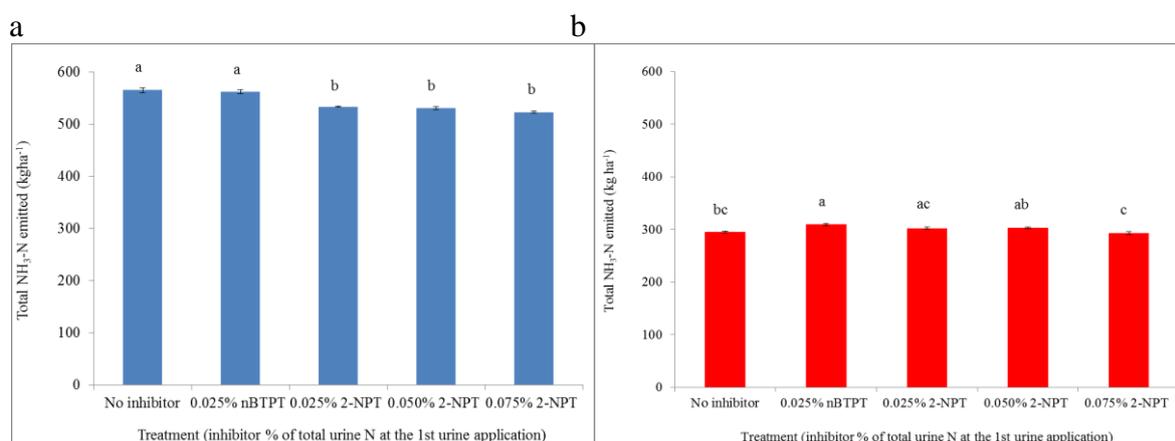
**Fig. 1** Changes in total NH<sub>3</sub>-N emissions from cow urine applied immediately before urease inhibitor application (first urine application) in the a) Rangitikei soil and b) Egmont soil (vertical bars indicate standard error values)

The inhibitors significantly reduced total NH<sub>3</sub> emissions by 4.2 to 13.4% when urine was applied 29 days after inhibitor application to the Rangitikei soils (Fig. 2a). The reduction with 2-NPT was significantly greater than with nBTPT. However, treatment effects were not significant in the Egmont soil (Fig. 2b).



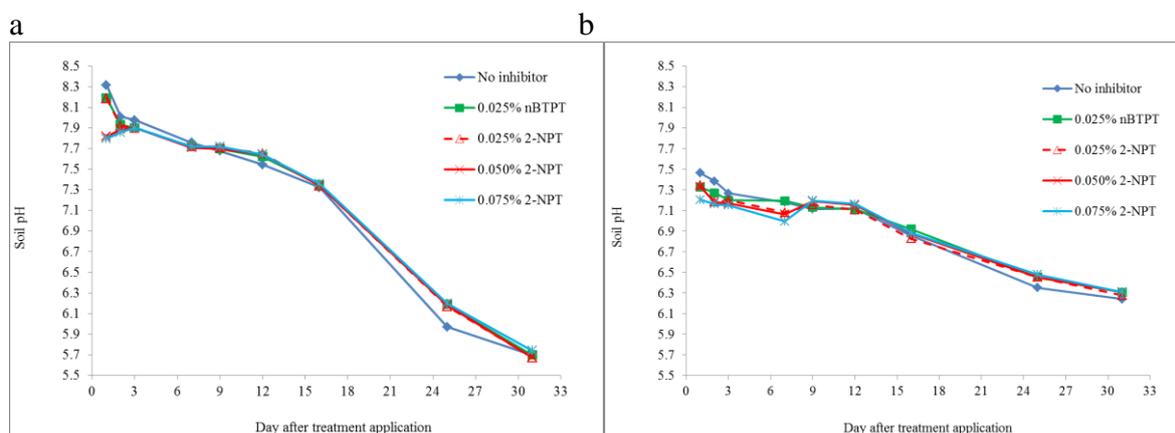
**Fig. 2** Changes in total NH<sub>3</sub>-N emissions from cow urine applied 29 days after urease inhibitor application in the a) Rangitikei soil and b) Egmont soil (vertical bars indicate standard error values)

The 2-NPT continued to reduce NH<sub>3</sub> emissions in the measurement period following the urine application at 56 days after inhibitor application on the Rangitikei soil with a reduction of 5.6 - 7.4% (Fig. 3a), but not with nBTPT. Treatment effects were not significant with the Egmont soil at this time either (Fig. 3b.). When urine was applied at 29 days after inhibitor application and reapplied at 60 days, there was no difference in NH<sub>3</sub> emissions from the second urine application between the treatments.



**Fig. 3** Changes in total  $\text{NH}_3\text{-N}$  emissions from cow urine applied 56 days after urease inhibitor application in the a) Rangitikei soil and b) Egmont soil (vertical bars indicate standard error values)

The changes in soil pH following urine applied immediately before inhibitor application to the Rangitikei and Egmont soils are shown in Fig. 4. The changes in surface soil pH was greater in Rangitikei soil and reached maximum of 8.3 on day 1 and then dropped down gradually for the no inhibitor control treatment (Fig. 4a). While with the Egmont soil, pH reached a maximum of 7.5 on day 1 and then dropped down gradually for the no inhibitor control treatment (Fig. 4b). By day 31, soil pH had almost returned to the background level of 5.6 with the Rangitikei soil for all the treatments. However, for Egmont soil the pH was still higher than background level of 5.7 by day 31 with the all treatments.



**Fig. 4** Effect of applying cow urine immediately before urease inhibitor application on soil pH of a) Rangitikei soil and b) Egmont soil

## Discussion

The significantly higher total  $\text{NH}_3$  emitted from the Rangitikei soil compared to the Egmont soil for the no inhibitor control treatment is attributed to lower buffering capacity and higher changes in the pH of Rangitikei soil (up to 8.3) compared with the Egmont soil (up to 7.5) (Fig. 4). Soil pH has influences on  $\text{NH}_3$  emissions by facilitating the dissociation of  $\text{NH}_4^+$  to gaseous  $\text{NH}_3$  in soil solution, which is ultimately emitted into the atmosphere (Saggar et al. 2004). Furthermore,  $\text{NH}_3$  concentration in soil solution is influenced by soil cation exchange capacity (CEC) via the reaction of  $\text{NH}_4^+$  with the negatively charged cation exchange sites (Bolan et al. 2004; Saggar et al. 2004). Lower CEC in the Rangitikei soil compared to Egmont soil, resulted in increased  $\text{NH}_4^+$  concentration in soil solution and ultimately enhanced  $\text{NH}_3$  emissions. Thus, resulting in higher  $\text{NH}_3$  emissions from Rangitikei soil than Egmont soil.

Results from this study indicated that the longevity of 2-NPT at inhibiting soil UA is extended compared with nBTPT. The application of 2-NPT significantly reduced  $\text{NH}_3$  emitted from applied urine in the Rangitikei soil up to 56 days, but not with nBTPT. Similar results have been reported by Domínguez et al. (2008) who found no urease inhibitions with nBTPT but 12% inhibition with 2-NPT after a 30 day incubation of soil with a pH 4.5. The lesser longevity of 2-NPT in the Egmont soil compared with the Rangitikei soil could partly be attributed to higher soil UA in Egmont soil relative to Rangitikei soil that may have exhausted the activity of the inhibitor faster. However, when urine was applied at 29 days after inhibitor application and reapplied at 60 days; there was no treatment effect on  $\text{NH}_3$  emissions during the measurement period following the second urine application to both soils. This is likely to be due to the activity of the inhibitors being exhausted by the first urine application.

## Conclusions

The urease inhibitor 2-NPT has the ability to extend the longevity of urease inhibition and reduce total  $\text{NH}_3$  emissions compared to the currently used inhibitor nBTPT.

## Acknowledgements

This research is supported by Manaaki Whenua Landcare Research New Zealand Limited with funding from Ballance Agri-Nutrients Limited.

## References

- Adhikari KP, Saggar S, Hanly JA, Guinto DF (2017) Understanding the ineffectiveness of Cu and Zn in reducing urea hydrolysis in grazed dairy pasture soils. In: Science and policy: nutrient management challenges for the next generation. (Eds L.D. Currie and M. J. Hedley). <http://flrc.massey.ac.nz/publications.html>. Occasional Report No. 30. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand
- Bolan NS, Saggar S, Luo J, Bhandral R, Singh J (2004) Gaseous emissions of nitrogen from grazed pastures: processes, measurements and modelling, environmental implications, and mitigation. *Advances in Agronomy* 84:37-120. doi: 10.1016/S0065-2113(04)84002-1
- Creason GL, Schmitt MR, Douglass EA, Hendrickson LL (1990) Urease inhibitory activity associated with N-(n-butyl)thiophosphoric triamide is due to formation of its oxon analog. *Soil Biology and Biochemistry* 22:209-211. doi: [http://dx.doi.org/10.1016/0038-0717\(90\)90088-H](http://dx.doi.org/10.1016/0038-0717(90)90088-H)
- Domínguez MJ, Sanmartín C, Font M, Palop JA, San Francisco S, Urrutia O, Houdusse F, García-Mina JM (2008) Design, synthesis, and biological evaluation of phosphoramidate

- derivatives as urease inhibitors. *Journal of Agricultural and Food Chemistry* 56:3721-3731. doi: 10.1021/jf072901y
- Hemida SK, Omar SA, Abdel-Mallek AY (1997) Microbial populations and enzyme activity in soil treated with heavy metals. *Water, Air, and Soil Pollution* 95:13-22. doi: 10.1007/BF02406152
- Hendrickson LL, Douglass EA (1993) Metabolism of the urease inhibitor n-(n-butyl)thiophosphoric triamide (nbpt) in soils. *Soil Biology and Biochemistry* 25:1613-1618. doi: [http://dx.doi.org/10.1016/0038-0717\(93\)90017-6](http://dx.doi.org/10.1016/0038-0717(93)90017-6)
- Hucke A, Niclas HJ, Wozniak H, Michel HJ, Schuster C (2010) N-phenylphosphoric acid triamides, method for the production thereof and their use as agents for regulating or inhibiting enzymatic urea hydrolysis: Google Patents.
- Laubach J, Taghizadeh-Toosi A, Sherlock RR, Kelliher FM (2012) Measuring and modelling ammonia emissions from a regular pattern of cattle urine patches. *Agricultural and Forest Meteorology* 156:1-17. doi: <http://dx.doi.org/10.1016/j.agrformet.2011.12.007>
- Livestock Improvement Corporation Limited, DairyNZ Limited (2016) New Zealand dairy statistics 2015-16.
- Manunza B, Deiana S, Pintore M, Gessa C (1999) The binding mechanism of urea, hydroxamic acid and N-(N-butyl)-phosphoric triamide to the urease active site. A comparative molecular dynamics study. *Soil Biology and Biochemistry* 31:789-796. doi: [http://dx.doi.org/10.1016/S0038-0717\(98\)00155-2](http://dx.doi.org/10.1016/S0038-0717(98)00155-2)
- Ni K, Pacholski A, Kage H (2014) Ammonia volatilization after application of urea to winter wheat over 3 years affected by novel urease and nitrification inhibitors. *Agriculture, Ecosystems and Environment* 197:184-194. doi: <http://dx.doi.org/10.1016/j.agee.2014.08.007>
- Saggar S, Bolan NS, Bhandral R, Hedley CB, Luo J (2004) A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. *New Zealand Journal of Agricultural Research* 47:513-544. doi: 10.1080/00288233.2004.9513618
- Saggar S, Singh J, Catto W, Stafford A, Blennerhassett J, Zaman M (2009) Desktop study of emission factors for urease inhibitors for nitrogen fertiliser.
- Saggar S, Singh J, Giltrap DL, Zaman M, Luo J, Rollo M, Kim DG, Rys G, van der Weerden TJ (2013) Quantification of reductions in ammonia emissions from fertiliser urea and animal urine in grazed pastures with urease inhibitors for agriculture inventory: New Zealand as a case study. *Science of the Total Environment* 465:136-146. doi: <http://dx.doi.org/10.1016/j.scitotenv.2012.07.088>
- Schraml M, Gutser R, Maier H, Schmidhalter U (2016) Ammonia loss from urea in grassland and its mitigation by the new urease inhibitor 2-NPT. *The Journal of Agricultural Science* 1-10. doi: 10.1017/S0021859616000022
- Schraml M, Gutser R, Schmidhalter U (2009) Abatement of NH<sub>3</sub> emissions following application of urea to grassland by means of the new urease inhibitor 2-NPT. Paper presented at the International Scientific Centre of Fertilizers
- Selbie DR, Buckthought LE, Shepherd MA (2015) The challenge of the urine patch for managing nitrogen in grazed pasture systems. *Advances in Agronomy* 129:229-292. doi: <http://dx.doi.org/10.1016/bs.agron.2014.09.004>

- Sherlock RR, Jewell P, Clough T (2008) Review of New Zealand specific FracGASM and FracGASF emission factors.
- Wyszkowska J, Kucharski J, Lajszner W (2006) The effects of copper on soil biochemical properties and its interaction with other heavy metals. Polish Journal of Environmental Studies 15:927.
- Zaman M, Nguyen ML, Matheson F, Blennerhassett JD, Quin BF (2007) Can soil amendments (zeolite or lime) shift the balance between nitrous oxide and dinitrogen emissions from pasture and wetland soils receiving urine or urea-N? Soil Research 45:543-553. doi: <http://dx.doi.org/10.1071/SR07034>