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**Availability of data and material**

Upon reasonable request, the datasets of this study can be available from the corresponding author.

**Authors’ contributions**

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This article does not require IRB/IACUC approval because there are no human and animal participants.

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Potential Application of Urease and Nitrification Inhibitors to Mitigate Emissions from the Livestock Sector: A Review

Abstract
Human activities have caused an increase in greenhouse gas emissions, resulting in climate change that affects many factors of human life including its effect on water and food quality in certain areas with implications for human health. CH$_4$ and N$_2$O are known as potent non-CO$_2$ gases. The livestock industry contributes to direct emissions of CH$_4$ (38.24%) and N$_2$O (6.70%) through enteric fermentation and manure treatment, as well as indirect N$_2$O emissions via NH$_3$ volatilization. NH$_3$ is also a secondary precursor of particulate matter. Several approaches have been proposed to address this issue, including dietary management, manure treatment, and the possibility of inhibitor usage. Inhibitors, including urease and nitrification inhibitors, are widely used in agricultural fields. The use of urease and nitrification inhibitors is known to be effective in reducing nitrogen loss from agricultural soil in the form of NH$_3$ and N$_2$O and can further reduce CH$_4$ as a side effect. However, the effectiveness of inhibitors in livestock manure systems has not yet been explored. This review discusses the potential of inhibitor usage, specifically of N-(n-butyl) thiophosphoric triamide, dicyandiamide, and 3,4-dimethylpyrazole phosphate, to reduce emissions from livestock manure. This review focuses on the application of inhibitors to manure, as well as the association of these inhibitors with health, toxicity, and economic benefits. Keywords: Livestock emissions, GHG emissions, Urease inhibitor, Nitrification inhibitor, Particulate matter
1. Introduction

Anthropogenic activities have led to the production of large amounts of greenhouse gases (GHGs) such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), which has resulted in climate change and global warming. Human activities are estimated to have caused global warming of approximately 1 °C above pre-industrial levels, ranged between 0.8 °C to 1.2 °C [1]. The total amount of GHG emissions in 2018 for developed countries (Annex 1 parties) was 16,794,455.9 kt CO₂ equivalent (CO₂-eq) [2]. CH₄ is an extremely potent GHG, responsible for approximately 30% of warming since pre-industrial times [3]. It has a global warming potential of 38 CO₂-eq over a 100-year time horizon, making it an important non-CO₂ GHG. Human-caused CH₄ emissions are predominantly from three sectors: fossil fuels, waste, and agriculture. N₂O is another potent GHG because of its 100-year global warming potential of 100 CO₂-eq. N₂O damages the environment and is known to contribute to the depletion of the ozone layer.

Global climate change affects human health, livelihoods and ecological and human systems, resulting in global monetary damage. The IPCC [1] indicated five reasons for concern that point up the risk of global warming at different level, including its impact on human, economies, and ecosystem. At present, the risk transitions of global warming range from moderate to high risk, between 1 °C and 2 °C. The Paris Agreement in 2015 was adopted to set the long-term goals to limit the global temperature increase to 2°C in this century while also attempting further limitation to 1.5 °C [4]. To be on track toward the Paris Agreement, global GHG emissions should reduce by 7.6% each year between 2020 and 2030 [3].

According to Annex I countries’ CH₄ and N₂O emissions, livestock sector (manure management and enteric fermentation) was responsible for 38.24% and 6.70% of total CH₄ and N₂O emissions, respectively, where CH₄ from livestock sector was responsible of approximately 95.4% of the total agricultural CH₄ emissions [2]. Also NH₃ emitted from livestock sector was the source of indirect N₂O emissions and NH₃ is a secondary precursor of particulate matter (PM) and contributes to the overall PM burden [5]. The sustainability of livestock production is necessary for continuity of human life and by targeting non-CO₂ for mitigation, agricultural CH₄ and N₂O emissions could be reduced; therefore, the mitigation of GHG emissions from this sector is crucial and in urgent need of being addressed.

2. Emissions from the Livestock Sector

2.1. Direct Emissions
Agriculture is one of the main contributing sectors of CH$_4$ and N$_2$O. Agriculture contributed approximately 9.27% of the total global emissions in 2019 (Annex 1 parties) [2]. Among the emissions from agriculture, 50.18% came from livestock. Emission from the livestock industry is a by-product of the digestive system of ruminants, in the form of CH$_4$ through enteric fermentation and as CH$_4$ and N$_2$O through manure handling. Livestock product demand is predicted to grow by 70% in 2050, resulting in significant increase in GHG emissions from livestock [6]. Therefore, it is important to mitigate emissions from the livestock industry.

CH$_4$ production is caused by microbial fermentation that hydrolyze carbohydrates, and is an energy loss [7]. Methanogenesis generates CH$_4$ and methanogens, a group of obligate anaerobic archaeabacteria that are chemoautotrophs [8], are responsible for this process [9]. These methane producers are strict anaerobes and pH sensitive, with an optimum pH range of 6.8 to 7.4, and function best at 95 °F [10].

In 2018, the contribution of GHG emissions from enteric fermentation reached 85% of the total livestock’s GHG emissions [11]. CH$_4$ from enteric fermentation is a byproduct of the digestive system in ruminants and is released during eructation; approximately 87%-90% is formed in the rumen and the remaining 13%-10% in the large intestine [12]. CH$_4$ emissions from cattle is seven times higher than that from sheep and nine times higher than that from goats [12]. CH$_4$ production in the rumen is affected by dietary factor and genetic factor [13].

Nitrification is a process that converts NH$_4^+$ to NO$_3^-$ through microbial action [14]. This is a two-step chemolithotrophic process whereby NH$_4^+$ is first oxidized to NO$_2^-$ by NH$_3$-oxidizing bacteria, followed by oxidation to NO$_3^-$ by nitrate-oxidizing bacteria [14]. Denitrification requires the conversion of NO$_3^-$ to NO$_2^-$ in the absence of oxygen by the enzyme nitrate reductase, then nitric reductase converts NO$_2^-$ to NO. Then, NO is converted to N$_2$O by nitric oxide reductase, and finally, N$_2$O is converted to N$_2$ gas by nitrous oxide reductase.

Low pH inhibits reductase enzyme and compare to the other enzymes, it is even more sensitive to oxygen in the denitrification pathway [9].

Manure handling and storage are the source of livestock CH$_4$ and N$_2$O emissions. However, on pastures or rangeland, N$_2$O losses is more considerable than CH$_4$ emissions that can be very low [15]. However, CH$_4$ emissions may be highly significant in housed indoor house or on feedlots, and manure storage may be required. N$_2$O emissions originate largely from denitrification of N soils arising from fertilizers and urinary deposits, and to a lesser extent from sources of N resulting from leaching, runoff, and volatilization.

2.2. Indirect Emissions
In addition to direct emissions, livestock also contributes to indirect emissions in the form of N\textsubscript{2}O emissions. Indirect N\textsubscript{2}O emissions account for one-third of the total global agricultural N\textsubscript{2}O sources. In theory, indirect emissions consist of five different sources, including volatilization and subsequent atmospheric deposition of NH\textsubscript{3} and NO\textsubscript{x} [16]. Indirect N\textsubscript{2}O emissions may arise through deposition of NH\textsubscript{3} volatilized from manure. The indirect N\textsubscript{2}O emissions, however, may also arise from the NH\textsubscript{3} deposited and NO emitted during manure management and application [17]. NH\textsubscript{3} is generated through urea hydrolysis during manure deposition. Urea is very stable, and it degrades so slowly without urease that its degradation is negligible. Urea in synthetic fertilizer does not come into contact with urease until it is applied to the field [18]; therefore, the application of livestock manure as an organic fertilizer is likely to accelerate NH\textsubscript{3} volatilization because of the urease present in feces. Urea hydrolysis also occurs in the presence of urease produced by bacteria in the soil, which results in the emission of NH\textsubscript{3}. Urea hydrolysis occurs when urinary urea is catalyzed by urease in feces, resulting to the conversion of urea to NH\textsubscript{3} and CO\textsubscript{2}. Urinary urea N is the source of NH\textsubscript{3}-N, and microbial urease in feces hydrolyzes it to NH\textsubscript{3} and CO\textsubscript{2} [19]. The mixing of feces and urine promotes hydrolysis [20] and occurs rapidly within 1 to 2 days of excretion [21]. Urease concentration is known to be the highest in chicken manure, compared to that in pig and cattle manure, during the initial composting process [22]. The concentration of urea N ranges between 50% and 90% of the total N [21,23].

Ruminants excrete nitrogen in which can be loss as NH\textsubscript{3} for more than 50%. This significant amount of NH\textsubscript{3} emissions is attributed to the formation of PM with an aerodynamic diameter smaller than 2.5 µm (PM\textsubscript{2.5}) [24]. The contribution of PM\textsubscript{2.5} to air pollution occurs through complex process. Primary particles interact with gaseous precursors, followed by photochemical transformation pathways and lastly, transport and deposited as PM\textsubscript{2.5} by meteorological process [25]. Organic carbon and sulfate control the formation of PM when NH\textsubscript{3} presents in excessive amount [26]. Livestock operations contribute to PM\textsubscript{2.5}, and PM\textsubscript{10}. PM\textsubscript{10} is a term for particles with an aerodynamic diameter ≤ 10 mm. Direct PM\textsubscript{10} is emitted as dust, and the reaction of NH\textsubscript{3} with nitrate and sulfuric acids forms indirect PM\textsubscript{10} [24]. In the atmosphere, NH\textsubscript{3} can bind to other gases, such as SO\textsubscript{2} and nitrogen oxides (NO and NO\textsubscript{2}) to form NH\textsubscript{4}\textsuperscript{+} containing fine PM [27]. This fine PM affects health when inhaled. PM\textsubscript{2.5} formed by NH\textsubscript{3} can penetrate deeper into the respiratory system of humans and animals where they damage tissues [28]. Although the average effect on lung function is modest, peak exposures of NH\textsubscript{3} may cause airway symptoms in vulnerable subjects [29].
Studies on the PM$_{2.5}$ reduction through NH$_3$ control have been performed. Over the eastern USA in July and January, a 4% and 9% decrease in PM$_{2.5}$ was caused by the reduction of NH$_3$ by 50% [30], whereas in Italy, [31] showed that a reduction of 50% in NH$_3$ emissions from agriculture could result in a decrease in PM$_{2.5}$. Pozzer et al. [25] also showed that a 50% decrease in NH$_3$ emissions could reduce the annual, geographical average of near-surface PM$_{2.5}$ concentration by 2% to 11%. These studies confirm that the reduction in NH$_3$ emissions is the most effective control strategy for mitigating PM$_{2.5}$.

3. Mitigation of Emissions from Livestock

3.1. Dietary Management

The single most effective way to mitigate GHG emissions is to increase animal productivity. Thus, reducing animal numbers may provide the same edible product output with a reduced environmental footprint [32]. Dietary management has been widely used and is the most effective method to reduce CH$_4$ from enteric fermentation. Overall dietary manipulation by selection and utilization of high quality forages, strategic supplementation of forages, changing concentrate, proportion with special emphasis on changing carbohydrate composition should be considered as an immediate and sustainable methane mitigation approach of enteric CH$_4$ emitted from ruminant livestock [33]. Methane emissions decrease in all regions when amended diets are adopted because more forage-based diets are less digestible than more concentrate grain-based diets [34].

Haque [33] divides dietary strategies into two categories: 1) improving forage quality and changing the diet proportion, and 2) dietary supplementation with feed additives. Although these strategies have been demonstrated to be effective, some obstacles are encountered. For instance, adding more grain in ruminant ration can be profitable because this strategy increases milk production, meat production, and also reduce the environmental footprint of livestock; however, the sustainability of this approach in the long term is questionable [32]. In some regions, grazing management may not be the best option to improve animal productivity due to poor pasture quality, in that case, improvement in productivity must come through feeding preserved forage or concentrate [32]. Some feed additives, known as inhibitors, are used to reduce methanogenesis by inhibiting methanogen activity. This includes supplementing with anti-methanogenic agents (e.g., antibiotics that reduce the methanogen population) or supplementing with electron (H$^+$) acceptors, such as nitrate salts [35]. Among additives, the most promising results have been with nitrate and 3-nitrooxypropanol; however, more research is needed to fully document the implications for environmental and animal health [36]. Although demonstrated to be effective in
reducing CH\textsubscript{4} emissions, these strategies may disrupt natural rumen processes, and pose potential health and other welfare challenges [35]. High-concentrate supplementation increases milk production and utilization of genetic potential of the animal. However, when the price of milk is lower than feed cost, this system may not be economically feasible [32].

3.2. Manure Treatment

NH\textsubscript{3} and GHG emissions from animal facilities are influenced by several factors, i.e. method of collecting manure, type of manure storage, type of housing, manure separation, and manure processing [15]. Mitigation practice for GHG emissions from stored manure mostly includes reducing storage time, air circulation (aeration), and stockpiling. These practices are intended to shorten fermentation process before land application [15]. These practices are found to be effective, but the practices is unclearly economically advantageous [15].

3.3. Inhibitors

In recent years, mitigation technologies, including the use of inhibitors such as urease inhibitors and nitrification inhibitors, have been explored to reduce emissions and nitrogen losses from agricultural fertilizer usage, and inhibitors have already been approved and are currently in the market [37]. Livestock manure is a rich source of organic compounds. Owing to this nutritional content, livestock manure is commonly used as fertilizer on agricultural soil or land. However, this practice may accelerate NH\textsubscript{3} volatilization because of the higher urease content in the manure than in the soil, which promotes the formation of indirect N\textsubscript{2}O. The loss of nitrogen affects the nitrogen content of the soil, which may result in low yield production. Due to significant nitrogen losses from manure management systems, estimating the remaining amount of nitrogen in the manure is important, mainly for soil application or other purposes such as feed, fuel, or construction [38]. According to IPCC [38], N\textsubscript{2}O emissions generated by manure in the pasture system, range, and paddock occur directly and indirectly from the soil.

4. Urease and Nitrification Inhibitors

4.1. Urease Inhibitors

The main principle of urease inhibitors is to deactivate urease, which hydrolyses urea into NH\textsubscript{4}\textsuperscript{+}, so that the hydrolysis of urea is delayed; hence, in the interim, several treatments can be performed to reduce the potential of
nitrogen loss. There are several types of urease inhibitors. N-(propyl) thiophosphoric triamide (NPPT) is known to improve NH$_3$ volatilization; however, the application of NPPT is mostly in combination with N-(n-butyl) thiophosphoric triamide (NBPT), and several studies have revealed that NPPT is suspected to be a reproductive toxin [37]. N (2-nitrophenyl) phosphoric triamide (2-NPT) is a new urease inhibitor that is under development. The application of NPT has been shown to lower NH$_3$ volatilization by 89% after 19 days of incubation [39], and depending on the soil characteristics, it also has greater longevity than NBPT [40]; therefore, the inhibitory effect of 2-NPT may last longer than that of NBPT. However, currently, 2-NPT is still on a laboratory-scale production; therefore, it is not easily available in the market and for field purposes.

NBPT is currently the most widely used urease inhibitor. NBPT blocks three active sites of the urease enzyme to form a tridentate bond. This bond consists of two nickel centers and one oxygen atom from the carbamate bridge linking both metal ions, which reduces the probability of urea reaching the active nickel center of the urease enzyme. NBPT must be converted into N-(n-butyl) phosphoric triamide (NBPTo), as it is not a direct inhibitor. The factors influencing this conversion are not clear, but the reaction is rapid in soils under aerobic conditions (occurring in minutes or hours) but can take days under anaerobic conditions. The direct application of NBPTo is inefficient because it degrades faster than NBPT.

NBPT is pH labile, and chemical hydrolysis appears to be an essential function of its breakdown under acidic conditions. This study concluded that under acidic conditions, chemical hydrolysis is likely the dominant pathway for NBPT and NBPTo breakdown. Under alkaline conditions, the biotic breakdown of these compounds via microorganisms became more significant [37].

NBPT mitigates NH$_3$ volatilization by controlling the rise in pH that occurs during urea hydrolysis, resulting in the production of two units of NH$_4^+$ and CO$_2$ and reducing the soil concentration of NH$_4^+$ around the urea granule [37]. These processes affect the equilibrium of NH$_4^+$ (soil), NH$_3$ (soil), and NH$_3$ (gas), which results in slow urea hydrolysis and ultimately allows ample time for the fertilizer to be incorporated into the soil via rainfall or irrigation, thereby protecting the applied N from volatilization [37]. Inhibition of urease by NBPT usually lasts 3 to 7 days as new urease enzyme production overwhelms the inhibitor [37].

The NBPT shows a relatively short period of protection. The ideal situation for the performance of urease inhibitors is through mechanical incorporation, followed by rain or irrigation occurring within 5 to 7 days after fertilization with NBPT-contained urea. In this period, depending on soil moisture or temperature, inhibitory
potential of NBPT is still high [41]. The results of field studies showed reductions of > 85% in NH$_3$ volatilization as a result of NBPT application and rain event within 5 days after urea application [41].

A study conducted by Engel et al. [42] showed that application of urea of wet or damp soil, NH$_3$ loss was significant. This study indicates that NH$_3$ loss was significantly affected by water conditions. Application of NBPT delayed the peaks of NH$_3$ loss until 7 to 9 days, whereas without NBPT application, the highest loss occurred on day 3. Not only delayed the peak of NH$_3$ loss, NBPT also reduced the peaks of NH$_3$ loss [43]. The conversion of urea to NH$_3$ is prevented by the addition of NBPT, resulting to urea buildup in the manure. NBPT, on the other hand, has limited effect as time passes, therefore, in order to hydrolyze the build-up urea, more NBPT may be required [44]. Previous research has shown that high temperature affects the inhibiting ability of NBPT; Pereira et al. [45] reported that at a temperature of 20 °C, NBPT was inhibited in a short time.

4.2. Nitrification Inhibitors

Nitrification inhibitors are chemical compounds that delay the bacterial oxidation of NH$_4^+$ to NO$_2^-$ in the soil, called nitrification. Nitrification inhibitors work by slowing down nitrifying bacteria that produce ammonia monoxygenase, hydroxylamine oxidoreductase, and nitric oxide reductase [37]. Delays in nitrification result in less NO$_3^-$ formation, which is considered to be the source of nitrogen losses through leaching and denitrification (N$_2$O); therefore, the use of nitrification inhibitors not only reduces environmental problems but also increases the efficiency of nitrogen-based fertilizer. Several studies also demonstrated that CH$_4$ emissions could be reduced as a side effect of nitrification inhibitor application (Table 1).

Some examples of nitrification inhibitors are dicyandiamide (DCD), 3-4, dimethylpyrazole phosphate (DMPP), nitrapyrin, and thiosulfate. Thiosulfate may delay urea hydrolysis for up to 4 days and retard the conversion of NO$_2$-N to NO$_3$-N, thus resulting in a substantial amount of NO$_2$-N in the soil. However, to achieve a significant reduction in N$_2$O, high concentrations need to be applied, making it inefficient in reducing N$_2$O emissions.

Nitrapyrin is widely used in the United States. It has been shown to reduce GHG emissions by 30%–50% [46]. It can also be retained in water for 7 to 10 days, whereas in soil it remains for 3 to 35 days [47], which implies that it can inhibit GHG emission production over an extended period. Even so, the use of nitrapyrin should be limited because of its low water solubility, and the results of nitrapyrin application differ depending on environmental conditions. Nitrapyrin is categorized as a moderate oral toxin and moderate dermal irritant [47].
There are conflicting results regarding the influence of nitrification inhibitors on CH₄ emissions. Bronson and Mosier [48] and Crill et al. [49] reported that nitrification inhibitors may increase CH₄ emissions, whereas Weiske et al. [50] reported that the addition of DCD either reduced the emissions or had no effect on CH₄ emissions. Another study by Villarrasa-Nogué [51] showed that the application of DMPP tended to reduce CH₄ oxidation, resulting in high CH₄ emissions.

### 4.2.1. Dicyandiamide

The breakdown of DCD results in NH₃, NO₃, H₂O, and CO₂, which may also contribute to increased N availability for microbial growth, as indicated by augmented CO₂ [52]. The kinetics of DCD degradation are highly influenced by temperature [52]. Minet et al. [53] found that DCD was still active after 6-month post application. Moreover, DCD did not affect the composition of the slurry during the period and cumulative N₂O-N emissions from DCD treatment was 88% lower than without DCD, which implies that DCD effectively reduced N₂O emission. The stability of the DCD (during the 6-month period) indicates that DCD does not degrade when the slurry is stored under anaerobic conditions. Mixing stored slurry with DCD could be a means to mitigate N₂O emissions at high-risk times such as in autumn, winter, and early spring, where N₂O emissions could be at their highest. DCD addition to slurry could be highly preferred, cost-effective, and efficient for widespread adoption of N₂O mitigation using nitrification inhibitors by the agricultural sector [54].

DCD was more effective in reducing N₂O emissions and NO₃⁻ leaching from urine depositions during autumn than during summer or spring [55]. DCD in solid form is suggested to be applied at rates of 0.44% to 0.88% of the dry matter of composting piles (swine slurry with sawdust) with reapplication within 15 to 23 days to prevent later N₂O emissions as DCD concentrations decrease during the composting process [52]. The application of DCD with urine in both autumn and winter was effective in reducing the peak N₂O fluxes and the total amount of N₂O from urine application [56]. Increased DCD application rates would be required to sustain DCD concentrations in the surface soil above the critical level for extended periods in order to achieve a significant reduction in N₂O emissions from urine patches [56]. Application of DCD through mixing with animal urine prolonged the presence of NH₄⁺ in the soil by approximately 3 to 6 weeks, which led to a reduction in the concentration of NO₃⁻-N by approximately 70%–85% [56].

Theoretically, inhibiting nitrification with DCD might also inhibit CH₄ oxidation to CO₂; however, the result from Minet et al. [53] showed that DCD application to slurry displayed lower CH₄ cumulative net flux than slurry
without DCD application. The application of NBPT, phosphoroamide (PPD), and DMPP together with pig manure resulted in significantly reduced cumulative CH$_4$ emissions, because the addition of inhibitors further influenced the existing forms of nitrogen, which is beneficial to the growth of methanotrophic organisms and results in increased CH$_4$ oxidation [57]. Anaerobic conditions may prolong DCD persistence, and although the reasons for this are unclear, DCD degradation is unlikely to occur under anaerobic conditions [53].

### 4.2.2. 3-4 Dimethylpyrazole Phosphate

The DMPP with non-split application resulted in a more efficient reduction of N$_2$O losses than split application [58]. The DMPP treatment seemed to stimulate CH$_4$ oxidation more than DCD treatment because the soil clearly acted as a CH$_4$ sink rather than as a source [50]. Significant reduction due to inhibition of nitrification may take more than a week after DMPP addition [59]. DMPP could increase soil N retention, improve plant N use efficiency, and potentially stimulate the shoot yield of tea trees [59]. Although data related to DMPP are limited, DMPP has potential as an alternative nitrification inhibitor.

The application of DMPP was found to be more efficient than that of DCD. Compared to DCD, DMPP applied at very low rates (one-third application rate) resulted in comparable or improved inhibitory effects on N$_2$O emission [60]. DMPP decreased the amount of N$_2$O released on average by 49%, whereas DCD reduced N$_2$O emissions by only 26%, although DMPP was applied at rates ca. 10 times lower than that of DCD [50]. At high N doses, mitigation of DMPP was not observed, possibly because nitrogen has a priming effect that if microbial activity increases sufficiently, the surplus N threshold is reached above which the effectiveness of DMPP application is lost [51].

### 4.3. Toxicity and Safety Concerns

#### 4.3.1. Toxicity in Plants

DMPP is safe and without any phytotoxic damage. A study conducted by Zerulla et al. [61] revealed that an overdose of DMPP (8 times higher than the recommended application rate) did not cause any symptoms, while pronounced symptoms were found in the plant with overdose application of DCD. Tindaon et al. [62] concluded that the use of DCD and DMPP is environmentally compatible and safe. In addition, the recommended application rate of DCD is 10 kg DCD per ha per application and that for DMPP is 1.84 kg active ingredient/mg urea or 0.71
microgram DMPP/kg soil [62]. Both DCD and DMPP may affect non-target microbial soil only at high concentrations.

4.3.2. Residues in Agricultural and Animal Products

Despite the fact of benefits associated with the use of urease and nitrification inhibitors, safety related to their residues in agriculture and animal products is debatable. In 2013, food safety concerns were raised regarding the use of DCD, which appeared as a residual contaminant in dairy products (Table 2) [37]. The MPI [63] reported that low-level residues of DCD were found in milk powder; however, there were no other reports on residues in other animal products. A study demonstrated that administration of DCD to dairy cows at 3 or 30 g DCD/cow/day was predominantly recovered in urine (61%–82%), feces (10%–19%), and milk (1.2%) [64]. This may be because of the residence time of DCD in plants. The residence time of DCD in plants was long in tall plants and under low rainfall conditions; therefore, the consideration in plant height and rainfall should be taken when selecting DCD application time to maximize the effectiveness of DCD [65]. Thus, contamination of animal products with DCD may be avoided when the animal eats the grass after DCD is fully degraded. Cai et al. [55] recommends to apply inhibitors before urine excretion. This method would be more efficient than other application method, i.e. at other timing.

In contrast, the NBPT is safe and has no influence on animal products (Table 2). A study conducted by Van De Ligt et al. [66] showed that there was no residue found in milk and bovine tissue from dairy cows fed with 1, 3, and 10 mg/kg body weight NBPT. The dose of NBPT was assumed from the maximum tolerable amount of urea (approximately 1 g/kg body weight) that a cow can consume on a daily basis and the maximum concentration of commercial NBPT for urea (0.1 % w/w NBPT in urea) [67].

4.3.3. Hazards to Animal and Human Health

Urease and nitrification inhibitors are not considered harmful, either to animals or humans; however, several precautions are needed when handling the substance owing to its possible hazard risk (Table 2). A study by Van De Ligt et al. [66] concluded that a high dose of NBPT fed to dairy cattle did not result in any harm. The possibility of urea toxicity to occur is rare, despite the fact that consuming NBPT in high level causes urea toxicity [36]. In 2006, NICNAS [67] reported that two workers became ill after handing NBPT with the trade name AGROTAIN® with the following symptoms: nausea and nose bleed. The following investigation revealed that
there was no mechanical exhaust in the room during installation and calibration of AGROTAIN®-urea spray application system. Although the workers were wearing respirators with the recommended cartridge, they reported that after several hours of work, they could smell the product. The work was continued, and the same cartridges were used for two and half days. The ensuing investigation revealed that because of the saturated cartridges, the respirator failed to perform. No exposures were reported by the employees, and no symptoms were reported by the production workers. Following the event, the company amended the current product label to read “Apply product with coarse spray only. Do not atomize.”

The ECHA [68] lists DCD under the name cyanoguanidine. According to ECHA [66], DCD is relatively low-hazardous for short-term or long-term exposure. However, caution is needed because it is an eye irritant. DMPP is non-hazardous, but it is considered to be low-hazardous if swallowed (oral exposure) and an eye irritant. Therefore, increased caution is needed. However, even though NBPT is low-hazardous, it is considered safe to use.

The DCD has a log octanol-water partition coefficient of -1 and is highly water soluble; therefore, it is unlikely to be taken up by fish gills or across other biological membranes [68]. However, DCD is not regarded as readily biodegradable in water; thus, the accumulation of DCD may occur, which may harm aquatic life. Information on bioaccumulation in aquatic environments or sediments is unavailable; thus, further research is needed to meet these criteria. NBPT is not considered to have a low potential for bioaccumulation [68].

4.4. Potential Use of Inhibitors in the Livestock Sector

The global population is estimated to increase to 9 billion people by 2050, and to ensure global food security, global agricultural production is expected to increase by approximately 100% [69,70]. Chemical fertilizers and organic manure are often applied in exceeding amount, leading to nitrogen loss, accounting for approximately 55% of the total applied N [71]. A significant amount of nitrogen loss not only has major consequences on human and environmental health, but also a significant economic loss for farmers.

The use of enhanced efficiency fertilizers prepared with coatings of low-permeability materials with an inhibitor attached as an additive may be used to reduce nitrogen loss and increase N uptake by plant and soil microbial populations [71]. Several studies have shown that with the addition of urease or nitrification inhibitors, plant yield is increased more than that without the use of such inhibitors. Adding DMPP at a rate of 120 kg/N resulted in a 7% increase in rice yield [72] and a 13% increase in wheat yield [73]. Other studies have shown that the addition
of NBPT increased rice yield by approximately 1%–3% [74] and increased wheat yield by as much as 1% [75].

The addition of DCD also increased yield. Kakabouki et al. [76] concluded that cotton yield increased by approximately 364 kg/ha or 8% more than that without DCD. The addition of inhibitors is not only a feasible mitigation option, but also economically beneficial if applied correctly. Laboski [77] showed that when N is relatively inexpensive, if a 20% nitrogen loss occurs, the return would be maximized with additional N application; however, in a situation where N is expensive, adding NBPT (AGROTAIN®) is more likely to be profitable.

Modern agricultural practices have been well documented to impart negative impacts on human health as well as on farms, and the practice of irrational and excessive use of chemical fertilizers and pesticides has inspired the search for alternatives [78]. The use of manure as fertilizer has become increasingly common in the past few years, and is known to be environment-friendly because the application of manure as fertilizer can improve soil composition. Manure plays an important role in regulating plant growth, potential nutrient input, and microbial decomposition activity. This role can largely mediate the soil nutrient and soil micro-environment, which have a strong influence on crop growth. In addition, manure could also result in increased microbial biomass and changes in community structure, which provide an improved environment for crop growth [79]. Hua et al. [80] revealed that the application of manure resulted in considerable beneficial income, both in terms of yield and N uptake. This is owing to the increase in nutrient and organic matter availability in the soil as a result of manure as a nutrient source. Moreover, with long-term applications, the use of organic fertilizer can maintain nutrient balance and soil physical properties. In tomato plants, the addition of poultry manure significantly influenced tomato stem girth and the mean weight of the fruit [81]. Long term application of dairy manure (> 5 years) to soil resulted in significant increases in C, N, and microbial biomass, and changes in the microbial community structure. Practices that enhance soil carbon and provide slowly mineralizable nutrients may result in a larger and potentially more robust microbial community.

A laboratory study conducted by Varel [82] implied that the addition of urease inhibitor in cattle and swine waste was very effective in inhibiting urease activity. The addition of phenyl phosphorodiamidate (PPDA) prevented up to 70% urea hydrolysis in cattle waste and up to 92% in swine waste [82]. Prolonged inhibition can be obtained by the weekly addition of inhibitors [82]. This result was validated in a field study indicating that NBPT can be successfully used to inhibit urease activity in cattle feedlot manure [83], especially because the results obtained in the study with the open environment of the feedlot surface were encouraging. The open environment is more difficult to control due to exposed weather elements than other manure-handling systems, such as enclosed
environments (pits with slotted floors). For instance, NBPT application to pit slurry is less complicated than application to a feedlot [83]. Application of NBPT causes urea build-up of urea in manure [83]. Dairy cows fed with DCD resulted in media concentrations of DCD in urine patches and were found to significantly reduce NO$_3$-N leaching and N$_2$O emissions by ±45% [56]. Slurry in mixture with DCD in long period of storage weaken the methanogens yet strengthen the methanothrophs [53]. Several manure treatment practices tend to produce more N$_2$O while reducing CH$_4$, in particular, treatment that includes air infusion, such as aerobic digestion or composting. N$_2$O emission mitigation by nitrification inhibitors can only be effective when the nitrification activity is essential, and the control of N$_2$O is in favor of emissions [84]. The addition of nitrification inhibitors to several manure treatment practices may be useful to reduce N$_2$O emissions and reduce CH$_4$ emissions as a manure treatment function.

5. Conclusions

The use of urease and nitrification inhibitors has been recognized as a mitigation tool to reduce nitrogen loss in agricultural soils. The application of inhibitors in agricultural soils decreases NH$_3$, N$_2$O, and CH$_4$ as a side effect; and yet, increases plant yield and nitrogen use efficiency. Although several concerns related to health and toxicity, either to humans, animals, or the environment, have been raised, both inhibitors have potential for long-term mitigation. However, further studies are required to confirm the safety of these inhibitors. Sufficient number of studies are lacking to understand the mechanisms of inhibitor application to livestock manure. In contrast, the use of livestock manure as fertilizer has been shown to be as effective as chemical fertilizers; moreover, such application is also known to improve soil composition and properties. However, manure application may accelerate NH$_3$ volatilization and, as a result, promote N$_2$O emissions. Several studies have also shown a positive effect of the application of inhibitors to manure on reducing emissions from livestock. Therefore, the use of inhibitors is likely to be effective and is considered to be an alternative mitigation method to reduce emissions from the livestock industry, either as an additive in organic fertilizer from manure or as an additive to manure treatment.

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<table>
<thead>
<tr>
<th>Inhibitor Type</th>
<th>Target</th>
<th>Fertilizer Form</th>
<th>Reduction Effect (%)</th>
<th>Application Rate (g/kg N)</th>
<th>Application Frequency</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urease Inhibitor</td>
<td>NBPT</td>
<td>Land Urea</td>
<td>NH$_3$</td>
<td>50-78</td>
<td>X</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Land Cow urine</td>
<td>NH$_3$</td>
<td>48</td>
<td>X</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Land Cow urine</td>
<td>CH$_4$</td>
<td>X</td>
<td>X</td>
<td>45-80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Land Cow slurry</td>
<td>CH$_4$</td>
<td>X</td>
<td>X</td>
<td>47-88</td>
</tr>
<tr>
<td></td>
<td>DCD</td>
<td>Land Swine slurry</td>
<td>CH$_4$</td>
<td>X</td>
<td>X</td>
<td>70</td>
</tr>
<tr>
<td>Nitrification Inhibitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Land Urea</td>
<td>N$_2$O</td>
<td>X</td>
<td>X</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Land Urea</td>
<td>N$_2$O</td>
<td>X</td>
<td>X</td>
<td>30-49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Land Urea</td>
<td></td>
<td>X</td>
<td>X</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Land Ammonium Sulfate</td>
<td></td>
<td>X</td>
<td>X</td>
<td>48.9-74.9</td>
</tr>
</tbody>
</table>

NBPT, N-(n-butyl) thiophosphoric triamide; DCD, dicyandiamide; DMPP, 3-4 dimethylpyrazole phosphate
Table 2. Hazards and ecotoxicology of nitrification and urease inhibitors

<table>
<thead>
<tr>
<th>Type of Inhibitor</th>
<th>Hazard Risk</th>
<th>Ecotoxicology</th>
<th>Residues in Animal Products</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urease Inhibitor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBPT</td>
<td>a. Causes serious eye damage(^{[95]})</td>
<td>Low acute in aquatic and terrestrial(^{[95]})</td>
<td>No residues were found on milk and bovine tissue from dairy cow(^{[65]})</td>
</tr>
<tr>
<td></td>
<td>b. Suspected of damaging fertility or the unborn child(^{[95]})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCD</td>
<td>Low hazard potential(^{[67]})</td>
<td>Low toxicity(^{[67]})</td>
<td>Minute residues in milk was found in 2013 in New Zealand(^{[62]})</td>
</tr>
<tr>
<td><strong>Nitrification Inhibitor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Harmful if swallowed(^{[94]})</td>
<td>a. No hazards identified for air(^{[94]})</td>
<td>Administration of DCD to dairy cow at 3 or 30 g/cow/day was 1.2% recovered in milk(^{[63]})</td>
</tr>
<tr>
<td></td>
<td>b. Causes serious eye irritation(^{[94]})</td>
<td>b. No potential for bioaccumulation for predators(^{[94]})</td>
<td></td>
</tr>
<tr>
<td>DMPP</td>
<td>c. Suspected of damaging fertility or the unborn child(^{[94]})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>d. May cause damage to organs through prolonged or repeated exposure(^{[94]})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source:\(^{[68]}\), \(^{[66]}\), \(^{[64]}\), \(^{[63]}\), \(^{[95]}\), \(^{[96]}\)

NBPT, N-(n-butyl) thiophosphoric triamide; DCD, dicyandiamide; DMPP, 3-4 dimethylpyrazole phosphate