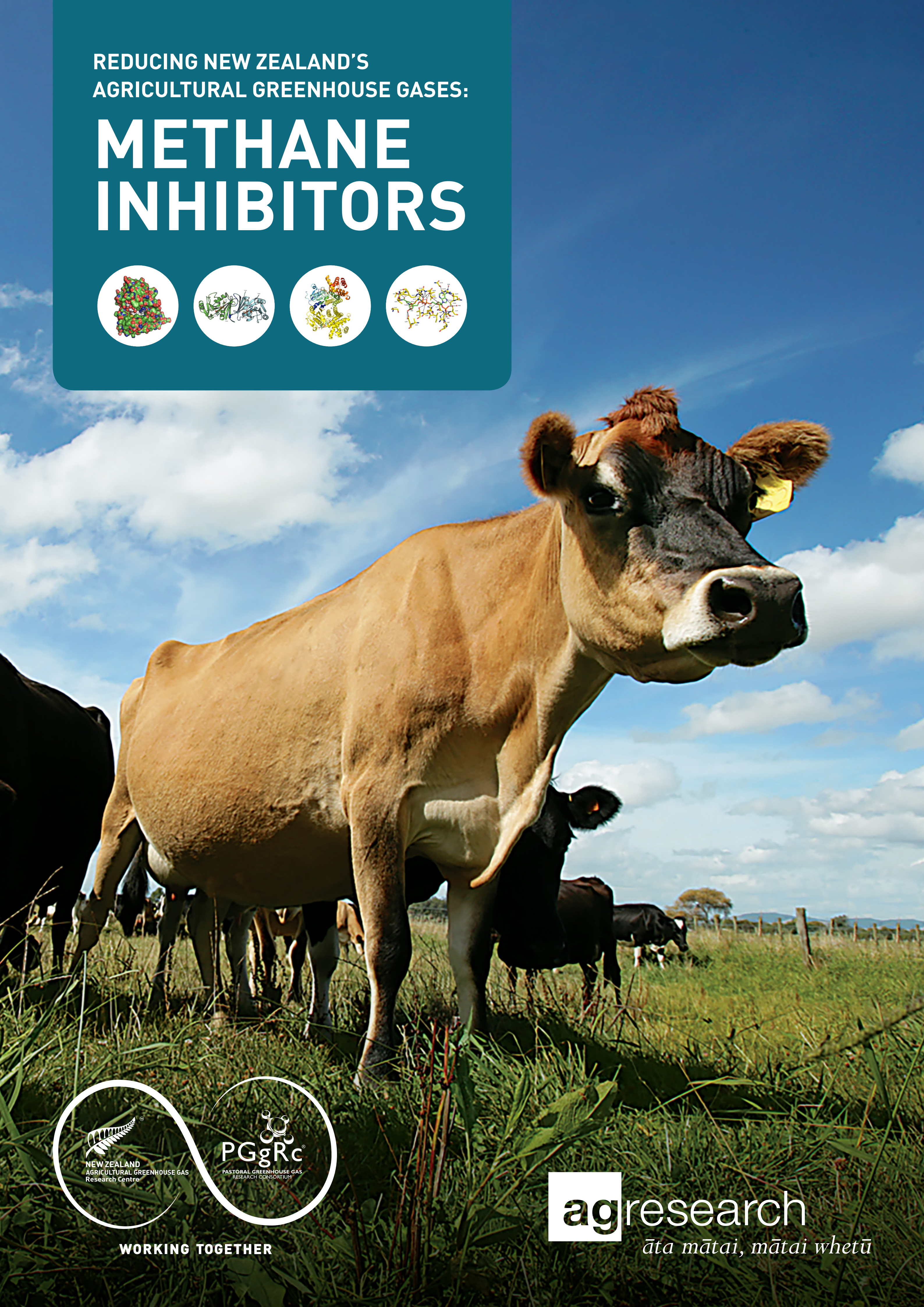
REDUCING NEW ZEALAND'S AGRICULTURAL GREENHOUSE GASES:

METHANE INHIBITORS



NEW ZEALAND AGRICULTURAL GREENHOUSE GAS Research Centre

PGgRc PASTORAL GREENHOUSE GAS RESEARCH CONSORTIUM

WORKING TOGETHER

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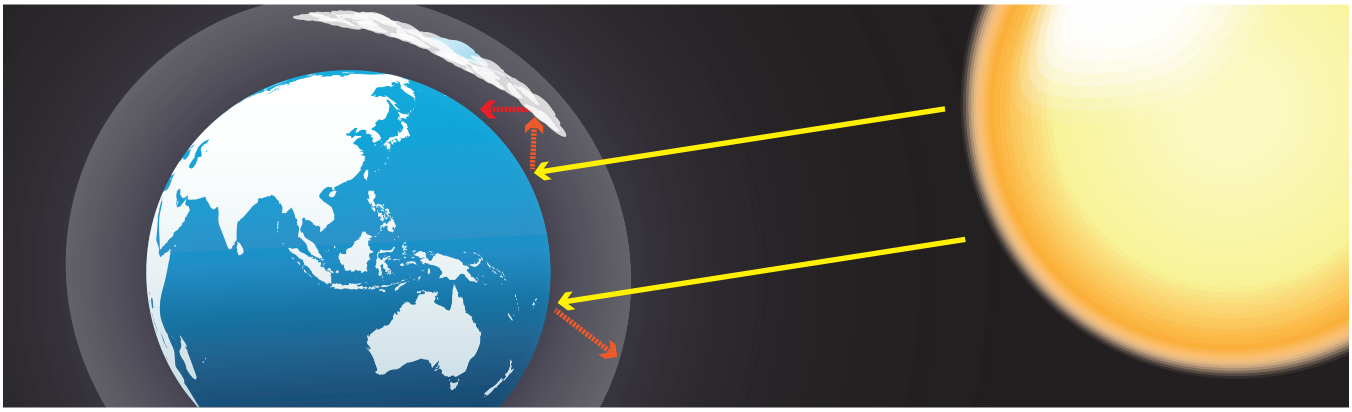
METHANE AS A GREENHOUSE GAS

Methane absorbs much more heat than carbon dioxide, but once it enters the atmosphere, it lasts on average about 12 years before being broken down. A small proportion lasts for decades. In contrast, carbon dioxide remains in the atmosphere for many centuries.

The metric used for reporting under the United Nations Framework Convention on Climate Change is called the Global Warming Potential (GWP), which is based on calculations of the total amount of heat trapped by a greenhouse gas in the atmosphere over a defined period of time. Over 100 years, the current GWP of methane is 25. This means that emitting one tonne of methane has the same warming effect, averaged over the next 100 years, as emitting 25 tonnes of carbon dioxide.



SOURCES OF METHANE

Methane is produced naturally as well as through human activities. Methane concentrations in the atmosphere have been relatively stable over the past two thousand years but have more than doubled during the last two centuries as a result of human activities, with agriculture as the largest source of emissions worldwide.

Most of the methane from agriculture comes from ruminant animals and, to a lesser degree, the flooding of rice paddies. Landfills and biomass burning also contribute to global methane emissions.

Methane is also a fossil fuel and, in some industrialised countries, a significant amount of the gas leaks into the atmosphere during the extraction of oil, gas and coal. Globally, the amount of methane from such fossil sources is roughly three quarters of that generated from agriculture.

Wetlands are the largest natural source of methane. The gas is produced when microbes break down organic matter in the absence of oxygen. Additional smaller contributions come from deposits of frozen methane (clathrates) on the seafloor, wild fires and the digestive tracts of some plant-eating animals, such as termites.

New Zealand is unusual among developed countries with its strong base in primary production and a high proportion of electricity generated from renewable sources. As a result, almost half of the country's greenhouse gas emissions come from agriculture, in the form of methane and nitrous oxide. The agriculture sector contributed 48 percent of New Zealand's gross emissions in 2014. Methane accounts for 43 percent of all emissions (from all sources). More than 80 percent of New Zealand's total methane comes from ruminant farm animals – cattle, sheep, goats and deer – mainly as a result of enteric fermentation. The great majority of that comes from the rumen, the enlarged modified foregut of ruminant animals. Only about three percent comes from animal manure.

In recognition of the fact that methane from ruminant livestock makes up most of New Zealand's agricultural greenhouse gas emissions, the Government and the pastoral industry are jointly investing in research to lower methane production in the rumen. The research takes a four-pronged approach:

- to investigate low-methane feeds;
- to breed for low-emitting animals;
- to develop a vaccine that targets methane-producing microbes in the rumen;
- to develop inhibitors that target methane-producing microbes in the rumen.

This fact sheet focuses on the development of chemical inhibitors that can reduce methane production in the rumen significantly without affecting the rest of the microbial community or animal productivity and health, or food safety.

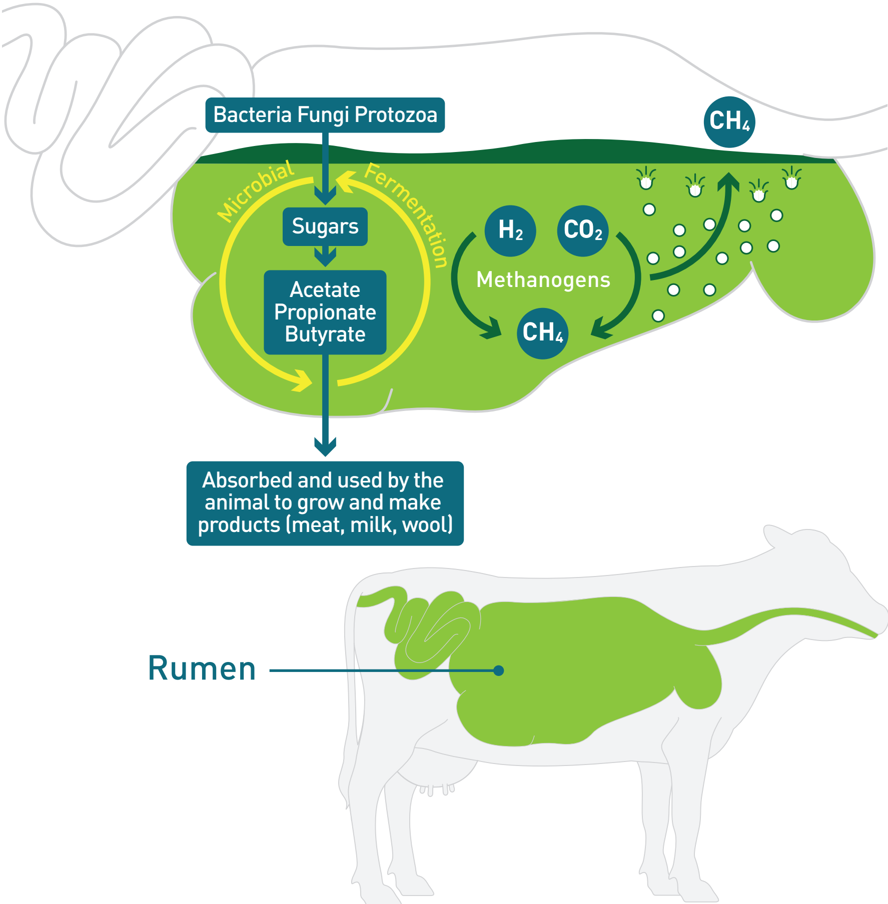
THE RUMEN: ONE OF THE MOST EFFICIENT FIBRE DECOMPOSING SYSTEMS

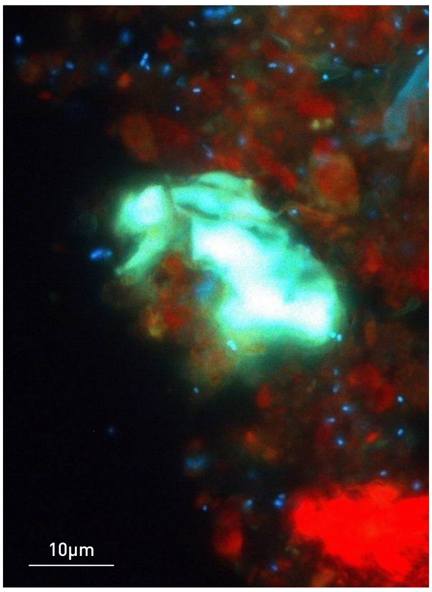
The rumen first evolved 50 million years ago, giving mammals access to plant foods they would otherwise not be able to digest.

The rumen is the first and largest part of the multi-chambered stomach of grass-eating ruminant animals. It acts as a fermentation vat where microbes break down plant cellulose into smaller compounds that deliver energy to the animal. This process is known as enteric fermentation.

Inside the anaerobic (oxygen starved) conditions of the rumen, a complex and highly adapted microbiome has evolved that includes different types of microbes: bacteria, archaea, fungi and protozoa.

The majority of these microbes live in symbiosis with the ruminant animal. The microbes partially ferment the feed which generates energy for themselves and delivers volatile fatty acids, water-soluble vitamins and high-quality proteins to the animal. In turn, the animal host maintains oxygen-free conditions in the rumen and provides an ideal environment for the microbes to thrive.





THE ROLE OF METHANOGENS

Methanogens belong to a group of ancient microbes known as archaea. They have evolved hundreds of millions of years ago and occupy oxygen-free niches such as peat bogs and anaerobic gut systems.

In the rumen, methanogens modify the fermentation process, but they are not thought to be essential to the host animal. They are opportunists, taking advantage of one of the by-products of fermentation in the rumen: hydrogen gas. This provides an energy source and methanogens combine it with carbon dioxide to produce methane and water. The methane is released into the atmosphere when the animal belches. The methanogens' use of hydrogen represents a loss of dietary energy to the animal. The small blue cells in the image to the left are methanogen cells in the gut contents of a sheep.

METHANE INHIBITORS AS A MITIGATION STRATEGY

More than 100 of the countries that have signed up to the Paris Agreement have pledged to reduce agricultural greenhouse gas emissions.

To meet the Agreement's goal of limiting average global warming to 2°C or less, the global agricultural sector would need to reduce emissions by 1 gigatonne per year in 2030. However, existing mitigation options can only deliver up to 40 percent of the mitigation effort required.

The suppression of methane production in the rumen through chemical inhibitors is a promising strategy. A successful inhibitor is hoped to deliver significantly more reductions than what would be achievable through low-methane feeds and animal breeding alone.

Methanogens are ancient organisms and are evolutionarily and biochemically distinct from bacteria and all other lifeforms. Many of the enzymes involved in a range of fundamental metabolic processes, e.g. methane production, cell wall synthesis and energy supply, are very similar across groups of methanogens but different from those in any other organism. This makes these enzymes excellent targets for chemical inhibitors, and since methanogens are not essential for the ruminant animal, their suppression or even complete removal is a feasible strategy.

Experiments have already shown that it is physiologically possible to raise lambs completely free of methanogens (see sidebar) and that long-term inhibition of methane production by 30 to 50 percent allows the rumen to continue functioning. Methane inhibition frees up extra energy that may even afford an advantage to the host animal.

Global Rumen Census: global reach for methane inhibitors

Giraffes in North Africa, buffaloes in China, bison in America, cattle and sheep in New Zealand – these are just some examples of animals whose rumen microbes have been surveyed as part of the Global Rumen Census project.

The analysis of hundreds of samples from 32 domestic and wild ruminant species from 35 countries found similar microbes present in rumens across a wide range of animal diets. Similar bacteria and archaea dominated in nearly all samples, and methanogens in particular were surprisingly similar across all animals, regardless of host species, diet or geographic location. This universality and limited diversity suggest that it could be possible to mitigate methane emissions by developing strategies that target the few dominant methanogens and that any inhibitor technology developed in one country should be applicable across the globe.

Methane-free sheep

In addition to its greenhouse gas contributions, methane from livestock represents a loss of roughly six percent of food energy that would otherwise be available to the animal. Several studies have shown that methanogens are not an essential component of the rumen microflora. Studies of lambs that have been kept in microbe-free conditions and inoculated with a complex rumen microflora without methanogens have demonstrated that fermentation continues in the absence of methane-producing microbes.

During earlier research, methanogen-free lambs have been reared for short periods of less than three months, but in 2007, a team of French and AgResearch scientists showed that it is possible to rear lambs to adulthood without methanogenic archaea in their rumen. The lambs with a methanogen-free rumen grew well. Their feed intake and the concentration of volatile fatty acids were lower but not markedly dissimilar to those in conventional lambs reared on the same fibrous diet.

This study confirmed that microbes already resident in the rumen can replace methanogens to sustain a functioning rumen. This provides evidence that chemical inhibition of methanogens is a physiologically viable approach to reducing methane emissions from livestock without affecting productivity.

Developments in rumen microbial taxonomy and understanding of rumen diversity

For more than a century, scientists have known that methane is a product of the fermentation process in the rumen, but it wasn't until American microbial ecologist Robert Hungate developed the first culturing techniques for anaerobic microbes during the 1940s that methanogens could be studied in detail. For a long time, only three methanogen species that were known from the rumen could be cultured successfully. Only one of these isolates represented a species that dominates in the rumen. Since then, at least 40 different strains of methanogens have been isolated from the rumen or other intestinal environments.

In 2010, AgResearch with funding by the Pastoral Greenhouse Gas Research Consortium (PGgRc) became the first organisation to sequence the genome of the prominent rumen species, *Methanobrevibacter ruminantium*. Further sequencing efforts soon revealed that there are four major groups of methanogens in the rumen, and that each includes several species.

Today, molecular techniques allow scientists to sequence species that cannot be kept in culture, and this has provided a better picture of the diversity of the methanogen community in the rumen. Twelve genomes that span the taxonomic diversity are now available to help scientists identify the best targets for inhibitors.

One important finding of the sequencing projects was that essential metabolic processes in rumen methanogens are very similar to those of methanogens living in different environments. Rumen methanogens differ in the number of additional proteins they use to interact with other microbes in the rumen, but they share many enzymes necessary for fundamental metabolic functions with methanogens from other anaerobic ecosystems. These findings support the approach that metabolic enzymes that are very similar across methanogens are the best targets for inhibitors.

THE INHIBITOR DEVELOPMENT PIPELINE

Archaea are ancient organisms and many of their enzymes are very different from other microbes.

The sequencing of methanogen genomes has revealed hundreds of genes that are virtually identical across all rumen methanogens. The enzymes that these genes code for represent possible targets for a methanogen-specific inhibitor technology that would allow other rumen microbes to continue their normal digestive functions.

The development pipeline is a complex sequence of screening assays and tests to identify compounds that suppress methanogens effectively without causing unwanted side effects. The first steps happen on the computer screen or in the laboratory. The research investment has supported the screening of five million compounds by computer analysis, which investigates the crystal structure of each enzyme and scores the interaction of each compound at the active site to identify the most promising inhibitors. These candidate inhibitors can then be tested in bioassays against methanogen enzymes or in test tubes against pure methanogen cultures. The goal is to identify inhibitors with high efficacy to reduce methane emissions at low concentrations.

The next step in the selection process is to test the potential inhibitor substance in a flask of rumen fluid, which contains methanogens and all other microbes that are part of the rumen microbiome. This probes for any impacts on the fermentation process.

Ultimately, any compounds that show promise as methane inhibitors have to be tested in ruminant animals, but before that can happen, each has to go through a toxicity test to rule out any unwanted effects beyond the rumen. Even if a compound shows some toxicity, it may remain in the discovery pipeline if it is possible to synthesise chemical analogues, or derivatives, that remove the toxic component. The analysis of derivatives could also discover some that are more effective, cheaper to make or more stable.

Finally, each compound is reviewed by an expert chemist to assess any potential for any other long-term effects on the animal. All these steps are taken to identify the most promising compound and to reduce the number of animals required for trials.

Finally, the compounds are tested in sheep or cattle in respiration chambers that allow scientists to monitor feed intake and methane production continuously.

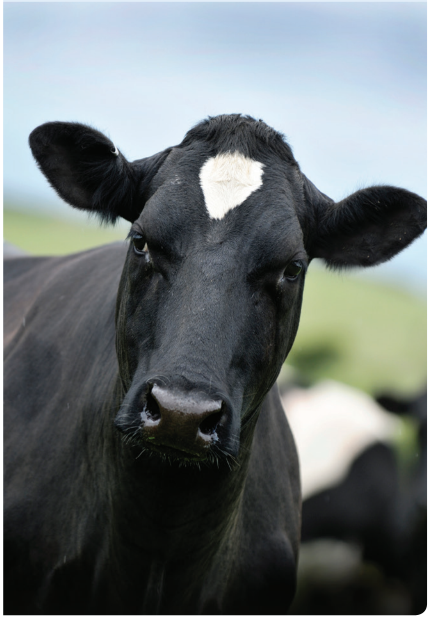
Depending on the route of discovery, some inhibitors might target pathways that are essential to methane formation while others target enzymes that are found only in some methanogens in the rumen. The overall aim is to find the right mechanism and dose that allows the rumen to function while inhibiting methane production by at least thirty percent.

A collaborative effort

The overall goal of this research effort is to develop a New Zealand solution to the global challenge of reducing methane emissions from livestock.

The research is supported by a three-way partnership. Funding comes from government and the pastoral industry represented by the PGgRc. The research is delivered by collaborations of scientists, led by AgResearch but with important inputs from Auckland and Otago universities.

International collaborations are supported by the Global Research Alliance on Agricultural Greenhouse Gases.



WHERE HAVE WE GOT TO SO FAR?

Our inhibitor development pipeline acts like a funnel. From millions of potential candidates, tens of thousands of compounds have been whittled down in laboratory tests to a small number of candidates that are safe to be used in animals.

Two-day respiration chamber trials in sheep helped to refine the selection even further and have identified five substances that showed methane inhibition of 30 percent without affecting general rumen function.

Based on these trials and further chemical analyses, several classes of compounds have undergone 16 day respiration chamber trials in sheep. For these longer experiments, the animals are first adapted to the trial feed for 10 days, before a baseline measurement of methane is made in a respiration chamber. Then, the inhibitor is added to the feed and additional measurements are taken immediately. The animals are kept in a controlled environment for two weeks and measurements taken at the end of the each week to monitor methane production. The trial duration is an industry standard method that provides enough time to detect any short term adaptations in the rumen microbiome, e.g. some microbes beginning to metabolise the inhibitor or the methanogens developing chemical resistance (similarly to bacteria developing antibiotic resistance).

The list of compounds that go forward to longer term respiration chamber trials is constantly revised as new promising candidates are added via multiple discovery pipelines.

At this stage, four classes of inhibitors, some of which contain more than one specific chemical compound, have been identified.

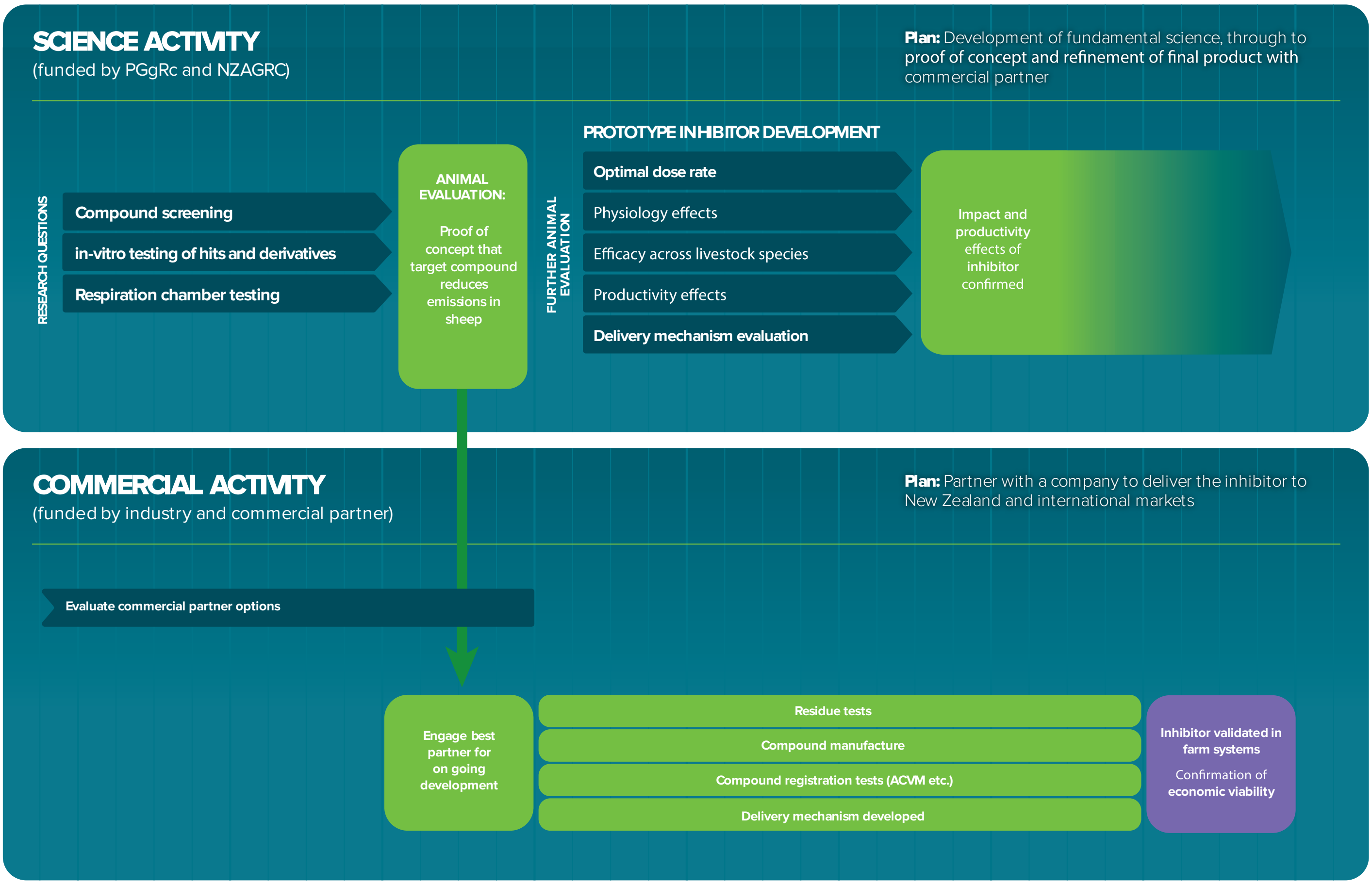
The best compounds emerging from the 16-day trials will have to be tested in longer production trials on-farm to determine whether any of the inhibitors affect the health, welfare and productivity of the animals in any way or leave any residue in the animal products.

As most farm animals in New Zealand are managed in grazing systems, on pasture-based diets, and are not seen by farmers every day, a concurrent challenge is to develop a delivery mechanism. For the respiration chamber trials, the inhibitor was mixed in with the feed, but on the paddock, this is not likely to be a feasible option. Instead, formulations and delivery mechanisms such as bolus capsules (a slow-release system that farmers are already using in other applications) are under investigation as a mechanism of delivering an inhibitor slowly and continuously, over long periods of time. The challenge is that for this approach to be effective, an inhibitor has to work at a very low dose rate.

Adaptation of promising methane inhibitor

The Swiss based company DSM Nutritional Products (DSM) has developed and tested an inhibitor against a specific enzyme found in methanogens. Animal trials have shown that the inhibitor (3-nitrooxypropanol) can successfully reduce methane emissions from sheep, beef and dairy cattle. During a 12-week dairy cow trial in the USA, emissions were reduced by 30%, the cows showed an increase in body weight, but no change in their feed intake or milk production. In its current form, this inhibitor is suitable for livestock managed in systems where the inhibitor can be mixed with feed and consumed continuously. DSM is currently working with research organisations globally, including some in New Zealand, to test its applicability and efficacy in pasture based systems.

RESEARCH PIPELINE: **METHANE INHIBITOR**



WHEN MIGHT AN INHIBITOR BECOME COMMERCIALLY AVAILABLE?

The widespread adoption of effective methane inhibitors could significantly reduce agricultural emissions of methane.

The 16-day trials of a suite of compounds have delivered the proof of concept, and if combined with an appropriate delivery mechanism, this approach could eventually work on New Zealand farms and globally.

The Global Rumen Census project has demonstrated that an inhibitor could work across the world, but we know that practical delivery may require inhibitors with different properties, particularly to work in low-intensity pasture-grazing systems.

As the most promising compounds are optimised and confirmed, further on-farm tests to establish the long-term efficacy and safety are planned. Conversations with potential commercial partners are underway.

The industry-led PGgRc will drive the commercial strategy to deliver this mitigation solution to New Zealand and globally. The key objective is to establish a commercial partnership with a global reach by 2019. The goal is to deliver inhibitors that have been shown to reduce methane emission from livestock by 30 percent or more.

Any such compound has to be tested thoroughly to rule out any impact on the animals' health, welfare and productivity. There cannot be any residue or food safety concerns and any inhibitors will require economic manufacture and distribution processes to ensure they are cost effective.

The exacting requirements of global food and animal products along with animal welfare set high standards, creating a substantial risk of striking any of the current lead classes of inhibitors out of contention as they develop towards delivery.

Therefore it is expected to take until 2023 before the best of the current suite of inhibitors from this investment could be released on the market.

More information

This fact sheet has been produced by NZAGRC and PGgRc.
There is more information on our websites, or contact us:

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