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NEW ZEALAND AGRICULTURAL GREENHOUSE GAS RESEARCH CENTRE

THE POTENTIAL USE OF BROMOFORM

AS A METHANE MITIGATING TECHNOLOGY IN AOTEAROA NEW ZEALAND FARM SYSTEMS

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Authors:

Robin Kelly	Principal Author, BioPacific Partners
Margot Bethell	Project Lead, BioPacific Partners
Dr Ian Boddy	Independent Animal Health Consultant
Dr Andrew Kelly	Executive Director, BioPacific Partners
Katherine Kemshall	Independent Regulatory Consultant, Veterinary Medicine
Alice Jones	Analyst, BioPacific Partners
Melissa Wong	Senior Consultant, BioPacific Partners

NEW ZEALAND
AGRICULTURAL GREENHOUSE GAS
Research Centre

AUTHOR'S NOTE

The literature review for this report was completed in October 2023. The understanding of bromoform as an inhibitor and regulatory requirements for inhibitors in New Zealand farm systems are both rapidly evolving. The information and commentary expressed here represent the outcomes of the authors' research and understanding of the regulatory environoment in Aotearoa New Zealand in 2023.

Throughout this report, the terms "methane inhibitor", "methane mitigator", and "methane-mitigating technology" are used interchangeably. The terms "technology" and "product" are often used to refer to the final formulation of a bromoform-containing inhibitor intended for use in agricultural systems and should be understood as distinct from bromoform as a chemical compound - referred to as the active pharmaceutical ingredient (API). The technology or product must be registered for use under the Agricultural Compounds and Veterinary Medicines Act and it is therefore the responsibility of the product developer/manufacturer/importer to provide the data required for regulatory approval.

Introduction

This report seeks to explore the potential of bromoform as a methane mitigating technology in Aotearoa New Zealand farming systems.

Aotearoa New Zealand's substantial reliance on primary exports from cattle and sheep products and the corresponding size of its livestock population mean that agricultural methane makes up a high proportion of the country's greenhouse gas emissions. This means that there is a pressing need to develop methane mitigation tools for the commercial livestock sector. In this context, bromoform has emerged as a compound showing promise as a methane inhibitor.

Both *in vitro* and *in vivo* research demonstrate the effectiveness of bromoform as an inhibitor of methanogenesis in the rumen[1,2] and multiple bromoform-containing technologies - both globally and in Aotearoa New Zealand - are nearing or have achieved final product formulations with which to proceed to market. Along with other inhibitor compounds such as 3-NOP, bromoform-containing technologies stand to be among the first methane inhibitors approved for use in Aotearoa New Zealand.

The purpose of this report has been to investigate the evidence regarding bromoform's safety and efficacy as a methane mitigating technology, and to detail the portfolio of evidence required to enable the practical use of bromoform-containing technologies in Aotearoa New Zealand farming systems.

Use of methane inhibitor technologies in Aotearoa New Zealand is regulated under the Agricultural Compounds and Veterinary Medicines Act (ACVM, 1997)[3]. This report examines the data required for registration of a bromoform-containing inhibitor under this regulation and also identifies knowledge gaps which may impact the path-to-market for any such inhibitor product.

The report also investigates the potential impact of bromoform use on Aotearoa New Zealand's international trade, especially regarding Codex Alimentarius international food standards, and identifies the research and data required to better understand the risk of bromoform use to Aotearoa New Zealand's export markets.

The report identifies critical data required to address outstanding questions and accelerate the development of a bromoform-based methane inhibitor. The authors also present a series of insights and recommended actions to facilitate the development of this emerging tool to reduce livestock emissions.

1 Stefenoni et al. (2021). "Effects of the Macroalga *Asparagopsis Taxiformis* and Oregano Leaves on Methane Emission, Rumen Fermentation, and Lactational Performance of Dairy Cows." *Journal of Dairy Science* 104(4).

2 Eason, C. T., and Fennessy, P. (2023). "Methane Reduction, Health and Regulatory Considerations Regarding *Asparagopsis* and Bromoform for Ruminants." *New Zealand Journal of Agricultural Research*.

3 Ministry for Primary Industries. "Agricultural Compounds and Veterinary Medicines Act 1997: Version as at 30 November 2022". https://www.legislation.govt.nz/act/public/1997/0087/latest/DLM414577.html#DLM414576. [Accessed September 18, 2023].

Bromoform Background

Introduction to bromoform

Bromoform is a trihalomethane with the chemical composition $CHBr_3$. The primary sources of naturally-occurring bromoform are marine micro- and macro-algae. Human populations are typically exposed to bromoform through the consumption of chlorinated drinking water (as bromform is a by-product of chlorination[1]). Research into bromoform as a ruminant feed additive has primarily focused on feed supplements derived from the marine red algae species *Asparagopsis taxiformis* or *A. armata*, both of which have been shown to contain relatively high concentrations of naturally occurring bromoform. Bromoform is also able to be synthesised as a chemically-identical molecule to that found from natural sources.

Bromoform in ruminants

A. taxiformis has been show to reduce methane production by 99% when 0.2g was included in a rumen fluid incubation[2]. Bromoform present in the seaweed has been found to inhibit the action of two key enzymes in the methanogenic pathway: methyl-coenzyme M reductase (MCR) and coenzyme M methyltransferase[3,4,5] (Figure 1).

Investigations into bromoform as a methane inhibitor in livestock have focused on five main formats for delivery:

(a) Feeding whole seaweed biomass as a mixed ration with feed

(b) Feeding freeze-dried seaweed as a mixed ration with feed

(c) Feeding a canola oil emulsion of seaweed with or without the algal biomass removed

(d) Synthetic bromoform as liquid mixed with feed (with or without stabilizing excipients)

(e) Synthetic bromoform in a slow-release bolus inserted into the animal's rumen

Aspects of trial design such as target species, basal diet, length of intervention, dosage, formulation, delivery method and measurement of various endpoints vary significantly between studies.

Of the cattle trials using *Asparagopsis* that have measured both bromoform concentration and methane production, the dose of bromoform has ranged from approximately 23.6mg/day[6] – 480mg/day[7], and methane production was reduced between 9% and 98%. The longest published trial at the time of writing was conducted in feedlot Wagyu beef cattle over 275 days[8], and found methane production was reduced by 28% in animals fed 25 mg/kg DM over the whole feeding period. However, this trial also recorded a reduction in overall feed intake and reduced liveweight in cattle across the course of the trial. Once these factors were taken into account, bromoform did not appear to significantly reduce the yield of methane per product unit (g methane per kg liveweight gain).

1 U.S. EPA, "Bromoform". *U.S. Environmental Protection Agency.* https://www.epa.gov/sites/default/files/2016-09/documents/bromoform.pdf [Accessed June 29, 2023].

2 Kinley et al. (2016). "The Red Macroalgae *Asparagopsis Taxiformis* is a Potent Natural Antimethanogenic That Reduces Methane Production During *in vitro* Fermentation With Rumen Fluid". *Australian Journal of Experimental Agriculture* 56(3).

3 Smith et al. (1962). "Partial Synthesis of Vitamin B12 Coenzyme and Analogues". *Nature* 194(1175).

4 Johnson et al. (1972). "Some Effects of Methane Inhibition in Ruminants (Steers)". *Canadian Journal of Animal Science* 52(4).

5 Glasson et al. (2022). "Benefits and Risks of Including the Bromoform Containing Seaweed *Asparagopsis* in Feed for the Reduction of Methane Production from Ruminants." A*lgal Research* 64.

6 Kinley et al. (2020). "Mitigating the Carbon Footprint and Improving Productivity of Ruminant Livestock Agriculture Using a Red Seaweed". *Journal of Cleaner Production* 259.

7 Alvarez-Hess et al. (2023). "Twice Daily Feeding of Canola Oil Steeped with Asparagopsis Armata Reduced Methane Emissions of Lactating Dairy Cows". *Animal Feed Science and Technology* 279.

8 Cowley et al. (2023a). "Effect of Asparagopsis Extract in a Canola Oil Carrier for Long-Fed Wagyu Cattle". *Meat and Livestock Australia*.

A variety of delivery methods and formulations will be required to implement methane inhibition technologies in Aotearoa New Zealand's livestock industry due to the variability of infrastructure and feeding practices within different livestock subsectors. For instance, pasture-grazed dairy cattle may reliably be treated with a supplemented feed twice-daily during milking, whereas pasture-fed beef cattle have less regular access to a controlled food source, meaning that a bolus technology may be more effective.

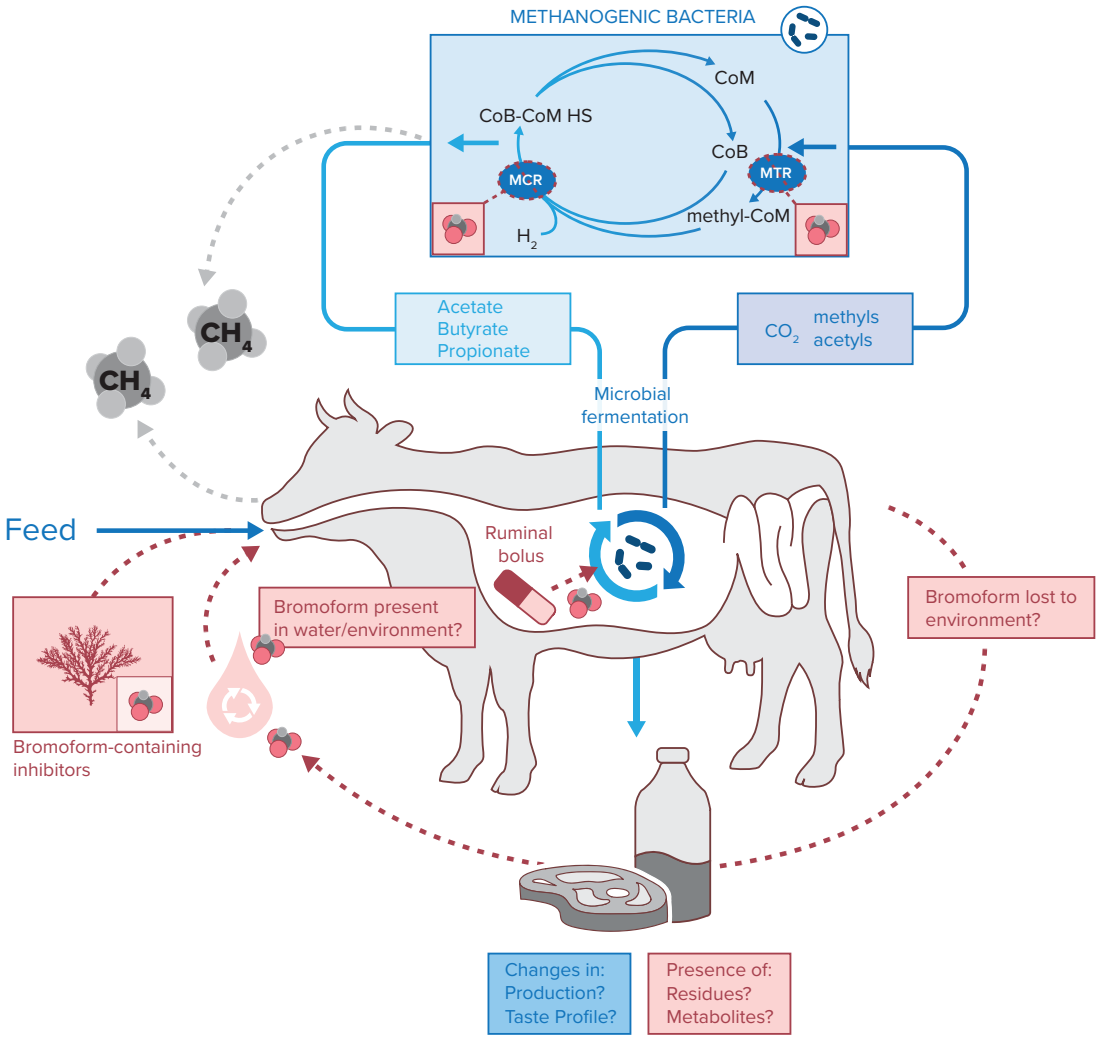


Figure 1. Mechanism of action of bromoform-containing inhibitors and lifecycle of bromoform in agricultural livestock
MCR = methyl-coenzyme M reductase, MTR = coenzyme M methyltransferase, CoM = coenzyme M, CoB = coenzyme B, CH_4 = methane

Tissue residues

The literature is inconclusive as to whether bromoform or related residues transfer into systemic circulation or accumulate in animal tissues. This is in part due to the limited number of trials that have been conducted, and the variation among them in parameters such as dosage, formulation, product format (seaweed, oil emulsion, bolus etc), basal diet, length of dosing regime, washout periods and handling of tissues.

Residues of bromoform have been detected in the milk of cattle fed *Asparagopsis*-derived feed supplements[9,10] however similar trials have shown either no significant increase in bromoform concentration[11] or significant variability in residue levels between treated animals[12]. From the small number of studies available, there are reports of altered milk fat, protein, and other nutritional compounds in the milk of *Asparagopsis*-treated animals compared to controls. The reason for these variations in milk composition is not yet fully understood.

At time of writing, six published studies have investigated the transfer of bromoform, and its metabolites, to skeletal muscle tissue and offal in ruminants, including beef steers, lactating dairy cattle, and sheep[13,14,15,16,17,18]. Bromoform has not been reported to accumulate in the muscle or organ tissues of animals being supplemented with *Asparagopsis*.

Bromide and iodine elevations have been found in the kidneys of beef steers dosed with an oil-based formulation of *Asparagopsis*[94], however not in skeletal muscle.

Bromoform and human health

There are several factors regarding bromoform's potential effect on human health. Most studies stem from the fact that bromoform is often elevated in drinking water as a by-product of disinfection with chlorine. Experts and meta-analysis have suggested the most compelling risks of bromoform to human health are increased risk of bladder cancer, and low birth weights as seen in regions where bromoform is elevated in drinking water.

Trihalomethanes (THMs), such as bromoform, may be carcinogenic when consumed in high quantities[19]. As a result, the United States' Environmental Protection Agency enforces a maximum contaminant level of 100µg/L of total THMs in drinking water[20]. Aotearoa New Zealand follows the World Health Organisation recommendation that drinking water not exceed 100 µg/L of bromoform.

The International Agency for Research on Cancer classifies bromoform as *not classifiable as to its carcinogenicity to humans*[21], whereas the US EPA (1986) classifies bromoform as a *probable human carcinogen*[22].

9 Krizsan et al. (2023). "Effects on Rumen Microbiome and Milk Quality of Dairy Cows Supplemented the Macroalgae *Asparagopsis Taxiformis* in a Grass Silage-Based Diet.", *Frontiers in Animal Science* 4.

10 Alvarez-Hess et al. (2023).

11 Roque et al. (2019). "Inclusion of Asparagopsis Armata in Lactating Dairy Cows' Diet Reduces Enteric Methane Emission by over 50 Percent". *Journal of Cleaner Production* 234.

12 Muizelaar et al. (2021). "Safety and Transfer Study: Transfer of Bromoform Present in *Asparagopsis Taxiformis* to Milk and Urine of Lactating Dairy Cows". *Foods* 10(3).

13 Roque et al. (2021). "Red Seaweed (*Asparagopsis Taxiformis*) Supplementation Reduces Enteric Methane by Over 80 Percent in Beef Steers". *PLOS ONE* 16(3).

14 Kinley et al. (2020).

15 Cowley et al. (2023b). "Efficacy and Safety of *Asparagopsis* Extract in a Canola Oil Carrier for Feedlot Cattle". *Meat and Livestock Australia*.

16 Muizelaar et al. (2021)

17 Li et al. (2016). "Asparagopsis Taxiformis Decreases Enteric Methane Production From Sheep". *Animal Production Science* 58(4).

18 Cowley et al. (2023a).

19 Theiss et al. (1977). "Test for Carcinogenicity of Organic Contaminants of United States Drinking Waters by Pulmonary Tumor Response in Strain A Mice". *Cancer Research* 37(8).

20 DeMarini, D.M. (2020). "A Review on the 40th Anniversary of the First Regulation of Drinking Water Disinfection By-Products". *Environmental and Molecular Mutagenesis* 61(6).

21 IARC. (2019). "Monographs on the Identification of Carcinogenic Hazards to Humans". *International Agency for Research on Cancer.* https://monographs.iarc.who.int/wp-content/uploads/2019/07/Preamble-2019.pdf [Accessed July 11, 2023].

22 U.S. EPA (2014). "Risk Assessment for Carcinogenic Effects". *U. S. Environmental Protection Agency*. https://www.epa.gov/fera/risk-assessment-carcinogenic-effects [accessed July 11, 2023].

Whether or not bromoform-associated residues in animal products are considered a health risk will depend in part on the concentration of these compounds in animal products[23]. In particular, if tissue residues are seen to be elevated above background levels in bromoform-treated animal tissues, they may have a negative impact on human health and therefore be subject to regulation by food safety standards such as Codex Alimentarius.

23 Eason and Fennessy (2023).

Key Knowledge Gaps

The authors performed a comprehensive literature review and analysis of the state-of-the-art of bromoform research to identify key knowledge gaps. The literature review was supplemented by interviews with stakeholders including developers of bromoform-containing products, animal trial experts, and end-user farmer collectives.

These gaps and challenges are ranked below by general scale of impact. The impact analysis considered the relevance of knowledge gaps to:

- product development - the highest impact gaps would affect any bromoform-containing technology in development,
- Aotearoa New Zealand markets and farm systems - gaps with a particular impact on major markets and farm systems e.g. pasture-grazed dairy cattle, and
- Researchers and industry bodies - the impact analysis prioritised knowledge gaps which could be practically addressed by coordinated action between industry collectives and research groups.

Background levels of bromoform

Bromoform is present in drinking water, from both natural and anthropogenic sources. Although it is known that environmental bromoform concentrations are high in regions proximal to indsutrial water treatment facilities, there is little known about the standard international levels of environmental bromoform. The presence of background levels of bromoform in milk is unclear, though inhibitor studies in lactating cows have reported trace levels of bromoform in the milk of control animals[24,25].

Determination of the levels of environmental bromoform in local and export markets will be crucial in determining the necessity of maximum residue limits (MRL) under Codex Alimentarius. Determination of a Codex MRL for bromoform may be a critical step in the adoption of these mitigating technologies in Aotearoa New Zealand.

Bromoform toxicity

Historical studies have produced ambiguous results as to the toxicity profile of bromoform. Significantly, it is listed by the US EPA as a probable human carcinogen, however the IARC lists it as “not classifiable”. This ambiguity relates to the lack of comprehensive evidence regarding bromoform’s human health impact. More data around bromoform toxicity will be required for Codex to set an MRL based on the toxicity and carcinogenicity of bromoform in human populations.

Should improved data indicate bromoform as a probable human carcinogen, further research will be required to determine its carcinogenic mechanism of action. Historic research indicates that bromoform has a genotoxic mechanism of action[26]. For genotoxic carcinogens, any level of human exposure could be associated with some degree of risk, meaning that a maximum residue limit (MRL) is not appropriate[27]. Critically, this would mean that if bromoform is shown to be a genotoxic carcinogen and an MRL is required, any product whose use results in bromoform residues being detected would either not be viable, or need to establish a withholding period which resulted in a demonstrated absence ofany residues in the final export product.

24 Stefenoni et al. (2021).
25 Roque et al. (2019).

26 Morimoto, K. and Koizumi, A. (1983). “Trihalomethanes Induce Sister Chromatid Exchanges in Human Lymphocytes in Vitro and Mouse Bone Marrow Cells in Vivo”. *Environmental Research* 32(1).
27 International Programme on Chemical Safety. (2009). “Hazard Identification and Characterization: Toxicological and Human Studies”. In *Environmental Health Criteria 40: Principles and Methods for the Risk Assessment of Food* (Food and Agriculture Organization of the United Nations/World Health Organization.

Impact of bromoform as an ozone-depleting substance

It is uncertain whether bromoform produced at scale through either synthetic processes or cultivation of *Asparagopsis* would be subject to the Montreal Protocol on Substances That Deplete the Ozone. Recently, experts have called for regulation of bromoform from other anthropogenic sources, such as ship ballast water[28]. Restriction under this agreement could have an impact on the further development of bromoform-based methane inhibitor technologies operating at an industrial scale. However, the balance of harm may be considered, given the mutual goal of environmental action of both the Montreal Protocol and the use of methane inhibitors.

Productivity and rumen dynamics

Research evidence shows that bromoform is able to achieve a significant reduction in rumen methane production, however, this inhibition efficacy may be confounded over a sustained application period by reducing productivity[29] and/or possible adaptation of the rumen microbiome to counteract bromoform's anti-methanogenic properties.

At the time of writing this report, the longest field study of a single bromoform technology showed that methane production was reduced by 28% over 275 days in beef steers[30]. However, dry matter intake and liveweight (LW) gain was also significantly reduced in these animals, leading to no difference in methane intensity (g/kg LW gain) between treatments. Registration of a methane inhibitor under ACVM regulations will require evidence of a reduced methane yield and intensity, so DMI and liveweight gain will be factored in to the data analysis. If new results are consistent with the above study, efficacy claims will be limited.

ACVM registration of an inhibitor product requires that the efficacy period of any approved inhibitor be validated. This means either:

- A new bromoform technology claiming methane inhibition will need to be registered with an efficacy statement claiming activity for only as long as the longest field trial showing efficacy of that technology (e.g., "reduces methane emissions by 60% over a maximum of 3 months' treatment"), or
- Further research is required to understand the biodynamics of rumen adaptation based on the dosage of bromoform, in order for scientific generalities to be derived and applied to comparable products.

Determining rumen adaptation mechanisms will be critical for efficacy claims for bromoform-containing inhibitor products to register for use.

Dose-dependent standard and target animal efficacy ratio

Comparisons between trials to establish a dose-dependent efficacy prediction are difficult due to many variables including product substrate (freeze-dried seaweed, seaweed-oil emulsions, bolus, etc.), lack of consistency in bromoform quantification and stability evidence, and widely varying delivery protocols ranging from pulse feeding, total mixed ration (TMR), and slow-release bolus. Validation of a dose-dependent standard could significantly provide valuable reference materials to support registration applications and significantly decrease efficacy study requirements.

In order for any technology to achieve regulatory approval for methane inhibition claims, efficacy trials will have to be done in the target animal population, however, efficacy claims could be referenced across species if equivalence or a consistent ratio of methane reduction is established. Using a standardised delivery methodology across beef and dairy cattle, and sheep and goats, an inter-species efficacy ratio may be determined, i.e., there may be sufficient statistical evidence to suggest that bromoform is more or less effective in dairy vs beef cattle, or beef cattle vs sheep.

28 Yue Jia et al. (2023). "Anthropogenic Bromoform at the Extratropical Tropopause". *Geophysical Research Letters* 50(9).

29 Cowley et al. (2023a).

30 Cowley et al. (2023b).

Validated analytical methods for bromoform and tissue residues

Any regulatory application will need to provide evidence of a validated analytical method with sufficiently low reporting limits for detecting residues of bromoform and its metabolites in the relevant tissues. There is the potential for the development of publicly available analytical methods which satisfy ACVM requirements, however these would need to be verified or partially validated in each individual lab.

During stakeholder consultation with national and international research laboratories, it emerged that many analytical labs may not be able to develop an economically-viable radiolabelled methodology for bromoform tissue depletion studies (necessary for a Codex maximum residue limit to be set) due to the volatility and small size of the molecule. Unless this issue is resolved or an alternative methodology is developed, Codex may not issue a MRL for bromoform.

Formulation and dosage schedules

Different animal feeds result in a different baseline methane yield. Additionally, feed composition may impact the methane inhibition efficacy of bromoform[31]. Generally, high forage diets result in higher methane production, and appear less sensitive to methane inhibitors[32]. More data is needed to determine the quantitative impact of different basal feed composition and bromoform dosage schedule (e.g., part of the TMR or pulse-fed) in order for emergent technologies to design effective trials for specific formulation. A body of literature in this area would support more robust efficacy claims for new products.

Additionally, the format of the bromoform product will have a significant impact on efficacy. As mentioned, there is considerable heterogeneity in approaches to administering bromoform, from whole-seaweed feed supplements to slow-release bolus technologies. Research into each of these formats is still in its relative infancy and data is lacking on the stability of bromoform products in different formats, the impact of product format on pharmacokinetics, and the impact of format on efficacy data.

Industry practices

The views and practices of influential industry bodies are crucial to the successful implementation of inhibitors. Plainly, there will be no bromoform products passing through regulatory approval if there is no market for them.

Consultation with exporters and local end-user groups revealed significant concerns which could impede inhibitor uptake, including:

- Impact on animal reproductive health
- Productivity impact
- Food contamination impacts on manaakitanga

These concerns will not be satisfied by data meeting the minimum threshold for product registration. Instead, industry bodies may require further assurance of bromoform's safety and efficacy, leading to further trial costs for product developers.

Export risks

Aotearoa New Zealand's export sector is sensitive to the risks of trade disruption due to tissue contamination. When interviewed, export industry stakeholders frequently mentioned this concern with reference to the impact of DCD residues being detected in milk export products in early 2013[33].

This indicates that futher assurance of export

31 Roque et al. (2021).

32 Dijkstra et al. (2018). "Short communication: antimethanogenic effects of 3-nitrooxypropanol depend on supplementation dose, dietary fiber content, and cattle type". *Journal of Dairy Science* 101(10).

33 Ministry for Primary Industries (2013). "New Zealand Government assures safety of country's dairy products". *Ministry for Primary Industries - Manatū Aha Matua.* https://www.mpi.govt.nz/news/media-releases/new-zealand-government-assures-safety-of-countrys-dairy-products/ [accessed August 13, 2023].

markets will be required to ensure the adoption of bromoform-containing products in Aotearoa New Zealand's farm systems.

Market considerations

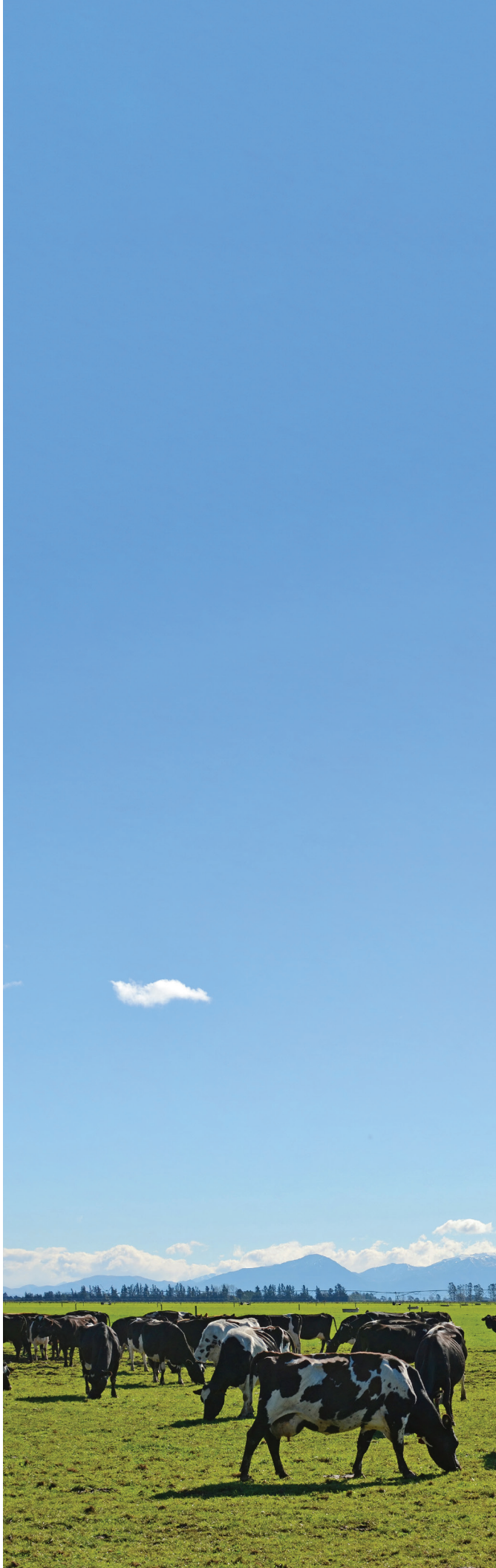
Due to the relative infancy of the inhibitor industry, and the lack of market validation of bromoform-containing inhibitor products, little is yet known of the market dynamics for such a product. Factors to consider in a thorough business case analysis will include the economics of production/extraction of the API (e.g. seaweed farming at industrial scale, chemical synthesis, excipient and product formulation costs), cost of registration, market appetite, economic impact to end-users, and consumer hestiancy.

The Agricultural Greenhouse Gas Inventory

Discussions from a recent AgriZeroNZ workshop[34] indicated that the GHG Inventory team was likely to require more evidence of efficacy than an initial registration with the ACVM. The ACVM has the ability to approve specific claims whereas the Inventory needs evidence of the inhibitory effect at a national level (in various farming systems and geographies) to determine the inhibitory effect.

The innovator company will therefore also need to work with the GHG Inventory team to determine a plan for capturing suitable activity data on an ongoing basis.

34 AgriZeroNZ. "Regulatory path to registration: Trial requirements for methane inhibitor efficacy label claims" [workshop]. July 25, 2023.



Further Data Requirements

No bromoform-containing inhibitor product has, to date, been registered for use in Aotearoa New Zealand. In addition to addressing the key knowledge gaps presented above, any product-developer seeking to bring such a product to market will require extensive data to support a registration application.

A summary of the trial requirements for registration of a bromoform-containing methane inhibitor and the determination of a maximum residue limit are given below.

Given there are no MRLs established for bromoform either locally or overseas, MRLs will need to be set by both the ACVM and Codex to allow international trade of treated animal products, if residues are above natural background levels. This may prove a crucial point for the regulation of any bromoform-containing product.

ACVM Registration

The registration of a methane inhibitor with the ACVM requires data for all the fields listed below. The majority of this data must be generated in compliance with Good Laboratory[35], Clinical[36], and Manufacturing[37] Practice standards (GLP, GCP, and GMP, respectively). Data already released to the public domain may be used to support regulatory applications, however due to the requirements for full data sets and use of the final product formulation, data may have to be generated *de novo* if historical data does not meet regulatory standards.

A summary of the data required for ACVM registration is presented in Figure 2, opposite.

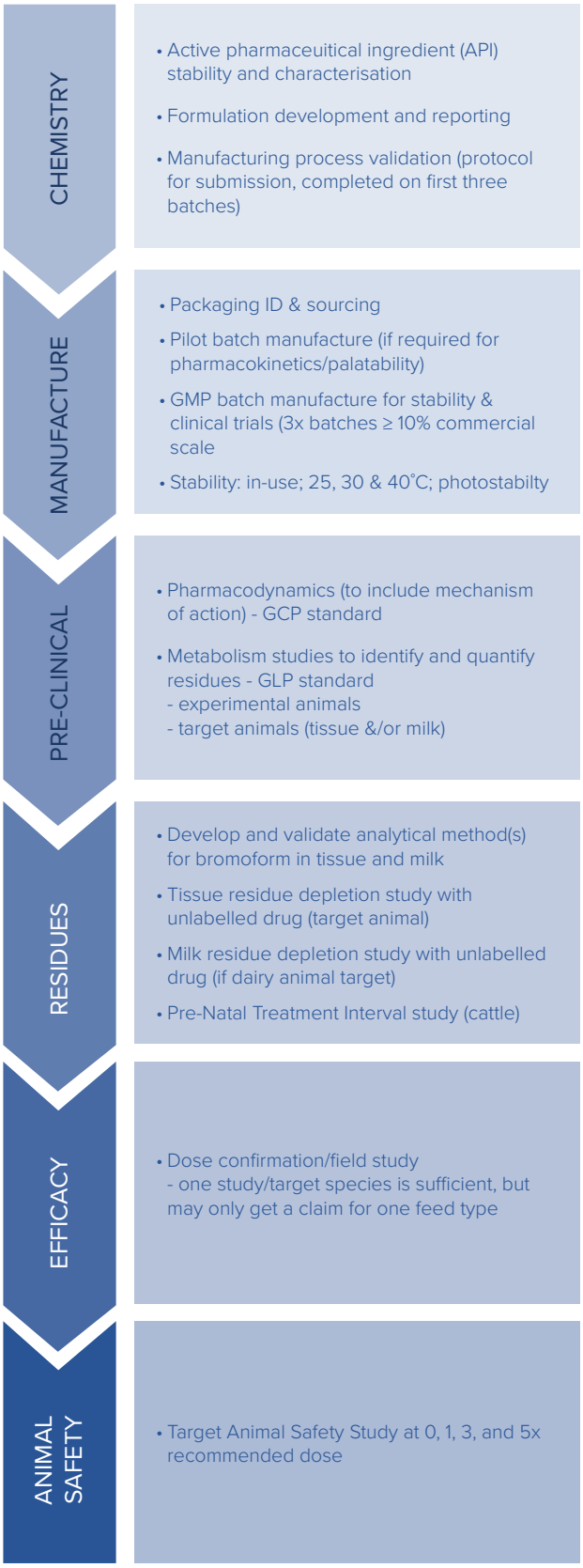


Figure 2. Data requirements for ACVM registration of a bromoform-containing methane inhibitor

35 OECD. "Good Laboratory Practice (GLP)". *Organisation for Economic Co-operation and Development.* https://www.oecd.org/chemicalsafety/testing/good-laboratory-practiceglp.htm [accessed September 22, 2023].

36 VICH Steering Committee (2000). "Good Clinical Practice" *VICH International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products.*

37 ACVM. "Guideline: Good Manufacturing Practice". *New Zealand Food Safety Authority.* https://www.mpi.govt.nz/dmsdocument/1379-ACVM-Guideline-for-Good-Manufacturing-Practice- [accessed September 22, 2023].

Codex Maximum Residue Limits

Codex, as the Codex Alimentarius Commission is known, sets Maximum Residue Limits (MRLs) for all food and animal feed.

Codex MRLs are important to Aotearoa New Zealand as they provide assurance that its animal products will be accepted in export markets. If bromoform residues are elevated above background levels, the current lack of bromoform MRLs worldwide necessitates the establishment of a Codex MRL to ensure the detection of bromoform residues does not disrupt trade.

Should background levels of bromoform in tissues be established and these levels are not significantly impacted by treatment with bromoform at the recommended dosage, there will be no need for Codex to establish MRLs for bromoform. This would significantly reduce the regulatory burden for developers.

Should Codex determine that an MRL is required, determination of the residue limit will require metabolism and residue data and toxicology trials. These data must be generated in compliance with GLP standards and are summarised in Figure 3.

Figure 3. Data requirements for determination of a Codex maximum residue limit (MRL)
VICH = International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products, GL# = guideline #

Codex metabolism and residues requirements

- Metabolism and residue kinetics studies – target animals (VICH GL46) (sheep and cattle)
- Metabolism and residue kinetics studies – laboratory animals (VICH GL47)
- Tissue residue depletion studies – non-radiolabelled drug (VICH GL48) (sheep and cattle)
- Milk residue depletion studies – non-radiolabelled drug (VICH GL48) (sheep and cattle)
- Analytical method validation (VICH GL 49)

Codex toxicology requirements

- General systemic toxicity:
  - range finding study,
  - 90-day study in rats or combined 2 year chronic toxicity/carcinogenicity study in rats
  - 1 year study in dogs
- Genotoxicity
  - In vitro test battery (ie. *Salmonella*/microsome assay and one or two tests in mammalian cells detecting point mutations or chromosome damage)
  - Confirmatory in vivo genotoxicity test if any of the results above are positive
- Carcinogenicity
  - 2 year rat carcinogenicity study (the chronic systemic toxicity study could be incorporated into this, as noted above)
  - 18 month mouse study
- Reproductive and developmental toxicity
  - Two-generation reproductive toxicity study in the rat
  - Developmental toxicity study in the rat
  - Developmental toxicity study in the rabbit (unless clear teratogenicity was observed in the above study, unless teratogenicity in the rat was the critical effect for the setting of the ADI
- Endocrine toxicity
  - Endocrine disruption sensitive endpoints are included in the Two-generation reproductive toxicity study
  - Hazard assessment should be completed to determine what further studies may be required in this area
- Neurotoxicity
  - Only required when indicated from structure-activity considerations or results of other toxicity tests.
- Immunotoxicity
  - Local lymph node assay in mice (to evaluate skin sensitising potential)
  - Further specific immunotoxicity studies should be conducted if a risk is identified or suspected.

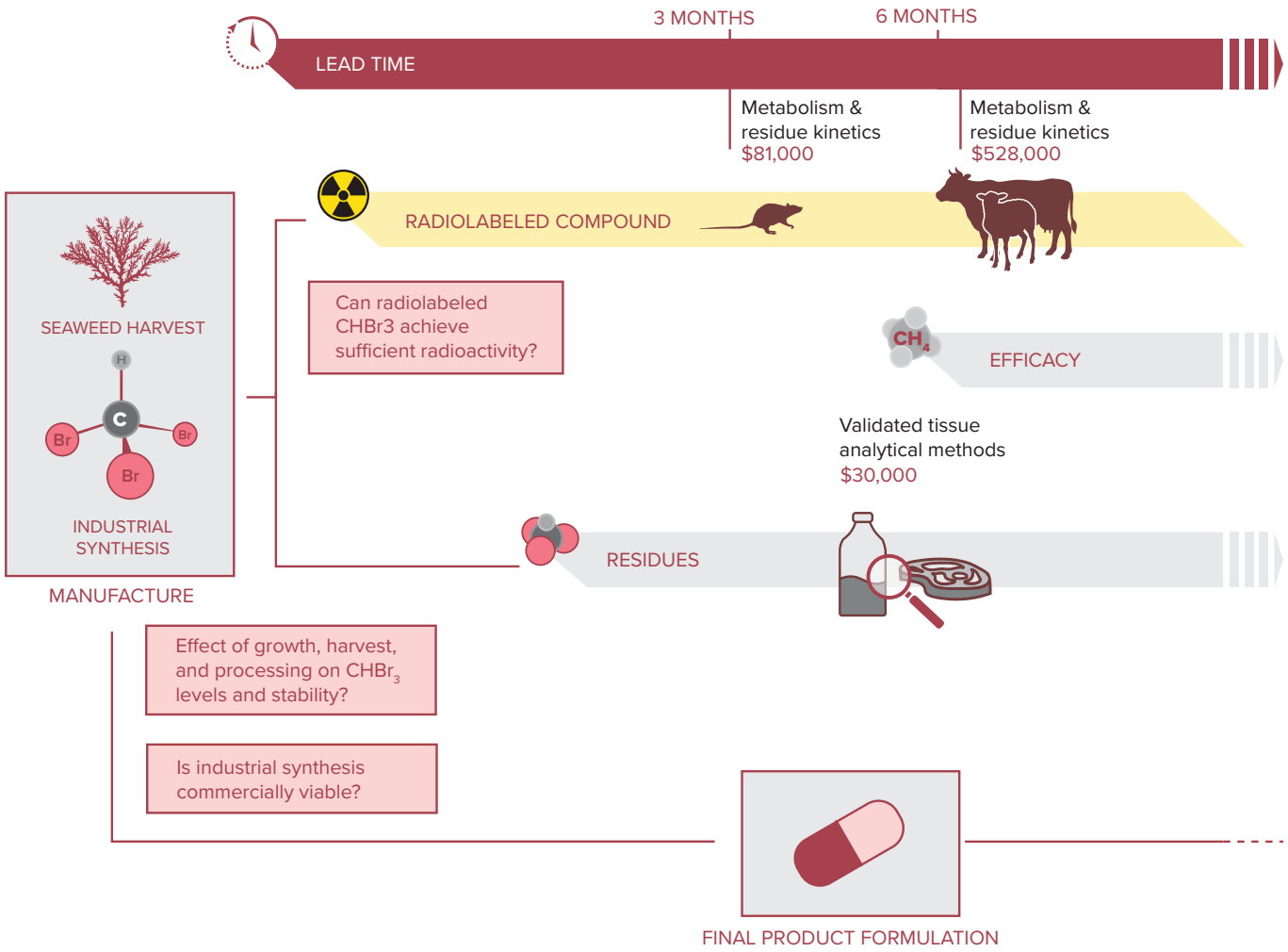
Indicative Research Costs

The flowchart below models the trial requirements for a bromoform-containing inhibitor product in the registration phase of its product development journey. Bromoform product developers can expect to spend a minimum of NZ$2 million to gather the data with which to achieve regulatory approval of a product for cattle with limited claims in one feed type. This cost assumes minimum formulation development and only one in-vivo dose determination study for a feed supplement to be administered daily. It also assumes a Codex MRL is not required.

Costs are estimates only and a ±20% contingency should be applied. Final cost will be dependent on the final study design. Time and cost savings may be achieved by conducting studies in a coordinated programme.

Lead times quoted do not include the required approvals (animal ethics, ACVM, EPA etc), but instead give an indication of the current capacity at the stated provider as of late 2023.

Bromoform is likely to be delivered either in-feed or via an intra-ruminal capsule. An in-feed product would need to be administered once

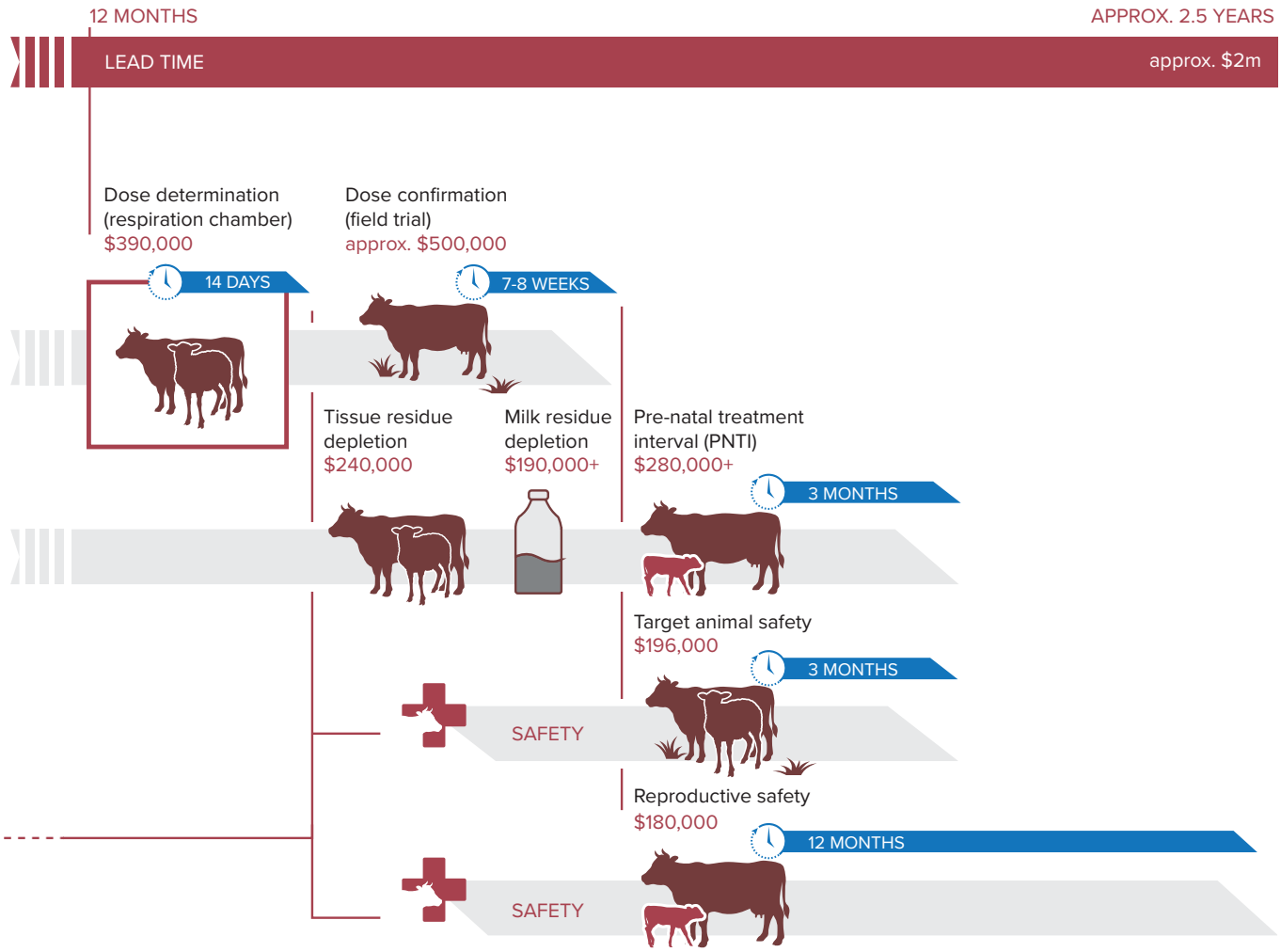


to twice daily, whilst the intra-ruminal capsule could be expected to deliver a continuous dose over a certain period. The dose form will not significantly change the regulatory requirements; however, costings have been based on an in-feed product due to the additional manpower required for dosing.

Note the costs indicated below represent one stage of the product development journey - assembling a portfolio of data to support the product registration. Further trial data will be required to achieve efficacy claims through the GHG Inventory.

Costs provided in consultation with:

- Invetus: www.invetus.com
- Analytica Laboratories: www.analytica.co.nz
- DairyNZ: www.dairynz.co.nz
- AgResearch: www.agresearch.co.nz
- Charles River Laboratories: www.criver.co



Insights and Next Steps

Methane mitigation is a rapidly evolving field. During the preparation of this report, new research data has emerged, various regulatory policies have been clarified or updated, and industry-wide forums have been held to progress the regulation of methane inhibitors. As far as possible the authors have sought to capture the latest information from our engagement with research literature, regulatory experts, and the wider industry.

Through this engagement, many insights emerged. The report presents these insights and suggested ‘next steps’ below. In some instances individuals, groups, or government agencies may have already begun this work or have similar historic workstreams in place. In instances where this work is ongoing, the authors simply encourage the continuation of these efforts and industry-wide discussion of the results obtained.

Insight	Next Steps
Timelines and costs of registering bromoform under Aotearoa New Zealand regulation are greater than many industry participants perceive.	All parties should seek to minimise both time and cost by coordinated action.
Early stages of the inhibitor development journey are common across many bromoform products.	A coordinated national research & development work programme may accelerate path-to-market for all bromoform inhibitor products.
The long-term nature of bromoform efficacy data could delay registrations and increase costs for regulatory compliance.	Consideration should be given by product developers to a staged registration, starting with short-term and extending to longer-term efficacy claims as data is obtained.
There is potential for greater alignment among stakeholders than has already been achieved, and for coordinated actions to be commissioned between them.	Efforts to communicate and align towards common goals should be continued or expanded.
Common interests in methane inhibitors exist between Australia & Aotearoa New Zealand.	High-level cooperation between Australia and Aotearoa New Zealand, such as data sharing, may accelerate both countries' bromoform programmes.
Regulatory approaches to bromoform-containing products internationally are rapidly evolving.	Maintaining close intelligence on the international landscape for bromoform regulation, particularly in jurisdictions covering Aotearoa New Zealand's major markets and agricultural competitors, will be valuable.
Some stakeholders perceive significant market risk from bromoform even if registered.	Stakeholders should further expand their existing efforts to align on these high-level concerns.
There is a possibility that bromoform will not be able to be registered under the existing framework, or that the market will not incentivise product developers to perform the necessary trials to achieve regulatory compliance.	Detailed analysis of the costs, benefits, and the likelihood of bromoform achieving successful registration, continuing from this report's findings, should be undertaken.