

# GE ANIMALS in New Zealand



## GENETICALLY ENGINEERED ANIMALS: THE FIRST FIFTEEN YEARS

GE Free NZ in Food and Environment has compiled this report from information gathered through AgResearch annual reports and Official Information Act requests (OIA).



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# EXECUTIVE SUMMARY

"GE Animals in New Zealand: the first fifteen years" documents the world's first field trials of transgenic cows. These cows have been bred to express one of six transgenic protein traits in their milks for use as bio-pharmaceutical products (biologics). AgResearch has carried out these "bio-pharming" trials for the last fifteen years, 2000 - 2015, at their Ruakura facility in Hamilton, New Zealand.

The information is obtained from Official Information Act (OIA) requests and the comprehensive health details in the AgResearch reports that were submitted annually to the Environmental Risk Management Authority (ERMA), now the Environmental Protection Authority (EPA).

These annual reports catalogue a sad and profoundly disturbing story of illness, reproductive failure and birth deformities that have consistently afflicted the genetic engineering (GE) trials.

Both the surrogate and transgenic cows suffer from chronic illness, reproductive losses, sudden unexplained deaths and severe deformities, relating to the foreign DNA inserted in the embryos used in the artificial insemination programme. Most of the transgenic cows are not able to reproduce past the first generation. The transgenic cows that have produced a second generation have borne sterile offspring.

After fifteen years of experimentation, from the many thousands of transgenic embryos the cows have carried, the average live birth rate has ranged from 0 - 7%. These embryos have been predominately developed offshore in the private partnership laboratories. In December 2014, there were a total of 19 transgenic cows survive at the Ruakura facility.

Clinical trials on transgenic proteins have resulted in allergic reactions in subjects causing the trials to be terminated early. It is noteworthy that, the proteins that these animals have been modified to express are available on the market today, made from simpler non-transgenic processes or produced in genetically engineered bacteria in laboratory containment.

Omission or carefully selected reporting of important experimental data to the media has enabled AgResearch to avoid scrutiny into the tragic results of using animals as bioreactors. Questions need to be asked as to how the Ruakura Animal Ethics Committee, of which the SPCA is a member, reviews and approves GE animal research activities, with particular reference to animal welfare concerns. There are serious gaps in the management of the experiments and a collective silence on the treatment of animals.

The animals' suffering has been going on for many years, hidden from public view. Research must not be able to continue to over ride the moral or ethical responsibilities that arise from scientific endeavors.

Recently, AgResearch has announced that they have significant and ongoing funding challenges. This report questions whether the GE animals trials has led to some of the problems they are facing. Regardless, these costly GE trials are a failure and should be closed down immediately to stop further animals' suffering.

Claire Bleakley  
President of GE Free NZ in Food and Environment  
23 October 2015

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## A MESSAGE FROM STEFFAN BROWNING

Kia ora

Thank you for the opportunity to comment on this report on the genetically engineered animal research at Ruakura. This report highlights my long concerns about the AgResearch GE animal experiments.

In my previous role as spokesperson for the Soil & Health Association (2003-2011), I closely followed the GE animal field trial at AgResearch's Ruakura facility and regularly participated as a submitter in the authorisation process and hearings.

The report outlines each animal experiment with its considerable deformities and animal welfare problems. It is of great concern that the scientists and regulatory bodies responsible for the oversight of animal welfare continually ignore these results.

The chapter in this report, "Site testing for Horizontal Gene Transfer," explains a complete dereliction of duty and integrity by the AgResearch scientists. In its approval decision for AgResearch's GE animal program, the Environmental risk Management Authority (ERMA – now EPA) said that HGT was the biggest environmental risk and that if it was found, the program must stop. The scientists, AgResearch, Ministry for Primary Industries, and ultimately the EPA have not ensured the appropriate level of research, monitoring or care to meet their responsibilities.

High rainfalls in the Hamilton region culminate in surface flooding of pastures that have not only been grazed by GE animals, but are subject to spray irrigation with transgenic milk and animal blood. During my farm visit and during other observations, I noted that this surface flooding drained in part through various ditches off property, through the surrounding rural countryside, and through or under Hamilton City to the Waikato River.

The trials face continued public opposition due to animal welfare and GE issues. AgResearch reported through Parliament's 2013 Financial Review process that it was spending 10% of its livestock research budget on GE animals and 25% of its forage research budget on GE forage.

Considering that the proteins intended from GE animal production are invariably available from non-animal industrial processes, AgResearch's GE animal program fails to forward New Zealand's scientific direction. Scarce research funding would be better directed to genuinely sustainable and acceptable agricultural programs.

This report will be a valuable research for academics, regulators and the general community as an overview of the AgResearch genetically engineered animals program.

Steffan Browning MP | Green Party of Aotearoa New Zealand  
Spokesperson for Organics, GE, Biosecurity, Pesticides and Food Safety

# BACKGROUND TO GE ANIMALS TRIALS IN NEW ZEALAND

Cows, sheep and goats (producing human proteins in their milk)  
This document does not deal with the AgResearch cloning trials<sup>1 2 3</sup>

In the early 1990's biotechnology corporations in Europe invested in the development of transgenic livestock for the production of nutraceutical and bio-pharmaceutical proteins. Prior to this, biologics such as GE insulin, were produced in laboratory micro - fermentation vats.

After the scrapie / BSE outbreak in England, New Zealand was chosen as a safe country for the further commercial growth of the animal transgenic bioreactor industry, also known as 'bio-pharming'. This was due to its internationally recognised disease free status.

In 1992, New Zealand's Interim Assessment Group (IAG),<sup>4</sup> was set up, prior to 1998, to recommend approval of genetically engineered / modified organisms (GMO's) in New Zealand. In 1994, the IAG received an application from Scottish company PPL Therapeutics to import 37 transgenic rams to breed into a "manufacturing" transgenic ewe flock (IAG34).

The Minister of the Environment, Simon Upton, turned down the application, as New Zealand had no formal legislation to monitor or regulate GMO's.

In 1996, the Hazardous Substances and New Organisms Act (HSNO)<sup>5</sup> was passed into law. The same year Mitchell Partners – PPL Therapeutics NZ, in Whakamaru, reapplied and gained approval for its application (IAG40), allowing the importation of the 37 transgenic rams to be held in quarantine until the procedures for managing and monitoring the animals were in place.

It was not until 1998, that the HSNO Act regulations governing the risk assessment and monitoring procedures for the GMO approvals came into force.

## THE ENVIRONMENTAL RISK MANAGEMENT AUTHORITY (ERMA) 1996-2012

The Environmental Risk Management Authority (ERMA) was finally set up in 1998. The function of ERMA, a quasi-judicial body, was to regulate GMO's under the HSNO Act, by placing protocols and controls on the monitoring, inspection routines, staff entry, and accidental release on GE applications. In 2012, the Environmental Protection Authority (EPA) subsumed ERMA and took over their processes under the HSNO Act.

The application processes are set out in the following six stages:

1. Importation into approved containment facilities (not open for public submissions)<sup>6</sup>
2. Development in containment (not open for public submissions)
3. Outdoor Development (public submissions are discretionary)
4. Field Tests (open for public submissions)
5. Conditional Releases (open for public submissions since 2005)
6. Full Release (open for public submission)

<sup>1</sup> Animal death toll ends cloning trials <http://goo.gl/7S0RUh>

<sup>2</sup> Wells DN., Misica PM. & Tervit HR. (1999) Production of cloned calves following nuclear transfer with cultured adult mural granulose cells. *Biology of Reproduction*. 60: 996-1005.

<sup>3</sup> Ravelich R, et al. (2004) Insulin-Like Growth Factor-I and Binding Proteins 1, 2, and 3 in Bovine Nuclear Transfer Pregnancies. *Biology of Reproduction*. 70, 430-438.

<sup>4</sup> GMO's prior to 1998 IAG <http://www.epa.govt.nz/Documents/is-GMOs-prior-1998-IAG-pdf>.

<sup>5</sup> Hazardous Substances and New Organisms Act (HSNO); <http://goo.gl/Wnk757>

<sup>6</sup> New Organisms and HSNO Act <https://goo.gl/I9BcKr>

# EPA APPLICATION PROCESS FOR A GE ORGANISM APPROVAL UNDER HSNO

The HSNO stages are further defined below -

1 & 2 - Import and Development in containment stage - all GE research is to be conducted in an enclosed laboratory called a PC 2 laboratory.

3 - Outdoor development - an outdoor development is limited to two generations of animals and requires no environmental testing.

4. A field test can have multiple GE plant or animal generations and is seen as a scientific data gathering trial. Field tests are conducted in fields from 5 - 500 acres, considered as PC1 containment. Experiments are undertaken on the effects of the organism under conditions similar to those of the environment into which the GMO is likely to be released, but from which the organism, or any heritable material arising from it, could be retrieved or destroyed at the end of the tests.

5 - A Conditional Release stage entails similar testing as field trials but in multiple sites and has never been sought in New Zealand.

6 - A Full Release means that there are no controls placed on the release to the environment of GE organisms. They are no longer considered a new organism under the HSNO Act. Responsibility for any effects ensuing from such release would fall to Councils under the Resource Management Act. A Full Release of a GE organism has never been sought.

The three principal methods used for the creation of transgenic animals are:- DNA micro-injection, embryonic stem cell-mediated gene transfer and retrovirus-mediated gene transfer.

On receiving an application for a GE organism, an EPA staff member is assigned to work with the applicant to ensure the application meets the minimum criteria as set out in the HSNO Act. If the application is for a field test or an outdoor development trial, the application is opened to the public for submissions. The agency staff members then consider all the submissions and present their assessments in an Evaluation and Review Report, which sets out the evidence of all parties and makes recommendations to the HSNO committee who either approve the application with controls or decline it.

The HSNO committee is made up of Government appointed members. A hearing is conducted and is open to all submitters who want to be heard. The Authority approves or declines the application. If the outdoor development or field test is approved, conditions / controls relating to the containment facility standards, movement of people into or out of the facility, health records of animals, disposal of all animals that have carried transgenic embryos or are transgenic themselves and protocols for the end of the experiment.

## GE PROCESS OVERVIEW

- Applications for GE research animals in NZ sought after scrapie epidemic in UK.
- IAG initially approved GE animal trials in NZ (1982-1996).
- HSNO Act 1996 – legislation for risk assessment and methodology for Hazardous Substances (chemicals) and the New Organisms (plants, animals, GE/GM organisms)
- ERMA – initial administrator of application regulating procedures for HSNO
- EPA – the new administrator of applications regulating HSNO began in 2012.



# THE FIRST ANIMAL FIELD TRIAL: PPL THERAPEUTICS (NZ) WHAKAMARU



Figure 1: Whakamaru GE sheep ash disposal pits 2014

In 1997, ERMA formally approved the Mitchell Partners – PPL Therapeutics NZ transgenic sheep trial, application GMF98001<sup>7</sup> located on 500 acres in Whakamaru. A flock was to be developed from the earlier importation of transgenic rams carrying the recombinant human alpha-1-antitrypsin (rhAAT) genes, from a “Danish woman”. The transgenic ewe progeny were to express rhAAT protein in their milk, which would then be isolated from the sheep milk, to develop an experimental drug for patients with cystic fibrosis. The trial had permission to establish a manufacturing flock of 10,000 sheep. The flock grew to over 3000 transgenic ewes in the 7 years.

Bayer<sup>8</sup>, a partner of PPL, conducted trials in mice, the protein isolate was discovered to cause fatal anaphylaxis in laboratory mice<sup>9</sup>, despite this, clinical human trials commenced. They were subsequently halted during the Phase II trials due to a significant number of “dropout” subjects who suffered severe wheezing<sup>10</sup>. The failure of the clinical trials led to the closure of the Whakamaru research site in New Zealand. As a result, the flock was destroyed. Animal carcasses were incinerated and their ashes were buried in a pit. (See figure 1 and call out box).

The site was sold in 2004, immediately after the animals were disposed of. The PPL Therapeutics portfolio was sold to Dutch biotech company Pharming NV for \$854k (€710k) in cash. There was no monitoring or inspection for transgenic contamination prior to the sale of the land. Public concerns over the possibility of contamination from transgenic sheep led to a High Court case and parliamentary questions.<sup>11</sup> The site was later sold to Transpower as a base to operate from whilst they developed the National power grid.

## ALPHA-1-ANTITRYPSIN SHEEP RESULTS

- Surrogate East Friesian sheep, 150% birth rate, top milk producers
- Anti-alpha Trypsin (hAAT) protein gene to be expressed in the milk of progeny
- Surrogate sheep and progeny were prone to disease and unexplained death
- Live birth rate was very low, (less than 6%)
- Clinical trials: Failed – Rec: hAAT isolate – lung distress and severe wheezing
- 3000- transgenic sheep incinerated,
- PPL went bankrupt sold IP to Pharming (NV)

<sup>7</sup> Decision GMF 98001 <http://www.epa.govt.nz/Documents/GMF98001-decision.pdf>

<sup>8</sup> Bayer Corporation and PPL Therapeutics Announce Collaboration Development of a Recombinant Aerosol Formulation for AAT Deficiency [www.investor.bayer.com/secured/9681](http://www.investor.bayer.com/secured/9681)

<sup>9</sup> Lu Y. et al. (2008) Human alpha 1-antitrypsin therapy induces fatal anaphylaxis in non-obese diabetic mice. <http://goo.gl/r9IQXY>

<sup>10</sup> Doubts over “pharming” technology <http://goo.gl/bmLZNS>

<sup>11</sup> Transgenic Sheep – Environment; Whakamaru Farm, 18 May, 2014, New Zealand Parliament <http://goo.gl/nC9xyB>



## NEW ZEALAND'S ROYAL COMMISSION ON GENETIC MODIFICATION (2000)



The Royal Commission on Genetic Modification (RCGM)<sup>12</sup> in 2000 was charged to investigate and recommend strategic options, using legislative, regulatory and policy measures, which would enable New Zealand to address present and future safety concerns for genetic modification in the environment and in products (RCGM 2001b,p.158).

The Commission set out 49 recommendations for safeguards to ensure the wellbeing of communities and the environment" (RCGM 2001a, p.322). Two of the recommendations specific to animals stated:

**7.5** "That, wherever possible non-food animals, or animals less likely to enter the food chain be used as bioreactors rather than animals that are a common food source".

And

**7.6** "That, wherever possible, synthetic or mammalian homologue's of human genes be used in transgenic animals to avoid the use of genes derived directly from humans".<sup>13</sup>

As documented by Sustainable Future (McGuinness Institute) in their 2008 review,<sup>14</sup> seventeen of the 49 recommendations have not been implemented. The failure to adhere to the RCGM recommendations has impacted on the humane treatment of sentient animals. The subsequent disestablishment of the Bioethics Council, a recommendation of the RCMG, by central government has further removed government ethics oversight.

<sup>12</sup> Report of the Royal Commission on Genetic Modification <http://goo.gl/9D0EVw>.

<sup>13</sup> Report Of The Royal Commission On Genetic Modification 2001 Chapter 15 p.335 <http://goo.gl/Y4fL3o>

<sup>14</sup> Review Of The Forty-Nine Recommendations of The Royal Commission on Genetic Modification. (2008) *Sustainable Future* p.107

## AGRESEARCH RUAKURA FACILITY, NEW ZEALAND GE ANIMALS APPLICATION GMF 98009 (1999-2010)

In 1999, a New Zealand Crown Research Institute, AgResearch, applied to ERMA<sup>15</sup> to create genetically engineered cows to express three types of transgenic proteins in their milk<sup>15a</sup>. The three traits were:- insertion of an extra casein gene, (C+), the deletion or knockdown of the Beta- lactoglobulin gene (BLG -) and the insertion of a human myelin basic protein gene (rhMPB).

In February 2000, ERMA approved two of the three traits: C+ and BLG-. In June 2000, following much discussion with Maori, the third trait, rhMBP, was approved. The decision by ERMA to approve the use of rhMBP embryos engineered with human genes in the cows was challenged in court.

Justice Joan Goddard upheld the challenge, finding ERMA had failed to state key safety criteria in the methodology.<sup>16</sup>

***"I consider there was an error of law in failure to state criteria in the methodology relied on in the decision, and that such error was material" J. Goddard 2/5/2001***

Whilst the case was under consideration, AgResearch went ahead with the MBP embryo transfer (ET) impregnating 60 surrogate/recipient cows. When the court decision found in favour of the challengers, the application was referred back for reconsideration to ERMA. Only seven pregnancies remained.

At this time two studies showing new information on adverse effects of the MBP by Bielekova et al<sup>17</sup> and Kappos et al<sup>18</sup> (2000) were published. The studies documented a 2-½ year clinical phase II Multiple Sclerosis (MS) trial on the recombinant MBP protein, involving 7 countries and 142 patients with relapsing - remitting MS. Patients suffered severe hypersensitivity reactions, chest pains, flushing, shortness of breath and severe cytokine storms demonstrating that it had the potential to cause encephalitis. There was also an increase in nerve lesions in 9% of patients. The trial was halted after 26 months.

These articles on MBP and its adverse effects were brought to the attention of ERMA whilst they were reconsidering the approval. However ERMA refused to discuss the findings with the scientists who had conducted the research and went on to approve the MBP cow field trial, with added controls.

These were:- a requirement to provide animal health records and conduct horizontal gene transfer testing. AgResearch provides this information in annual reports to ERMA.

<sup>15</sup> AgResearch Application to create transgenic cows, GMF98009 <http://goo.gl/nbAuu6>

<sup>15a</sup> Transgenic cows making therapeutic proteins <http://goo.gl/HnXzak>

<sup>16</sup> P177/00 Appeal against the decision GMF98009 of the Environmental Risk Management Authority between Claire Bleakley and ERMA. Reserved decision of Goddard J, 2/5/2001.

<sup>17</sup> Bielekova et al. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83-00) in multiple sclerosis: Results of a phase II clinical trial with an altered peptide ligand. *Nature Medicine*, 2000. 6: 10 1167-1175

<sup>18</sup> Kappos et al. Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of altered peptide ligand in placebo controlled, randomised phase II trial. *Nature Medicine*, 2000. 6:10:1176-1182.

# CASEIN PLUS (C+) COWS

Casein plus (C+) cows express an extra transgenic casein gene that increases the levels of the casein protein in their milk, highly sought after in cheese making. AgResearch developed seven transgenic C+ cell lines carrying two copies of the transgenic casein. These cell lines were sourced from the rare, naturally- occurring variants, kappa and beta casein proteins. The bovine fetal fibroblast (BFF) host cell line was isolated from the lung tissue of an aborted fetus from a Friesian cow. The BFF cells were transfected with the two casein protein constructs, which resulted in multiple copies of co-integrated transgenes. The seven transgenic lines carry up to 4-5 casein copies each. However, the casein levels in milk tested varied considerably, the TG 3 line had almost 20% more kappa casein than non-GE dairy cows, but no difference in the beta casein. The TG 5 & 7 line did not produce detectable levels of K-casein.<sup>19</sup>

Of the initial transgenic C+ embryo transfers implanted in 1999, eight calves were born (Casein 501-508). One calf was stillborn (502) and another calf (503) was oversized and had no bladder. Its pericardial sac was fused to the chest wall and it died in the first 24 hours. The remaining calves blood tests showed liver, spleen and white and red blood cell abnormalities.<sup>20</sup>

In the years 2000 - 2002, a total of 636 transgenic C+ embryos were implanted, of which, 366 developed to the first 56 days, 24 pregnancies reached full term and 6 heifer calves survived to generate the first founder casein calves.

**TABLE 1**

C+ embryos implanted	C+ survived to day 56	Fetuses full term	Heifer calves at weaning
636	366	24	6

Valuable information was gathered from the Casein+ cow experiment relating to the movement of transgenic fetal blood across the placental barrier. In collaboration with Dr. Turin, Milan University of Veterinary Medicine, AgResearch traced the leakage of transgenic C+ fetal blood into the maternal circulation of non-transgenic recipient cows (fetal-maternal microchimerism) whilst in pregnancy and post-calving. Transgenic Casein DNA from the fetus was found to persist in the maternal circulation for up to two years post calving.<sup>21</sup> This study raised the possibility the transgenic process could “enhance transfer across the placenta” (Turin, 2007. p.490) and the transgenic fetal blood might engraft itself into the maternal lymphoid tissue or bone marrow with unknown implications.

The dangers posed to the environment and animals from this transgenic trial could have been averted if consideration of a more natural source of high casein levels had been researched. The milk of the rare heritage breed of Modenese cattle from Northern Italy have high levels of casein proteins, calcium and phosphorous and less chloride in their milk than transgenic cows.<sup>22</sup>

There are 14 living C+ transgenic animals in the facility.

## CASEIN+ TRIAL RESULTS

- Deformities and abortions common
- 62% of embryos survived development
- 17% of pregnancies reached full term
- 6% live birth survival
- 14 C+ cows alive after 14 years of experiments (2000-2014)
- Transgenic casein fetal blood leakage into maternal blood system
- Transgenic fetal blood persists for two years in maternal circulation post calving
- Chronic lameness, chronic arthritic changes in weight bearing joints

<sup>21</sup> Brophy B. *et al* (2003) Cloned transgenic cattle produce milk with higher levels of B-casein and K-casein. *Nature Biotechnology*. Vol: 21:157-161

<sup>20</sup> AgResearch Lab Reports of transgenic calves (1999-2000) <http://www.gefree.org.nz/reports-and-submissions/>

<sup>21</sup> Turin L. *et al*. (2007) Bovine fetal microchimerism in normal transfer pregnancies and its implications for biotechnology applications in cattle

<sup>22</sup> Summer A. *et al*. (2002). Structural and functional characteristics of Modenese cow milk in Parmigiano-Reggiano cheese production. *Ann.Fao.Medio.Vet di Parma*. Vol:22:163 -174

## BETA-LACTOGLOBULIN KNOCKDOWN (BLG-) COWS



Figure 2: Daisy, AgResearch file photo

Beta-lactoglobulin knockdown (BLG-) trial - in 2000, ERMA approved the Beta-lactoglobulin knockdown (BLG-) trial the cow's milk expressed no Beta-lactoglobulin(BLG-) protein. The approved method failed to create viable embryos.

After 11 years of failure to create viable embryos using DNA cloning, AgResearch refocused their research on a technique using MiRNA mouse and sheep genes to silence the unwanted gene. In December 2010, 103 transgenic BLG- embryos were implanted into recipient cows. All embryos had aborted by day 52.

In February 2011, 107 BLG- embryos were implanted. In March, there were five pregnancies and by June, there were only two pregnancies remaining. Of the two remaining pregnancies, one of the cows started to abort. She was slaughtered and the live fetus was “recovered”. The cells were used to re-engineer new embryos.

The remaining cow gave birth prematurely to “Daisy” at 255 days. (The normal gestation period is 285 days). Both Daisy and her mother suffered from excessive abdominal fluid (hydrops). Daisy still suffers from a swollen abdomen and has pelvic deformities and no tail. She developed an outward curve of her lower front limbs with a collapse of her inner digit of the medial hoof, and she has been given “walkease” blocks to alter her gait. Daisy has regular two weekly harvesting of her eggs to produce embryos for future use. (AgResearch Annual Report 2013)<sup>23</sup>

In September 2012, 70 BLG- embryos were transferred to recipient cows, resulting in six pregnancies. Four of the recipient cows were euthanased after developing hydrops and aborting their calves. Three of the aborted calves were recovered. The fourth cow was culled for fetal cell line collection. Of the remaining two pregnancies, one lasted until January when the cow developed hydrops. She was induced and her calf was born dead and she died soon afterwards. The remaining cow whose fetus was to be used for cell collection was euthanased in March 2013, after 164 days gestation. Her calf was unviable and no cells could be harvested, but it did have a tail.

Camel milk contains no Beta-lactoglobulin and could be used as a viable alternative, instead of the GE cows, for the health effects sought.

The facility in 2014 has one living transgenic BLG- calf named Daisy.

### **BETA-LACTOGLOBULIN KNOCKDOWN RESULTS**

- Reproductive failure and abortions common
- Unable to create viable embryos in first 11 years
- 210 embryo transfers, 1 live birth survival (2010-2014)
- 1 B L G -cow surviving after 14 years of experiments (2000-2014)
- Routine oocyte (ova) recovery and harvesting of eggs, the embryo yield low

<sup>23</sup> Annual report to ERMA New Zealand for Activities under GMF 98009, GMD02028 & ERMA 200223, 2013 (p.8-12)

# MYELIN BASIC PROTEIN (rhMPB) COWS



Figure 3: AgResearch transgenic cow

Myelin Basic Protein (rhMPB) cows<sup>24</sup> – These cows' milks express a recombinant human Myelin Basic (rhMBP) protein, to be used for pharmaceutical drug development. Myelin Basic Protein is the major protein constituent of the myelin sheath that surrounds the nerves. Demyelination of these sheaths occurs in illnesses like multiple sclerosis.

In 2000, 60 embryo transfers were performed. Of these, six surrogate cows carrying 7 calves came to term. Four calves were born live. Twin calves were stillborn. The first twin was 28kg and had started to decompose. It had enlarged thyroids. The second twin weighed 33 kg and had excess fluid in its peritoneal cavity, an enlarged and mottled liver, congested lungs and kidney s, and no bladder. A third calf died soon after birth, from liver failure.

By 2002, another 60 embryo transfers with four more calves carrying the rhMBP were born.<sup>25</sup>

In 2005, a new technique was used to create the rhMPB embryos. All the pregnancies, from the 130 embryo transfers, failed to produce any live births and did not meet expectations.<sup>26</sup>

The oldest of the founder (F0) cows is 11 years old one further cow has been born creating a first generation (F1). After 7 years of matings, the F1 progeny are unable to reproduce. The existing cows have been induced into milk and in 2007, the rhMPB milk was sent to the Malaghan Institute for a study on mice. Massey University PhD student Al Ghobashy (2009) thesis<sup>27</sup> found the cows' milk protein expression of MBP was different to humans.

Ghobashy (2009) gives further detail on the implications of the post-translational modification. Fifteen years later, from the hundreds of embryo transfers there are now only three surviving animals.

The experiment is on hold and the three remaining animals are living out their lives.

## MYELIN BASIC PROTEIN RESULTS

- Deformities, mastitis, pregnancy complications and abortions common
- 1% of pregnancies reach full term
- Founder (F0) generation only
- rhMBP Progeny reproduction failure
- rhMBP isolate was modified to a casein-like protein, not similar to human MBP
- 3 rhMBP cows alive after 14 years of experiments (2000-2015)

<sup>24</sup> <http://www.epa.govt.nz/search-databases/Pages/applications-details.aspx?appID=GMF98009#>

<sup>25</sup> Claire Bleakley OIA request 2003. <http://goo.gl/MgyyqQ>

<sup>26</sup> AgResearch Annual Report 2005 <http://goo.gl/4Yrm6Y>

<sup>27</sup> Al-Ghobashy MA. (2009) Downstream Purification and Analysis of the Recombinant Human Myelin Basic Protein Produced in the Milk of Transgenic Cows, Massey University (Palmerston North) <http://goo.gl/4i4FXL>



## APPLICATION GMD02028

In 2002, a generic outdoor development GM application to create a range of transgenic cows expressing human proteins, was approved by ERMA (GMD02028). A High Court case was taken by Mothers Against Genetic Engineering (MAdGE)<sup>28</sup> to challenge the generic nature of the experiment. The case was unsuccessful and MAdGE was charged with costs of \$32,000, which caused it to fold.

There have been three pharmaceutical constructs developed under this approval.



Figure 4: MAdGE poster Keep our milk GE Free

## NGA KAIHAUTU TIKANGA TAIO

Ngā Kaihautū Tikanga Taiao is a statutory (legally mandated) Māori Advisory Committee that is appointed by the EPA Board. Ngā Kaihautū Tikanga Taiao committee have considered, from a Maori perspective, all the GE animal applications and assessed their possible effects on tangata whenua.<sup>28a</sup>

When approving the GM animal applications, Ngā Kaihautū advised the ERMA decision panel that the risk to the relationship of Maori [particularly Ngāti Wairere] with their taonga [treasures] is likely to be significant".<sup>28b</sup>

To minimise the risk the ERMA controls mandated that representatives from Ngāti Wairere, the tangata whenua of Ruakura, set up a monitoring group to work constructively with AgResearch. Together they were to implement and oversee culturally appropriate protocols on the GM animal trials.

There are many Maori who consider genetic modification to be contrary to their tikanga because of the interference with the whakapapa and the mauri of all species.<sup>28c</sup> These views were aired in a pre-application hui in 2008,<sup>28d</sup> where there was strong opposition to transgenics for a variety of reasons including: the effect on the whakapapa of the research animals; potential, unforeseen effects; potential horizontal gene transfer; and the risk of containment being breached. There was recognition that there might be some potential health and economic benefits for New Zealand, tempered with reservations as to whether those benefits would be realised.

The monitoring groups were Ahi Ka and Te Kotuku Whenua. They were disbanded in 2010.

<sup>28</sup> High Court Mothers Against Genetic Engineering vs. Minister of the Environment CIV.2003-404-673  
<http://www.gefree.org.nz/assets/pdf/MAdGE-v-Minister-for-the-Environment-copy.pdf>

<sup>28a</sup> Ngā Kaihautū Tikanga Taiao [http://www.epa.govt.nz/te-hautu/who-we-are/Nga\\_Kaihautu/Pages/default.aspx](http://www.epa.govt.nz/te-hautu/who-we-are/Nga_Kaihautu/Pages/default.aspx)

<sup>28b</sup> Simon Collins Human genes in cows 'jumps the gun' 2002 [http://www.nzherald.co.nz/nz/news/article.cfm?c\\_id=1&objectid=787579](http://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=787579)

<sup>28c</sup> Issues of Significance to Māori <http://www.parliament.nz/resource/0000146804>

<sup>28d</sup> Pre-Application Consultation Hui With Maori On Transgenic Research 2008 <http://goo.gl/gQqC8R>

# LACTOFERRIN (LF) COWS

In 2005, Pharming NV announced the partnership with AgResearch<sup>29</sup> for the manufacture of transgenic cows expressing the recombinant human lactoferrin gene (rhLF). ERMA approved the experimental development amid controversy<sup>30</sup> concerning irregularities with the importation from the Netherlands of embryos containing the rhLF transgene for implementation.

In 2006, there were 233 rhLF embryo transfers (ET), which produced 12 live female calves. In 2007, there were 96 ET's and 18 births. Fourteen of these were euthanased, due to being male or for humane reasons. In 2008, 20 cows received ET's resulting in two pregnancies. These were medically aborted at day 31 and day 40 and the fetuses harvested to "generate additional new cell lines" (Annual Report 2008, p.41). As of August 2013 there were 10 transgenic rhLF founder cows existing in the facility.

In the years 2006-2009, the herd grew to 23 founder (F0, first generation cows. Of the transgenic rhLF animals at the facility there were only F0 animals and no further generations. The transgenic cows have a high abortion rate and suffer from sterility, heart abnormalities, mastitis, ligament problems and early arthritis and many have had to be euthanased for humane reasons.

Lactoferrin regulates the absorption of iron, zinc and copper in the intestine and is important for the delivery of these elements to the cells. Mother's milk contains the highest levels of lactoferrin and aids in protecting breastfed babies against bacterial infections. It is also used for those with digestive problems. Other animals and plants have been engineered to express or produce recombinant rhLF. It is produced in commercial quantities isolated from cow's colostrum and is an important commodity for Fonterra.

Clinical studies on the safety of recombinant human lactoferrin from the NZ experimental cows milk have not been conducted. In 2011, a Chinese study by Yu et al<sup>31</sup> compared the recombinant human lactoferrin with human breast milk. They found the rhLF molecule differed in the post - translational modifications and glycosylation processes, distorting how the protein folded, compared with the lactoferrin in human breast milk. These changes in the rhLF affected its degradation and digestive absorption. The glycosylation residues from other molecules could lead to immune reactions not seen with breast milk lactoferrin (p.220).

The Goven *et al* (2008) report<sup>32</sup> clearly defines the meaning of "post-translational modifications and glycoproteins" (Box 2, p.28).

As of 2014, AgResearch has no rhLF animals in the facility as the funding has been discontinued.

## HUMAN LACTOFERRIN RESULTS

- Founder generation created
- No ensuing progeny
- Reproductive failure of progeny
- High abortion rates (92%)
- Heart abnormalities
- Mastitis
- Ligament and arthritis problems
- All animals killed 2014

<sup>29</sup> Pharming Announces Partnership with AgResearch for Human Lactoferrin, <http://goo.gl/s48Zh>

<sup>30</sup> AgResearch GE Cow Application Contravenes Law, <http://goo.gl/ICXUQr>

<sup>31</sup> Comprehensive characterization of the site-specific N-glycosylation of wild-type and recombinant human lactoferrin expressed in the milk of transgenic cloned cattle. <http://goo.gl/oplULA>

<sup>32</sup> Goven J., Hunt L., Shamy D. and Heinemann J (2008) Constructive Conversations/Kōrero Whakaaetanga (Phase 2): Animal Biopharming in New Zealand Drivers, Scenarios and Practical Implications [www.conversations.canterbury.ac.nz/documents/animalbioreport.pdf](http://www.conversations.canterbury.ac.nz/documents/animalbioreport.pdf)



## FOLLICLE-STIMULATING HORMONE (FSH) COWS



Figure 5: Follicle stimulating hormone calf -photo Steffan Browning

In November 2005, under a delegated approval, the Ruakura Animal Ethics Committee (RAEC) approved the trial to produce cows expressing milk that contained copies of the transgenic human follicle-stimulating hormone (rhFSH) gene for drug therapy. Normally, FSH is produced and secreted by the ovaries and testes and regulated by the anterior pituitary gland, it is essential for the development, growth, pubertal maturation, and reproductive processes of the mammalian body. Trials on the rhFSH<sup>33</sup> did not lead to an improvement of conventional or sperm parameters or an increase in pregnancy rates.

On the 19<sup>th</sup> May 2006, AgResearch veterinarians transferred 28 rhFSH embryos to recipient cows, all of the resulting pregnancies failed. A second run of 28 embryo transfers was undertaken on 11th August 2006. At gestational day 42, 27 pregnancies had failed and only one cow was carrying a calf. The fetus was aborted and its cells were used to rederive new rhFSH cell lines using somatic nuclear transfer.<sup>34</sup>

Due to a mishap during the recovery procedure, the fetus was “destroyed in a way that no cellular material suitable for in vitro cell culture could be recovered”.<sup>35</sup> The process of “rederiving cell lines” is performed because genetically modified embryonic cells stop dividing and cannot be cultured in a laboratory (ex vivo) beyond day seven. Regenerated transgenic fetal cell lines are reported to have “undiminished vigor”.

In 2007, after two years of failing to produce rhFSH calves, 226 embryo transfers were implanted into non transgenic recipient cows and 11 cows maintained pregnancy until day 213, when five calves were aborted on humane grounds. A cow’s gestation lasts about 275-285 days. The remaining 6 recipient cows needed assistance with calving, as the calves were large.

There were six live calves born; of these, two were euthanased. Of the four remaining transgenic calves, one did not express the transgene, but was kept as a control. The three calves that expressed the rhFSH gene showed male-like muscular development, enlarged abdomens, precocious udder development and faster heart and respiratory rates and one had severe deformities in the hind limbs. It was determined the abnormalities were triggered from the high levels of rhFSH expression resulting in raised levels of oestradiol, causing a hyper-stimulation of the endocrine system, due to the transgenic hormone “leaking” into the blood. This contradicted AgResearch’s belief the GE hormone could be restricted to expression in the milk.

The calves also had early fusion of their growth plates, causing them to become knock-kneed and making it difficult for them to support their extra weight. Three of the calves also suffered from abnormally large ovaries and all animals were infertile.

<sup>33</sup> Kamischke A. et al. Recombinant human follicle stimulating hormone for treatment of male idiopathic infertility: a randomized, double-blind, placebo-controlled, clinical trial. Hum Reprod. 1998 (3):596-603. <http://www.ncbi.nlm.nih.gov/pubmed/9572419>

<sup>34</sup> Re-derived cell lines – aborted fetuses from selected transgenic events are the basis for further genetic engineering either by classic gene targeting, gene editing or recombinase-mediated transgene integration to create new cell lines. AgResearch OIA.

<sup>35</sup> Committee and applicants notes attached to the #10724 AE Application. Re Interim 211 (03/11/2006) Received under OIA request.

*Heart and respiratory rate elevated as a likely side effect of the increased estrogen levels associated with the hFSH gene input"*

*Veterinary Report 8/7/2009*

At five months old, one of the calves was found dead in the field. This was caused by rupture of the umbilical artery due to the "stretching and distortion" of the oversized ovaries. (Normal ovaries of calves at this age are approximately the size of a thumbnail. However, the ovaries of the rhFSH calves were the size of tennis balls). A second calf was found dead and the autopsy revealed the oversized ovaries had become twisted and separated from the uterus (Watson & Beedle, p.7).<sup>36</sup>

The third rhFSH calf was euthanased, October 2010, due to its poor health, respiratory distress and enlarged ovaries. The fourth calf had an enlarged head, shortened and bowed front legs that affected standing, polycystic ovaries and a high circulating level of rhFSH (see figure 5).

#### **HUMAN FOLLICLE STIMULATING HORMONE**

- Years 1-2: failure to produce a live calf
- Year 3: 226 embryos were transferred
- Six hFSH calves born, four calves were live
- Animals were infertile and had abnormally large ovaries
- After 2 years all 4 surviving calves had died or were euthanased
  - One from rupture of the umbilical artery
  - One from over sized ovaries twisting and separating from the uterus
  - One euthanased due to poor condition, respiratory distress and enlarged ovaries
  - One euthanased due to enlarged head, shortened bowed front legs that affected standing

#### **GOVERNMENT REPORTS ON DEATHS OF rhFSH CALVES**

The Minister of Research, Science and Technology at the time, Wayne Mapp, requested an inquiry into the deaths of the cows. The report "Deaths of Transgenic Calves at AgResearch's Ruakura Facility"<sup>37</sup> by Sir Peter Gluckman, the Chief Science advisor to the Prime Minister, detailed many concerning omissions in the experimental procedures and assessments. a summary of the findings were that

1. There were questions early in the research on the commercial viability of the targeted proteins (rhFSH).
2. The rhFSH protein is available and in use, but there was no assessment that the extraction and clinical development would be successful.
3. AgResearch believed they could restrict the GE hormone to the milk. However, the genetic construct behaved differently than expected. When it was discovered there was leakage into the blood system, the correct veterinary care was delayed, because the test procedures had been outsourced.
4. No external scientific advice, that may have addressed the problems associated with this research, was sought. An external scientific review by clinical specialists could have provided expertise on the hormone's adverse symptoms.
5. Independent Scientific Advisory Boards are now in place.

<sup>36</sup> Deaths of Transgenic Calves at AgResearch's Ruakura Facility Dr. Jim Watson and Dr. Alan Beedle 6/10/2010.<http://goo.gl/Qlcgo3>

<sup>37</sup> Deaths Of Transgenic Calves At AgResearch Ruakura Facility, report to Minister of Research, Science and Technology 6/10/2010.  
<http://www.gefree.org.nz/assets/pdf/Agresearch-and-Biopharma.pdf>

## OMNIBUS: WIDE RANGING GENERIC FOUR PART APPLICATION DECLINED (2010)

In 2010, AgResearch submitted four wide-ranging, generic applications to ERMA to import and develop, within indoor or outdoor containment facilities, 18 domestic and exotic animal species. These animals were to be genetically engineered with a range of transgenes. The transgenes were intended to be used for research, breeding and for the production of proteins with potential commercial applications for an unlimited duration at unspecified locations<sup>38</sup>.

GE Free NZ lawyer, Tom Bennion<sup>39</sup>, successfully challenged this case in the High Court. AgResearch appealed the decision to the Appeal Court<sup>40</sup> who ruled that the challenge had been taken too early and did not leave room for ERMA to use their discretion as to whether the application met the approval criteria.

The application was sent back to ERMA to rule on, and the agency staff made a recommendation to decline the application due to an absence of adequate information<sup>41</sup>. AgResearch withdrew the application before it went to the Authority for ruling. The ERMA Authority has never turned down an application for a GM development or field trial.

<sup>38</sup> New Zealand's GM cattle under fire: Research delayed by court battles Branwen Morgan. *Nature* <http://goo.gl/18W3GA>

<sup>39</sup> CIV.2008-485-2370 <http://goo.gl/DWTFkm>

<sup>40</sup> CA380/2009 <http://goo.gl/YgcbxN>

<sup>41</sup> Applications: GMC07012, GMD08012, GMD07074 AND GMF07001. <http://goo.gl/l1yiV8>

## APPLICATION GMD200223 (2010-2015)

AgResearch submitted a smaller application ERMA200223<sup>42</sup> that replaced the withdrawn larger four trial applications; this was approved by ERMA in 2010. There has been one new GM cow trait developed under this application, though all the previous approvals have been moved to this application.

### ERBITUX COWS



Figure 6 "Erbie" with non-transgenic friend

Erbitux is a pharmaceutical drug available on the market for the treatment of cancer.<sup>43</sup> In 2009, transgenic Erbitux nuclear transfer embryos were developed and up to four runs of embryo's implanted into recipient cows with no pregnancies resulting.

In 2010, further runs were undertaken, all embryos in run 1 failed to develop past day 66. In run 2, there were three recipient cows carrying viable fetuses, two were aborted at 3 months. The fetal cells were re-engineered to create a second round of embryos. The third recipient cow developed excessive abdominal fluid (hydrops), was induced early, and underwent a caesarean section. The premature calf suffered from excessive abdominal fluid and respiratory distress. It died after two hours and the recipient cow was euthanased. In May and August, another 92 embryos were implanted and two pregnancies resulted.

In 2011, of the two pregnancies one surrogate suffered from fluid abnormality and delivered a premature dead calf.

One calf, Erbie, was born with a slight back leg deformity. She was induced into lactation at 12 months and her milk expresses 1 copy of the Erbitux gene.

#### ERBITUX RESULTS

- Reproductive failure and abortions common
- Pregnancy complications
- Skeletal deformities in progeny
- 200 embryo transfers, 1 live birth survival (2009-2015)
- 1 Erbitux cow surviving after 5 years of experiments (2009-2015)

<sup>42</sup> ERMA200223 <http://goo.gl/WKxu2C>

<sup>43</sup> Erbitux data sheet [http://packageinserts.bms.com/pi/pi\\_erbitux.pdf](http://packageinserts.bms.com/pi/pi_erbitux.pdf)

## SUMMARY TRANSGENIC COWS

There have been over 2000 transgenic embryo transfers between the different trait lines. All GM animal pregnancies suffered from a high level of spontaneous abortions, and sterility of ensuing progeny is common.<sup>44</sup> The live birth rate of cows carrying transgenic embryos has ranged from 0-7% depending on the engineered event. (Table 1).

Hundreds of cattle of varying ages, transgenic and non-transgenic have been routinely euthanased or have died suddenly. They were euthanased on veterinary advice due to deformities or sickness, identified as surplus to requirements or unsuitable for further experimental work.

After 14 years of illness and reproductive losses there are 19 GE cows surviving in the experimental facility. Of these, only five cows carry one of three biologic pharmaceutical proteins in their milk. Most of the transgenic lines are not able to reproduce and are still founder (F0) generations. The GE cow lines that have produced a second generation (F2) are sterile. The milks containing GE proteins are not human specific, and the trial data remains unpublished. Clinical trials on recombinant milk proteins have resulted in allergic reactions in the subjects. Furthermore, the proteins these animals have been modified to express are available on the pharmaceutical market today through simpler non-transgenic processes or are made in contained fermentation vats using genetically engineered bacteria.

### IN SUMMARY

- Total failure to create some GM cell lines
- Overall < 4 % live birth for embryo transfer
- F1-F2 generations have low conception
- Unusual deformities congenital and heritable
- Internal organs missing
- Uterine and ovarian rupture
- Hormonal and metabolic problems
- Heart abnormalities
- Limbs imperfectly formed or fused together

Transgenic cows suffered from

- Internal organ problems
- Lack of diaphragm.
- No bladder
- Patent foramen ovale. (Hole in heart)
- Bladder and pericardia fused
- Ascites.
- Ovarian and uterine structural abnormalities.
- Squamous cell carcinoma
- Deformities in limbs.
- Calf club foot and fused neck.
- Endocrine disruption
- Rear fetlocks bent back
- Bilateral medial strip contracted
- Growth plates early fusion

Transgenic calves suffered from

- Sterility, worsening over generations,
- High Abortion / slips
- Reproductive and pregnancy problems
- Low percentage (0-7%) of live births
- Increased disease susceptibility
- Liver and umbilical abscesses
- Post partum bladder paralysis
- Metabolic problems
- Gangrenous mastitis
- Respiratory problems
- Arthritis, joint and cartilage malformations
- Carcinoma of the eye

<sup>44</sup> AgResearch Annual report to ERMA New Zealand for Activities under GMF 98009, GMD02028 & ERMA 200223, 2000 - 2014



# TRANSGENIC WASTE TO LAND MANAGEMENT

ERMA controls require that the milk is denatured. This is carried out by fermenting the milk in 1000 litre Industrial Bulk Containers (IBCs) until the pH levels drop to below pH4 and no viable cells are recoverable. Once the milk has been denatured it is stored until ground conditions allow irrigation onto the fields, in the containment facility. Waste blood products are also mixed in with the milk. There has been 3000 - 79,000 litres of denatured milk sprayed on the fields every year.

AgResearch Ruakura Facility (dotted purple), the GE animal containment area (pink), area where transgenic milk and blood products are sprayed (stripped lines). (See picture below)

**AgResearch GE Animal containment facility showing discharge of waste products.**





## SITE TESTING FOR HORIZONTAL GENE TRANSFER



Figure 7: Carcass pits – photo by Steffan Browning

AgResearch is required to conduct horizontal gene transfer (HGT) testing on the carcass pits where dead animals are buried, but not the fields where they spray the waste milk and blood products, as part of the reporting requirements. The pits are situated at the top of a hill.

The carcass pits are 7 metres deep and 1 metre wide. Animal carcasses are disposed of to the depth of 5 metres and then the remaining 2 metres is filled with soil. Any subsidence is topped up with more soil. (See Diagram 1 B.)

In 2008, in support of the AgResearch multi species generic application, GMD200223, HGT tests were submitted to ERMA. The HGT test report, called “Microbial Characterisation of Soil in the Offal Pits at AgResearch 2004-2009” was part of the prior environmental testing programme.<sup>45</sup>

Though AgResearch had stated there was no sign of HGT, their report noted that in the first year of testing unexpected microbial populations, that might have bacteria carrying the antibiotic-resistance marker gene for puromycin resistance, were detected. They were still awaiting the right probes to detect if this was the case.

GE Free NZ applied under the OIA to obtain the HGT report from ERMA, who had not seen it, so the request was transferred to AgResearch.



Figure 8: Carcass pit covers and subsidence holes

<sup>45</sup> Microbial Characterisation of Soil in the Offal pits at AgResearch 2004-2009. HGT study received under OIA 2010. <http://www.gefree.org.nz/reports-and-submissions>



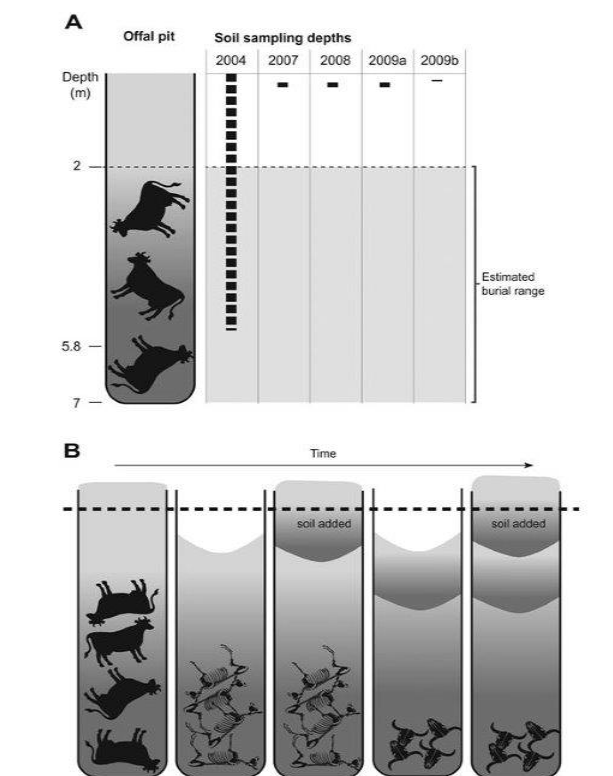
GE Free NZ had concerns with the information in the report and asked Professor Jack Heinemann and his team at The Centre for Integrated Research in Biosafety (INBI) in the School of Biological Sciences at Canterbury University to conduct a review into the AgResearch HGT report. The INBI report, published in the Journal of Organic Systems (2011)<sup>46</sup> on the “Microbial Characterisation of Soil in the Offal pits at AgResearch 2004-2009” found:

1. The experiments suffered from a design incapable of detecting HGT with the sensitivity necessary to detect bacteria that might cause the adverse effects of concern to the Authority, including but not restricted to bacteria developing antibiotic resistance.
2. The sampling depth in all but one year was in the range of 2-6 m above the soil interface with the carcasses. Importantly, no study confirmed the samples were taken from soil in contact with carcasses”. (Diagram 1.)
3. The suitability of control sites and the efficacy of the sampling were not demonstrated.
4. The design and standards of follow-up on observations and determining causes of negative results (e.g. particularly from routine molecular work such as sequencing and PCR) was below what would be sufficient for assurance that risk management controls were met.

The report went on to say

*NBI finds that these experiments were irretrievably flawed for providing baseline data for future soil analysis, effectively monitoring HGT as a risk management strategy or influencing the assessment of the risk of HGT in future application”. INBI report (2011)*

Diagram 1: Sampling depths by the year



<sup>46</sup> Jack Heinemann et al (2011) Evaluation of Horizontal Gene Transfer monitoring experiments conducted in New Zealand between 2004-2009. Journal of Organic Systems Vol: 6 (1) <http://goo.gl/juylvc>

## OIA – PHOTOGRAPHS OF THE ANIMALS

In January 2013, GE Free NZ wrote to AgResearch under the Official Information Act requesting copies of photos of the GE cows and calves born at Ruakura from 2000-2013. The reply was turned down stating –

“AgResearch is withholding all photographs under the EPA approvals requested. This information is withheld under section 9.2(j) to enable AgResearch holding the information to carry on, without prejudice or disadvantage in negotiations (including commercial and industrial negotiations)”.

This reply was referred to the Ombudsmen who looked carefully into the appeal in the light of public interest. She upheld AgResearch's decision not to release the photographs as the photographs belonged to “Industry partners” and their release could be used to “display biased representation of genetic modification”. If the photographs were released there would be no way of controlling how the information could be used and distortion of the research could jeopardise public and commercial investment and AgResearch needed to keep its options open for the future.” She saw the Ruakura Animal Ethics Committee (RAEC), the internal body who oversees animal research activities, as minimizing public interest concerns.

Below are two quotes from the reply written by Dame Beverly Wakem (the Chief Ombudsmen), dated 2/8/2013, explaining why photographs of the GM animal experiments would not be released to the public.

*"It is AgResearch's view that genetic modification is a sensitive topic which elicits emotive responses and that the release of these photographs could be used to display a biased representation of the effects of genetic modification. It does not consider that this is in the public's interest, as a distortion of the research could jeopardise public and commercial investment in this area. It is maintained that although genetic modification through genetic engineering is not used in the commercial sense in New Zealand as present, it does need to keep its options open for the future given its current common use in many parts of the World".*

*"Without making a value judgment on the credibility, future or otherwise of genetic engineering, I can understand and accept, the thread of AgResearch's position that the public interest would likely be damaged should the photographs be made available."*

*Dame Beverly Wakem (the Chief Ombudsmen)*

Concern has been raised over the money AgResearch, which is now working on forages, spends on GE projects.<sup>47</sup>

The McGuinness Institute<sup>48</sup> has published a report on genetic modification in New Zealand with observations, and recommendations on the way forward.

## COST TO THE TAXPAYER

With regard to the total cost of GM applications to the New Zealand taxpayer for regulatory assessment of each application, ERMA states: “The full cost may not necessarily rest with the applicant” (Official Information Act request, 1 April 2011). For example, the cost to ERMA for the application process of a GMO field test and outdoor development between 2007-2010 was \$400,600 and the cost to ERMA for the application process of one GMO notified conditional release during the same period was \$197,300 (ERMA's Annual Report 2010, p.23). These costs identified do not include the private partnership money or laboratory development of GM animals.

<sup>47</sup> AgResearch quizzed over GM cattle trials <http://www.pressdisplay.com/staging/timesonline/viewer.aspx>

<sup>48</sup> Report 16, An overview of Genetic Modification in New Zealand. The first forty years, 1973-2013 [http://mcguinnessinstitute.org/Site/Publications/Project\\_Reports.aspx](http://mcguinnessinstitute.org/Site/Publications/Project_Reports.aspx). 20

## COST OF GE EXPERIMENTS IN NEW ZEALAND

Pure Hawkes Bay, a group of local food producers committed to building the region's global reputation for safe sustainable and high quality food, has recorded all the trial sites and costs associated with the GE field trials in New Zealand before ERMA became the EPA.<sup>49</sup>

**Table 2: Cost of GE experiments**

Animal	Applicant	Protein construct	Outcome	Trial length	Cost to applicant /taxpayer	Size of Trial site
Cattle (GMF98009) Part I  Part II	AgResearch Ruakura, Hamilton 1999  2000	Human casein and lactoglobulin  Myelin Basic Protein	Deformities, high Mortality and abortions. Sterility Nil outcome	Ended. Cattle living out their lives	\$15,892.02	45 ha, 200 cows max
Cattle GMD02028	AgResearch 2002	Generic Application Lactoferrin, follicle stimulating hormone	Deformities, high mortality, failure to maintain pregnancy. Sterility Nil outcome	No current activity	\$107,043.13	
GMD07074 GMD08012 GMC07012 GMF07001	AgResearch 2008	Multiple Animal Species (cattle, buffalo, sheep, pigs, goats, llamas, alpacas, deer and horses		Application withdrawn before hearing	ERMA \$56,250 AgResearch - \$717,236.42 + \$347,511.16 legal costs	Ruakura. Unidentified locations in NZ
ERMA200223 Livestock cattle, sheep, goats.	AgResearch 2010	Generic Human proteins in milk	Deformities, high mortality, failure to maintain pregnancy.	20 yrs	\$67,500 \$266,173.95	

<sup>49</sup> GM Trial Activity, collated by Kate White <http://goo.gl/VLTaUI>

# NUMBER OF TRANSGENIC COWS AT RUAKURA FACILITY EACH YEAR (2000-2014)

Table 2 GM Animals at Ruakura –born from 2000-2014

Tg Cows	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
C+ Yr. Tot			18	53	64	74	55	64	67	72	69	64	30	20	14
ET/NM	65	96	22:18	62:10	30	18	20	31	15	13	-		-	11	
Born (GM.F)	4	18/11	4/1	14	15/4	22/8	14/8	13/7	21/14	16	11/8	0	2	DAB	0
MBP Yr. Tot				9	13	17	13	12	16	10	7	7	4	4	3
ET/NM	60	60	60:9	6	-	29	6	4	2	2	4	-	-	2	-
Born (GM.F)		4	9/4	5/1	-	0	0	5/2	0	0	0	1	2	DAB	0
LF Yr. Tot								12	16	20	19	25	21	10	0
ET/NM						154	73	187	9	13	0	-	-	-	
Born (GM.F)						0	14	18/14	7/5	6/2	0	10/4	8/4	-	
FSH Yr. Tot										5	2	0	0	0	0
ET/NM							92	168	140	0	0	-	0	-	0
Born (GM.F)							0	0	9/5	0	0	-	0	-	0
ERBIT Yr.Tot													1	1	1
ET/NM											60	104	66	?	?
Born (GM.F)											0	1	0	?	?
BLG- Yr. Tot													1	1	1
ET/NM												107	0	70	0
Born (GM.F)												1	0	0	0
Facility Total			18	62	77	91	68	88	99	107	97	98	57	36	19

C+ = Casein +(plus) carry an extra transgenic casein gene

MBP = Myelin Basic Protein (MBP) carry an extra transgenic human MBP gene

LF = human LactoFerrin, cows carry the transgenic human hLF gene

FSH = human Follicle Stimulating Hormone, cows carry the transgenic hFSH gene.

ERBITUX = cows carry the transgenic ERBITUX gene.

BLG- = Beta-Lacto-globulin minus transgenic cows have the BLG gene silenced.

DAB – dead at birth

ET/NM - the number of embryo transplants (ET) or natural matings (NM).

Born (GM/F) Calves born but died 24 hours after birth, were male or killed as or surplus to requirements. (GM/F) The number of GM female calves.

Facility Total -Total number of transgenic animals in the facility at the end of each year.<sup>1</sup>

<sup>1</sup> AgResearch Annual reports to ERMA /EPA 2000-2014. <http://www.gefree.org.nz/reports-and-submissions/>

## FUTURE OF AGRESEARCH ANIMALS

Proposals have been in place to downsize the AgResearch Ruakura facility since 2013. This will leave only 86 staff at the Hamilton site. There are no plans to relocate any transgenic animals in the near future and the cows are living out their natural lives on the site. Further trials appear to be on hold.

Since 2010, the research has moved to breeding transgenic goats, some of which are engineered with the same proteins as the cows. Reports show the abortion rate is 85%. In addition, from the 150 embryo transfers typed as female, 19 kids born. Four were females and 15 were hermaphrodites, goats with female traits but male genitalia (see figure 9). Though the goats were sterile, they underwent hormonal treatment to induce them into lactation, but this was unsuccessful in producing the desired protein.

The 2013 Annual report notes that of the 25 goat kids born, only 7 survived. From the cell line GN388 there were no pregnancies from the 25 embryo transfers (ET's). From the cell line GN97; out of 93 ET's there were only four live kids born at term and one of these died at birth. From the cell line GN451 there was only one surviving kid from 37 ET's. The deaths of goats and their kids from deformities and hydrops and respiratory distress syndrome were similar to the cows. Milk from transgenic "Erbitux" goats produced minimal volumes of the low-grade recombinant protein.<sup>50</sup>

The 2014 Annual report records embryo survival to term was 4 out of 37 (11%) however 1 out of 37 (3%) of kids survived.



Figure 9: Sterile female goats with male genitalia

<sup>50</sup> 2013 AgResearch cattle, sheep and goats Annual report. <http://goo.gl/3tCTbx>



## CONCLUSION

After 15 years, these transgenic animal experiments have been an expensive failure. Members of the public have expressed serious concerns about the cruelty and unnecessary suffering the sentient animals have endured, which has largely been ignored by ERMA/EPA.

The grotesque deformities and health problems that these transgenic animals have been subjected to, are not noted in the AgResearch final reports.<sup>51</sup>

*“There have been no unforeseen effects to the environment, public health, Maori culture, the economy or society from the research identified during the period of the GMD02028 approval” p.9.*

*Final EPA report for activities under GMD02028*

Omission or carefully chosen reporting of important experimental data and the provision of only selected photographs to the media fails to depict the tragic results of using animals as bioreactors. Questions need to be asked as to how the Ruakura Animal Ethics Committee, of which the SPCA is a member, reviews and approves GM animal research activities, with particular reference to animal welfare concerns.

It appears there are serious gaps in the management of the experiments and there is a collective silence on the horrific treatment of animals. The censorship of vital visual information to the media and regulators has denied the public the right to discuss the moral or ethical implications of such research.

The transgenic animal experiments were not for the benefit of people per se, as the drugs were already on the market. They were, it seems, intended as a competitive and cost effective solution to the production of pharmaceutical proteins extracted from the milk of GE animals. The Bio ethics Council<sup>52</sup> set up to advise on cultural, ethical, and spiritual aspects of biotechnology was disbanded in 2009, leaving a void in the ethical and moral issues of using animals as bioreactors.

It is time New Zealand re-evaluated these experiments and closed down the facility, retiring the animals from experimentation so they can die naturally. Research money must be put into agricultural research, which would benefit New Zealand farmers, a clean environment, and sustainable farming practices.

We would like to thank all those who have helped in the writing, editing, and design of this report with a special thanks to Kyra Xavia, Heidi Clarke, Steffan Browning, Dr. Bob Jones and Dr. Elvira Dommissie. This project was the result of the successful court challenge in 2001, and ERMA required AgResearch to report annually on the health outcomes and survival statistics of the research cows. This has allowed us to follow the sad and disturbing story of reproductive failure and birth deformities that genetic engineering has wrought on these animals.

<sup>51</sup> Final report for activities under GMD02028, <http://goo.gl/AKox7>

<sup>52</sup> <http://www.bioethics.com/archives/6178>

# GLOSSARY OF TERMS

## MEDICAL MEANINGS

**Ascites** - the accumulation of fluid in the peritoneal cavity, causing abdominal swelling.

**Abscess** - a lesion within body tissues, containing an accumulation of pus.

**Arthritis** - a painful inflammation and stiffness of the joints.

**Bio-reactors** – Animals that produce proteins through transgenic modification. (GE processes).

**Biopharmaceuticals** – the production of pharmaceutical drugs through recombinant/transgenic/modification of animals or plants.

**Bio-pharming** - the development of transgenic animals to produce pharmaceutical products.

**Bladder** - a muscular sac stores urine for excretion.

**Bladder paralysis** – The loss of nerve conduction between the bladder and the brain resulting from spinal injury or nerve damage. Bladder paralysis in livestock is characterised by an inability of the bladder to empty and serves as a source of bacterial growth as well as other complications.

**Bovine fetal fibroblast (BFF)** – a bovine fetal cell that contributes to the formation of connective tissue fibres in the lung.

**Carcinoma** - a malignant tumor derived from epithelial tissue.

**Clubfoot** - a deformed foot, which is twisted so that the sole cannot be placed flat on the ground.

**Diaphragm** - a large flat muscle that separates the lungs from the stomach area.

**DNA** - deoxyribonucleic acid the carrier of genetic information, main constituent of chromosomes.

**DNA - Recombinant** - A DNA molecule that has been recombined from one or more, bacterial, viral, fungal, plant, mammalian organisms, creating sequences not naturally found in nature.

**DNA - Transgenic** - an animal or plant containing artificially introduced genetic material from unrelated organisms from one or more species.

**Early Growth plate fusion** – the premature closing in the growth region of a long bone

**Endocrine disruption** - the disturbance of the hormonal system processes, which can cause cancerous tumors, birth defects, and other developmental disorders.

**Fetlocks** - small bones at the back of a cows leg above the hoof.

**Fetus** - unborn offspring of a mammal.

**Gangrenous mastitis** - an acute or chronic infection of the udder marked by blue/dark colour of dead and decaying tissue, this can lead to the animal's death if untreated.

**Homozygous** - identical pairs of genes for any given pair of hereditary characteristics.

**Metabolic disorder** - the disruption of cellular metabolic processes that convert food to energy.

**Metabolism** - the process of breaking down food nutrients into energy.

**Miscarriages/ slips** - the spontaneous or loss of a fetus before it can survive independently.

**Neospora caninum** - a cyst-forming coccidian parasite.

**Ovarian and uterine structural abnormalities** - Abnormalities in the uterus.

**Patent foramen ovale (Hole in Heart)** - A hole in the wall between the right and left atria

**Pericardia Ascites** - abnormal accumulation of serous fluid in the membrane enclosing the heart.

**Post partum** - After childbirth.

**Respiratory problems** - breathing difficulties.

**Somatic Cell** – the cell of a living organism other than the reproductive cells.

**Somatic cell nuclear transfer (SCNT)** - technique in which the nucleus of a somatic cell, is transferred to the cytoplasm of a donor egg that has had its nucleus removed (enucleated egg).

**Sterility** – inability to unable to conceive young, especially through natural means.



## TRANSGENIC BOVINE CONSTRUCTS:

**AAT** - Alpha-1-Anti Trypsin protein - lacking in patients with cystic fibrosis and emphysema

**BLG Beta Lactoglobulin** -  $\beta$ -Lactoglobulin is the major whey protein of cow and sheep's milk and other mammals, a notable exception being humans.

**Casein protein** – phosphoproteins found in mammalian milk, making up 80% of the proteins in cow milk.

**Erbix** - a recombinant drug, used for the treatment of metastatic colorectal, head and neck cancer.

**Follicle Stimulating Hormone** - A hormone secreted by the anterior pituitary gland, which promotes the formation of ova or sperm.

**Lacto-Ferrin** - a protein present in milk and other secretions, with bactericidal and iron-binding properties.

**Myelin Basic Protein** - a protein important in maintaining the myelin sheath which functions as an insulator to greatly increase impulse conduction of nerves.

**Synthetic human analogue** - laboratory compound engineered from genetic material that is structurally similar to its human equivalent.

## MAORI TERMS

**Mauri** - the essential nature and vitality of a being or entity.

**Taonga** - an treasured being, object or natural resource.

**Tikanga** - The correct way to carry out Maori customs

**Whakapapa** - the history and knowledge that binds and maps the animate and inanimate, known and unknown phenomena in the terrestrial and spiritual worlds.

## ORGANISATION ACRONYMS

**AgResearch** – A Crown Research Institute serving agricultural and biotechnology industry sectors.

**ERMA** - The Environmental Risk Management Authority 1996-2011.

**EPA** - Environmental Protection Authority (EPA).

**Genetic Engineering** - The science of altering and cloning genes to produce a new trait.

**GMO's** - Genetically Modified Organisms.

**HGT** - Horizontal Gene Transfer – the non-sexual transfer of DNA, from one organism to another.

**HSNO** - Hazardous Substances and New Organisms Act.

**IAG** - Interim Assessment Group (IAG) – A group that approved the trials of GE before 1996.

**Ngā Kaihautū Tikanga Taiao** - Māori Advisory Committee to the EPA

**Pharming NV** – A corporation developing transgenic drugs, for the treatment of genetic disorders.

**RCGM** - Royal Commission on Genetic Modification.