

Regulation and Commercialization of Genetically Engineered (GE) or Transgenic Animals in the Livestock Sector

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http://animalscience.ucdavis.edu/animalbiotech



An experiment in genetic engineering turns harmless sheep into blood-thirsty killers that terrorize a sprawling New Zealand farm.

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Animal breeders have been genetically modifying animals for faster growth and improved feed conversion for many years

## 1957 vs. 2001 chickens





Havenstein, G., Ferket, P. and Qureshi, M. (2003). Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. *Poultry Science* 82, 1500-1508.

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# In some cases using the process of recombinant DNA (rDNA)

1992 Nature Publishing Group Founder female produced in 1989

### GROWTH ENHANCEMENT IN TRANSGENIC ATLANTIC SALMON BY THE USE OF AN "ALL FISH" CHIMERIC GROWTH HORMONE GENE CONSTRUCT

### Shao Jun Du, Zhiyuan Gong, Garth L. Fletcher<sup>1</sup>, Margaret A. Shears<sup>1</sup>, Madonna J. King<sup>1</sup>, David R. Idler<sup>1</sup> and Choy L. Hew<sup>\*</sup>

Research Institute, The Hospital for Sick Children and Departments of Clinical Biochemistry and Biochemistry, University of Toronto, Toronto, Canada M5G 1L5. <sup>1</sup>Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7. \*Corresponding author.

We have developed an "all fish" growth hormone (GH) chimeric gene construct by using an antifreeze protein gene (AFP) promoter from ocean pout linked to a chinook salmon GH cDNA clone. After microinjection into fertilized, nonactivated Atlantic salmon eggs via the micropyle, transgenic Atlantic salmon were generated. The presence of the transgene was

transgenic Atlantic salmon by using an "all fish" transgene consisting of a promoter derived from an ocean pout antifreeze protein (opAFP) gene<sup>9</sup>, and the GH cDNA clone from chinook salmon<sup>10</sup>.



#### University of Toronto/Memorial University of Newfoundland, Canada

Nature Biotechnology. 1992. 10:176 – 181.





# Fish reach adult size in 16 to 18 months instead of 30 months

### Growth Curves (Growout) Founder female in 1989



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#### Event

September 1995

Date

#### AquaBounty submits Investigational New Animal Drug (INAD) application with FDA for fast-growing salmon with intent to commercialize

September 2010

 Public Veterinary Medicine Advisory Committee (VMAC) meeting to consider data on safety and efficacy of AquAdvantage salmon Held in Washington DC





#### The public VMAC meeting held in Washington, DC was intended to increase transparency, clarity, and public confidence in the GE animal regulatory process





Obama's FDA is regulating genetically engineered salmon, a genetically modified organism (GMO) that is the first of its kind, not as an animal, but as an animal drug.

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# Timeline of AquAdvantage regulatory process

#### Year Event

1989

1995

2001

2009

2010

2011

2012

2014

- Founder AquAdvantage fish produced in Canada
  - FDA review of AquAdvantage salmon begins (INAD)
  - First regulatory study submitted by Aqua Bounty Technologies to U.S. FDA for a New Animal Drug Applications (NADA)
  - FDA guidance on how GE animals will be regulated
    - FDA approval of first GE animal pharmaceutical
    - Final AquAdvantage regulatory study submitted to FDA
- FDA VMAC meeting on AquAdvantage salmon (9/20/10)
  - Political efforts to defund FDA, ban fish, delay approval
  - FDA released "FONSI" finding of environmental assessment
  - AquaBounty Total R&D investment > \$60 million to develop and bring the AquAdvantage salmon through the regulatory approval process <u>thus far</u> (D. Frank, CFO, AquaBounty, pers. comm.)
    - Still waiting for regulatory decision on AquAdvantage salmon
    - Development of GE animal technology moving to other countries with more predictable policy environments



How can \$60+ million be warranted to bring a fast-growing fish to market, when conventional fish (and other animal) breeders routinely develop all manner of fast-growing animals that are associated with the same set of risks?



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It is often stated that AquAdvantage is precedent setting – but many people already think they eat genetically modified animals – and they do – it is just that modifications were not done with rDNA



https://fbcdn-sphotos-f-a.akamaihd.net/hphotos-ak-ash3/p480x480/936235\_497070347014003\_426756402\_n.jpg Van Eenennaam PAG 1/11/2014 Animal Biotechnology and Genomics Education



### There is no scientific case for a blanket approval of all uses of GE. But equally there is no scientific case for contrived safety testing

There is always the issue of novel proteins or compounds with no history of safe use. These will need to be tested for toxicity and allergenicity – be they introduced by GE or conventional breeding techniques.

A large amount of safety testing and expense is to detect the unknown "unintended" changes resulting from the GE process

Continued testing using ever more-expensive techniques including emerging "omics" for these "*unintended" and* unknowable effects of GE process on food composition is scientifically dubious as the **biological relevance** of a "statistically significant" compositional change in whole foods is unclear – especially in the absence of analogous data on the variability that exists in conventional foods.



# Unintended effects of GE have not materialized

It seems more scientifically defensible to be able to state that certain likely effects (e.g. novel allergens and toxins, positional insertion effects) have been assessed and found absent, than to admit that one did not know quite what to look for – but found it absent nevertheless

"Skeptics who remain fearful sometimes respond that "absence of evidence is not the same thing as evidence of absence". Yet if you look for something for 15 years and fail to find it, that must surely be accepted as evidence of absence. It is not proof that risks are absent, but proving that something is absent (proving a negative) is always logically impossible\*"

\* Paarlberg, R. 2010. GMO foods and crops: Africa's choice. New Biotechnology 27:609-613



AquAdvantage regulatory delay has been occasioned by factors including: the use of a process-based risk assessment arbitrarily triggered by the use of rDNA rather than the novel phenotype and attributes of the product; misrepresentation and questionable interpretation of data by special interest groups; a risk assessment paradigm that does not consider the known risks associated with existing production systems; continued political interference; lack of predefined timeline

- Focus risk assessments on those **unique risks** associated with the GE animal application and evaluate them in relation to known risks associated with existing production systems.
- Require **hypothesis-driven studies** for regulatory evaluation detailing the biologically relevant issue(s) based upon the novel traits or phenotype(s) associated with the species/gene/insertion event combination.
- Following submission of all pre-defined required data, impose **finite response times** for agency decisions at each point in the evaluation process to provide developers and investors with a predictable regulatory timeline for GE animals.

Van Eenennaam, A.L., W. M. Muir, and E. A. Hallerman. 2013. Is Unaccountable Regulatory Delay and Political Interference Undermining the FDA and Hurting American Competitiveness?: Food and Drug Policy Forum. Volume 3(13).



## What does the future look like Targeted gene editing

More sophisticated gene editing and knockout techniques have been developed in the 25 years since the founder AquAdvantage<sup>®</sup> fish was made using pronuclear microinjection.

Recently, targeted nucleases such as zinc-finger nucleases (ZFN), meganucleases, transcription activator-like effector nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats System (CRISPR) have increased targeted gene mutation efficiency

Use of these techniques will challenge the definition of "regulated article," as the resulting animals will not harbor rDNA constructs nor any foreign DNA .....

Van Eenennaam, A.L., and A. Bruce. 2014. Regulatory Issues Associated with Genetically Engineered Farmed Animals" Chapter in "*Genetically Modified Animals"*. H. Sang (ed.) John Wiley & Sons, Inc. *In press* 

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## What is regulated article? It depends....

<u>Codex:</u> Recombinant-DNA Animal" is defined as an animal in which the genetic material has been changed through *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles.

The Food and Drug Administration defines "genetically engineered (GE) animals" as those animals modified by rDNA techniques, including the entire lineage of animals that contain the modification, and regulates based on the use of rDNA techniques. All GE animals are captured under these provisions, regardless of their intended use. Thus although the review is product based, the process used to produce the genetic change that results in the product (i.e. rDNA techniques) has implications for triggering regulatory oversight.





## What is regulated article? It depends....

The Cartagena Protocol definition of 'living modified organisms" resulting from modern biotechnology'.... living organisms produced by (a) modern biotechnology such as <u>recombinant DNA technology</u> including self cloning and/or recombinant DNA techniques using genetic material (host, vector and foreign genes) derived from an organism between which natural gene exchange is possible ....

<u>EU definition of GMO</u> (included in Directive 2001/18/EC) definition an 'organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination'. ...including recombinant nucleic acid techniques ..... Techniques not considered to result in a genetic modification include *in vitro* fertilisation, polyploidy... and techniques of GM excluded from the directive including <u>mutagenesis</u>.



Hypothetical example comparing intragenic fastgrowing Atlantic salmon with an rDNA Atlantic growth hormone promoter expressing an Atlantic growth hormone gene; and the same phenotype made by selecting a naturally-occurring gene duplication mutant

Attribute	GE salmon	Gene duplication salmon
Fast growth?	YES	YES
Possible environmental impacts if escape and interbreed with native salmon?	YES	YES
Possible differential levels of growth hormone expression?	YES	YES
Regulated	Yes in the U.S.; EU, New Zealand, and Australia	NO
Regulatory costs	>\$USD 60 million?	\$0



Some animal biotechnology applications, including GE animals, would seem to align with many sustainability goals including improving animal well-being – will they be permitted to do so given current regulatory policy?





- Naturally polled cattle
- Trypanosome resistance
- Sex selection for ♀ in dairy and egg industries



Use of rDNA to introduce a site specific polled mutation into Holstein cattle versus repeated backcrossing from polled breed into Holstein to obtain the same phenotype through introgression of the polled mutation into Holstein germplasm

Attribute	Polled Holstein through rDNA	Polled Holstein through introgression
No horns	YES	YES
Mutation uniquely detectable	NO	NO
Food safety concerns associated with phenotype	NO	NO
# generations taken to achieve polled >15/16 Holstein	ONE (FAST)	MANY (SLOW)
Linkage drag?	NO	YES
Improved animal welfare	YES	YES
Regulated?	Depends on definition of regulated article	NO
Likely to happen	Not if costs >\$60 million	NO

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## Kevin Wells (MO) summarizes the current situation succinctly

"The basic premise for regulation appears to be that any genotype produced by breeding is safe, and that any genotype produced intentionally via rDNA technologies cannot be allowed to go to market.

The fact is that every animal produced by natural mating (excluding identical twins) also has a unique genotype, and therefore is also different from every historic genotype that has ever been consumed as food."

Wells KD: Natural genotypes via genetic engineering. Proc Natl Acad Sci U S A 2013, 110:16295-16296.



Now is an opportune time to review the current process-based regulatory framework where the trigger for regulatory review is rDNA, rather than the unique characteristics and attributes of the resulting animal.

To complicate the regulatory oversight and segregation of targeted gene mutation animals further, there will likely be no molecular approach to detect and uniquely identify genetic changes made by these approaches.

The techniques and processes being used to make genetic modifications in animal genomes comprise a rapidly evolving field, and the line between animal breeding and "genetic modification" is becoming increasingly blurred.

Van Eenennaam, A.L., and A. Bruce. 2014. "Regulatory Issues Associated with Genetically Engineered Farmed Animals" Chapter in "*Genetically Modified Animals"*. H. Sang (ed.) John Wiley & Sons, Inc. *In press* 



# It is time to reconnect the GE animal regulatory framework to regulate risk rather than process?

"Difficult regulatory regimes for GE animals produce, to varying degrees, a negative, reinforcing cycle of regulatory inertia, lack of investment, policy ambivalence, lack of research funding and lack of commercial products."

"Increasingly sophisticated and discriminating innovation in the methods available for producing GE animals raises questions about the current state of development of regulatory regime in the EU and USA; about the appropriateness of regulations that have been derived in the context of previous generations of GE technology; and about the relevance of regulatory systems developed for GE crops and micro-organisms to GE animals."

Bruce A, Castle D, Gibbs C, Tait J, Whitelaw CB. 2013. Novel GM animal technologies and their governance. Transgenic Res. Aug;22(4):681-95.

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### Sites working on GE livestock for food – 1985 North America, Europe and Australasia



#### Graphic developed by Dr. J. Murray, UC Davis

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Sites working on GE livestock for food - 2012 Asia and South America are moving forward with this technology in their animal agriculture



#### Graphic developed by Dr. J. Murray, UC Davis

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## **Parting thoughts**



Regulatory processes should be consistent across products that have equivalent levels of risk. Regulations based on how products are made are inconsistent with science-based risk assessment unless there is something inherently risky about the process, as compared to existing methods

The trigger for regulatory review should be the novelty of the introduced trait (regardless of how or when it was derived), and *not* the process used to introduce the trait

GE animal regulatory burdens are disproportionately high and are associated with unaccountable delay and considerable uncertainty. These regulatory burdens are not justified by scientific evidence or experience

While regulation to ensure the safety of new technologies is necessary, in a world facing burgeoning demands on agriculture from population growth, economic growth, and climate change, overregulation is an indulgence that global food security can ill afford



"I now say that the world has the technology either available or well advanced in the research pipeline — to feed on a sustainable basis a population of 10 billion people. The more pertinent question today is whether farmers and ranchers will be permitted to use this new technology? While the affluent nations can certainly afford to adopt ultra low-risk positions, and pay more for food produced by the so-called 'organic' methods, the one billion chronically undernourished people of the low income, food-deficit nations cannot."



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