GM animals: what are the issues, what's in the pipeline, and what are the risks?

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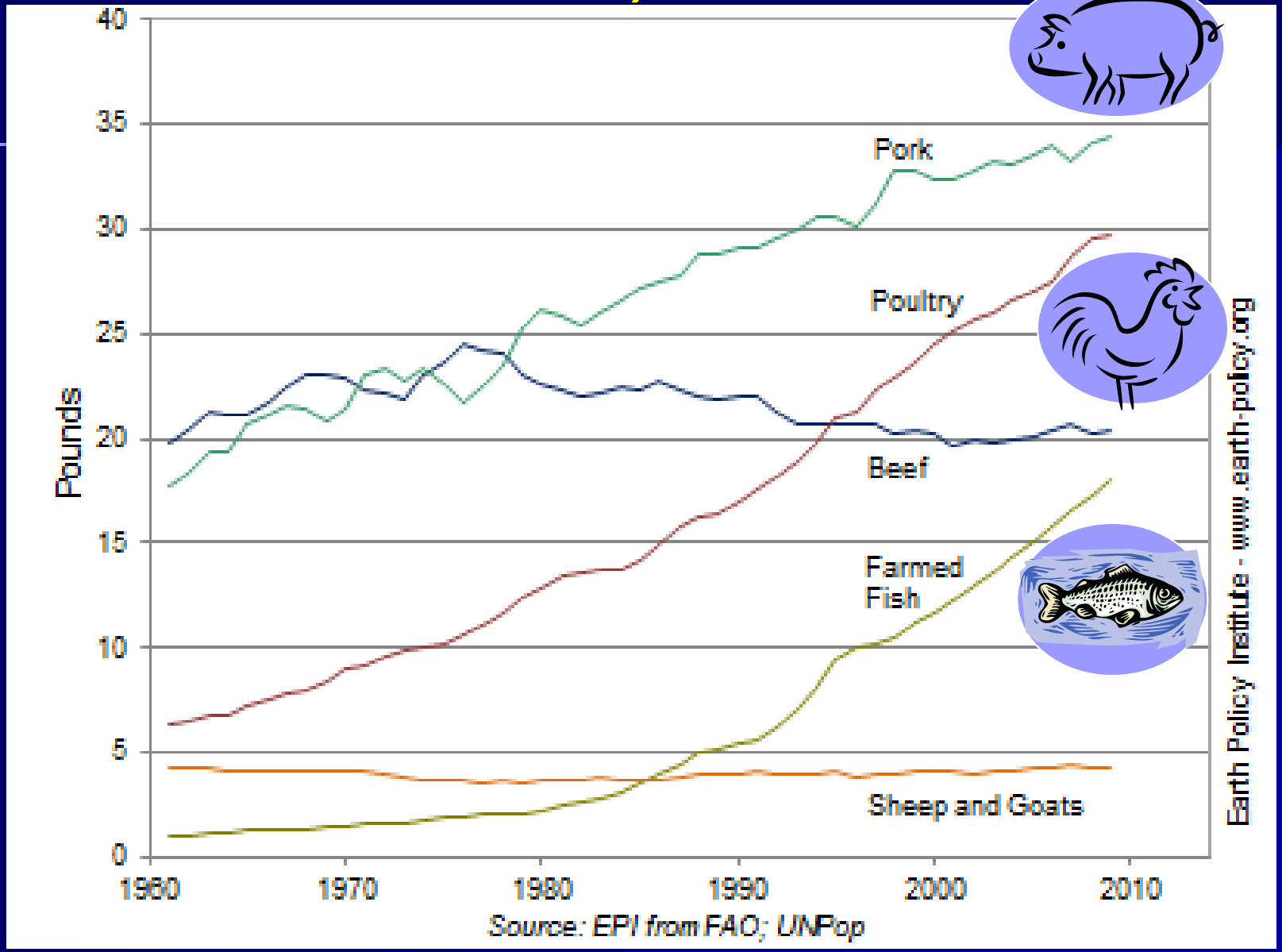
"The mission of the animal genomics and biotechnology extension program is to provide broad, science-based extension programming on the uses of animal biotechnologies in livestock production systems."

http://animalscience.ucdavis.edu/animalbiotech

Overview

- The livestock revolution (bigger than the Green revolution!)
- What have animal breeders been up to?
- Genetically modified (GE) animal pipeline
- The AquAdvantage GE salmon story
- Effect of regulatory gridlock on investment, US competiveness, geographical location of research and application of GE technology for animal agricultural applications

World Animal Protein Production Per Person, 1961-2009



The livestock revolution

- By 2050 nearly twice as much meat will be produced as today
- Unlike the supply-led Green Revolution, the “Livestock Revolution” is driven by demand resulting from population growth, rising affluence in developing countries and urbanization
- From the early 1970s to the mid-1990s, the volume of meat consumed in developing countries grew almost three times as much as it did in the developed countries
- For more than a decade, the strongest increases in animal protein production have been in the developing world
- Since 1995 developing countries produce more meat and dairy products than are produced in developed countries
- **Demand for livestock products is expected to continue growing strongly through the middle of this century**

Delgado, C. L. 2003. Rising consumption of meat and milk in developing countries has created a new food revolution. Journal of Nutrition 133:3907S-3910S

Global livestock populations are major consumers of GE feed

- 70-90% of harvested GE biomass is fed to food producing animals
- 80% of all feed fed to livestock in the EU is imported
- 98% of EU soybean meal is imported from Brazil, the USA, and Argentina; ~ 2/3 of this imported soybean meal animal feed is GE
- If the EU were not able to import soybean protein from outside the EU it would only be able to replace 10-20% of imports by high protein substitutes, resulting in a substantial reduction in animal protein production, exports and consumption, and a very significant increase in animal protein imports and cost*
- The proportion of GE in animal feed is likely higher in the US where 93% of soy and 88% of corn grown were GE varieties in 2012

* Directorate-General for Agriculture and Rural Development. 2007. Economic impact of unapproved GMOs on EU feed imports and livestock production. http://ec.europa.eu/agriculture/envir/gmo/economic_impactGMOs_en.pdf

POPULATION

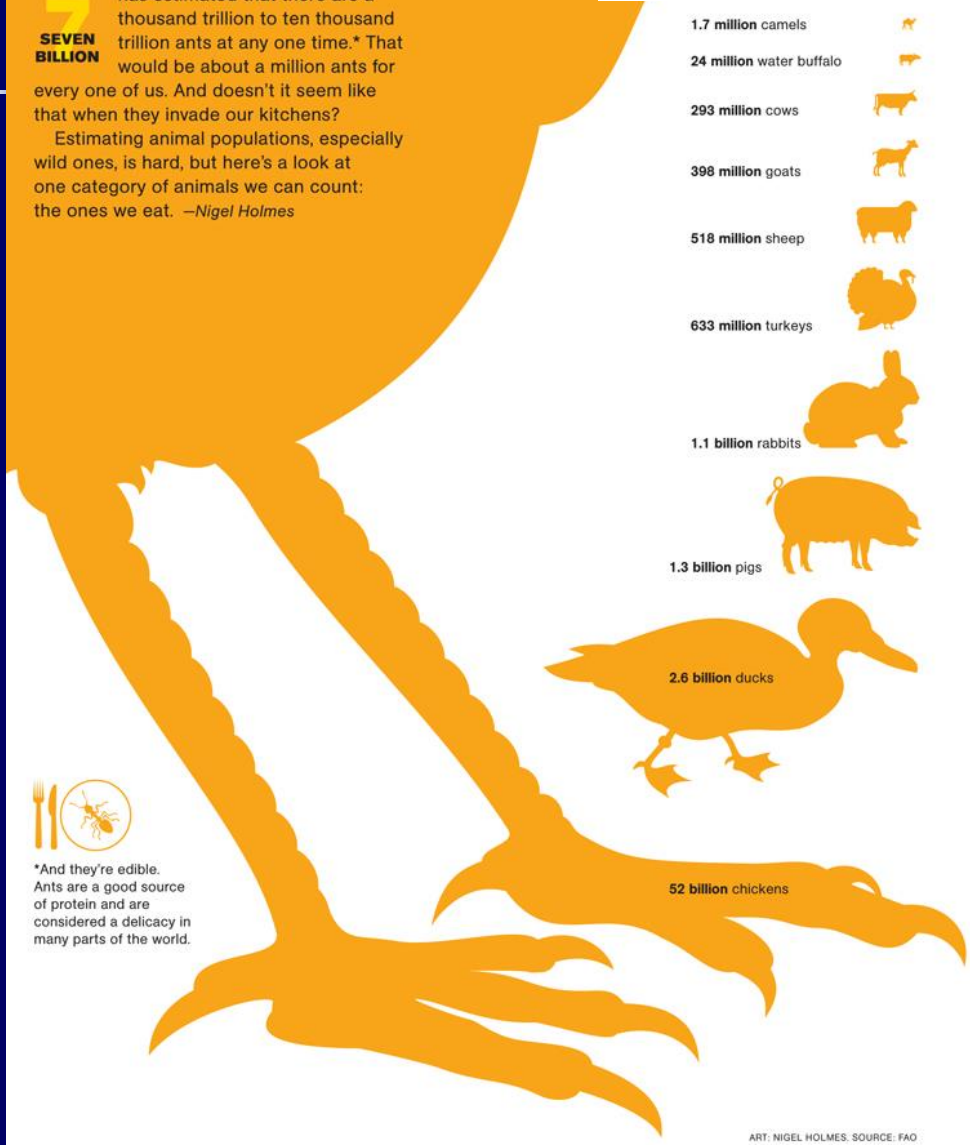
Food for Thought

There will soon be seven billion humans on Earth, but how does that number compare to other species on the planet? We are certainly outnumbered by ants. Harvard biologist and ant expert Edward O. Wilson has estimated that there are a thousand trillion to ten thousand trillion ants at any one time.* That would be about a million ants for every one of us. And doesn't it seem like that when they invade our kitchens?

7 SEVEN BILLION

Estimating animal populations, especially wild ones, is hard, but here's a look at one category of animals we can count: the ones we eat. *–Nigel Holmes*

*And they're edible. Ants are a good source of protein and are considered a delicacy in many parts of the world.



We are vastly outnumbered by chickens !!

1.3 billion pigs

2.6 billion ducks

52 billion chickens

- 59 million tons eggs
- 90 million tons meat

The 8-week old body weight of broiler (meat) chickens has increased from 0.81 kg to 3.14 kg over the period 1957 to 2001, and approximately 80% of this four-fold increase has been the result of genetic selection.

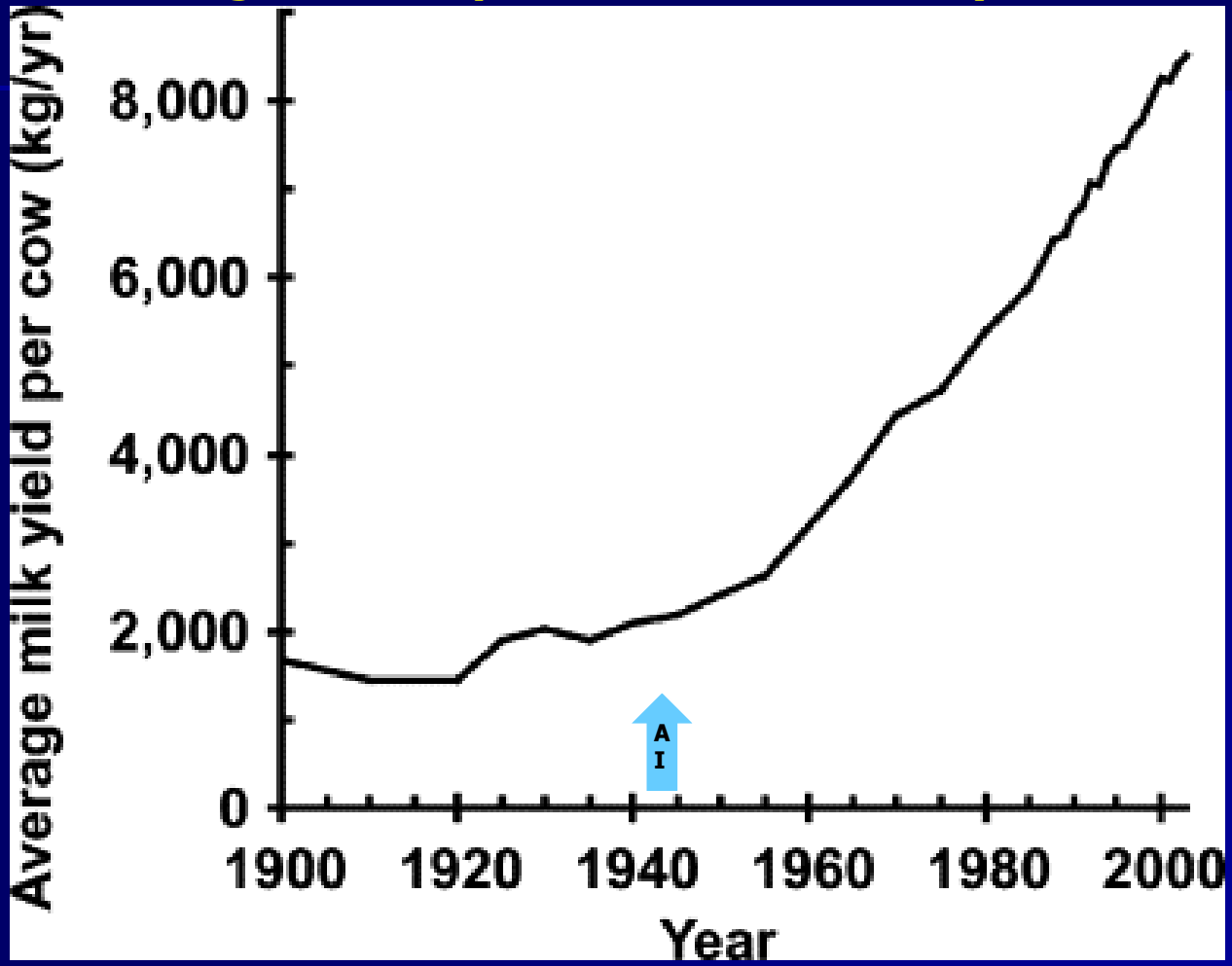
Havenstein, G., Ferket, P. and Qureshi, M. (2003). Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. *Poultry Science* 82, 1500-1508.

Animal breeders have been contributing to sustainability

- Advanced breeding and feeding technology has spurred significant productivity growth, especially in the poultry, pork and dairy sectors
- Genetic advances are much faster in short-cycle animals, such as poultry and pigs, than in species with a longer generation interval, such as cattle
- In all species, feed conversion and related parameters, such as growth rate, milk yield and reproductive efficiency, have been major targets for breeding efforts
- The speed and precision with which breeding goals can be achieved has increased considerably over recent decades due to the adoption of technology
- The use of technologies like hybridization and artificial insemination has accelerated the process of genetic improvement

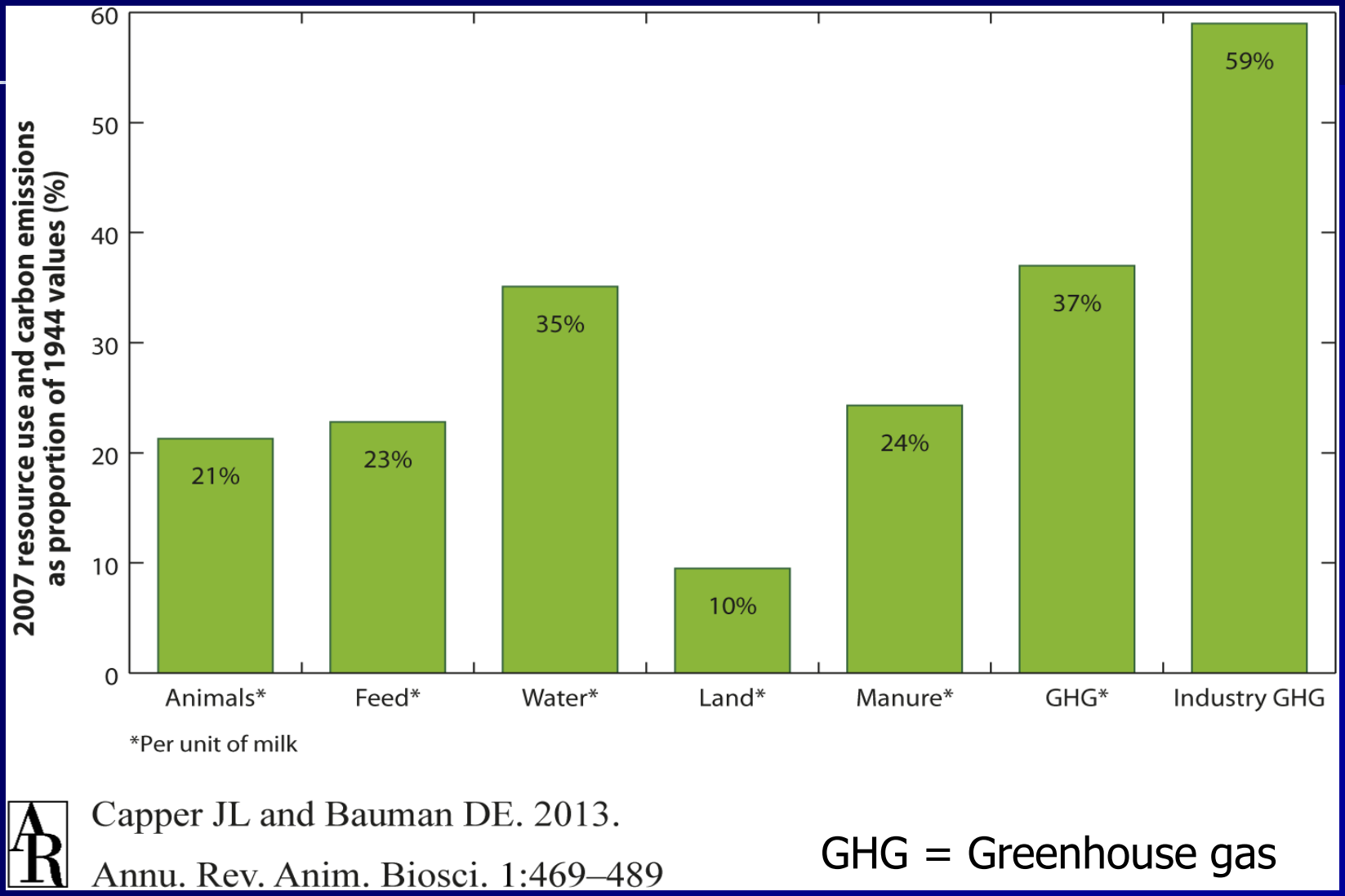
US DAIRY INDUSTRY: 1944: **25.6** million cows; **53.1** billion kg milk annually
1997: **9.2** million cows; **84.2** billion kg milk annually

About half of this 369% increase in production efficiency is attributable to genetic improvement enabled by Artificial Insemination



VandeHaar, M.J. and St-Pierre, N. (2006). Major Advances in Nutrition: Relevance to the Sustainability of the Dairy Industry. *Journal of Dairy Science* 89, 1280-1291.

Resource use and waste outputs from modern US dairy production systems typical of the year 2007, compared with historical US dairying (characteristic of the year 1944).

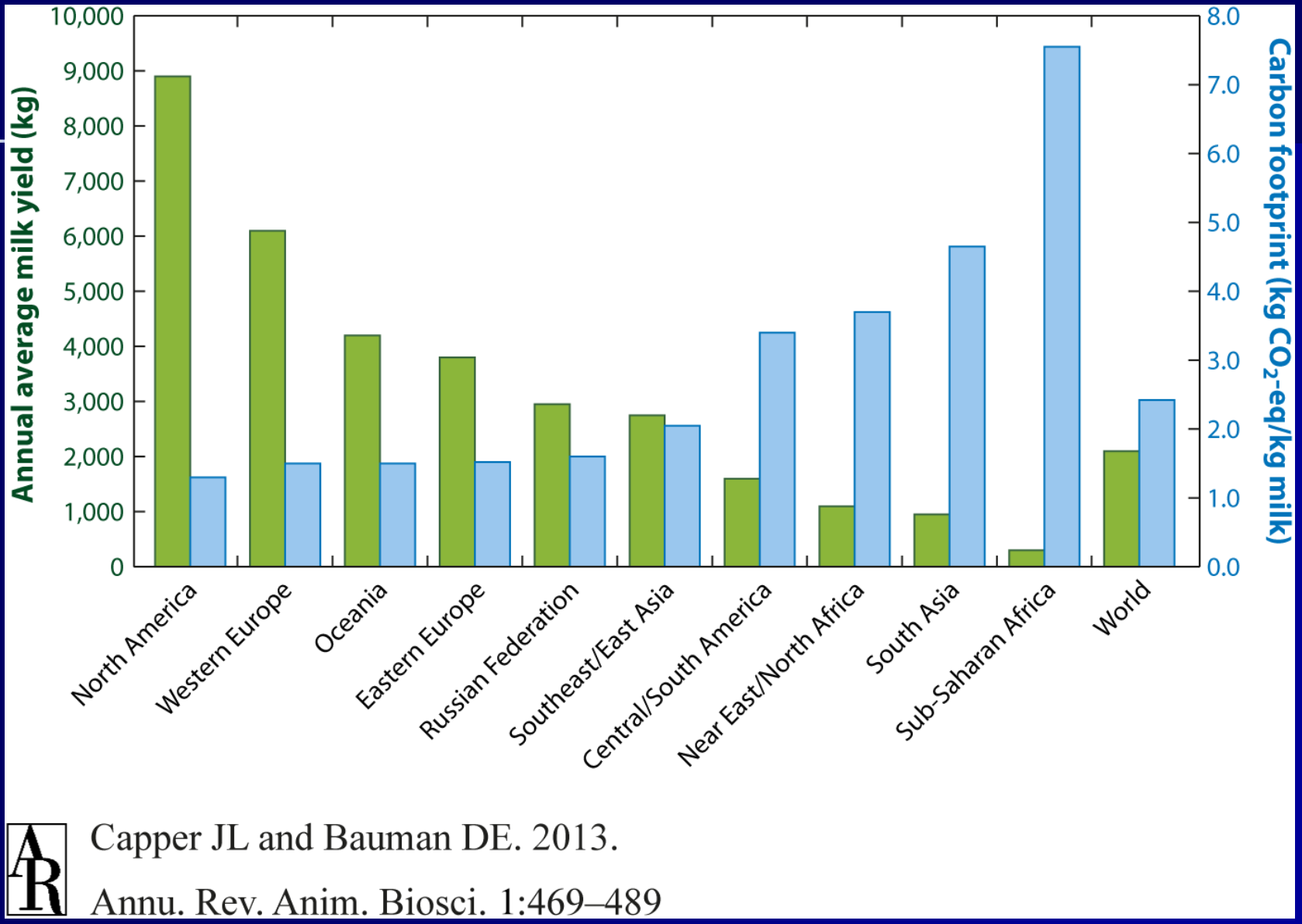


Capper JL and Bauman DE. 2013.

Annu. Rev. Anim. Biosci. 1:469–489

GHG = Greenhouse gas

Average annual milk yield and carbon footprint per kg milk - across global regions. Data adapted from FAO.



Capper JL and Bauman DE. 2013.

Annu. Rev. Anim. Biosci. 1:469–489

Recombinant rDNA gene construct

embryo

transgenic animal

Research
- disease models

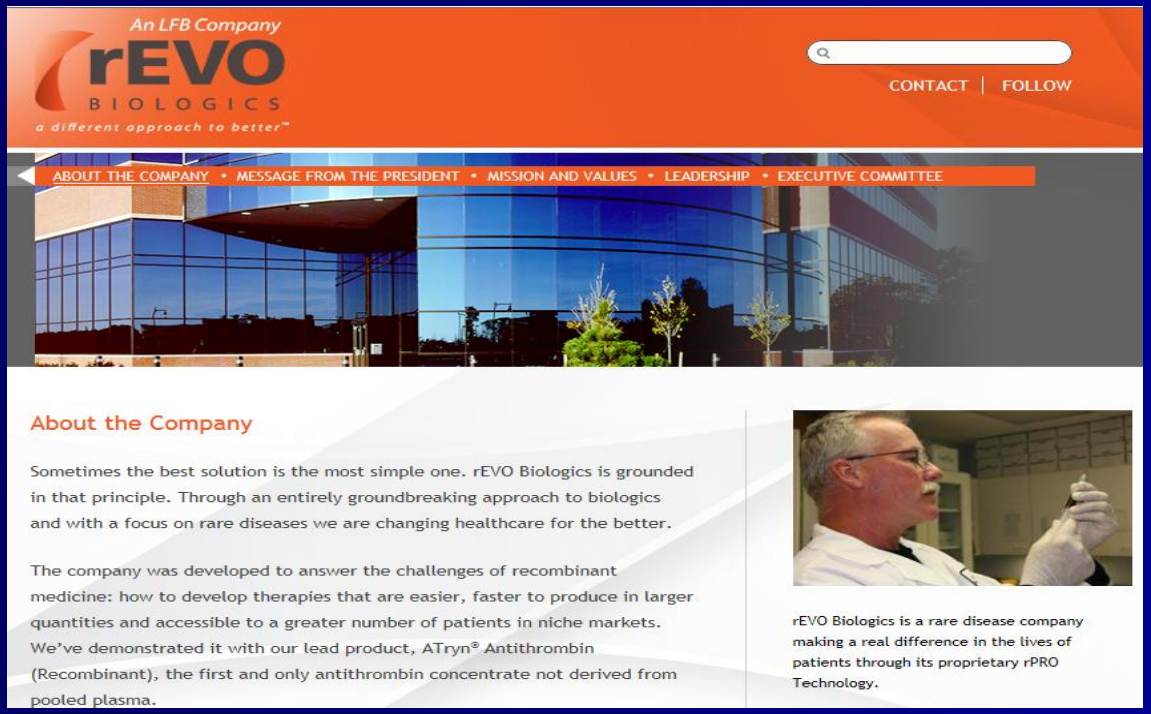
Biomedical
- pharmaceuticals
- xenotransplantation

Industrial
- spider silk

Agriculture
- none on market to date

Pharma and industrial applications of GE (or a combination of cloning & GE) have proven less controversial

The first product from a transgenic farm animal to become a registered drug was Antithrombin III from rEVO Biologics , USA, produced in the mammary gland of transgenic goats for heparin resistant patients to prevent blood clots



February 2009, First GE Animal Product

www.gtc-bio.com

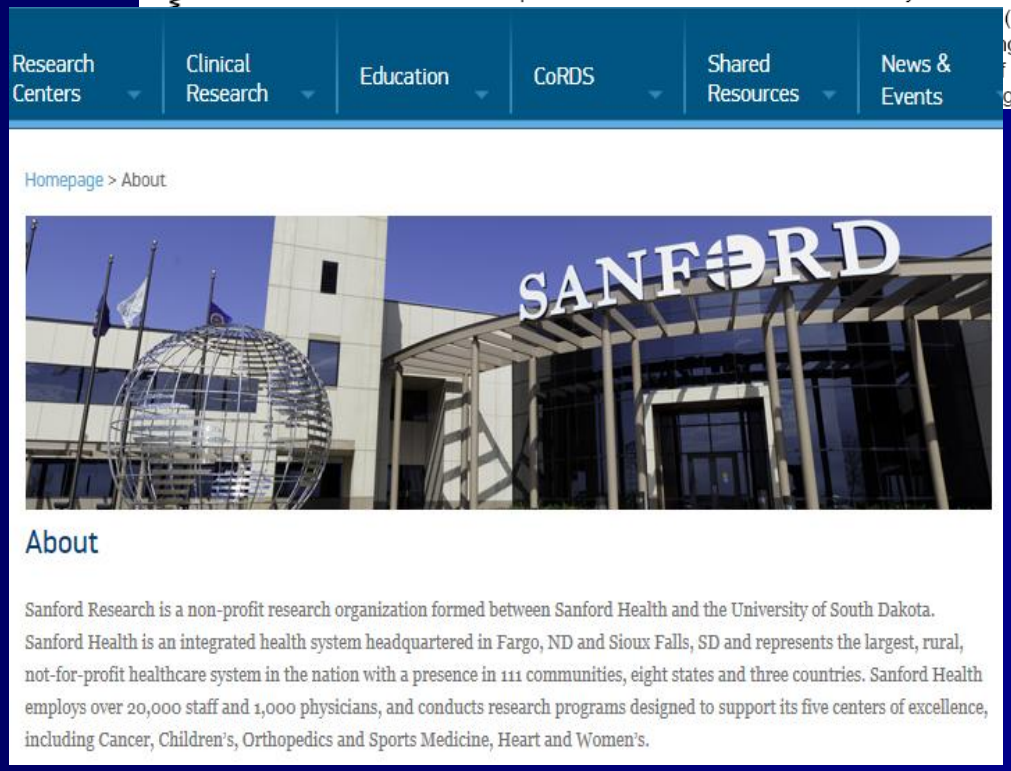
Cloned transchromosomic calves producing human immunoglobulin

Yoshimi Kuroiwa[1], Poothappillai Kasinathan[2], Yoon J. Choi[3], Rizwan Naeem[4], Kazuma Tomizuka[1], Eddie J. Sullivan[2], Jason G. Knott[2], Anae Duteau[3], Richard A. Goldsby[3], Barbara A. Osborne[5], Isao Ishida[1]*,

www.nature.com/naturebiotechnology

Nature Biotechnology 20:889-894. 2002

Human polyclonal antibodies (hPABs) are useful therapeutics, but because they are available only from human donors, their supply and application is limited. To address this need, we prepared a human artificial chromosome (HAC) vector containing the entire unrearranged sequences of the human immunoglobulin (h*Ig*) heavy-chain (*H*) and lambda (*λ*) light-chain loci. The HAC vector was introduced into bovine primary fetal fibroblasts using a microcell-mediated chromosome transfer (MMCT) approach. Primary selection was carried out, and the cells were used to produce cloned bovine fetuses. Secondary selection was done on the regenerated fetal cell (Tc) calves. The HAC was retained at a ngement and expressed diversified tran- newborn calves. The production of Tc g therapeutic hPABs.



http://www.sanfordresearch.org

Transchromosomal cattle carry a human artificial chromosome harboring the entire sequence of the human major histocompatability complex . These animals were cloned from bovine fibroblasts after transfection with the additional chromosome.

Xenotransplantation 2010: 17: 48–60
Printed in Singapore. All rights reserved
doi: 10.1111/j.1399-3089.2009.00564.x

XENOTRANSPLANTATION

Pigs as organ donors - xenotransplantation

Structural characterization of α1,3-galactosyltransferase knockout pig heart and kidney glycolipids and their reactivity with human and baboon antibodies

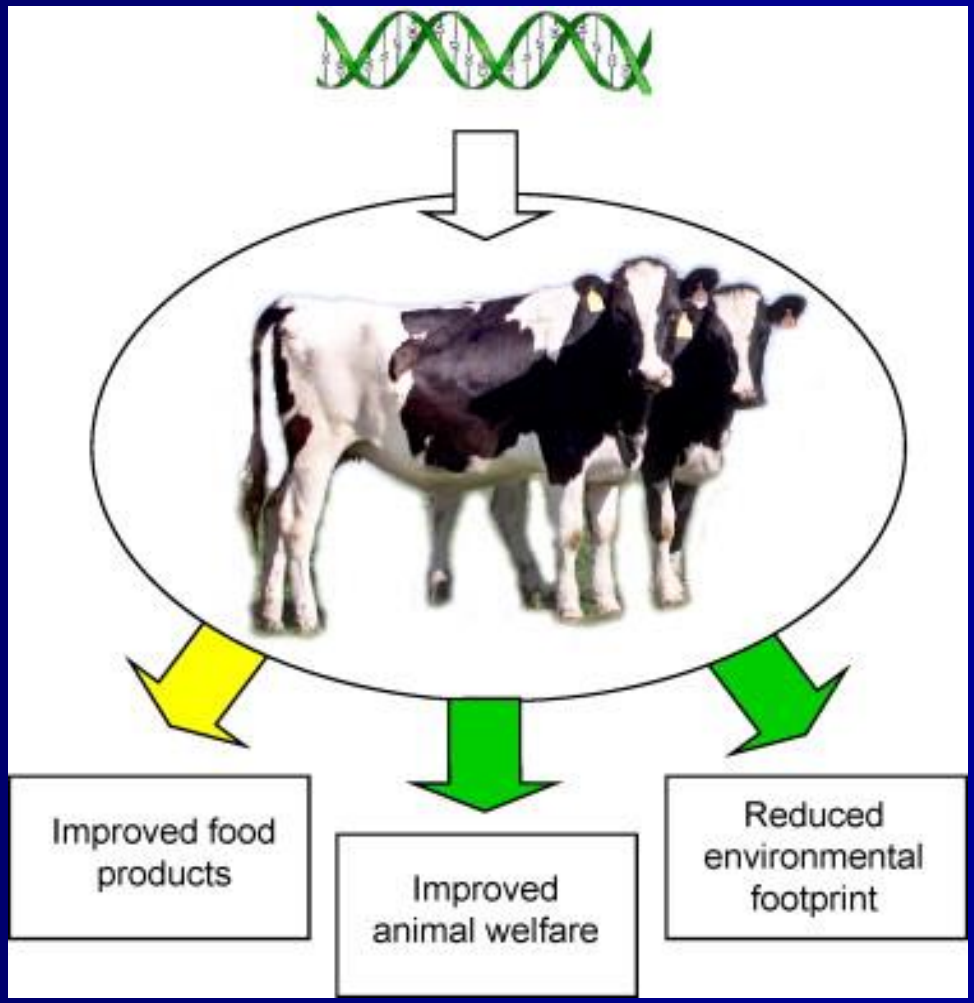
Diswall M, Ångström J, Karlsson H, Phelps CJ, Ayares D, Teneberg S, Breimer ME. Structural characterization of α1,3-galactosyltransferase knockout pig heart and kidney glycolipids and their reactivity with human and baboon antibodies.
Xenotransplantation 2010; 17: 48–60. © 2010 John Wiley & Sons A/S.

Mette Diswall,[1] Jonas Ångström,[1] Hasse Karlsson,[2] Carol J. Phelps,[3] David Ayares,[3] Susann Teneberg,[2] and Michael E. Breimer,[1]

[1]Department of Surgery, Institute of Clinical

www.revivicor.com

Agricultural applications of GE



Mastitis-resistant cows
(inflammation of mammary gland)

nature biotechnology

ARTICLES

Nature Biotechnology 23:445-451. 2005

Genetically enhanced cows resist intramammary *Staphylococcus aureus* infection

Robert J Wall[1], Anne M Powell[1], Max J Paape[2], David E Kerr[3], Douglas D Bannerman[2], Vernon G Pursel[1], Kevin D Wells[4], Neil Talbot[1] & Harold W Hawk[1]

Mastitis, the most consequential disease in dairy cattle, costs the US dairy industry billions of dollars annually. To test the feasibility of protecting animals through genetic engineering, transgenic cows secreting lysostaphin at concentrations ranging from 0.9 to 14 mg/ml in their milk were produced. *In vitro* assays demonstrated the milk's ability to kill *Staphylococcus aureus*. Intramammary infusions of *S. aureus* were administered to three transgenic and ten nontransgenic cows. Increases in milk somatic cells, elevated body temperatures and induced acute phase proteins, each indicative of infection, were observed in all of the nontransgenic cows but in none of the transgenic animals. Protection against *S. aureus* mastitis appears to be achievable with as little as 3 mg/ml of lysostaphin in milk. Our results indicate that genetic engineering can provide a viable tool for enhancing resistance to disease and improve the well-being of livestock.

http://www.nature.com/naturebiotechnology

Enviropig™ (Low-phosphorus manure)

RESEARCH ARTICLE

Nature Biotechnology 19, 741–745 . 2001

Pigs expressing salivary phytase produce low-phosphorus manure

Serguei P. Golovan[1,2], Roy G. Meidinger[2], Ayodele Ajakaiye[3], Michael Cottrill[1], Miles Z. Wiederkehr[4], David J. Barney[4], Claire Plante[5], John W. Pollard[5], Ming Z. Fan[3], M. Anthony Hayes[6], Jesper Laursen[7,8], J. Peter Hjorth[7], Roger R. Hacker[3], John P. Phillips[2,*], and Cecil W. Forsberg[1,*]

To address the problem of manure-based environmental pollution in the pork industry, we have developed the phytase transgenic pig. The saliva of these pigs contains the enzyme phytase, which allows the pigs to digest the phosphorus in phytate, the most abundant source of phosphorus in the pig diet. Without this enzyme, phytate phosphorus passes undigested into manure to become the single most important manure pollutant of pork production. We show here that salivary phytase provides essentially complete digestion of dietary phytate phosphorus, relieves the requirement for inorganic phosphate supplements, and reduces fecal phosphorus output by up to 75%. These pigs offer a unique biological approach to the management of phosphorus nutrition and environmental pollution in the pork industry.

"reduces fecal phosphorus output by up to 75%"

www.uoguelph.ca/enviropig

Omega-3 Pigs
(Pigs cloned after genetically engineering cell)

BRIEF COMMUNICATIONS

nature biotechnology

Nature Biotechnology 24:435-436. 2006

Generation of cloned transgenic pigs rich in omega-3 fatty acids

Liangxue Lai[1,2,8], Jing X Kang[5,8], Rongfeng Li[1], Jingdong Wang[5], William T Witt[6], Hwan Yul Yong[1], Yanhong Hao[1], David M Wax[1], Clifton N Murphy[1], August Rieke[1], Melissa Samuel[1], Michael L Linville[3], Scott W Korte[4], Rhobert W Evans[7], Thomas E Starzl[6], Randall S Prather[1,2] & Yifan Dai[6]

Meat products are generally low in omega-3 (*n*-3) fatty acids, which are beneficial to human health. We describe the generation of cloned pigs that express a humanized *Caenorhabditis elegans* gene, fat-1, encoding an *n*-3 fatty acid desaturase. The hfat-1 transgenic pigs produce high levels of *n*-3 fatty acids from *n*-6 analogs, and their tissues have a significantly reduced ratio of *n*-6/*n*-3 fatty acids ($P < 0.001$).

The health benefits of long chain *n*-3 fatty acids, found mainly in fish oils, are well recognized. Meat products normally contain small amounts of *n*-3 fatty acids and large amounts of *n*-6 fatty acids[1]

University of Missouri/Massachusetts General Hospital and Harvard Medical School

GE Chickens That Don't Transmit Bird Flu

Breakthrough could prevent future bird flu epidemics

Suppression of Avian Influenza Transmission in Genetically Modified Chickens

Jon Lyall,[1] Richard M. Irvine,[2] Adrian Sherman,[3] Trevelyan J. McKinley,[1] Alejandro Núñez,[2] Auriol Purdie,[3]* Linzy Outtrim,[2] Ian H. Brown,[2] Genevieve Rolleston-Smith,[3] Helen Sang,[3]† Laurence Tiley[1]†‡

Infection of chickens with avian influenza virus poses a global threat to both poultry production and human health that is not adequately controlled by vaccination or by biosecurity measures. A novel alternative strategy is to develop chickens that are genetically resistant to infection. We generated transgenic chickens expressing a short-hairpin RNA designed to function as a decoy that inhibits and blocks influenza virus polymerase and hence interferes with virus propagation. Susceptibility to primary challenge with highly pathogenic avian influenza virus and onward transmission dynamics were determined. Although the transgenic birds succumbed to the initial experimental challenge, onward transmission to both transgenic and nontransgenic birds was prevented.

The diversity of avian influenza viruses (AIVs) and their propensity for interspecies transmission make them a global threat to animal and public health communities. Cross-species transmission of influenza viruses mediate host species that amplify and diversify virus populations, notably domestic chickens, ducks, and pigs (*1*). Although control of AIV infection in its wild aquatic bird reservoir is impractical, control of AIV in domesticated hosts is

The diversity of viral antigenic sub-

Science 331:223-226. 2011 SCIENCE VOL 331 14 JANUARY 2011 223

www.roslin.ed.ac.uk/public-interest/gm-chickens

Fast growing salmon

The founder female was generated in 1989 – 24 years ago

Nature Biotechnology 10:176 – 181. 1992

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

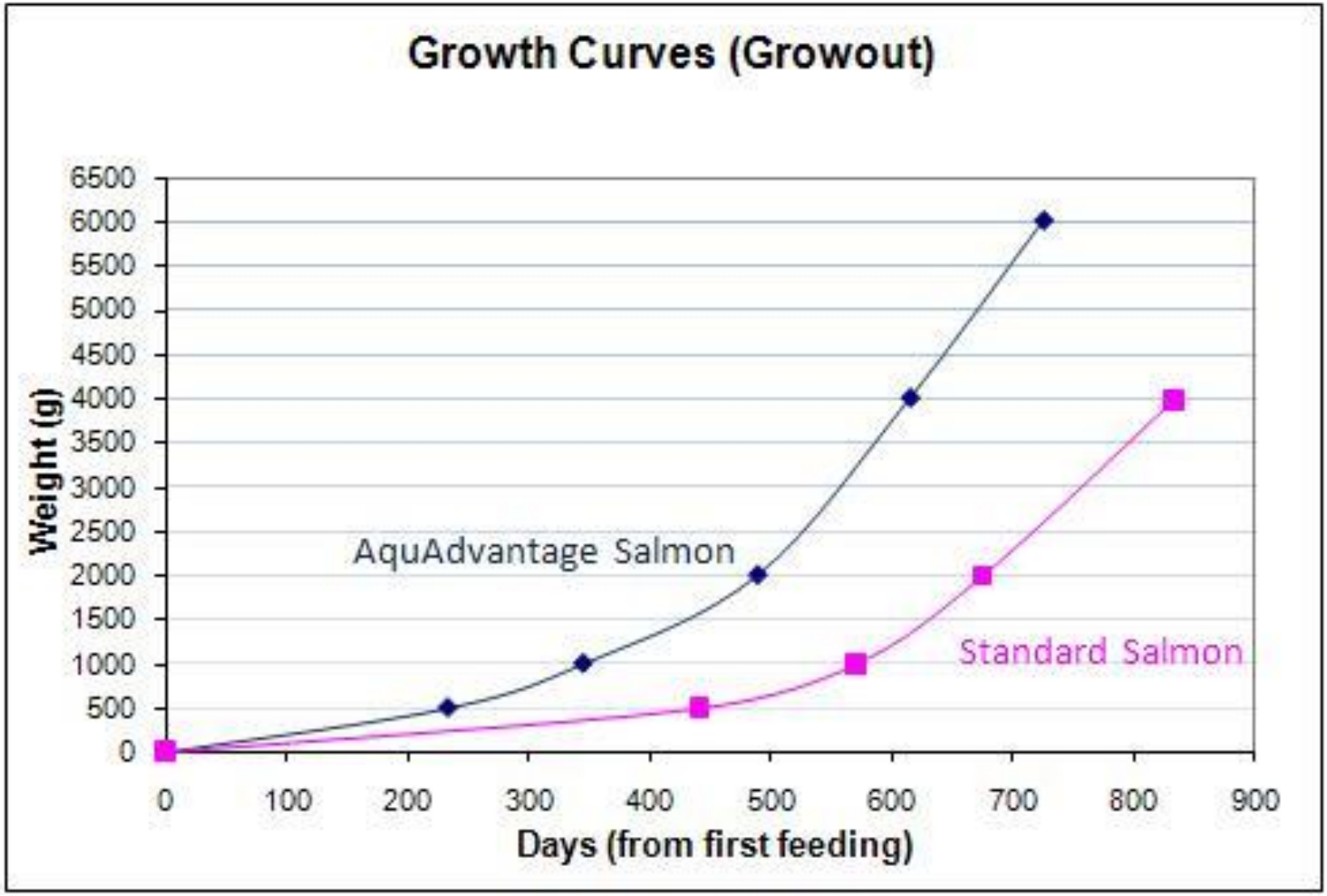
Shao Jun Du, Zhiyuan Gong, Garth L. Fletcher[1], Margaret A. Shears[1], Madonna J. King[1], David R. Idler[1] and Choy L. Hew*

Research Institute, The Hospital for Sick Children and Departments of Clinical Biochemistry and Biochemistry, University of Toronto, Toronto, Canada M5G 1L5. [1]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

University of Toronto/Memorial University of Newfoundland, Canada

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



In a letter to the FDA dated April 26, **1993**, AquaBounty Technologies (then A/F Protein) initiated discussions with the FDA seeking regulatory guidance for development and approval of a GE Atlantic salmon intended to grow faster than conventionally bred Atlantic salmon.

- In January 2009, the Food and Drug Administration issued a final guidance for industry on the regulation of genetically engineered (GE) animals (had 28,000 comments on draft!!)
- FDA plans to regulate GE animals under the new animal drug provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA), and the National Environmental Policy Act (NEPA)

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Guidance for Industry

Regulation of Genetically Engineered Animals

Containing Heritable Recombinant DNA Constructs

Final Guidance

http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf

Basis for FDA regulatory oversight is use of rDNA process

- The FDA considers the recombinant DNA (rDNA) construct to be a "new animal drug" because it is "***an article intended to alter the structure or function***" of the animal.
- As with all new drug applications, the sponsor is responsible for conducting [and paying for] the studies required in order to obtain regulatory compliance data on food safety and drug efficacy.
- Sponsors must receive formal approval before GE animals (or any new animal drug) can be introduced into commerce.
- **Conversely, the United States has no specific legislation expressly regulating animal breeding so fast growing or more productive animals produced using other technologies like AI and hybridization are not subject to regulatory review.**

Council for Agricultural Science and Technology (CAST). 2011. *The Science and Regulation of Food from Genetically Engineered Animals*. CAST Commentary QTA2011-2. CAST, Ames, Iowa.

Date	Event
September 1995	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

Product Definition for the AquAdvantage Salmon

Product Identity

Triploid hemizygous, all-female Atlantic salmon (*Salmo salar*) bearing a single copy of the transgene.

Claim

Significantly more of these Atlantic salmon grow to at least 100 g within 2700 deg C days than their comparators.

Limitations for Use

These Atlantic salmon are produced as eyed-eggs for grow-out only in the FDA-approved physically-contained fresh water culture facility.

Food and Drug Administration (FDA). 2010. *2010 VMAC Briefing Packet*, http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/UCM224762.pdf.

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

Paradoxically it often seems that the arguments for and against GE animals for food purposes overlap

- Groups opposed to the technology argue that the risks GE animals pose to food safety, animal health, and the environment are too great to allow the technology to move forward.

- Proponents of the technology see the potential benefits for GE animals to produce safer food, improve animal health, and reduced environmental impact as too great to forgo the use of this technology in animal agriculture production systems.

Timeline of AquAdvantage regulatory process

24+ years from discovery to application?

Year	Event
1989	• Founder AquAdvantage fish produced in Canada
1995	• FDA review of AquAdvantage salmon begins
2001	• First regulatory study submitted by Aqua Bounty Technologies to U.S. FDA for a New Animal Drug Applications (NADA)
2009	• FDA guidance on how GE animals will be regulated • FDA approval of first GE animal pharmaceutical • Final AquAdvantage regulatory study submitted to FDA
2010	• FDA VMAC meeting on AquAdvantage salmon (9/20/10)
2011	• Political efforts to defund FDA, ban fish, delay approval
2012	• FDA released "FONSI" finding of environmental assessment
2013	• **AquaBounty has expended over $60 million to bring the AquAdvantage salmon through the regulatory approval process *thus far*** (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

It is time to reconnect the GE regulatory framework to the best available science

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?

Is this level of scrutiny aligned to science-based risks associated with this technology, or is this level of regulatory oversight and associated regulatory compliance costs making the deployment of this valuable technology unworkable, and providing an opportunity for unwarranted political interference to the detriment of food security globally?

Dr. Calestous Juma, Harvard's Kennedy School of Government, at a 6/23/11 hearing to examine the benefits of agricultural biotechnology held by the House Agriculture Committee's Subcommittee on Rural Development, Research, Biotechnology, and Foreign Agriculture

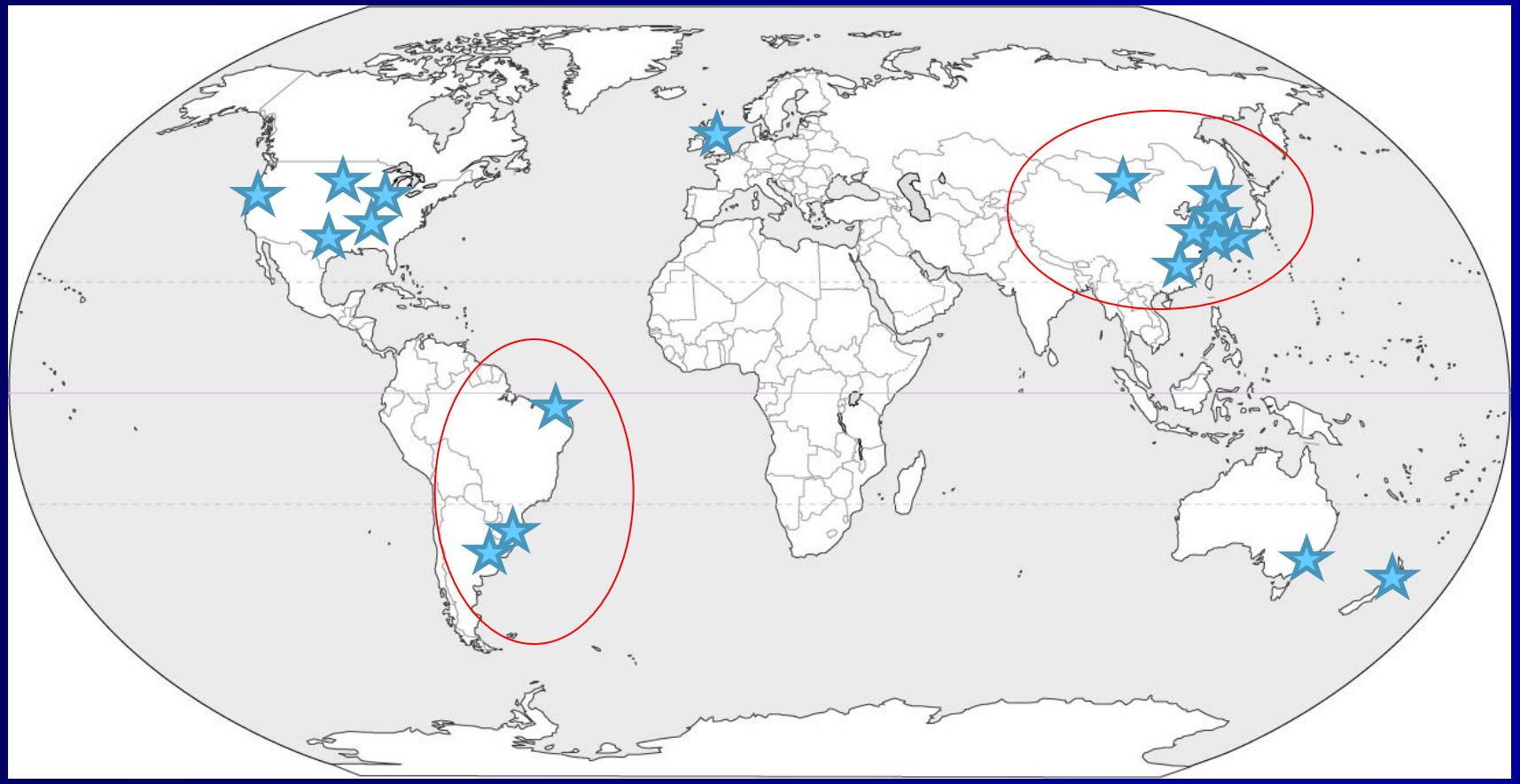
". . It is not this particular fish that is at stake. It is the principle behind the amendment (to prohibit use of FDA funds to evaluate any application for approval of genetically engineered salmon) and its wider ramifications. It sends the message to the rest of the world that the science-based regulatory oversight as embodied in the FDA review process is subject to political intervention.

Furthermore, it signals to the world that the United States may cede its leadership position in the agricultural use of biotechnology. . . I believe it is imperative that the United States stay the course it has set in not letting politics interfere with its science-based regulatory system that is truly the envy of the world."

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

Graphic developed by Dr. J. Murray, UC Davis

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



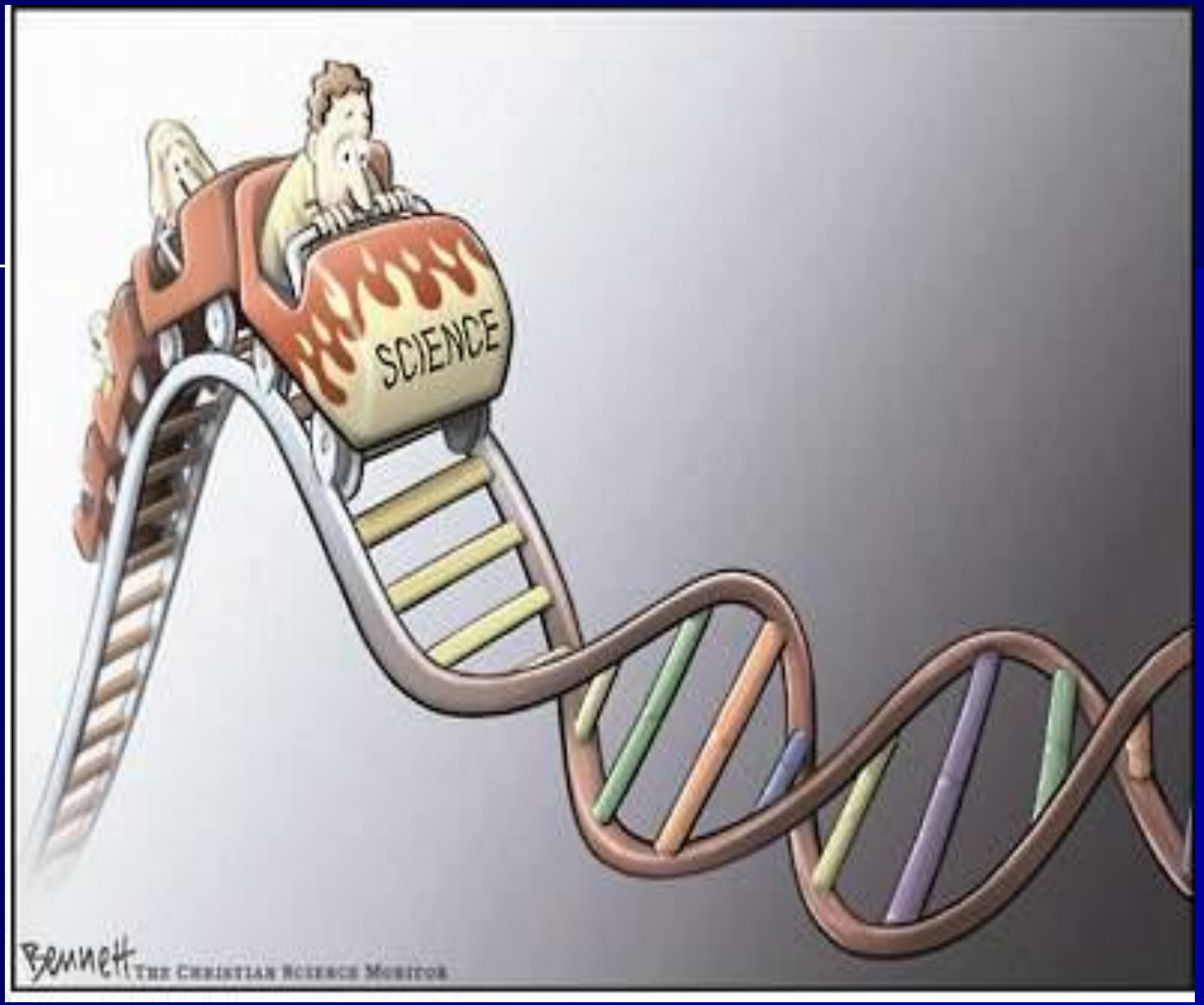
Graphic developed by Dr. J. Murray, UC Davis

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

"…genetic engineering is a key technology, which will be vital for meeting the world's future food needs. While animal genetics alone will not solve the world's future food problems, to fail to apply the best available technologies to the solution of contemporary and future food shortages would be morally reprehensible."

Fahrenkrug *et al*. 2010. Precision Genetics for Complex Objectives in Animal Agriculture. *Journal of Animal Science* 88:2530-9


SCIENCE
Bennett
The Christian Science Monitor