HIGHER-ORDER ALCOHOLS AS BIOFUELS: AN INTERDISCIPLINARY EXAMINATION

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I. Introduction

The prospect of transportation fuels produced on a large scale without the use of petroleum feedstock has received prominent attention in recent years, motivated by several potential economic, environmental, and geopolitical advantages. Among possible gasoline replacements, ethanol has been the focus of the greatest attention thus far, building on centuries-old knowledge developed through its production for human consumption. At this juncture however, its commercial viability as a fuel appears tenuous due to several intrinsic challenges, including low energy content, miscibility in water, and corrosivity to existing engines. Looking ahead, higher-order alcohols are an attractive alternative, for while they share with ethanol a high compatibility with native microbial metabolism, their chemical properties suggest they are more likely to be commercially viable. It should be noted though, that certain environmental, techno-economic, and governance issues still remain outstanding, issues that are best addressed in the near-term while the technological development and infrastructure investment are at an early stage.

It is in this context that this project is situated, seeking to answer such questions as:

- How do different platforms for higher order alcohol bioconversion compare in terms of technological and economic barriers and opportunities?
- What is the scope of environmental impacts that may arise and how do these compare with one another?
- Using genetic engineering as a case study, how do prior perceptions and regulations constrain evaluations of risk and uncertainty of new fuel technologies?

Sections II through V will introduce the technological, economic, and environmental background and representative companies that provide a basis for the discussion that follows. Section V sets forth several levels of comparisons that form the heart of our analysis: a comparison of gasoline and alcohols as fuels; a comparison of ethanol and higher-order alcohols; and a comparison of three broad approaches to industrial production of higher-alcohol fuels. In Section V we point out some of the governance challenges that arise from these comparisons, both in the context of managing the potential for societal harm and the role of the public sphere in promoting new technology development.

II. Technology

In this section an introduction to alcohol biosynthesis will be given, along with a discussion of techniques that allow the engineering of organisms. This will enable a presentation of three core technologies for generating higher alcohols. Finally these three technologies will be compared based on their inherent limitations and benefits, as well as their current level of technological development.

WHAT MAKES *BIO*ALCOHOLS?

The present analysis is targeted toward *bioconversion* technologies for generating higher alcohols from carbon-based feedstock. The main potential carbon inputs are natural organic matter (e.g., forest debris), farmed organic matter (crops), waste (sewage), or 'abiotic' carbon sources (CO2, bicarbonate). Bioconversion entails that living species, or enzymes derived from them, will catalyze the reactions converting the chemical feedstock into alcohol products. The enzymes responsible for generating higher alcohols are catalysts for specific chemical steps in a reaction pathway leading from starting chemicals to alcohol products. Such enzymatic pathways generally involve several steps. Enzymatic reaction pathways are presumed to occur in the organisms that produce these enzymes, but in theory could be performed in abiotic settings.

NATURE'S PRECEDENT: NATIVE ALCOHOL BIOSYNTHESIS

The pathways that humans have available for controlled higher alcohol biosynthesis are composed of evolved enzymatic transformations. While modification of evolved enzymes could enable new transformations, the basic structure of all the available enzymes comes from the bank that nature has provided. In some species, the metabolic network is arranged such that significant flux through higher alcohol biosynthesis pathways is observed. This is the case for Clostridia bacteria (Moholkar 2012). In other cases, enzymes for higher alcohol biosynthesis are present, but the metabolic network is such that these pathways have very low flux. This is the case for most species, and is a phenomenon well characterized in yeast (Rabinovitch-Deere 2013). A final case includes species that don't have a complete pathway to higher alcohols, and thus no flux is possible in native organisms (e.g., *E. coli*). The last two cases often are only weakly distinguishable from each other without detailed proteomic and metabolomic knowledge, since the end result is nearly the same.y

There are multiple pathways to higher alcohols, which rely on different enzymes and to some extent on different energy sources and carbon based starting compounds. Large portions of these pathways are derived from different areas of central carbon metabolism (Figure 1). An acetyl CoA dependent pathway leads from pyruvate to acetyl CoA to straight chain alcohols through a series of NADH dependent steps. A second keto-acid pathway (based partially on the Ehrlich pathway) leads from amino acids (e.g. threonine) or keto acids (e.g. pyruvate) to linear or branched alcohols through a series of NADPH dependent steps. Both of the above pathways can be connected in tandem with routes to keto acids such as pyruvate. This allows species to in use carbohydrates or amino acids as input to either pathway, and allows CO2 input to either pathway via carbohydrate generation from CO2. For these reasons, pyruvate serves as a node linking multiple of the pathways under discussion (Figure 2).

Figure 1. Central Carbon Metabolism

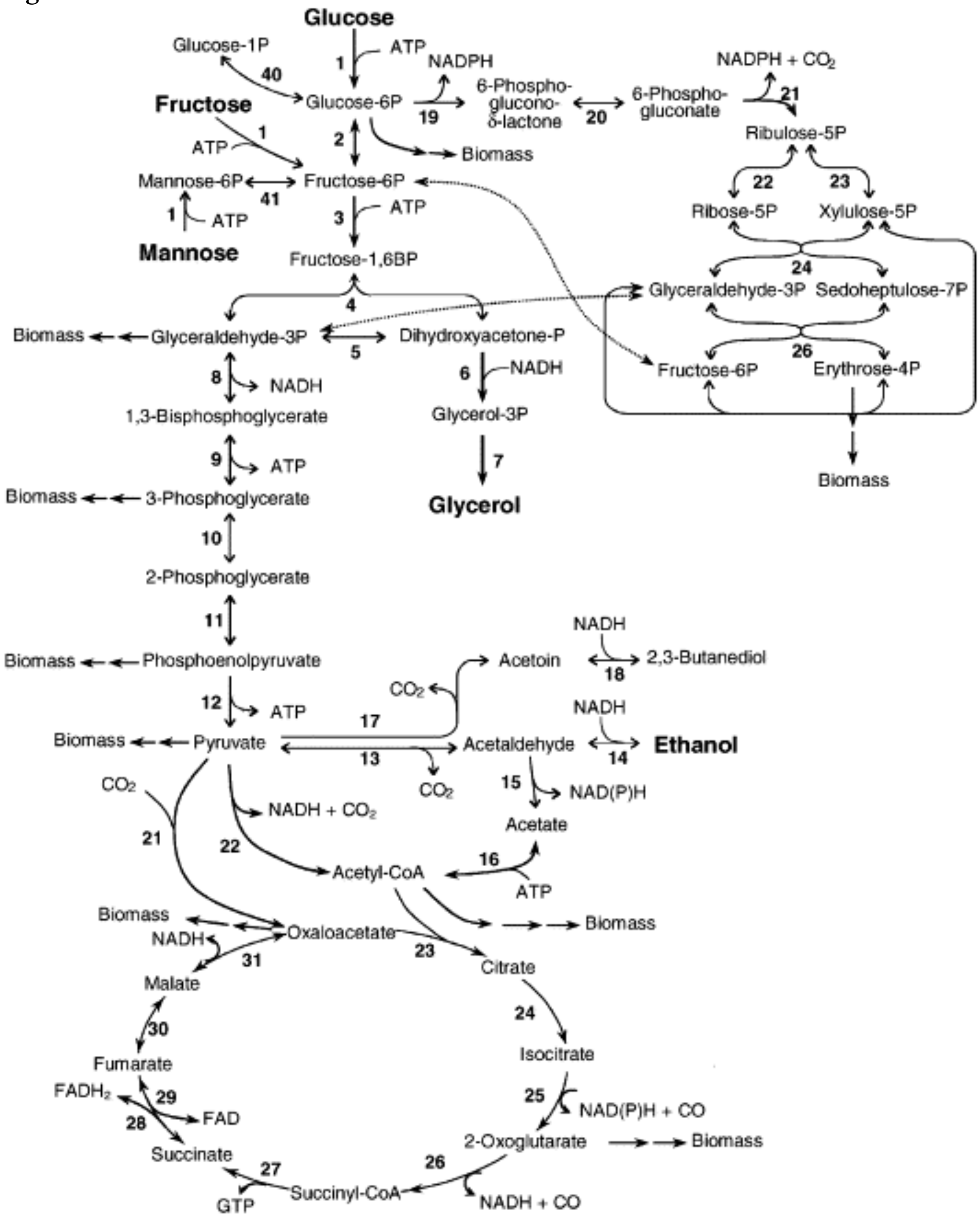
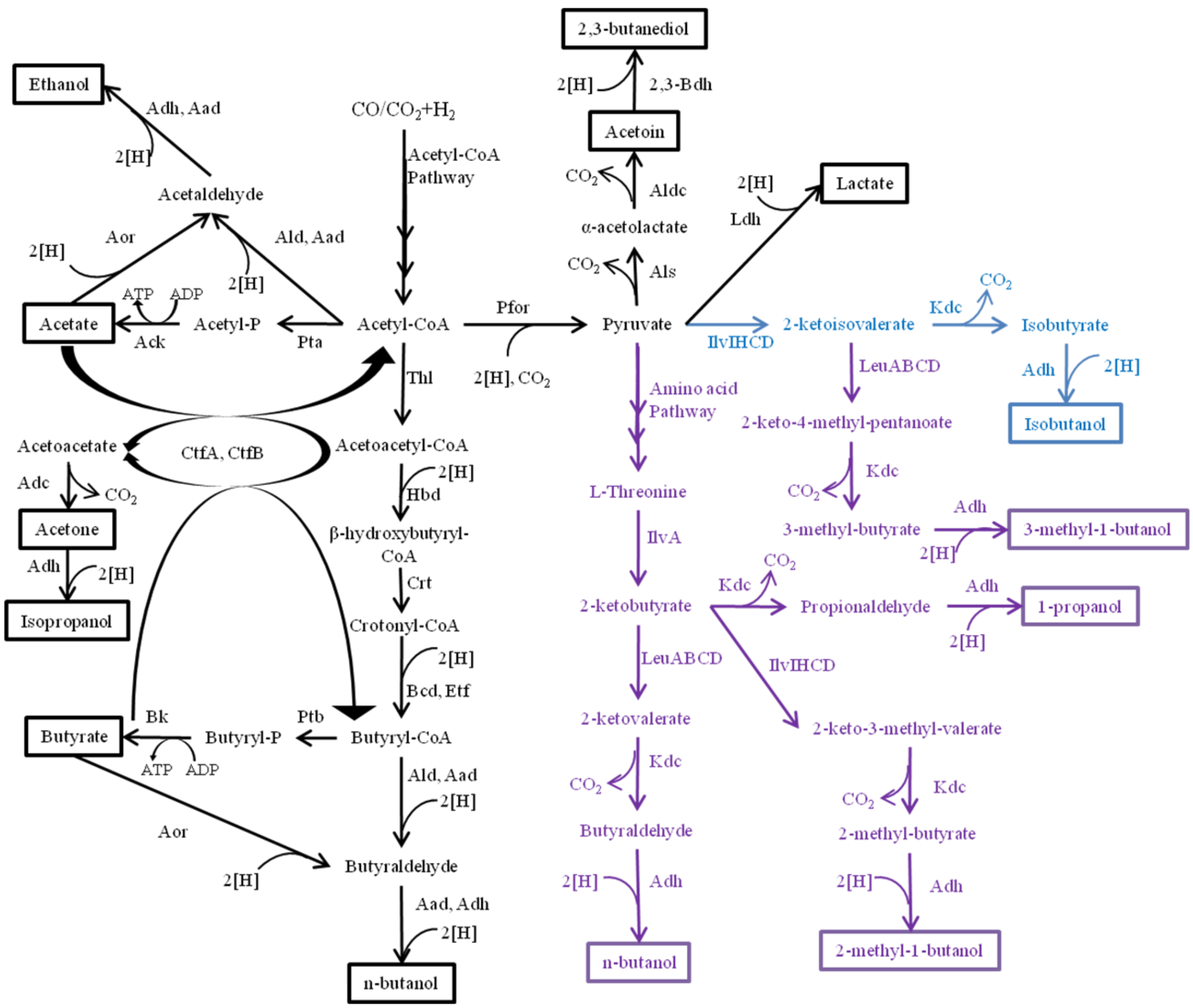


Figure 2: Pathways from Pyruvate



ROUTES TO HIGHER ALCOHOLS: THREE REPRESENTATIVE TECHNOLOGIES

Three technologies represent humans' most promising effort to biologically generate higher alcohols: the ABE process, engineered *E. coli* and yeast, and engineered cyanobacteria. These three technologies draw separately from the set of available approaches for employing microbes in controlled biosynthesis, and differ in their stage of development. The ABE process was founded by employing naturally evolved species and their native biosynthetic pathway. It has been improved upon by classic strain selection and process engineering, and more recently genetic engineering. *E. coli* and yeast are the best studied organisms, and scientists and engineers are actively using this knowledge to establish non-native metabolic pathways to higher alcohols using genetic engineering. The engineering of cyanobacteria represents a broadening of the scope of humans' species focus, as they can use CO2, an abundant non-biomass feedstock.

Clostridia and the Acetone-butanol-ethanol (ABE) Process

Controlled anaerobic fermentation of carbohydrates by Clostridia bacteria has been done for well over 100 years. *Clostridium acetobutylicum* cultures were isolated and identified around 1912, and the industrial ABE process was established within a decade (Moholkar 2012). This technique began using organisms derived from nature, and was improved through the first 80 years of its industrial period by strain selection and process engineering. Today it is well developed and optimized and arguably the most market competitive biological route to higher alcohols. The main biomass inputs have been carbohydrates such as molasses, corn, wheat, and rye (Woods 1986). Using batch fermentation, classic strains could produce 20 g/L of solvent, and more advanced 'hyper-butanol-producing' strains can produce 33 g/L. These numbers are limited by Clostridia growth intolerance to higher solvent concentrations. Substrate concentrations are generally 60-80 g/L, so solvent yields run in the range of 30-40%. Clostridia use the Coenzyme A dependent pathway reliant on NADH to convert pyruvate to butanol (Figure 3).

Figure 3: Clostridia Pathway

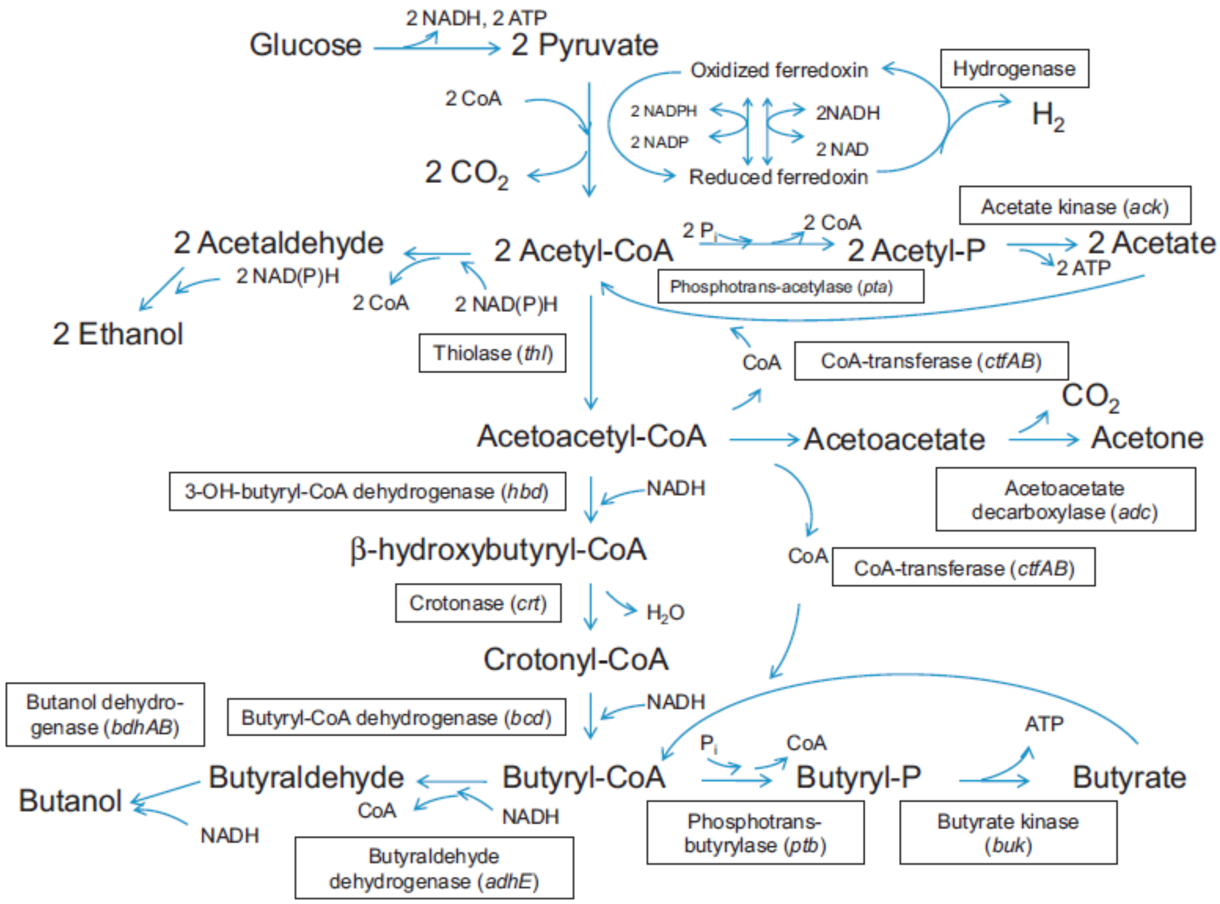


Fig. 1. Acidogenic and solventogenic metabolic pathways in clostridia. The enzymes involved are shown in boxes with the corresponding gene in parenthesis.

Engineered Escherichia coli and yeast

E.coli and yeast (*Saccharomyces cerevisiae*) are the best understood organisms, and effective mass balance, metabolite concentration, and enzymatic reaction flux models exist for these organisms. A high degree of pathway understanding is established and many of these species' enzymes are well characterized. This enables a somewhat rational approach to pathway design and genetic engineering in these species for the production of higher alcohols. Since these species did not evolve to produce large amounts of solvent product, however, challenges in rewiring their metabolism and enabling product tolerance are significant, and engineering efforts rely largely on inputting heterologous pathways from other species.

E.coli have been used as an effective host species for reconstructed metabolic pathways from alpha-keto acids to a diverse array of alcohol products (Atsumi 2008; Bastian 2011). Pyruvate and 2-ketobutyrate are the best representative direct inputs to alcohol synthesis by this decarboxylative pathway, and are themselves commonly derived from glucose and from other amino acids (threonine). Enzymes of the Ehrlich pathway enable conversion of the keto-acid to an aldehyde (the decarboxylation), which in turn may be reduced to an alcohol by NADPH or oxidized using NAD(P)+ to a carboxylic acid (side product).

E. coli have also served as a platform for the heterologous expression of the CoA dependent pathway from Clostridia (Inui 2008, Atsumi 2008). With various gene deletions for competing CoA utilizing pathways, NADH boosting engineering steps, and growth in microaerobic conditions, a butanol titer of 552 mg/L was achieved (up from 13.9 mg/L from just pathway input alone).

Saccharomyces cerevisiae naturally produces isobutanol, albeit in low levels, and has a fairly good tolerance to higher alcohols in growth media (Giudici 1990, Knoshaug 2009). Furthermore it is quite tolerant to environmental stresses such as pH and temperature fluctuations. These attributes, combined with human's good understanding have made this yeast a target for genetic modification to produce higher alcohols. By employing overexpression of native alcohol biosynthesis genes along with deletion of genes for competing pathways, and minimal heterologous gene input, isobutanol titers of 143 mg/L have been achieved in microaerobic conditions (Kondo 2012).

Engineered Cyanobacteria

Cyanobacteria can fix CO2 from the air using sunlight as an energy source. For this reason they have attracted much attention as candidates for engineering to produce biofuels (Lan 2012, Wargaki 2012, Rabinovitch-Deere 2013). Efforts to use cyanobacteria to generate higher alcohols hinge on genetic engineering to input biosynthesis pathways from CO2 derived starting compounds to the end-product alcohols. One approach has been to input the CoA dependent pathway from Clostridia, to generate butanol from CO2 derived carbohydrates, although this approach suffered initially from a cofactor mismatch, as Cyanobacteria are rich in NADPH, but the pathway relies on NADH to drive the reductive reactions. The next section will detail these fundamental design issues.

DISTINGUISHING BETWEEN THE THREE TECHNOLOGIES

In this section, several fundamental research and development tools and basic challenges in metabolism will be discussed, so that deeper distinctions can be drawn between the three focus technologies. On this foundation, these distinctions will be further developed in the next section where connections to environmental and economic variables will be drawn.

Genetic modification and other tools

Genetic modification for altering metabolism is central to the future of using bioconversion for alcohol production. It is useful to consider as a group the reactions and enzymes that nature employs for the production of alcohols. This approach is justified by the ease with which genes may be transferred and expressed in model species, and the high degree of commonality between the metabolic networks of all known species (in general) and those relevant for alcohol production (specifically). Since many enzymes are shared, often just a few pathway modifications are sufficient to rewire metabolism towards new products (Huffer, S. 2012, Rabinovitch-Deere 2013). The most common ways to do this are importing genes, deleting genes, random and targeted mutagenesis, and directed evolution in selective conditions. Usually combination approaches are employed (Bastian 2011, Rabinovitch-Deere 2013).

The ABE process gained success without any genetic modification, so is unique in this regard. Now genetic modification is used to improve these strains and will likely serve best to aid selectivity for butanol over other solvents and improve product tolerance. Since Clostridia have the pathway to alcohol product in the native genome, however, engineering of this pathway is not required for success of this technology. On the other hand, the success of technologies based on E. coli, yeast, and cyanobacteria hinge on genetic engineering to create non-native pathways and to modify the rest of the metabolic networks in these organisms to allow growth and survival in light this massive perturbation of the natural metabolism.

In addition to presenting technical challenges, genetic engineering poses multiple ethical and policy challenges. The impact and regulation of genetically modified organisms (GMO) will be a case study in the Environmental Impacts and Policy section, to illustrate how environmental impact, social judgment, and legal policy intersect in developing higher order alcohol technology.

The constraints of Earths' metabolic pathways: yield, cofactors, energetics

The theoretical yield for alcohols (based on nutrient input) has been largely set by evolutionary selection for particular pathways and the overall requirements of biological metabolism. Thus this variable is quite inflexible. Perhaps the key challenge for pathway design and optimization lays in increasing the flux towards biosynthetic products, so that a given number of microorganisms can make more product in the same amount of time. If the carbon uptake and processing rates are higher, synthetic output will increase (González-Lergier 2006). Nevertheless, understanding where the theoretical yields (30-50%) for biosynthetic products in biology come from can aid in selecting pathway modifications (Bastian 2011, Rabinovitch-Deere 2013). The two carbohydrate based technologies have similar limitations on the theoretical yields of their product, and the product energy content per nutrient input is very similar for all carbohydrate based pathways. Pathways to higher chain species (such as to hexanol as opposed to butanol) will have lower theoretical yield, but the products will have higher energy content, a compensating effect. Starting from the CO_2-derived carbohydrates, the cyanobacteria will show the same trend. Yet since the true starting material for these species is the CO_2, and energy input in the form of light occurs to make the carbohydrates, the yield limitations based on carbohydrate do not define the overall yield limits in this system.

One huge factor in creating species able to make alcohol industrially is enabling the production of NADH or NADPH cofactors at high enough concentrations to drive continued alcohol biosynthesis. Whether NADH or NADPH is required depends upon the operative metabolic pathway and whether the conditions are aerobic or anaerobic. In carbohydrate driven metabolism (*E. coli*, yeast, clostridia), anaerobic conditions favor increased concentrations of NADH, while aerobic conditions supply both NADH and NADPH. Conditions that favor NADH production often limit NADPH production, unless direct conversion routes to NADH from NADPH are available. In CO_2 driven metabolism (cyanobacteria), NADPH is supplied in abundance from the photosynthetic light reactions, and only low levels of NADH are found (Wargacki 2012, Rabinovitch-Deere 2013).

This cofactor-species specificity couples with the specificity of particular metabolic pathways to produce a significant energetic challenge for the genetic engineering of organisms. The three technologies discussed here provide rich examples of these complexities. The CoA dependent alcohol biosynthesis pathway from Clostridia depends on NADH. This works nicely with the native Clostridia growth pattern in anaerobic environments (favoring NADH production), but causes problems when moved into organisms with aerobic-environment dependency (*E.coli*) or organisms that are naturally abundant in NADPH (cyanobacteria).

Efforts to address this NADH versus NADPH cofactor challenge follow several tracks. One approach is to mutate NADH dependent enzymes to become NADPH dependent. This approach was used to enable alcohol biosynthesis through the CoA dependent pathway input in cyanobacteria. Another approach involves input of transhydrogenase enzymes for conversion of NADH and NADP+ into NADPH and NAD+. This was employed to enable the operation of the keto-acid based modified Ehrlich pathway to higher alcohols in E. coli (Bastian 2011,Wendisch 2007). Also important are efforts to upregulate NADH and NADPH production in species by increasing flux through glycolysis and the pentose phosphate pathway, respectively. Often, microaerobic conditions are employed to enable NADH recycling (in conditions of excess) and some NADPH production in species with NADH-based alcohol synthesis pathways but NADPH dependence for growth and survival.

The mutant challenge: a new technological ballgame

Here several more abstract challenges related to bioengineering for alcohol production will be given to aid in understanding how uncertainty related to these technologies can be managed from a technical viewpoint. Naturally evolved microbes tend to have correlated control mechanisms for biosynthesis and morphology changes. Engineered microbes on the other hand are much more likely to have these processes uncoupled, as engineered changes are highly local in the metabolic network and long-range regulations are most easily achieved through evolutionary optimization.

In general, satisfying the energy requirements of cells with a minimum use of carbon input without sacrificing the rate of synthetic output is not trivial. Natural metabolic pathways are expected to operate at local maxima of optimization, and may generally suffer decreased efficiency if only small changes are made. This makes the current cut and paste gene transfer methods for species engineering appear a little haphazard from the evolutionary perspective. Likely the future will hold a better coupling of gene input/deletion methods with directed evolution, so that the metabolic network scale perturbations can be resolved by reoptimization of these metabolic systems.

Despite the above difficulty, synthetic pathways are becoming able to operate and be designed in organisms. Much discussion surrounds putting functional pathways into organisms with inherently high tolerance to use conditions. The usefulness of species for genetic engineering hinges on host metabolic compatibility with the changes; it may be that the metabolic adaptability and evolutionary adaptability of an organism is central to its tolerance of imposed changes in metabolite flux distributions.

CONNECTING THE TECHNOLOGIES TO BROADER CONSIDERATIONS

While the success of higher alcohols as fuels hinges on the level of basic understanding and technological development, the impact that higher alcohols could have in the environment and the marketplace is best analyzed by merging this technical approach with broader knowledge about the environment and the economy. In this section, connections will be drawn so that the reader can refer back to these three technologies as examples when reading the more general presentations in the next sections.

Connecting the technology to environmental impacts and economic forces

The environmental impact of the higher alcohol technologies above vary based on the feedstock they utilize. Since the ABE process and *E. coli* and yeast-based pathways use carbohydrate feedstock, they will require use of more or less arable and vegetation-rich land, a potential drawback. This could be ameliorated by using land that is poor for food crops and ideally of low biodiversity.

Feedstock differences will also influence process economics. The CO2 feedstock for cyanobacteria is potentially cheap, and possibly even of negative value near power plants that seek to dispose of it. If coupled with CO2 sources, the above discussed limits in conversion yield from feedstock to product could become trivial for this feedstock. On the other hand, the carbohydrate based feedstocks for the other two processes are expensive, so the fundamental yield limits of bioconversion may be a significant financial barrier in these cases.

Differences in the potential types of higher alcohol product between the keto-acid/Ehrlich based pathways and the CoA dependent pathway enable some distinction to be drawn in environmental impacts. The straight chain alcohols (especially of even carbon number) have the highest biodegradability, so are expected to have the shortest environmental lifetime, least environmental impact, and lowest toxicity. These can be produced by either pathway discussed. Branched alcohol products are expected to have longer environmental lifetimes and and increased toxicity relative to straight chain. The modified Ehrlich-based pathways can produce branched alcohol products, where the CoA dependent pathway does not.

The distinction between branched and linear alcohol products also has economic implications. Branched alcohol products have higher volatility for a given molecular weight, which enables the use of more energy dense molecules for gasoline applications. On the other hand, linear alcohols may have better applicability to the smaller commodity chemicals market. Thus commodity chemical applications of straight chain alcohols (butanol) are saturated, branched alcohols (isobutanol) are expected to have higher value.

In terms of predicting the potential novel environmental impacts of engineered species, it may be fruitful to consider their potential role in an evolving microbial ecology, where gene transfer and species evolution may take place. Engineered species often produce substances that were not detectably produced before, an 'all vs. nothing' type of change that could create new selection pressures. If designed species produce only products common in nature there is more precedent and direction in predicting impact. If substances were not previously found in nature, additional challenge is present. At some level this analysis merges with the environmental toxicological view of substances in nature. Here it is simply recognized that significant environmental exposure to new substances could originate from genetically modified species.

The ABE process is much better developed than the approaches founded in bioengineering, but with increased understanding of protein signalling and enzyme mediated metabolic networks, this could be subject to change. Perhaps the three main factors to consider when looking into the commercial viability of these bioconversion technologies are feedstock source/cost, conversion yield, and conversion flux (product mass produced per time).

CO2 is an ideal feedstock, and is ultimately the feedstock for and process, but carbohydrate based methods require an initial biomass generation step (not part of the alcohol generation process). Conversion yield is limited by nature to 30 to 50%, based on starting material, product molecular weight, and growth conditions. Flux is perhaps the variable most able to be manipulated by orders of magnitude with better bioconversion technology, and related directly to the rate a factory of a given size can supply product to the economy. For processes that have a value increase on product relative to starting material (taking yield into account), higher flux directly translates to increased profitibility.

II. Economics

Let us step back briefly and review some key economic concepts that pertain to biofuels.

TECHNOLOGY

Economic activity consists, at its heart, of the transformation of factor inputs into outputs. These inputs have classically been represented as land (natural resources), labor, and capital – a mutually exclusive and collectively exhaustive set. The conversion is what is meant by *technology*, and to the extent that this conversion evolves to become more efficient, that is, a higher ratio of output to inputs, this is what is meant by *technological advance* (Solow 1956).

Technology, at least in modern times, is also closely linked to science, the "harnessing of a phenomenon for some purpose." It is also best thought of not as any single element, but as a complex system, or more accurately as combinations of recursive subsystems (Arthur 2009). In the context of biofuels produced via bioconversion, there are large subsystems such as feedstock procurement, conversion, processing, use for transportation. Within one of these, conversion for instance, there is sugar production, microbe/enzyme development, fermentation engineering, separations, distillation, etc. Within microbe development, there is transport, pathway analysis, pathway engineering, energetics, heat and pH optimization, etc. These have to be properly coordinated with the other subsystems, for example heat optimization with fermentation engineering and distillation.

ECONOMIES OF SCALE AND LEARNING

As the production volume of a good increases, the per-unit cost of that production often decreases due to non-linear relationships between inputs and outputs. For instance, a fermentation facility producing 1000 gallons of ethanol per day may require ten workers, for all the different tasks to be completed efficiently, but to increase production from there to 10,000 galoons per day may require far less of a labor increase, perhaps a doubling. This is known as *economies of scale*. (Some inputs are still linear, of course, as with sugar volume required.) The economies of scale phenomenon may also have a purely physical basis, such as the so-called square-cube law, where the volume of an object increases in the third-order while the surface area increases only in the second order. This is particularly relevant in the context of liquid fuels, where much of the capital equipment is in the form of tanks whose costs are determined in large part by the area of material required for their construction.

A related but quite different mechanism by which per-unit costs decrease is *learning economies*, whereby the manufacturing workers, engineers, and managers of a plant gain tacit knowledge of more efficient operations, thus lowering costs as time goes by (Arrow 1962). Comparing this to economies of scale, its potential provides considerably less incentive to be the leader into new processes, to the degree that the gains it offers are easily appropriable by other following firms.

Path-dependency and Lock-In

We typically think of the competitive landscape of the market as providing a selection mechanism, where products that achieve the needs of users at the lowest cost succeed while those that do not meet these criteria fail. While this picture is accurate, it is not sufficient to understand reality. This does not take place in a static vacuum, but within an interdependent economy developed through a historical path and moving forward through time under the force of human actors. Selection takes place not on a level playing field, but in consideration of connections to complementary elements and already-sunk investments. This is the concept of *path dependency* (David 1985), and is a large contributor to *technological lock-in*, where economically suboptimal technologies persist, the system unable to escape the pull of local optima (Arthur 1988; Unruh 2000).

This is in large part driven by network effects. That is, there is a benefit derived from the fact that other consumers have gravitated towards one particular technology, causing non-price-based advantages. For example, in deciding on a new car, the customer may perform an analysis of fuel costs and performance characteristics and determine that a natural gas vehicle gives them the best value. But because very few other people are driving natural gas vehicles, the refueling stations are infrequently located on local roads. This is a network effect driven by the need for complementary system elements within the transportation fuel system to be aligned. Large fleets of vehicles can overcome this by switching all at once and by having a central fueling location, but this is not the case for the large majority of the driving public.

Externalities

Markets rely for their proper functioning on the balancing of producers' marginal costs with consumers' marginal benefits. Its great power as a system of social coordination lies in its harnessing of private incentives to maximize social value – trades will occur wherever there is value to each party, maximizing social utility and incentivizing entrepreneurs to develop new untapped forms of value creation. This system relies, however, on certain important assumptions, one of which being that private costs and benefits fully reflect social costs and benefits. Wherever this is violated and either is left *external* to the economic valuation, there will be a misallocation of resources and a loss of social value.

Environmental pollution is a classic case of externalities. Take for instance the pollution of a river by farmland: The farm owner improves the fields, seeds and fertilizers, and hires employees into various roles. The aggregate of these comprise the costs of production, which when set against consumers demand preferences and income levels determines price and a quantity produced. Social utility is maximized, or so it would seem. But what of the fertilizer and other effluent dumped out of the back and into the river? This have no cost to the farm owner, and so play no role in the setting of optimal price and quantity produced, but as they cause eutrophication of the river, killing fish and making the water unsafe for other uses, they have serious costs to the downstream communities and society as a whole.

FINANCE

How are new technologies developed and the plants required for their production built if there is no money to pay for these things? Entrepreneurs may have an invention or an idea but in order to bring it to commercialization they must purchase equipment, hire scientists and engineers, and keep the lights on over the course of sometimes many year R&D periods. Once a technology is ready for commercialization, huge expensive plants must be built and enormous amounts of inputs purchased, particularly in order to realize the economies of scale required to drive down costs to a competitive level. If this is taking place in a large firm, there is capital available on the balance sheet to pay for this, but it is still in competition with other uses towards which the firm could put it. In a small or start-up firm, even this is not available. This is the place of *financing*.

Focusing on the small firm for the moment, there are two broad categories of financing that are available: *debt* and *equity*. In debt financing the issuer, often a commercial bank or syndicate of banks (private debt) or bondholders (public debt), provides capital to the borrower under a contract whereby the borrower agrees to return this amount, plus interest, after some predetermined period. The rate of interest can be thought of as the cost of borrowing, or the cost of capital, and is variable, rising as the credit of the borrower is weaker or if as risks concerning technology, regulation, offtake, input costs, are considered to be higher. If the borrower cannot honor the debt contracts to which it is party, it must renegotiate their terms or, absent this, declare bankruptcy.

Equity financing is structured differently, with the financiers acquiring ownership rights to a piece of the firm in exchange for their capital. Here there is no risk of bankruptcy, but the original owners' ownership share, and so eventual share of profits, is diluted. Venture capital is a form of private equity, focused on early-stage companies, whereas stock markets are forums for the issuing of public equity.

Early stage biofuels companies would expect to get small infusions of equity funding along with grants from foundations or the government to finance their early operations. Once some proof-of-concept has been achieved, and amounts of approximately $1-5 million are required, they may look to venture capital firms. Subsequent rounds of veture capital investment will occur as capital requirements grow, but the percentage of equity the investors receive for the same dollar amount will decrease, reflecting the lower risk of as the technology becomes less uncertain. Once capital is required for the construction of production facilities, the technology risk will have shrunk to the point where debt financing is economical. To the extent that the macroeconomic environment is amenable, this is also the stage when a company would either issue public equity (stock) on a stock market, in the form of an IPO, or be sold to a competing or complementary firm. Either of these last events is known as an 'exit' whereupon the founders and equity investors split the sale cost according to their ownership shares.

These Concepts Applied to the Current Biofuels Marketplace

The current market for transportation fuels is dominated by huge companies of worldwide reach that comprise some of the largest business firms in the world, some of which are arms of national governments. ExxonMobil, the largest of the privately held firms, for instance, had revenues in 2012 of $482B, greater than the GDP of Austria, the world's 27^{th} largest economy (United Nations 2012; ExxonMobil 2012). A typical world-scale oil refinery complex may cost upwards of $9B to construct, with the expectation that it operate for 30 or more years. Needless to say, it is an industry that relies on scale economies and carries enormous sunk costs. While many oil companies are pursuing alternatives this petroleum-based system, it is easy to understand why they are not eager for change to come soon. Many oil refiners are back-integrated into exploration and extraction, but those that are not would be expected to be more interested in changes, as they are more exposed to the price volatility of the crude oil market.

From the early days of the 20^{th} century, the oil and gas industry has grown up alongside two other prominent industries, chemicals and automobiles. With chemicals, they share a common feedstock, with chemicals manufacturers utilizing the light fractions (C_5 and below) of crude oil distillate, increasing its value, and with automobiles, oil companies of course share a common customer. These are emblematic examples of complementary technological systems discussed above. With automobiles, we see a clear demonstration of path dependencies as well, with the engines these manufacturers have developed, designed for use with petroleum fuels. That ethanol (and to some extent higher alcohols), with corrosive hydroxyl groups and high hygroscopicity, is incompatible with current engine designs is nothing inherent to the internal combustion process, but is a result of the historical paths that engine design has taken.

Following the energy crises of the 1970s, concerns of depleting petroleum reserves, and repeating cycles of over- and under-capacity, a number of firms began pursuing ethanol production, with ethanol plants today dotting the landscape of the Midwest US and other countries, particularly Brazil. In the United States, however, ethanol production has not proven to be commercially viable, at least not without government mandates and supports, and so many of these ethanol plants sit unused, or "stranded" in the parlance of economics. This provides an opportunity to higher alcohol producers, as these plants can be acquired at a far lower cost than it would take to build a new plant. This greatly decreases the need for financing, particularly at the later development stages when a technology is not yet fully proven and so access to capital is difficult, a time referred to as the 'Valley-of-Death' when so many firms falter.

Related to this same 'Valley-of-Death' concept, particularly today when economic conditions make capital particularly inaccessible and investor faith in industrial biotechnology has been shaken, many biofuels developers are pursuing a business model where they license their technologies and do not get involved with the production of fuels. Coupled with this, many firms are modifying their technologies for the production of platform chemicals, whose lower volumes and higher profit margins make them much more attractive to produce in-house.

IV. Representative Companies

GEVO

Founded in 2005, Gevo combines an engineered yeast biocatalyst and proprietary separation technology with an ethanol plant retrofit strategy as it pursues a narrow focus on isobutanol production. The company was founded on technology developed at UCLA and California Institute of Technology and received early-stage financing from Khosla Ventures and Virgin Green Fund, as well as Malaysian Life Sciences Capital Fund and Burrill & Company. Early grants came from the DOE SBIR program and the DOE/USDA Joint Biomass Research and Development Initiative. Gevo issued an IPO in early 2011 (NASDAQ: GEVO), with its stock price initially in the $20 range. It sits today just below $2.

The company currently operates a 22 MGPY plant in Luverne, MN, which went online last summer and produced an initial run of 150K gallons of isobutanol. In addition to complications arising from Butamax's lawsuit (see below) production has been interrupted by higher than acceptable levels of bacterial contamination in their fermenters, forcing a short-term switch from isobutanol to ethanol production in order to draw some revenue. They are still very much 'learning-by-doing,' but are doing this with reduced costs, $12.6M in 1Q13 compared to $25M in 4Q12. Notable partnerships include Cargill (cellulosic technology licensing) and Total (equity investment and non-binding off-take), and the US Navy (offtake for up to 15K gallons of isobutanol-based jetfuel).

BUTAMAX

A joint venture between DuPont and BP, Butamax has entered into agreements with ethanol plant operators representing over 1 billion GPY capacity to retrofit their plants for biobutanol (n-butanol and isobutanol) production. With a demonstration plant in Hull, UK, the company expects to complete its first installation during 2013, with the technology available for full licensing to partners during 2014. Notable partners include BioArchitecture Lab, with whom Butamax is carrying out an $8.8M ARPA-E grant developing technology to convert sugars from macroalgae (seaweed) into isobutanol.
It is important to draw attention to a series of patent infringement lawsuits brought by Butamax against Gevo in 2012, alleging two separate patent infringements – one covering the deletion of pyruvate decarboxylase, an essential step in shutting down microbial production of ethanol in order to shunt the pathway towards other alcohols (US/8017375), the other challenging the distinction between NADPH- and NADH-dependent pathways (US/7993889). While the courts have now ruled in Gevo's favor in both cases, the process considerably slowed Gevo's development, taking time and resources, and restricted them from selling isobutanol in the automobile market during 5 weeks in Summer 2012.

JOULE

While focusing on a wide range of products, of which higher-order alcohols are only a minor focus, Joule Unlimited presents a worthwhile comparison to traditional fermentation-based technologies. Combining engineered cyanobacteria with complex bioreactors, Joule's production process requires only sunlight, CO_2 (flue effluent), and non-potable water as inputs. Their first product, ethanol, is currently slated for world-scale production in the 2015 timeframe at a target cost of $1.28/gallon, which would be achieved with a per acre yield of 25K gal/yr. They have to date achieved 15K gal/acre/yr in the lab and 8K gal/acre/yr outdoors.

SAFFRON EAGLE

Still in stealth mode, Saffron Eagle Biofuels was founded in 2012 by Jay Keasling, synthetic biology pioneer, Director of JBEI at UC Berkeley and co-founder of biofuels company Amyris. It is aimed at developing five-carbon alcohol fuels through the isoprenoid pathway.

V. Environmental Impacts and Regulation

Identifying the different types of environmental concerns and presenting the different ways proposed for addressing them is an important first step in defining the 'selection environment' that guides technological advance. Multiple indicators exist for comparing environmental impacts of higher order alcohol bioconversion, versus conventional petroleum-derived liquid fuel. These indicators vary both in terms of extent of impact vs. conventional fuel, and the difficulty in monitoring and quantifying them. In fact, there is little correspondence between the extent of impact of a technology, and how readily this impact can be measured (Table 1). This results in inconsistencies in extent of regulation across impact categories.

The generally favorable public perception of biobased technology for liquid fuels (Craig Vaughn, BP, *Pers. comm.*), combined with limited regulation in practice, illustrate how regulatory response is governed by community perceptions. For example, examining current regulations on ethanol plants, despite the wide range of potential effects (Table 1), the measurement and regulation of potential impacts of first-generation refineries in Kansas and Iowa is limited to Clean Water Act and Clean Air Act violations (Selfa 2010). Further, surveys of local community leaders and members, and regional environmental advocacy organizations indicate a general perception that environmental burdens are acceptable, versus more deleterious energy sources such as coal combustion.

Despite community perception that biobased liquid-fuel development for higher order alcohols will have net environmental benefits, LCA work to date paints a more equivocal picture. On the positive side, higher order alcohol development is anticipated to have clear benefits for life cycle energy use and GHG emissions, compared to conventional petroleum. Such was the finding of a LCA comparing corn-based butanol development versus conventional gasoline, indicating approximately 50% reduction in energy and GHG emissions (Wu et al. 2007, 2008). Additionally, as will be detailed in the next section, alcohols present much lower human and environmental health hazard than gasoline. However, there are multiple impacts that could be worsened due to biofuel development (including higher order alcohols), largely dependent on the feedstock.

Negative ecological impacts include land use conversion, as a result of cropping to grow feedstocks for fuel (Fargione et al. 2010), although development on currently underutilized agricultural lands would mitigate this impact (Fargione et al. 2008). Other environmental impacts are most likely to vary according to feedstocks and growing scenarios. For example, net impacts to human health as a result of air pollution vary across feedstocks (Fargione et al. 2010). Hill et al. (2009) determine that, compared to gasoline, cellulosic ethanol versus corn ethanol result in reduced vs. increased health impacts from particulate matter air pollution. As another example, use of corn grain to develop ethanol will have substantially greater water withdrawal impacts than conventional petroleum, ethanol from other sources (e.g., corn stover or *Miscanthus sp.*), or oil sands (Scown et al. 2011).

Table 1. Categorizing potential environmental and human health impacts of biobutanol technology.

Impact Type	**How Readily Measured or Quantified**[a]	**Anticipated Life Cycle Impact**[b]	**Current US Regulations**
Energy use	1	1 (Wu et al. 2007, 2008)	
GHG emissions	1	1 (Wu et al. 2007, 2008)	Clean Air Act
Hazardous air pollutants[c]	1	1-3 (Hill et al. 2009)	Clean Air Act
Water quantity	1	1-3 (Scown et al. 2011)	Safe Drinking Water Act
Surface water quality (eutrophication)	2	3	Clean Water Act
Solid and Hazardous Waste	1	1-2	RCRA
Pesticide release/exposure	1	2	Clean Water Act; FIFRA
Land use/occupation	1	2 – 4 (Fargione et al. 2008, 2010)	
Occupational injury	2	Unknown	OSHA
Nontarget/biodiversity risk (e.g., Species or GMO invasion)	2	2 – 4 (Andow and Zwahlen 2006)	Endangered Species Act
Aesthetic values (e.g., visual, odor)	3	2 (Selfa 2010)	
Gene flow from GMO	3	1 - 2	TSCA

a. 1 = Straightfoward to measure; precisely quantifiable. 2 = Possible to measure with some effort; quantifiable with some uncertainty. 3 = Very difficult to measure; high uncertainty.

b. Impact for entire life cycle (planting, harvesting, processing, transport, and end use) compared to petroleum derived liquid fuel. 1 = Beneficial (positive impact). 2 = Low negative impact. 3 = Moderate negative impact. 4 = High negative impact.

c. Including EPA mandated criteria air pollutants (O_3, SO_2, NO_2, Pb, CO, PM-10) as well as other toxic air emissions (e.g., benzene, toluene, heavy meta

Since use of microalgae for fuel is highly novel, there are a wide range of hazards that must be considered. In a framework for sustainability analysis, Zhu and Ketola (2012) described potential hazards including water overuse and pollution, ecosystem impacts of invasion, GHG emission via respiration, and potential for disease (e.g., mosquito-borne illness). Surprisingly, using currently available technology, the energy, GHG, and water quantity footprint of algal biomass is worse than corn (grain plus stover), switchgrass, and canola (Clarens et al. 2010). This result is largely due to the reliance on generation and release of large amounts of CO2 and nutrient-derived fertilizers, which are costly in these metrics. The energy and GHG impacts of algae could be mitigated by reuse of certain types of partially treated waste waters (e.g., activated sludge from sewage treatment works, or source separated urine), and carbon sources (e.g., from flue gas of power plants), which would require colocation with these other industries. Nevertheless, these findings of Clarens et al. (2010) emphasize that, in practice, upstream energy and materials requirements may cancel out potential efficiencies of synthetic biology. Therefore, higher order alcohol alternatives that rely on existing platforms (e.g., "drop in" fuels) and efficiencies will be at a competitive advantage on both environmental and economic fronts.

Life cycle assessments and impacts assessments to date focus largely on energy or resource use impacts of broad technologies. For higher order alcohols and associated technology, there are a number of areas for novel research. An interesting way to frame the evaluation would be to explicitly contrast impact of bioengineering vs. other forms of engineering technology in improving energy and GHG footprint. That is, what are the potential gains that could be achieved by each technology, and how do they compare to the current status quo. Another question is the environmental footprint of biotechnology. Using bioengineered organisms as an indicator, we could examine the percentage of the fuel production process that is attributable to bioengineering.

In summary, despite potential energetic and blend wall benefits of higher order alcohols as a fuel source, lifecycle environmental impacts will likely be governed by feedstock production, and warrant further investigation. Energy and resource intensive crops, such as corn grain and sugarcane, are likely to have high environmental impacts. Cellulosic fuels from low intensity crops (e.g., *Miscanthus sp.*) would have reduced impacts. In addition to general life cycle impacts to the environment, potential health impacts of the fuel types also warrant comparison. In fact, there is a profound contrast between conventional petroleum and bio-based alcohols in terms of health hazards associated with their use. This contrast is highlighted and described in the next section.

CONTRASTING IMPACTS CASE STUDY: HEALTH HAZARDS OF GASOLINE, ISOBUTANOL, AND N-HEXANOL

The twelve principles of green chemistry include the design of inherently safer chemicals that are readily degraded and pose lower risk and impact due to human accidents (Anastas and Eghbali, 2010). Higher alcohols as potential fuels are readily compared to gasoline, to aid in understanding their health and safety hazards relative to the current primary vehicle fuel source. Although hazards are present in all life stages of compound development, this section focuses on the chemical properties of the fuel products, themselves, as people will be more exposed to them (i.e., greater "exposure intimacy"; Nazaroff et al. 2012) than compounds used in their development.

Isobutanol, n-hexanol, and conventional unleaded gasoline illustrate the chemical behavior and risks of a range of potential fuel types. Isobutanol is a moderate molecular weight fuel with highly desirable potential energy output. Hexanol is one of the longer chain alcohols, which are in fact sometimes evaluated as a general class (OECD SIDS, 2006). Gasoline is the "status quo" compound to which these alternatives are compared. Gasoline is a mixture of compounds, including a range of hydrocarbon "blocks" (MacLeod et al., 2004), in addition to additives to oxygenate and raise octane level (i.e., MTBE, ethyl-TBE, and tertiary amyl methyl ether). Of note, these additives would be largely unneeded for higher order alcohols, which also serve as oxygenating agents. The alternatives were evaluated as single compounds; as such, all comparisons should be considered provisional, and contingent upon the mixture composition used for alternative fuels, and whether additives are needed.

In terms of physical/chemical properties (Appendix Table 1), isobutanol, n-hexanol, and gasoline are quite similar. They all exhibit low reactivity, high flammability, and moderate flammable range, having very low acute toxicity (oral and dermal and LD50 ranging from 0.7 to >5 mg/kg, similar to table salt at 4 mg/kg; Klaassen and Watkins III, 2010). To the extent that there are differences, isobutanol and n-hexanol are generally safer alternatives to conventional gasoline, in that they exhibit higher flash points (i.e., lower risk of flammable explosion), apparent lower skin irritation, and are not listed to cause lung damage when inhaled (Appendix Table 1). Additionally, the alcohols have lower toxicity and bioaccumulation potential than conventional gasoline (Appendix Tables 2 and 3), largely due to their greater polarity (shorter chain alcohols), and degradability by beta-oxidation and related enzymatic pathways to nontoxic products. Gasoline exhibits more indication of potential for neurotoxicity, greater developmental hazard, potential cardiovascular and renal effects, and may be fatal if swallowed (Appendix Table 2). Whereas apparent carcinogenicity in n-hexanol and isobutanol are low, gasoline exhibits moderate evidence of carcinogenicity. In particular, a rat gasoline inhalation exposure study resulted in kidney tumor development, and gasoline contains benzene, a known carcinogen. In summary, available evidence suggests reduced human health hazard would result from transitioning from gasoline to the C4 or C6 alcohols under consideration.

Of note, longer chain alcohols (e.g., 1-tridecanol and longer) have log Kows at 5.5 and greater, indicating the potential for bioaccumulation for these compounds. Nevertheless, they all have high metabolism in mammals, and very high measured environmental biodegradation rates, with the majority of compounds degraded > 60% within a 10 day window. As a result, the probability of biomagnification is very low (OECD SIDS, 2006). Efficient natural enzymatic pathways exist to extract energy from long chain alcohols. Thus, the environmental persistence of long-chain alcohols should be lower than alkanes and arenes of the types found in gasoline.

Most of the chemical output to the environment stemming from biofuel use will be either direct output of alcohol or alcohol-gasoline blend or exhaust output from combustion of these liquids. Although isobutanol, n-hexanol, and gasoline all have low environmental persistence, short term effects of environmental exposures are likely to be reduced by the replacement compounds (Appendix Table 3). In particular, gasoline appears to exhibit greater acute aquatic toxicity than the replacement compounds. For example, reported 48 hr *Daphnia* LC50 is 13.4 mg/L for gasoline, whereas 24-48 hr *Daphnia* EC50 is 200 and 1300 mg/L for n-hexanol and isobutanol, respectively (Appendix Table 3). The compounds also differ in multimedia partitioning, with gasoline predicted to rapidly partition into air, while isobutanol and hexanol mostly partition into water. Although surface water spills are likely to degrade rapidly in all cases, the potential for a large groundwater contamination event appears greater for the replacement alcohols. Groundwater contamination could result from their phase partitioning into water and the relatively low biodegradation rates that occur in groundwater. Potential for bioaccumulation is reduced for the replacement compounds due to lower Kow.

A last important comparison is needed between tailpipe emissions from gasoline fuel, blended alcohol-gasoline or alcohol-diesel fuel, and pure alcohol fuels. Studies are somewhat limited in number, but indicate that more alcohol in fuels will lower emissions of particulate matter and carbon monoxide, while raising emissions of NOx, and possibly raising unburned fuel emissions (Giakoumis 2013, Gravolos 2013).

A detailed look into different bio-production platforms for higher alcohols will help us predict their potential impact at scale. The three biological pathways to higher alcohols are distinct in their chemical and energy requirements and their chemical outputs. These differences will underlie their differences in impacts. The ABE process is well established industrially as a source of butanol, along with ethanol and acetone (a 2,3,4 carbon chain source) (Ranjan and Moholkar 2012). Process modifications could be achieved by genetic engineering of the responsible clostridia bacteria (usually *C. acetobutylicum*, which naturally performs ABE fermentation). Genetically engineered bacteria and yeast show promise for production of a whole host of alcohols: isobutanol, isopropanol, n-butanol, sec-butanol, isobutanol, 2-methyl-1-butanol 3-Me-1-pentanol (2-6+ carbon chain source). This approach has been enabled by extensive research on these species (Garcia et al. 2011, Huffer et al. 2012). The species used are not necessarily naturally fit for high flux higher alcohol synthesis, but genetic engineering has the potential to make them so. Engineered algae hold promise as a source of higher alcohols using sunlight for energy and CO2 as a carbon source, which would relieve chemical input requirements (Wargaki et al. 2012), although coupling with anthropogenic CO2 and nutrient sources would be required to reduce carbon and GHG burdens associated with obtaining these inputs (Clarens et al. 2010).

IMPACTS AND REGULATION CASE STUDY: REGULATION OF NOVEL BIOTECHNOLOGY

Successes in research and engineering to develop genetically modified bacteria and yeast for carbohydrate conversion into higher alcohols raise the potential for new technological advances and adoption of these new fuel sources. This raises the challenge of understanding how engineered-microbial fuel production will affect the chemical flux through human industry into the environment. There is also uncertainty regarding the perturbation of earth's microbiosphere by industrial scale use of engineered microbes (Tamis et al. 2009). Examination of biotechnology in biofuel development provides a useful case study for how culture, technology, and existing policies intersect to shape the application of new technology. This section reviews literature on the hazards of these technologies, how the hazards are perceived by different communities, how GMO are defined and regulated in different contexts, and current findings on the impacts in agricultural settings, for which considerable data exist.

Several potential impacts from use of these genetically modified organisms (GMO) must be considered. Since the production efficiency of GMO is expected to surpass that of unmodified species, their introduction would result in a greater scale of fuel production using bioprocessing methods. The feedstocks that can be utilized for fuels with such organisms will be key. If they can include high concentration C5 and C6 sugars that are less easily metabolized than glucose or fructose, or if polymeric sugars can be used, more diverse feedstocks can be sought than are currently employed. If these GMO could survive in nature, then they will have some influence in nature, since the sheer magnitude of their use will make sequestration extremely difficult. Despite the fact that they are in contained settings, accidental or intentional releases will periodically occur, and the impacts of these releases must be considered (Tamis et al. 2009).

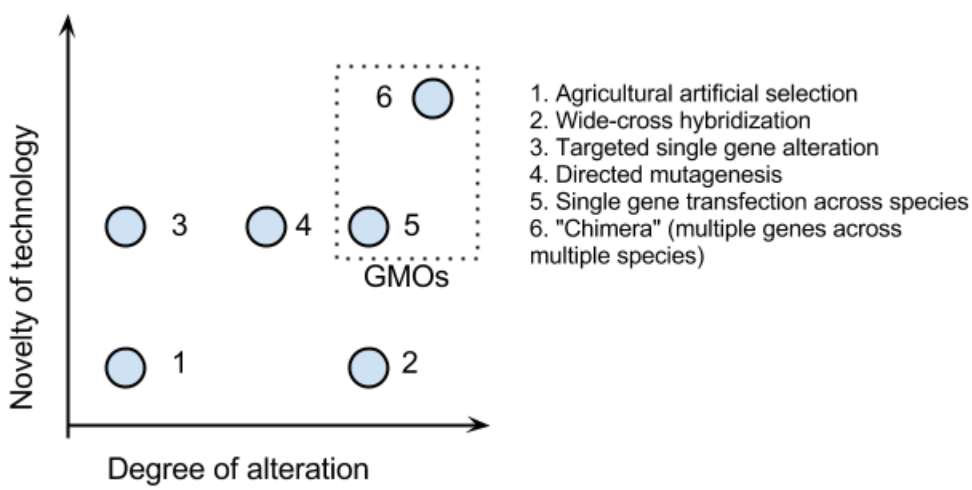
The range of technologies employed for bioengineering is broad, and whether different engineering methods entail qualitatively different risks warrants consideration (Tamis et al. 2009). The legal and regulatory criterion to define GMO is generally organisms containing genes transferred across species lines by recombinant DNA techniques (Winickoff et al. 2005; Miller 2010). For example, based on the Toxic Substances Control Act, the US EPA regulates intergeneric microorganisms in commerce or commercial research, although some exemptions exist (U.S. EPA 2013a). However, proponents of GMO argue that the alteration of heritable traits of organisms is not new; hybridizations across disparate taxa are achieved by humans, especially in plants (Miller 1995, 2010), with the only difference from GMOs being the absence of molecular targeting via currently available technologies.

CONCEPTUAL MODEL TO CATEGORIZE NEW GMO PRODUCTS

Given the diversity of technological innovations that exist, and the widespread use of bioengineering in research for fuel production and other technologies, the application of heuristic structures to categorize bioengineering may benefit its evaluation and regulation

(Tamis et al. 2009). A “taxonomy” of engineering could be organized across two axes (Figure 1): novelty of the technology, and degree of modification. The first axis, novelty, would separate historic methods, such as wide-cross hybridization and other widespread agricultural methods from more recent methods. Presumably more novel technologies may have greater uncertainty of impacts due to lack of precedent and study. For example, traditional single gene insertion has had widespread use in agriculture, providing at least some information on potential risks (Sayre and Seidler 2005; Andow and Zwahlen 2006; Tamis et al. 2009; Seralini et al. 2012). The second axis of GMO evaluation would be extent of genetic modification, which actually encompasses both the amount of heritable material that is altered (e.g., single genes vs. multiple genes), in addition to taxonomic boundaries crossed (e.g., inserting genes from a separate kingdom vs. altering existing genes in an organism). The most extreme example on both axes would be the as yet unachieved development of a “Chimera”, an organism which encompasses a blend of genetic material across taxa and no longer can be accurately described as a single organism (Anthes 2013).

Figure 4. “Taxonomy” of bioengineering based on two axes. Only products within the dotted box qualify under the current definition as genetically modified organisms.



In the context of this framework (Figure 1), we hypothesize that a greater degree of alteration is more likely to result in invasive organisms with high environmental impacts. Directed evolution, random mutation, or site directed mutagenesis are essentially iterative modifications from a known starting point, resulting in alterations to proteins or metabolic pathways already extent within a given organism. As such, the risks of widespread ecosystem alteration or invasiveness may be lower than combining fundamental processes across taxa in the interest of generating highly efficient species. For example, incorporating the Clostridia fuel production pathway into cyanobacteria, and making it NADPH dependent, and therefore able to utilize photosynthesis, could conceivably result in a species that is more efficient, and therefore more dangerous, than currently existing organisms.

Beyond these two axes, a conceptual model of hazard could also categorize biotechnology in terms of some of the assessment metrics currently used in chemical hazard evaluation. This could include anticipated toxicity and environmental persistence, as well as quantity in use and extent to which humans would be exposed (Muir and Howard 2006; Nazaroff et al. 2012). Similarly, Tamis et al. (2009) recommend risk-based testing and classification. Among the principles of green chemistry (Anastas and Eghbali 2010), "design for degradation", accident prevention, and real-time analysis for pollution prevention could also be considered in evaluating and minimizing hazard . To reduce hazard of invasion and consequent environmental impact, "suicide genes", perhaps triggered by the absence of an essential nutrient or element, could ensure that the GMO do not persist in natural settings (Gentry et al. 2004). Additionally, reporter genes can be added to facilitate environmental monitoring for survival in effluent (Gentry et al. 2004).

EXISTING GMO REGULATIONS

The above-described phenomenon of "lock-in" (Unruh 2000) plays a role in driving what environmental issues are currently considered, and the mechanism of evaluation. Historic regulatory infrastructures are in-place, and maintain a form of policy hysteresis that governs which environmental impacts are evaluated. Formal and informal institutions, and associated knowledge bases focus future environmental assessments into these paradigms. In the US, emerging fuel development technology evaluations have focused on existing regulations, such as TSCA, CWA and CAA (for water and air pollution discharge) (Selfa 2010). New biotechnology products including novel chemicals, biochemical compounds (e.g., enzymes), and now genetic materials are also regulated under the existing TSCA program. GMO-based pesticides are regulated separately under FIFRA (Sayre and Seidler 2005).

Although foci of the US review process seem appropriate, critiques have been leveled at the *ad hoc* case-by-case approach for impeding development and application of new technology. In a talk at UC Berkeley, Lindow (2013) indicated that the environmental evaluations have severely impeded ability to perform field-plot evaluations of the yield and safety of grapes genetically modified to be disease resistant. The presenter opined that environmental regulators were generally not focused on scientific concerns or risks, but rather, narrowly focused on implementing bureaucratic regulations to the letter of the law. Not surprisingly, TSCA approved microbial releases have been rather rare. Gentry et al. (2004) indicated that there had only been 11 approved microbial environmental releases since 1998, the majority involving the field release of *Bradyrhizobium japonicum* bacteria, to improve nitrogen fixation.

In contrast to the US, in the European Union (EU) new regulations were developed regarding GMOs, thereby reducing the potential for "lock in" into historical regulatory infrastructure. Another distinction between US vs. EU regulations is that US evaluation is based on the products, themselves, whereas EU evaluation considers the underlying production process (Winickoff et al. 2005). The EU has established separate regulations for GMO use in contained systems, the food supply, medical applications, transport across borders (both within and outside of EU), intentional release for research, and intentional release for commercial applications (LGC Limited 2006). GM crops in particular must be traced and labeled across the agricultural food chain. Prior to deliberate release, a broad risk assessment is prescribed, describing uncertainty regarding risks, and implementing market-based monitoring to evaluate hazards (Levidow et al. 2005). The precautionary principle (deFur and Kaszuba. 2002) is included in EU guidance documentation regarding potential risks, and unlikelihood of impact is not considered an acceptable basis to neglect potential risks (Levidow et al. 2005). However, the international Office of Economic Cooperation and Development (OECD) takes a much more sanguine view towards GMO in contained industrial applications than towards agricultural use or other intentional environmental release (Tamis et al. 2009).

Tamis et al. (2009) observed several flaws in the OECD's *de facto* policy guidance for contained industrial GMO microbe use. The guidelines were published in 1988 and 1992, and serve as the principal basis for current assumptions regarding contained industrial GMO biosafety. They generally draw from longstanding historical experience with industrial biotechnology, and thus could be considered to be a form of intellectual "lock-in" based on prior practices. The OECD guidelines draw a strong distinction between contained industrial use vs. outside deliberate release (e.g., in agriculture or bioremediation). The OECD guidelines indicate that contained uses pose little risk due to the containment, targeted nature of genetic alterations, absence of documented negative effects, and similarity to traditional industrial biotechnology which is considered safe. Among their critique of this interpretation, Tamis et al. (2009) indicate that these policy statements are based on multiple unexamined assumptions, for which factual supporting information is not provided. They were also able to find little recent research on environmental effects of industrial GMOs. Tamis et al. conclude that there is hazard of unintentional release via multiple routes (including air, water, and waste), DNA peristence and transfer, and ecological or public health effects. They recommend a research agenda to continue evaluating the hazards and uncertainties, including establishing biomarkers of GMO release into the natural environment, development of classification schemes, and formalized risk assessment procedures.

Turning back to regulation of non-contained GMOs (i.e., to be used in agriculture or other intentional environmental release), the EU has established new and apparently strict regulations, including evaluation of the production process, and explicit incorporation of the precautionary principle (Winickoff et al. 2005; LGC Limited 2006; Levidow et al. 2005). Given this, one would expect that EU regulation of non-contained GMOs would be protective and sufficiently account for uncertainty. In practice, van Asselt and Vos (2008) find that there is a lack of explicit consideration of uncertainty, which substantially impairs the transparency of GMO technology permitting. According to the authors, in a case study evaluation of the MON 863 maize strain genetically modified to express the *Bacillus thuringiensis* (Bt) endotoxin, the EU-required risk assessment would be better described as a "safety assessment" (van Asselt and Vos 2008). Performed by the industrial proponent (Monsanto), this assessment affirmed no effect, ignored uncertainty, and excluded results indicating potential adverse effects to rats. Government regulators proceeded to summarily accept this conclusion, not because they were corrupt and complicit with the industrial components, but rather because they were similarly unable or unwilling to incorporate uncertainty into their cognition, and the consequent assessment process (van Asselt and Vos 2008).

For GMO, as with any technologies, perception, guided by values and prior experience, strongly influence the likely response. What is striking for GMO technology is the clear disparity in perception between different regions, scientists versus laypersons, and even different communities of scientists. For example, the contrast between the US widely adopting the use of GMO in food crops and EU rejection of these applications until recently, coincided with broad public opposition to GMO in Europe in the 1990s, versus less controversy in the US (Winickoff et al. 2005). Within the scientific community evaluation of GMOs in industrial biotechnology, there appears to be a contrast between the sanguine view of industrial scientists and engineers, and a more concerned stance by conservation biologists and other environmental scientists. For example, in an overview of industrial biotechnology, Chotani et al. (2007) present little attention to risks, noting that regulations are in place, and briefly describing factors that reduce the probability of environmental impact. These include the fact that organisms are designed to not be viable in field settings, use of containment, designed to minimize release into the natural environment (Chotani et al. 2007). In contrast, reviews of GMO research performed by ecologists and environmental scientists detail both the lack of evidence for safety (Tamis et al. 2009) and existing cases where environmental impacts were observed (Sayre and Seidler 2005; Andow and Zwahlen 2006).

The contradiction in perception among scientific communities is important because social factors within institutions responsible for managing and mitigating risks, such as the shared perception and communication of risk, will ultimately impact the extent of risk (Winickoff et al. 2005). For this reason, it is important not to overlook the potential benefits of maintaining communication among scientists, community members, and other potentially affected parties, for ultimately managing the hazards posed by GMO and other novel technologies (Tamis et al. 2009). Further examination of the current perceptions of GMO in different applications by different technical and layperson communities, e.g., via survey instruments or structured interviews, may provide greater insight into the extent and potential impacts of the disparity of perceptions.

ENVIRONMENTAL HEALTH IMPACTS OF AGRICULTURAL GMO USE

Most of the literature on GMO impact assessment focuses on agricultural use, with a notable absence of research on risks of contained industrial release (Tamis et al. 2009). This presumably stems from the greater apparent risk of field applications resulting from lack of environmental containment, use of organisms intended to survive and grow well in natural settings, and potential entry into the human food supply. Given the abundant literature in this area, it is instructive to examine cases of impact. There are multiple findings of pesticide toxicity to non-target organisms. For example, the Cry1ab toxin, inserted into Bt corn, has been observed to increase larval mortality for different invertebrate orders from target pest organisms, including natural pest enemies (reviewed in Andow and Zwahlen 2006). Additionally, a review of 80 published laboratory studies found significant negative effects of transgenic crops and transgenes to natural predators of insect pests in 11% of cases evaluated (Lovei et al. 2009). This finding supports the potential for GMO to alter community dynamics, especially when direct toxicological mechanisms are at play. More relevant to releases of industrial biotechnology organisms, Sayre and Seidler (2005) review effects of microbial GMOs released into the natural environment. Two studies performed in 1991 found that herbicide degrading bacteria altered soil microbiota communities, and a 2000 examination found that a nitrogen-fixing bacteria designed to enhance alfalfa yield exhibited long term field persistance (Table 1 in Sayre and Seidler 2005).

Despite the general lack of research on effects of industrial release of microbial GMO (Tamis et al. 2009), existing information on agricultural application strongly suggests that there will be at least some alteration of existing microbial communities. Maintaining existing community composition is, in itself, a subjective value. The human societal response to alteration of microbial communities is of unknown, and perhaps questionable, strength. Although there have been no instances of GMO "superbug" invasions, invasive species generally have broad ecological and economic impacts (Pimentel et al. 2005), which could conceptually occur across taxa.

IV. Governance

To this point, the discussion has focused on the specifics of the technologies with the potential to produce higher-order alcohol biofuels and the private interests pursuing this goal, but there are also potentially significant societal benefits from such a transition within our transportation fuels regime. Along with the environmental externalities inherent in current petroleum-based fuels, there are also concerns that, to the extent that petroleum is imported from abroad, it supports interests not otherwise in line with US priorities and exposes our domestic economy to macroeconomic shocks as market prices change. For all these reasons, there is a role for government and other governance actors to consider policies and programs that will accelerate change and help overcome the technological lock-in particularly powerful in this highly technical and capital-intensive domain.

But as we have seen, there are multiple and variegated issues at play, many of which are incommensurate with one another, to the extent that they can even be quantified. This presents a particularly difficult governance challenge, the contours of which we will take this final section to present.

EXTERNALITIES

Earlier, we introduced the concept of externalities, but here we address some of the nuances that arise as attempts are made to address them and bring them inside economic decision making. First, as with any policy change, they must be implemented through a political process. Without getting bogged down in a discussion of the current US political climate, suffice it to say, there is little hope that meaningful laws along these lines will be enacted in the near-term. Compounding the issue is that for externalities which operate on a global scale, such as greenhouse gas-driven climate change, global-scale institutions are required – again something that seems unlikely in the near-term.

But putting aside these political considerations, there are real operational challenges that come with mechanisms addressing externalities. For instance: a difficulty in monitoring or measurement, such as with product ingredients that are claimed as confidential; difficulty in quantification of damages, such as with fertilizer runoff, whose impact is related not only to individual effluent levels but also to aggregate levels within an ecosystem (Carpenter et al. 1998); incomplete scientific agreement, as with endocrine disrupting chemicals (Rhomberg and Goodman 2012; Vandenberg et al. 2012); or ethical rather than scientific concerns, such as with some genetic technologies (Winickoff et al. 2005).

RISK, UNCERTAINTY, AND IGNORANCE

Decision makers are continually faced with scenarios where some future effect is unknown. But this can take several different forms – risk, uncertainty, and ignorance – and appreciating the differences among these forms is essential to understanding the different approaches that must be taken (Stirling 2010). *Risk* has two components: some potential harm and a probability that this harm will occur. The essential character of risk is that, at least in principle, both of these values can be known and so its 'actuarial value' can be determined by a decision maker. When society decides, for instance, how much NOx to allow from the tailpipe of a car, decision makers are making a determination that with these parameters the likelihood of harm is below some level we deem acceptable or that the benefits at this level of pollution outweigh the costs. In thinking about emerging technologies, however, we are often without sufficient information to determine risk, and so we must fall back to different positions, with different tools at our disposal. If we can identify a potential harm, but because of incomplete scientific understanding know nothing of its likelihood, we have *uncertainty*. This is the case we face today with the several global-scale threats to complex interdependent systems, such as our climate or ocean ecosystems. Moving one step further, if we know not even what harms to expect, nor their likelihood, we are said to have *ignorance*. Here the case of GMOs is emblematic.

How can governance respond to these different challenges? Addressing risk is something we already do quite often, so we will not discuss it here, except to say that it requires inherent value judgments that must be made transparent and explicit. Addressing uncertainty is more difficult. Rather than focusing on a single element that we are uncertain of, it is something that must be taken up at a systems level. In particular, systems within which it resides must be rich with diversity and flexibility. That is, no single solution should be relied upon and paths of development should be designed so that they are easy to back out of. Diversity in this sense must be thought of in the broadest frame possible. Multiple paths to higher-order alcohols as biofuels? Yes, just as we have seen here, the processes of technological change pursue many paths at once. But in just the same way that technological systems are composed of many levels of recursive subsystems, we must use the same concept to frame what is meant by diversity, traveling up through these levels. Not only alcohols, but other forms of biofuels as well Not only bioconversion, but gasification and chemical conversion as well. And the frame need not - should not - be confined to liquid fuels only. For what consumers and society wants is not the best liquid fuel; this is only a means to an end. What they want is transportation, or mobility, at the lowest (true) cost and with the greatest convenience. Even this is not far enough to go in expanding our mindset. If cities and patterns of life were redesigned so that less travel of any type were needed, this might be the best solution of all. It is this mindset that finally brings us back to the core of Green Chemistry and the holistic way of thinking it encompasses. This case of higher-order alcohol biofuels has provided an excellent case, but it is only one of many that need to be addressed as we seek to transition from the dirty technological systems of today to cleaner, more sustainable systems of the future.

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