Executive Summary

No federal laws have ever been passed to govern the regulation of genetically engineered foods and crops. The regulations in place, cobbled together under existing statutes, require no mandatory pre-market or post-market health testing. When the regulations were legally challenged in the 1980s, the court found they were flawed but did not set them aside, reasoning that they were only a beginning effort to set policy. Instead, the regulations remain largely in place, although weakened over time. One result of this lax oversight is that potentially unsafe practices, such as the inclusion of antibiotic resistance marker genes, have gone forward with far too little scientific and public debate and scrutiny.

Many genetically engineered crops on the market currently contain antibiotic resistance marker genes because of the imprecision of the gene insertion process. Scientists use these genes to determine whether a gene has inserted itself into a target organism. As a result of incorporating these antibiotic resistance genes, these crops threaten the already growing problem of antibiotic resistance, which the world medical community acknowledges as a serious public health concern. Infectious diseases are responsible for one-quarter of all the deaths in the world, second only to cardiovascular diseases.\(^1\) As new strains of bacteria and viruses emerge that are resistant to drugs and antibiotics, infections become more difficult to treat.

The market for genetically engineered crops hinges in large part upon their acceptance by food processors. Food companies such as Kraft Foods, the largest food company in the United States and the second largest in the world, can take the lead and tell biotechnology companies they do not want to put their customers at risk and join the call for an end to antibiotic resistance marker genes. Corporations have set a precedent for this type of action; McDonald’s and other large corporate consumers of chicken have played a significant role in reducing in the use of antibiotics fed to chickens for non-therapeutic purposes.\(^2\) If food processors—as potential customers—clearly articulate that antibiotic

resistance marker genes are unacceptable, manufacturers will have no incentive to continue their use.

Antibiotic resistance marker genes are just one example of how genetically engineered crops should be better regulated, so that products that should never make it to market do not, and so that health concerns are addressed before – and not after – products are commercialized. In order to accomplish this goal with regards to antibiotic resistance marker genes, products on the market with them should be removed, and no new products should be approved that contain antibiotic resistance marker genes. In addition, the State Public Interest Research Groups, along with our coalition partners in Genetically Engineered Food Alert, have issued the following call to action:

Genetically engineered food ingredients or crops should not be allowed on the market unless:

1) Independent safety testing demonstrates they have no harmful effects on human health or the environment,
2) They are labeled to ensure the consumer’s right-to-know, and
3) The biotechnology corporations that manufacture them are held responsible for any harm.
I. Introduction

Genetically engineered crops have hit the market in a legislative vacuum. The U.S. Congress has never passed any law that specifically regulates this radical new technology. In the absence of any directly relevant legislation, federal agencies created a regulatory framework for genetically engineered crops that was tucked into existing statutes not designed to handle genetically engineered crops and thus in many ways inappropriate. The framework for how to handle these new plants, focused largely on the United States Department of Agriculture (USDA), Environmental Protection Agency (EPA), and Food and Drug Administration (FDA), was flawed from its inception. In a public interest legal challenge to the framework in the late 1980s that sought to set it aside, the U.S. District Court for the District of Columbia decided that, although the regulatory framework “is not a model of clarity,” it would stand because it was “merely a first effort to aid in the formulation of agency policy.” Yet the FDA, EPA, and USDA have barely revisited their regulations, except perhaps to weaken them.

That proponents of genetically engineered crops have asked for and received self-policing status on essentially all aspects of genetically engineered crops is a fact that is not in dispute. Even proponents of genetically engineered crops acknowledge that industry has charted its own course. Industry has exerted tremendous influence on the executive branch where, as the former head of biotechnology issues at the Food and Drug Administration acknowledged, the “U.S. government agencies have done exactly what big agribusiness has asked them to do and told them to do.” Industry has also accomplished this feat in part by giving generous campaign contributions to members of Congress. One result of this lax oversight is that potentially unsafe practices, such as the inclusion of antibiotic resistance marker genes, have gone forward with far too little scientific and public debate and scrutiny.

II. What are antibiotic resistance marker genes, and why are they used?

The process of inserting a gene of interest into a plant is crude, haphazard, and random. Scientists cannot easily determine where a gene will land, or even if a gene has been successfully incorporated into a plant cell. There are two common methods of gene insertion. The first involves a “gene gun” that literally shoots microscopic particles covered with DNA at a high velocity into the target organism. The second method uses a type of bacteria, with the gene of interest attached, to infect a plant and thus insert the gene. Neither method is precise, as both methods provide no guarantee where the gene will land in the host organism, or even whether the gene of interest has been inserted into the host organism at all. Genetic engineers use these methods to insert the gene, but need an additional tool to determine if the gene of interest ends up inside the host organism. As a result of this imprecision, scientists use marker genes to determine which plant cells contain the inserted gene of interest. The marker gene is part of a genetic cassette, which also includes the gene of interest and a powerful promoter that functions as a genetic “on” switch. For example, if a genetic engineer seeks to incorporate a gene from a fish into a tomato plant, she could try to insert the fish gene, also attached to the promoter and a marker gene, into the tomato plant via a gene gun. She could determine whether or not the fish gene successfully landed in the tomato cell by looking for the marker gene.

The primary marker genes are either antibiotic resistance or herbicide tolerance. When an herbicide tolerance gene is used as the selectable marker, if herbicide is added to the plant culture media it kills the plant cells that did not incorporate the herbicide tolerance marker gene, while the few cells that did incorporate the gene survive. The same logic applies for the antibiotic resistance marker gene. When an antibiotic is added to the plant culture media, it kills the plant cells that did not incorporate the antibiotic resistance marker gene, and any cells that did incorporate the gene will live.

Beyond the laboratory, the antibiotic resistance marker gene serves no purpose. Yet they are found in many products currently on the market. Appendix A indicates which crops currently on the market contain antibiotic resistance marker genes, and to which antibiotics they are designed to be resistant.

III. What are the human health risks of antibiotic resistance marker genes?

The major concern with the use of antibiotic resistance marker genes is that they will diminish the efficacy of antibiotics in humans and animals. The large presence of antibiotic resistance genes in the environment and soil, as well in the food eaten by animals and humans, could pass the trait of antibiotic resistance rapidly and widely. This can occur through a transfer of antibiotic resistance marker genes to bacteria in the guts of animals or humans, or to bacteria in the environment. Many bacteria have the ability to pick up genes from their surroundings and to pass these genes on to other species of bacteria—including antibiotic resistance marker genes. Such genes might eventually find their way into disease-causing bacteria, resulting in antibiotic resistance and therefore making treatment more difficult.

That antibiotic resistance genes can be transferred to bacteria present in the human digestive tract is a real possibility, one that is only now beginning to be adequately explored. Preliminary research indicates that, in fact, transfer can happen, finding that the human mouth contains bacteria capable of taking up and expressing DNA containing antibiotic resistance marker genes.\(^5\)

Although it is often thought that DNA is rapidly broken down under normal conditions in pieces too small to be functional, there is evidence showing possible DNA uptake. Under certain conditions, direct feeding of free DNA obtained from bacteriophages (viruses that infect bacteria) to mice resulted in some pieces of DNA being taken up into cells in the mouse intestine and other tissues.\(^6\)

A major piece of new evidence on this issue comes from research conducted at the State Institute for Quality Control of Agricultural Products in Wageningen, The Netherlands. Scientists discovered that, in contrast to assurances from industry, antibiotic resistance marker genes could jump into bacteria in the guts of livestock and create


antibiotic-resistant pathogens. Their research demonstrated that DNA lingers in the intestine and confirms that genetically engineered bacteria can transfer their antibiotic resistance marker genes to bacteria in the gut. According to the organizer of the research, “It was a surprise to see that DNA persisted so long in the colon.” The scientist that designed the computer-controlled gut stated that, because the DNA had a half-life of six minutes in the large intestine, “This makes it available to transform cells.” This work adds weight to results from the University of Leeds, in which plant DNA was shown to survive mild processing.

Although antibiotic resistance marker genes are designed to confer resistance only to one antibiotic, the reality is that other antibiotics might be affected as well. Ampicillin resistance genes have undergone point mutations; the gene causes resistance not only to ampicillin, but to an extended range of antibiotics, including clinically important antibiotics. According to the Food Standards Agency of the United Kingdom, “If such point mutations occurred in antibiotic resistance genes used as selective markers, which subsequently transferred into gut micro-organisms, this could have implications for the clinical treatment of serious infections including meningococcal meningitis or any other disease.”

Kanamycin resistance genes also are approved for commercial use, despite concerns about cross-reaction between it and other related antibiotics. Kanamycin is a member of a family of antibiotics that includes streptomycin, gentamycin, and tobramycin. Scientists have demonstrated cross-resistance within this family of antibiotics, and all three of the antibiotics mentioned above are used to treat human diseases. Kanamycin also has a variety of medical applications.

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10 ibid.
12 ibid.
The United Kingdom’s Advisory Committee on Novel Foods and Processes (ACNFP) is self-described as a “body of independent experts whose expertise is acknowledged world-wide.”

Monsanto consulted the ACNFP to see if its aad gene (aminoglycoside adenyltransferase), an antibiotic resistance marker gene, was a human health concern. The Committee’s report criticizes Monsanto’s claims of aad’s safety on a number of different fronts. It faults Monsanto’s conclusions at points for being “at odds with the most recent studies,” and calls data extrapolations the company makes “unwise.” The report notes that the risk from the use of the aad gene, while small, does “give rise to serious concerns at the potential, in some cases life-threatening, implications should human pathogens, in particular Neisseria gonorrhoeae, acquire the aad gene, which confers resistance to streptomycin and spectinomycin.”

The report continues its cautious warning. It states that, although resistance to streptomycin and spectinomycin is widespread (among other factors), ACNFP “take[s] issue with the company’s assertion that this makes the marker safe to use.” In fact, one of the principal pathogens treated by these antibiotics “could potentially acquire the aad gene from a transgenic plant during infection of the alimentary canal. The consequences of this would be severe.” This is because spectinomycin is currently used for resistant strains of Neisseria gonorrhoeae, and diseases caused by this organism, such as endocarditis, septic arthritis, and others, would effectively become untreatable.

In addition to the risk of antibiotic resistance marker genes passing from genetically engineered food to gut bacteria, there is also concern about antibiotic resistance marker genes passing resistance to bacteria in the soil. These bacteria could then, in turn, transfer the resistance genes to other bacteria. Scientists have shown antibiotic resistance genes from genetically engineered tobacco can survive in soil for four months.

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13 More information can be found at <http://www.foodstandards.gov.uk/maff/archive/food/novel/acnfp.htm>


research has demonstrated that a species of soil bacterium could pick up the trait of antibiotic resistance from a genetically engineered sugar beet.\textsuperscript{16}

\textbf{IV. Overview of U.S. government, independent scientists, and industry on the use of antibiotic resistance marker genes}

\textbf{A. Food and Drug Administration}

FDA issued its first Statement of Policy with respect to genetically engineered foods in 1992.\textsuperscript{17} The Statement, which was not a regulation and thus contained no requirements for manufacturers of genetically engineered foods such as mandatory pre-market safety testing, did address the issue of using antibiotic resistance marker genes. The agency had already received a request in 1990 for an advisory opinion from biotechnology company Calgene to use a kanamycin resistance gene in its Flavr Savr tomatoes, as well as other crops. After publishing its Statement of Policy, FDA was not clear whether it would issue an advisory opinion or decide that antibiotic resistance marker genes were in fact food additives, requiring a food additive petition.

In a subsection of “Scientific Issues Relevant to Public Health” in the 1992 Policy titled “Antibiotic Resistance Selectable Markers,” FDA discussed the kanamycin resistance gene in particular and the subject of selectable marker genes in general. The agency acknowledged that the kanamycin resistance gene “serves no further useful purpose” outside of the laboratory and that antibiotic resistance marker genes “may reduce the therapeutic efficacy of the antibiotic when taken orally if the enzyme in the food inactivates the antibiotic.”\textsuperscript{18} FDA also acknowledged that it would be important to study the issue further, which it claims was already underway as a result of the Calgene petition.

\textsuperscript{16} Frank Gebhard and Kornelia Smalla. “Transformation of \textit{Acinetobacter} sp. Strain BD413 by Transgenic Sugar Beet DNA.” \textit{Applied and Environmental Microbiology}. Vol. 64, No. 4. April 1998.
\textsuperscript{18} 57 FR 22988.
FDA issued its final rule on the kanamycin resistance gene on May 23, 1994. FDA accepted the gene as safe, basing its decision on outdated research conducted in 1978 on the rapid digestion of DNA, which is contradicted by more recent research described above. The agency claimed that “it is highly unlikely that the [kanamycin resistance] gene could move from the plant genome into soil microorganisms via horizontal gene transfer,” a claim also contradicted by more recent research. FDA’s rule came despite recommendations from an Advisory Committee convened by FDA to examine the issue of the kanamycin resistance gene. Some members of the committee advised the agency that, if approved, the kanamycin gene should still not be considered safe for use on a wide scale because of concerns about horizontal gene transfer, but rather should be carefully evaluated on a case-by-case basis. This caution has been largely ignored by the agency.

The FDA’s lack of precaution was further compounded by their decision that, for the kanamycin resistance gene, “there is no need to set a tolerance for the amount of [the gene] that will be consumed.” It further stated that “there is no need to require quality control and assurance procedures to ensure that the [gene’s] level” is kept low. FDA claimed that the gene would be unable to interfere with clinically useful antibiotics. The agency also felt that “neither animal studies on the effects of ingestion of [kanamycin resistance genes] on the efficacy of the antibiotics, nor special labeling of foods…are necessary.” The agency was very confident and unequivocal in its conclusion that “compromise of clinical efficacy [of antibiotics] will not occur.”

The last time the agency formally visited this topic was its 1998 draft guidance entitled “Use of Antibiotic Resistance Marker Genes in Transgenic Plants.” The document did not depart in any significant way from the agency’s previous position on the use of antibiotic resistance marker genes, declaring them safe and the risks minimal if at all existent. However, the agency did acknowledge that if antibiotics are the only drug available to treat certain conditions, biotechnology companies should not use them as

20 ibid.
marker genes. FDA also stated that “it is important to consider the possibility that resistance to antibiotics in microorganisms might spread through potential horizontal transfer of antibiotic resistance genes from plants to microorganisms in the gastrointestinal tract or in the environment.” The agency has failed to act on recommendations from experts consulted for the document’s preparation, including recommendations to monitor for transferred antibiotic resistance and to survey present levels of antibiotic resistance among several microbial populations.

As described in this report, new research has cast doubt on the assurances offered by FDA about its own policies. Interestingly, in December 1998, a representative of the UK government’s Ministry of Agriculture, Food and Fisheries commented on the FDA policy, offering a number of risks involved in the use of antibiotic resistance genes that the FDA failed to adequately consider. The letter states: “The widespread use of transgenics carrying antibiotic resistance marker genes will involve a massive amplification of these genes in the biosphere. Whether or not these genes are expressed, amplification on the scale that will occur when transgenic crops are planted in large fields means that arguments about the rarity of possible transfer events will become less significant.”

B. Independent Scientists

Outside of industry laboratories, the medical and health communities share the unanimous opinion that antibiotic resistance marker genes are unnecessary and carry risks. The British Medical Association, for example, has made it clear that “there should be a ban on the use of antibiotic resistance marker genes in GM [genetically modified] food as the risk to human health from antibiotic resistance is one of the major public health threats we face in the 21st century.” The American Medical Association, which has taken a decidedly positive view of genetically engineered crops, has acknowledged

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that “most organizations have concluded that …the use of markers conferring resistance to clinically relevant antibiotics should be phased out.”24 An expert consultation of the Food and Agriculture Organization and the World Health Organization recommended that, for antibiotic resistance marker genes that confer resistance to important drugs, “the possibility of transfer and expression of these genes is a risk that warrants their avoidance in the genomes of widely disseminated GMOs [genetically modified organisms] and foods and food ingredients.”25

C. Industry

Given this widespread agreement among health professionals, crop biotechnology companies have pledged to phase out antibiotic resistance marker (ARM) genes. However, many are failing to do so. Companies engaged in genetic engineering issued a statement in 1998 that they would “remove antibiotic resistance marker genes, wherever possible, from the next generation of genetically modified products.”26 The statement claims that alternatives to the use of antibiotic resistance marker genes “are being developed,” and that it will “take some years before products that will be developed using any of these methods will reach the market place, at least for some crops.” The statement does not make it clear what companies engaged in genetic engineering consider to be the “next generation,” nor does it offer any indication of what will happen to products already commercialized that contain ARMs. And most importantly, it fails to acknowledge that alternative methods are and have been available for some time.

Many of the major companies engaged in research on genetically engineered plants, along with the Biotechnology Industry Organization, recently launched a several hundred million dollar public relations campaign under the auspices of the “Council for

In the Council’s position paper on antibiotic resistance marker genes, industry offers essentially the same arguments as the FDA, namely that “extensive research has concluded that the use of antibiotic resistance markers is safe.” Although industry acknowledges that gene transfer can occur and that DNA may not be broken down entirely by the digestive system, the position paper described these events as unlikely and of little relevance. The Council’s document also claims that no antibiotic resistance genes in use have any clinical value, meaning essentially that they are no longer medically relevant. This claim is discussed and refuted above in Section III. The document does not cite any research post-1998, nor does it state that companies are phasing out the use of antibiotic resistance marker genes. It merely states that industry is “exploring options for markers that do not use antibiotic resistance genes.”

Monsanto put out a document clarifying its position on the use of antibiotic resistance marker genes in 1999. The company claims that “responding to public concern, Monsanto is developing alternatives to antibiotic resistance marker genes and methods to remove them. These technologies are in the research stage and are not yet applicable for commercial products.”

The author contacted all the companies that are part of the Council for Biotechnology Information involved in the production of genetically engineered crops, as well as many other smaller companies, by mail. The purpose was to determine their use of antibiotic resistance marker genes with crops on the market, in their product pipelines, and their general views on the issue of antibiotic resistance marker gene use. Despite follow up with phone calls and e-mails, many companies, such as Aventis and Monsanto, declined to respond to the questions posed. DuPont and Pioneer (now wholly owned by DuPont), for example, were far more helpful. Both companies expressed severe skepticism of any concerns related to risks from the use of antibiotic resistance marker genes, but said that they either had removed products that used antibiotic resistance marker genes entirely (Pioneer) or were in the process of doing so (DuPont).

V. Current issues

Although some companies are saying they will move away from using antibiotic resistance marker genes, Monsanto seems to be investing in this technology. Monsanto was awarded a patent on technology in 2001 that “covers all practical methods of making transformed plants that employ antibiotic resistance markers.” According to the Director of Food Security for the Rockefeller Foundation, “It appears to be just another nail in the coffin of public sector researchers’ ability to produce transgenic plants with their techniques of choice.” Monsanto initially filed the patent in 1983, but a series of delays kept the Patent Office from granting the application until January 2001, long after the technology was in regular use.

Industry claims that alternatives to antibiotic resistance marker genes are in their infant stage and thus can not be used commercially. The reality is quite different; alternatives have existed for more than a decade. Research published in 1991 described one method of removing the marker gene after gene insertion; another method was described in research published in 1997. Other scientists have recently detailed several other methods for removing marker genes. The technology exists where industry’s will may not.

Scientists also are using antibiotic resistance marker genes in crops being field tested and created in laboratories. A search on USDA’s field testing database revealed, for example, that 542 field tests were conducted in 2001 alone with the NptII gene. That

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31 ibid.
number reflects 45.5% of all field tests conducted that year. Another concern regarding inadequate regulations of antibiotic resistance marker genes is reflected in a recent European Commission (EC) decision. Despite the decision of 12 out of 15 member states to remove a specific antibiotic resistance marker from a variety of genetically engineered corn before it could be sold, the EC overruled the member states and approved the crop. If there are not clear guidelines to eliminate their use, antibiotic resistance marker genes will continue to be marketed by manufacturers of genetically engineered foods.

VI. Conclusion and Recommendations

Genetically engineered crops are largely unregulated, and safety tests, if they have been conducted, are rarely subjected to the rigors of independent peer review. The system for monitoring the safety of genetically engineered crops before they come to market, instead of asking questions only afterwards, is weak. In order to bring credibility to the U.S. regulatory framework for genetically engineered crops, there must be a comprehensive, rigorous, and mandatory pre-market approval system that examines genetically engineered crops for their safety for human health and the environment.

No genetically engineered crops should be approved for commercialization that contain antibiotic resistance marker genes. The utility of these genes outside of the laboratory is nonexistent, and they pose risks that are significant. Alternatives are available, and to continue endangering public health for no reason is an illogical approach. All of the crops containing these genes that are currently on the market should be quickly removed.

In order to make consumers aware of the poor regulations in place for genetically engineered foods in this country, the State Public Interest Research Groups helped to form the Genetically Engineered Food Alert coalition (www.gefoodalert.org). The platform of the coalition, which would add significant strength to current oversight of genetically engineered crops, follows.

35 http://www.nbiap.vt.edu/cfdocs/fieldtests1.cfm
Genetically engineered food ingredients or crops should not be allowed on the market unless:

1) Independent safety testing demonstrates they have no harmful effects on human health or the environment,

2) They are labeled to ensure the consumer's right-to-know, and

3) The biotechnology corporations that manufacture them are held responsible for any harm.
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